Ц-РЕАКТИВЕН ПРОТЕИН-МАРКЕР НА ИНФЛАМАЦИЈА КАЈ ПАЦИЕНТИ СО ПНЕВМОНИЈА

C-REACTIVE PROTEIN –BIOLOGICAL MARKER OF INFLAMMATION IN PATIENTS WITH PNEUMONIA

Violeta Mircevska, Sonja Petrovska, Oliver Zafirovski and Maja Jovikj

PHI Institute for Respiratory Diseases in Children, Kozle, Skopje, Republic of Macedonia

Abstract

Introduction. The term biomarkers or biological markers generally refers to proteins measured in the circulation (blood), whose concentration indicates some normal or pathological state or condition. The biomarker is objectively measured and evaluated as an indicator of normal biological processes or pharmacological responses to therapeutic interventions. One of these biological markers is C-reactive protein (CRP).

Aim. To evaluate the efficiency of using adequate and appropriate biomarkers for diagnosis, treatment and monitoring of pneumonia in children.

Methods. This study included 30 patients with diagnosed pneumonia, who were admitted at our Institute. Blood samples were taken prior to initiation of treatment in patients with pneumonia and 7 and 14 days after the treatment. The control group included physically active and healthy individuals without family history of disease, aged from 1 to 14 years. All the results of the biochemical analyses were within the reference values, and none of the control individuals received therapy. In our country, for serum C-reactive protein, as well as for other parameters that determine the diagnosis of the patient, we use a common method for determining normal values, by choosing a huge representative sample from the screening population, consisting of healthy individuals without any disease, especially with no sign of this disease (the children who should be checked up). Further, normal values obtained in this way are taken as reference values.

Results. The average serum CRP protein levels prior to antibiotic therapy were the following: (1-2) $\bar{x} \pm sd = 34.94 \pm 20.44$ mg/L, (3-6) $\bar{x} \pm sd = 68.20 \pm 10.87$ mg/L, (7-11) $\bar{x} \pm sd = 65.59 \pm 12.01$ mg/L, whereas in patients with pneumonia after 7-day antibiotic treatment the values were the following: $\bar{x} \pm sd = 24.64 \pm 11.57$ mg/L/ $\bar{x} \pm sd = 40.10 \pm 6.83$ mg/L/ $\bar{x} \pm sd = 32.09 \pm 6.68$ mg/L ($P < 0.05$),

and after 14-day-therapy $\bar{x} \pm sd = 6.80 \pm 1.14$ mg/L/ $\bar{x} \pm sd = 7.20 \pm 0.82$ mg/L/ $\bar{x} \pm sd = 8.10 \pm 0.89$ mg/L ($P < 0.05$). Also, CRP in serum correlated with the severity of the clinical condition ($P < 0.05$).

Discussion and conclusion. This study is useful for showing the importance of C-reactive protein in serum for early diagnosis and follow-up of the pneumonia. CRP is very important as a biomarker, both from clinical and scientific aspect. It can be used for early rapid diagnosis, monitoring of pneumonia and can successfully be used in a large number of diagnosis. It opens new opportunities for further research in this field.

Keywords: biomarkers, CRP, children, pneumonia

Апстракт

Вовед. Со поимот биомаркери, во медицината се означуваат, најчесто, протеини измерени во циркулацијата (крв), чија концентрација укажува на нормален или на патолошки одговор на организмот, како и на фармаколошкиот одговор кон примената терапија. Еден од нив е и C-реактивниот протеин во серум (CRP).

Цел. Да се види дали Ц-реактивниот протеин како маркер е важен и корисен за брза дијагноза, терапија и следење на пневмонијата кај децата.

