Interleukin-6 and acute phase proteins as biomarkers of septic osteoarthritis

S. Magomedov, Yu. V. Polyachenko, M. P. Hrytsai, I. H. Litovka, V. I. Sabadosh, N. O. Dekhterenko, T. A. Kuzub

SI «National Institute of Traumatology and Orthopedics of the NAMS of Ukraine», Kyiv

Septic arthritis (SA) is a severe and rapidly progressive joint infection and a potentially life-threatening condition that can affect all age groups. Due to the lack of effective methods for early detection and assessment of treatment outcomes, measurement of biochemical markers (biomarkers) is a promising method for monitoring the disease. The aim of the study was to determine the diagnostic significance of inflammatory biomarkers (IL-6, CRP, haptoglobin, ceruloplasmin) in patients with septic joint inflammation of various localizations. Methods. The study analyzed blood serum from 54 male and female subjects. Of these, 18 were conditionally healthy and entered group I (control), and 36 patients were diagnosed with septic osteoarthritis of joints of various localization in the skeleton. Of these, 18 had SA of the knee joint; 13 patients had SA of the hip joint and 5 had SA of the ankle joint. Results. Patients with septic arthritis of the knee and hip joints had significantly increased levels of IL-6 and acute phase proteins in the blood serum. We believe that it is the enhanced synthesis of the pro-inflammatory cytokine IL-6 and acute phase proteins that plays a significant role in the pathophysiology of the inflammatory response, which initiates a chain of reactions that lead to cartilage degradation and further complication of inflammatory processes in the joint. Therefore, these biomarkers can be tools for diagnosing the progression of this disease in both preclinical and clinical studies. The results obtained emphasize the importance of identifying inflammatory biomarkers for diagnosing the progression of septic arthritis in both preclinical and clinical studies to establish the stage of the disease and predict clinical outcome.

Септичний артрит (СА) — це важка та швидко прогресуюча інфекція суглобів і потенційно небезпечний для життя стан, який може вплинути на всі вікові групи. Через відсутність ефективних методів раннього виявлення й оцінювання результатів лікування, вимірювання біохімічних маркерів (біомаркерів) є перспективним методом моніторингу захворювання. Мета. Визначити діагностичну значущість запальних біомаркерів (ІЛ-6, СРБ, гаптоглобіну, церулоплазміну) в пацієнтів із септичним запаленням суглобів різної локалізації. Методи. У дослідженні проаналізовано сироватку крові 54 осіб чоловічої і жіночої статі. Із них 18 були умовно здоровими і увійшли до I групи (контроль), а 36 хворих мали діагноз септичний остеоартрит суглобів різної локалізації в скелеті. Із них 18 — СА колінного суглоба; 13 — СА кульшового і 5 — СА надп'ятково-гомілкового. Результати. У пацієнтів із септичним артритом колінного та кульшового суглобів були значно підвищені рівні ІЛ-6 та білків гострої фази в сироватці крові. На нашу думку саме посилений синтез прозапального цитокіну ІЛ-6 та білків гострої фази відіграє значну роль у патофізіології запальної відповіді, за якої ініціюється ланцюг реакцій, що призводять до деградації хряща і подальшого ускладнення запальних процесів у суглобі. Тому ці біомаркери можуть бути інструментами діагностики прогресування цього захворювання як на доклінічних, так і в клінічних дослідженнях. Одержані результати підкреслюють важливість визначення біомаркерів запалення для діагностики прогресування септичного артриту як на доклінічних, так і в клінічних дослідженнях для встановлення стадії захворювання та прогнозування клінічного результату. Ключові слова. Інтерлейкін-6, білки гострої фази, септичний остеоартрит.

Keywords. Interleukin-6, acute phase proteins, septic osteoarthritis

Introduction

Septic arthritis (SA) is a severe and rapidly progressive joint infection [1, 2]. It is a potentially life-threatening condition that can affect all age groups. The annual incidence of SA ranges from 1 to 35 cases per 100,000 people in different countries [3]. The mortality rate can be high, ranging from 3 to 25 % [4]. Despite the severity of the disease, many patients do not have classic signs, symptoms, or laboratory abnormalities [4]. Diagnosis is complicated by the large number of conditions that can mimic SA, further complicating the diagnosis. Major risk factors include advanced age, pre-existing joint disease such as rheumatoid arthritis or osteoarthritis, diabetes, immunosuppression, recent joint surgery, intravenous drug use, and systemic infections. The increase in SA incidence is partly due to an aging population and an increase in comorbidities [2].

Each joint of the skeleton is characterized by a certain kinetics of the development of the pathological process, which is associated with symptoms of inflammation, stiffness and loss of mobility. Usually, SA develops in the knee, hip, talocrural joints and in the spinal vertebrae [5]. Diagnosis, which is based on clinical examination and radiography, provides little information about the onset and progression of the disease, metabolic changes in the tissues of the joint. Due to the lack of effective methods for early detection and analysis of treatment results, measurement of biochemical markers (biomarkers) is a promising method for monitoring the disease. Of particular interest are inflammatory biomarkers of SA, which are present in biological fluids such as blood, urine and synovial fluid, sources that are easily isolated from the body [6]. By examining these biochemical markers: cytokines (interleukin-6, (IL-6) and acute phase proteins (C-reactive protein (CRP), ceruloplasmin and haptoglobin) in the patient's serum, it is possible to accurately determine the level and activity of the inflammatory process. After all, it is known that the heterogeneity of the pathogenesis of AS is reflected in the combinations of different markers indicating the degree of joint degradation [7]. Cytokines are small proteins that are secreted by cells and have a unique effect on their interaction and communication. These signaling molecules are responsible for the regulation of immune responses and inflammation, and also cause cell growth and differentiation. Inflammatory cytokines are released in response to tissue damage or infection and have the potential to activate nerve fibers, contributing to the development of chronic pain [8].

Interleukins play an important role in modulating immune responses in various scenarios, from infectious diseases to pain and postoperative period. They act as pro- and anti-inflammatory and have a crucial role in the activation of immune cells, tissue repair and the overall balance of the immune system. Proinflammatory ILs, including IL-6, can lead to tissue damage if overexpressed [9–11]. The production of proinflammatory cytokines is primarily attributed to activated macrophages that induce inflammatory responses. The proinflammatory biomarker IL-6 initiates a chain of reactions that causes cartilage degradation and other inflammatory processes [12]. CRP, ceruloplasmin and haptoglobin are acute phase reactants that positively correlate with inflammation and joint pain in osteoarthritis and have been shown to predict pre- and post-operative outcomes [13–15]. The analysis of the above literature sources proves that AS is one of the most common causes of disability among people of all ages and can affect almost all joints of the skeleton. Each joint is characterized by certain kinetics of the development of the pathological process. However, the most vulnerable among them are the hip and knee. Determination of biochemical markers of inflammation demonstrates their variability for detecting pathological processes in the joints according to SA.

Purpose: to determine the diagnostic significance of inflammatory biomarkers (interleukin-6, C-reactive protein, haptoglobin, ceruloplasmin) in patients with septic inflammation of the joints of various localization.

Material and Methods

The study analyzed the blood serum of 54 male and female subjects. Of these, 18 were relatively healthy and included in group I (control), and 36 patients were diagnosed with septic osteoarthritis of joints of various locations in the skeleton. Of these, 18 (group II) had CA of the knee joint; 13 patients (group III) had CA of the hip joint, and 5 (group IV) had CA of the talocrural joints.

The study was carried out after approval by the Bioethics Committee of the Institute of Traumatology and Orthopedics (protocol No. 4 dated 11.17.2023) in accordance with the Declaration of Helsinki 2000, the European Community Directive 86/609 on the participation of humans in biomedical research and orders of the Ministry of Defense of Ukraine No. 690 dated 23.09.2009, No. 944 dated 14.12.2009, No. 616 dated 03.08.2012. All patients signed an informed consent form to participate in the study.