Методи. Во рамките на овој труд беа вклучени 30 пациенти со дијагностицирана пневмонија, хоспитализирани во нашиот Институт. Крв за анализа беше земена непосредно пред почнување со терапијата и по 7 и 14 дена по третманот кај пациентите со пневмонија. Во контролната група беа опфатени испитаници на возраст од 1 до 14 години, физички активни, здрави и без фамилијарна историја за одредена болест. Сите биохемиски анализи беа во границите на референтни вредности, ниту еден од испитаниците од контролната група не примаше терапија. Кај нас, за Ц-реактивниот протеин во серум, како и за другите параметри кои ја детерминираат дадената дијагноза кај еден пациент, се применува вооби-

чаената метода за одредување на нормалните вредности преку одбирање доволно голем репрезентативен примерок од испитуваната популација, составен од наизглед здрави испитаници и без клинички знаци на некакво заболување, особено без знаци за постоење на оваа болест (деца дојдени на контрола), така што добиените нормални вредности ги земаме како референтни вредности.

Резултати. Просечната вредност на SRP во серумот пред антибиотската терапија изнесуваше (1-2) $\bar{x} \pm sd = 34,94 \pm 20,44$ mg/L, (3-6) $\bar{x} \pm sd = 68,20 \pm 10,87$ mg/L, (7-11) $\bar{x} \pm sd = 65,59 \pm 12,01$ mg/L, додека кај возрастните групи со пневмонија, по 7 дена од антибиотски третман, изнесуваа $\bar{x} \pm sd = 24,64 \pm 11,57$ mg/L/ $\bar{x} \pm sd = 40,10 \pm 6,83$ mg/L/ $\bar{x} \pm sd = 32,09 \pm 6,68$ mg/L ($P < 0.05$), и по 14 дена $\bar{x} \pm sd = 6,80 \pm 1,14$ mg/L/ $\bar{x} \pm sd = 7,20 \pm 0,82$ mg/L/ $\bar{x} \pm sd = 8,10 \pm 0,89$ mg/L ($P < 0.05$).

Исто така, CRP во серум корелира со тежина на клиничката слика ($P < 0.05$).

Дискусија и заклучок. Од овој труд се гледа дека C-реактивниот протеин во серум е многу важен како биомаркер, и од клинички и од научен интерес, за навремено, брзо дијагностицирање и следење на пневмонијата, може успешно да се искористи за поголем број дијагнози, што отвора широки можности за натамошни истражувања на ова поле.

Клучни зборови: биомаркери, CRP, деца, пневмонија

Introduction

The term biomarkers or biological markers generally refers to proteins measured in the circulation (blood), whose concentration indicates some normal or pathological state or condition. The biomarker is objectively measured and evaluated as an indicator of normal biological processes or pharmacological responses to therapeutic interventions.

One of these biological markers is C-reactive protein (CRP), which is a protein in the human blood that belongs to the pentraxin family [1].

C-reactive protein or acute-phase protein belongs to a group of complex mucoproteins, e.g. complex proteins which are composed of more than 15% of carbohydrate. In addition, CRP in healthy individuals is found in low concentrations.

CRP is produced in the liver and by the mast cells (adipocytes) [2]. CRP is a glycoprotein, known as C-reactive protein, an acute-phase protein found in the blood which increases rapidly in response to inflammation; the CRP levels can increase up to 100 times for

24 hours during acute inflammation. Therefore, in the last few years CRP is frequently used for diagnosis instead of erythrocyte sedimentation, which was used for more than 50 years [3].

CRP is a protein with many physiological roles within the human immune system, such as binding phosphocholine expressed on the surface of dead or degraded cells (damaged by infection, disease or trauma), binding of nuclear antigens and certain pathogenic organisms, which bound in this way, activates the composition of a complement (which participates in the cell destruction) or activates macrophages or bound to a cell serves as opsonin [4]. In clinical practice, CRP is an essential marker for many pathological diseases, such as: malignant diseases, lymphomas, systemic lupus erythematosus, rheumatoid arthritis, vasculitis, pneumonia, heart attack, fungal infection, intestinal inflammation, rheumatic fever, tuberculosis, etc. [5,6].

C-reactive protein is known to be the most commonly analyzed protein of plasma in laboratory diagnostics, which serves as a marker of inflammation. CRP is considered to be an early indicator of infection or inflammation and a marker for disease activity.

CRP is a biomarker used for detecting the inflammation in the human body, but a CRP test cannot show where the inflammation is located.

CRP is essential in distinguishing viral and bacterial infection. In viral infections that are followed by rapid sedimentation and increased leukocytes, CRP levels are normally low in the blood [7,8].

CRP levels are quite increased in invasive bacterial infection.

Besides detection of acute inflammation, measurement of CRP concentration is useful for assessment of the progression of the disease or assessment of the effectiveness of therapy. For example, with successful antibiotic therapy the level of CRP concentration in the serum will fall faster than erythrocyte sedimentation rate (SE), and by monitoring its concentration can prevent unnecessary use of less effective or ineffective antibiotics, hence it gives time to replace ineffective antibiotics with other, more efficient and more effective ones [9]. CRP concentrations drop more rapidly in response to effective antibiotic treatment than erythrocyte concentrations (SE). In contrast, inadequate treatment is reflected in persistently high CRP levels. Monitoring of CRP concentrations can alert to complications of inadequate therapy and is a substantial aid in helping the healthcare practitioners make the right diagnosis and reach the correct treatment decision [9]. The levels of CRP are of crucial importance in diagnostic tests and monitoring of the treatment of infections, especially in premature babies and newborns.

CRP-reactive protein is used to discover whether there is an infection after surgery. CRP normally rises within 2 to 6 days after surgery and then it decreases by the third day after surgery. If CRP stays elevated for 3 days after

surgery, an infection may be present, as a complication of the surgery.

CRP can help in distinguishing bacterial and viral infections, in monitoring the progression of the disease, in evaluating the benefits of the treatment, but it can also be used as a risk factor.

Normal concentrations in healthy human serum are 10 mg/L. High levels of 10-40mg/L are found in pregnant women, mild infections and viral infections, smokers, etc. CRP levels of 40-200mg/L show acute infection, bacterial infection. CRP levels higher than 200mg/L are found in severe bacterial infection and burns [10].

Pneumonia is an inflammatory condition of the lungs and it is one of the most common lung diseases. Pneumonia is due to infection caused primarily by bacteria and viruses and less commonly by fungi and parasites [11]. Pneumonias of bacterial origin, which will be the subject of our investigation may be caused by gram positive and gram negative bacteria. In practice, despite microbiological examination that is used in detecting the etiologic cause, frequently biochemical and immunological methods for differentiation of pneumonia are used. Over the last decade there has been a significant progress in biochemical investigation, inflammation and sepsis. Examinations of the changes in proteins are of the greatest interest in clinical practice [12-14]. There are many acute phase proteins in medicine, but for the determination of acute phase proteins with rapid kinetics, with a short half-life and a wide range of variation in the acute period of the disease, we will broadly investigate C- reactive protein (CRP). This means that as a marker, it has a very large and important role in medical research and its application allows the perception of the mechanism and course of the disease [15,16].

Aim

Determination of the CRP concentrations in serum or whole blood in children suffering of pneumonia, with precise and sensitive immunofluorescence method for determining the concentration of C-reactive protein (CRP) in serum or whole blood (ELFA).

Materials and methods

Of all respondents involved in this study, blood sample was taken on an empty stomach early in the morning without giving anticoagulation agents. In the control

group were included subjects aged from 1 to 14 years, physical active, healthy individuals that had no family history for the particular disease. All biochemical analyses were within the reference values and these subjects did not receive any therapy. In our country, for serum C-reactive protein, as well as for other parameters that determine the diagnosis of a patient, we use a common method for determining normal values, by choosing a huge representative sample from the screening population, consisting of healthy individuals without any disease, especially with no sign of this disease (the children who should be checked up). Further, normal values obtained in this way are taken as reference values. The study group included 30 patients diagnosed with pneumonia.

Blood sample was taken immediately before the commencement of treatment and one month later in patients with pneumonia. In this analysis the quantitative method for determination of CRP in serum or whole blood was used, which is based on laser-epifluorescence immune response (ELFA). The reference values of CRP in serum or whole blood is less than 10 mg / L.