The concentration of IL-6 in the patients' blood serum was determined on the Cobas 411 analyzer using Roche Diagnostics test systems. The analysis of CRP, haptoglobin and ceruloplasmin was performed on the Cobas 311 biochemical analyzer using Roche Diagnostics test systems.

Statistical processing of the obtained results was carried out using the Origin Pro 8.5 program package. The mean values of the obtained indicators (M) with standard deviations (SD) were determined. The significance of the difference between groups with a normal distribution of comparison was assessed by the Student's t-test. Changes were considered significant at $p < 0.05$. In order to determine the statistical significance of the differences between groups for quantitative (with a distribution different from normal) and ordinal variables, the Kruskal-Wallace test was applied. Comparison of quantitative and ordinal variables in dependent samples was carried out using the Wilcoxon test. The data in the graphs are presented as medians and 5–95 percentiles.

Results and Discussion

According to our findings, the average concentration of IL-6 in the blood serum of individuals in the control group was (2.07 ± 0.22) pg/ml. In the re-maining patients, its significant and probable increase was noted. Thus, in group II, the concentration of IL-6 increased by 695.7 %, and in group III by 1884.1 % ($p < 0.05$; Table). In group IV, a tendency to increase this indicator was recorded.

The pro-inflammatory biomarker IL-6 plays a crucial role in the development of the inflammatory response in SA and initiates a chain of reactions that lead to cartilage degradation and further complication of inflammatory processes in the joint [12]. An increase in its concentration triggers the synthesis of acute phase proteins, such as CRP, ceruloplasmin and haptoglobin, and also activates the innate immune system [16].

The level of CRP in the blood serum increased the most in patients with SA of the hip joint by 2635.4 % relative to control values ($p < 0.05$; table).

Almost half as much, namely by 1126.02 %, the level of this indicator increased in SA of the hip joint ($p < 0.05$; Fig. 2). In the case of septic arthritis of the talocrural joint, a tendency to its increase was recorded.

Thus, an increased level of CRP in the blood serum reflects the activity and risk of progression of the disease of the knee and hip joints.

Table

**Concentration of interleukin-6 and acute phase proteins in patients
with septic joint inflammation of various localizations**

№	Indicator	Control	Joint		
			knee	hip	talocrural
1	Interleukin-6, pg/ml	2.07 ± 0.22	16.47 ± 3.42*	39.0 ± 8.81*	69.17 ± 52.93
2	C-reactive protein, mg/l	2.46 ± 0.75	30.16 ± 5.66*	67.29 ± 16.10*	65.82 ± 33.83
3	Ceruloplasmin, g/l	0.23 ± 0.01	0.36 ± 0.02*	0.33 ± 0.03*	0.28 ± 0.02*
4	Haptoglobin, g/l	1.16 ± 0.09	3.60 ± 0.48*	3.33 ± 0.44*	2.93 ± 0.64*

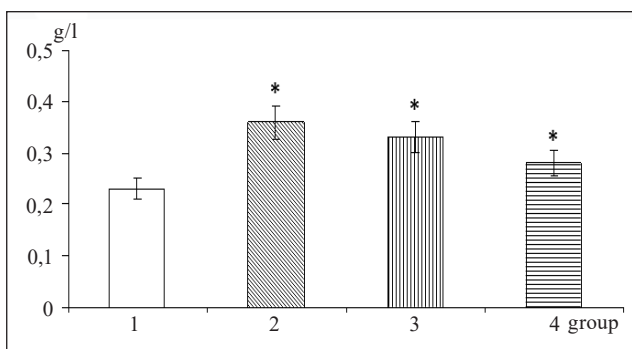*Note.* * — $p < 0.05$ realtive to control group.