Statistical analysis of data was done by using all variants of Student's t-test. Calculations were performed using the computer program EXCEL.

Values of *p* less than 0.05 were considered to be statistically significant.

Results

This study included 30 patients with pneumonia who were treated at the Institute of Lung Diseases in Children, Skopje. All participants were followed by doctors who work in the departments of pneumonia. Necessary diagnostic procedures for detection of specified disease and initiation of antibiotic therapy were made.

Table 1. Distribution according to age of patients with pneumonia

Age (years)	1-2	3-6	7-11	total
Number of patients	4	10	16	30

Table 1 presents the groups aged 1-11 years, patients with pneumonia, with mean age $\bar{x}=6.43$ while the age of control individuals was in the range from 1 to 14 years, mean age $\bar{x}=7.7$ years.

Fig. 1. Distribution according to sex in patients with pneumonia

Table 2. Serum CRP levels in patients with pneumonia

Number	Group	Age (years)	CRP mg/L
1	Controlled group	1-14	4.97±0.38
2	Group of patients with pneumonia before the treatment	1-2	34.94±20.44
		3-6	68.20±10.87
		7-11	65.59±12.01
3	Group of patients with pneumonia after 7 days of the treatment	1-2	24.64±11.57
		3-6	40.10±6.83
		7-11	32.09±6.68
4	Group of patients with pneumonia after 14 days of the treatment	1-2	6.80±1.14
		3-6	7.20±0.82
		7-11	8.10±0.89

*Mean ± standard deviation

Fig. 2. Serum CRP levels in patient with pneumonia

Figure 1 presents the predominating male gender 19 (63.33) among patients with pneumonia versus female gender 11(36.67).

Table 2 and Figure 2 show the concentration of C-reactive protein in serum in children with pneumonia one month after the application of antibiotics, where the average value of CRP in serum in patients before antibiotic treatment was $\pm sd=62.37 \pm 7.86$ mg/L, while in the group after antibiotic treatment it was $\pm sd=10.29 \pm 0.71$ mg / L.

There was an evident difference in the first group of children with pneumonia before treatment compared to the second group after treatment ($p<0.05$), which indicates the severity of the disease and the concentration of C-reactive protein in serum at the beginning. Furthermore, there was an evident upward trend of the ave-

rage values of C-reactive protein in serum as the disease progressed; the measured average concentrations were significantly higher in the control group ($p<0.05$). In children with pneumonia after antibiotic treatment, the average concentration of C-reactive protein in the serum did not differ significantly compared to the control group ($p>0.05$).

Table 3. The number of positive culture in patients with pneumonia

Positive culture	The number of enrolled patients
Moraxella catarrhalis	4
Haemophilus influenzae	6
Streptococcus pneumoniae	8
Escherichia coli	6
Others	6

Table 3 shows the number of tested patients and isolated bacteria. In the group of children with pneumonia before treatment there were 24 isolated bacteria, while the

number of the rest was either small or there were no isolated bacteria and were placed in the group of other bacteria.

Table 4. Distribution according to the severity of the clinical picture

	Number	Rate (%)	CRP (mg/L) $\bar{x} \pm \text{sd}$
Severe clinical diagnosis	17	56,6	91,55 $\pm$ 8,45
Moderate severe clinical diagnosis	13	43,3	24,23 $\pm$ 2,57
Severe clinical diagnosis-WBC $>16 \times 10^9$, GR $>70\%$, X-ray (massive findings), hypoxia

Fig. 3. Distribution according to the severity of the clinical picture

Table 4 and Figure 3 show the distribution of patients according to the severity of the clinical condition and the correlation of CRP with the severity of the clinical condition of the patients ($p < 0.05$).

Discussion

Increased respiratory infection in infants and young children affects on physical depending on the environment where they live. Unfavorable conditions of the external environment (air pollution, unventilated apartments, smoking) has a detrimental (harmful) effect more on the young than on the older children because the terminal parts of the lungs are less distant from the external environment thus easily enter microbial and no-microbial agents.

Despite exposure to large amounts of these harmful organisms, there are certain conditions which may lead to pneumonia in an individual. Certainly, the lack of normal anatomical structure may increase the risk of developing pneumonia.

Viruses may interfere with ciliary function, letting themselves or other harmful microorganisms (such as bacteria) to penetrate into the lower respiratory tract. Various chronic conditions predispose to infection with pneumonia. These include asthma, cystic fibrosis and neuromuscular diseases that can hinder closing the epiglottis. Esophageal disorders can cause spillage of substances from the stomach back into the esophagus. In childhood, one third of pneumonias are from viral etiology, one

third belongs to unknown etiology, and the rest are probably caused by bacteria [17].

In practice the cause of pneumonia is based on epidemiological, clinical, radiological and hematological tests as well as on the basis of pneumonic lesion response to antibiotics and other treatment. In other words, in practice non-specific, indirect methods of etiological differentiation of pneumonia are often used, not a microbiological examination which is the only authoritative method in detecting the etiologic cause. It is known that pneumonia in infants is more critical than in older children. The severity of pneumonia depends on the etiologic cause, the nature of the lesion, and the degree of intoxication and impairment of lung function which is important for prognosis and therapy.

Pneumonia is divided according to etiological and anatomical criteria [18]. This division is used in everyday work. It is often difficult to diagnose pneumonia, because most often the diagnosis is based on the etiological causes. There are few institutions, including our, where one can identify bacterial etiological factor for pneumonia in children. In the first stage in children with pneumonia of bacterial origin, which takes several hours, interalveolar capillaries expand and the alveoli begin to fill up with serous exudate, rare polymorphonuclear leukocytes and a huge amount of mucus, which has been confirmed by our results.

Also, the values of C-reactive protein as a biomarker in serum in patients with bacterial pneumonia, and the procalcitonin in the serum are confirmed in the studies

of many scientists [19], which state that later in the second phase the alveoli are filled with a number of neutrophils and fibrin; while in the last stage of the disease remains a heavy exudate, the number of macrophages and neutrophils increases and occurs necrosis. This is followed by an increase in sedimentation of the erythrocytes and leucocytes.

Cellular immunity is an important mechanism in defense agents of these intracellular pathogens [20], proving our values of neutrophils in blood. With the advent of penicillin and other antibiotics, modern surgical techniques and intensive care in the 20th century, the rate of death from pneumonia sharply declines. Highly evident is the need for vaccination of newborns, which has been confirmed by our research [21].

Vaccination of infants against *Haemophilus influenzae* type B began in 1988 and led to a dramatic decline in cases shortly thereafter. Vaccination against *Streptococcus pneumoniae* in adults began in 1977, and in children in 2000 and resulted in a similar outcome.

Concentrations of C-reactive protein in serum of patients with proven bacterial pneumonia after applying antibiotic therapy were highly reduced, and were confirmed in investigations of many scientists.

Reduction in use of antibiotics is an essential object in modern medicine, which will result in reduced costs. Avoiding the side effects of treatment will lead to reduction of bacterial resistance. Very often, after taking a detailed history, physical examination, laboratory tests and x-ray of the lungs, clinicians cannot set reliable diagnosis because of overlapping symptoms and signs in bacterial and viral infections. In order to eliminate the uncontrolled use of antibiotics, there is a need for fast and reliable differentiation of bacterial infection from a viral infection of the respiratory tract. In pneumonia which is defined as inflammation of the pulmonary parenchyma, often caused by bacterial cause, there is elevated CRP [23].

Conclusion

The value of the serum C-reactive protein correlates with the severity of clinical form and the evolutionary progress of the disease. Hence, the conclusion that C-reactive protein is an important biomarker of pneumonia as a diagnosis, as well as other diseases. On the other hand, this newly introduced method can be successfully used for multiple diagnoses, which opens up broad opportunities for further research in this field.

Conflict of interest statement. None declared.

References

- Mantovani A, Garlanda C, Doni A, Bottazzi B. „Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3” *J Clin Immunol* 2008; 28 (1): 1-13.
- Ridker PM, Hennekens CH, Buring JE, and Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med* 2000; 342(12): 836-843.
- Claeys R, Vinken S, Spapen H, et al. Plasma procalcitonin and C-reactive protein in acute septic shock: clinical and biological correlates. *Crit Care Med* 2002; 30(4): 757-762.
- Preas HL 2nd, Nylen ES, Snider RH, et al. Effects of anti-inflamminalory agents on serum levels of calcitonin precursors during human experimental endotoxemia. *J Infect Dis* 2001; 184(3): 373-376.
- Rifai N, Ridker PM. Proposed Cardiovascular Risk Assessment Algorithm Using High-Sensitivity C-reactive protein and Lipid Screening. *Clin Chem* 2001; 47: 28-30.
- Korac D. Klinicka imunologija I alergiski bolesti kod dece, 1983.
- De Dooy JJ, Mahieu LM, Van Bever HP. The role of inflammation in the development of chronic lung disease in neonates. 1: *Eur J Pediatr* 2001; 160(8): 457-463.
- Almirall J, Bolibar I, Toran P, et al. Contribution of C-reactive protein to the diagnosis and assessment of severity of community-acquired pneumonia. *Turkish Thoracic Society* 2004; 125(4): 1335-1342.
- Block S, Hedrick J, Hammerschlag MR, et al. Mycoplasma pneumonia and Chlamydia pneumonia in pediatric community-acquired pneumonia: comparative efficacy and safety of clarithromycin vs. erythromycin suspensions. *Pediatr Infect Dis J* 1995; 14: 471-477.
- Kindmark CO. The concentration of C-reactive protein in sera from healthy individuals. *Scand J Clin Lab Invest* 1972; 29: 407-411.
- Falk G, Fahey T. C-reactive protein and community-acquired pneumonia in ambulatory care: systematic review of diagnostic accuracy studies. *Fam Pract* 2009; 26(1): 10-21.
- Kruger S, Welte T. Biomarkers in Community-acquired Pneumonia. *Expert Rev Resp Med* 2012; 6(2): 203-214.
- Sanders S, et al. Systematic review of the diagnostic accuracy of C-reactive protein to detect bacterial infection in nonhospitalized infants and children with fever. *J Pediatr* 2008; 153(4): 570-574.
- Claeys R, Vinken S, Spapen H, et al. Plasma procalcitonin and C-reactive protein in acute septic shock: clinical and biological correlates. *Crit Care Med* 2002; 30(4): 757-762.
- Mitrevski A. Using the levels of CRP in inflammatory proceses of sepsis; 4th. National congres of medical biochemistry, 2005.
- Oh SW, Moon JD, Park SY, et al. Evaluation of fluorescence hs-CRP immunoassay for point-of-care testing. *Clin Chim Acta* 2005; 356: 172-177.
- Torres A, et al. Biomarkers and community-acquired pneumonia: tailoring management with biological data. *Semin Respir Crit Care Med* 2012; 33(3): 266-271.
- Muller B, Becker KL, Schachinger H, et al. Calcitonin precursors are reliable markers of sepsis in medical intensive care unit. *Crit Care Med* 2000; 28(4): 977-983.
- Coelho L, et al. Usefulness of C-reactive protein in monitoring the severe community-acquired pneumonia clinical course. *Crit Care* 2007; 11(4): R92.
- Garcia Vazquez E, et al. C-reactive protein levels in community-acquired pneumonia. *Eur Respir J* 2003; 21(4): 702-705.
- Jloncevikj I, Kanazir M. Immunization of children, 2010.
- Rifai N, and Ridker PM. High-Sensitivity C-Reactive Protein: A novel and Promising Marker of Coronary Heart Disease. *Clin Chem* 2001; 47(3): 403-411.
- General Recommendations on Immunization, MMWR, CDC, 2011.