Fig. 1. Ceruloplasmin concentration in the blood serum of conditionally healthy subjects (1) and patients with SA of the knee (2), hip (3), and talocrural joints (4). * — $p < 0.05$ relative to group 1
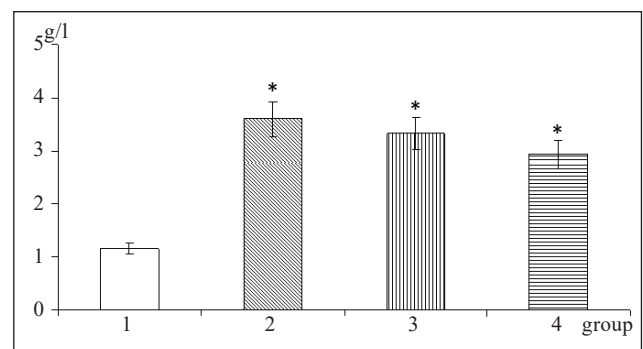

Fig. 2. Haptoglobin concentration in the blood serum of conditionally healthy subjects (1) and patients with SA of the knee (2), hip (3), and talocrural joints (4). * — $p < 0.05$ relative to group 1

The concentration of ceruloplasmin in the blood serum increased in all patients with SA of the studied joints. However, it was not as high as when determining IL-6 and CRP. The largest increase of 56.52 % was noted in SA of the knee joint (p < 0.05; table). In SA of the hip and talocrural joints, the level of this indicator significantly increased by 43.47 and 21.74 %, respectively (p < 0.05; Fig. 1) compared to the control.

The same trend was observed when determining the concentration of haptoglobin. The highest value was recorded when measuring in the blood serum of patients with SA of the knee joint. It was higher by 210.34 % compared to the control group (p < 0.05; Fig. 2).

In SA of the hip or talocrural joints, a significant increase in its level was observed by 187.07 and 152.59 % of the control values, respectively (p < 0.05; Fig. 2). According to our findings and literature data, patients with septic arthritis of the knee and hip joints had significantly increased levels of IL-6 and acute phase proteins in the blood serum [17, 18]. We believe that it is the enhanced synthesis of the pro-inflammatory cytokine IL-6 and acute phase proteins that plays a significant role in the development of the inflammatory response in SA. An increase in the concentration of IL-6 triggers the synthesis of acute phase proteins, primarily CRP, and initiates a chain of reactions that lead to cartilage degradation and further development of inflammatory processes in the joint. This is consistent with the results of other authors who observed a certain positive relationship between peak serum IL-6 and CRP levels in knee and hip osteoarthritis [12, 16]. Therefore, these biomarkers can be tools for diagnosing the progression of this disease in both preclinical and clinical studies, the main goal of which is to establish the diagnosis, stage of the disease and predict clinical outcome. The results obtained emphasize the importance of determining inflammatory biomarkers for the assessment and understanding of the inflammatory process.

Conclusions

Patients with septic arthritis of the knee and hip joints had significantly increased levels of IL-6 and acute phase proteins in the blood serum.

An increase in the concentration of IL-6 triggers the synthesis of acute phase proteins, primarily CRP, and initiates a chain of reactions that lead to cartilage degradation and further complication of inflammatory processes in the joint.

The results obtained emphasize the importance of determining inflammatory biomarkers for diagnosing the progression of septic arthritis in both preclinical and clinical studies to establish the stage of the disease and predict clinical outcome.

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INTERLEUKIN-6 AND ACUTE PHASE PROTEINS AS BIOMARKERS OF SEPTIC OSTEOARTHRITIS

S. Magomedov, Yu. V. Polyachenko, M. P. Hrytsai,
I. H. Litovka, V. I. Sabadosh, N. O. Dekhterenko, T. A. Kuzub

SI «National Institute of Traumatology and Orthopedics of the NAMS of Ukraine», Kyiv

✉ Sadrudin Magomedov, MD, Prof.: alexander@magomedov.kiev.ua; https://orcid.org/0000-1234-5678-9101

✉ Yuriy Polyachenko, MD, Prof.: info@ito.gov.ua

✉ Mykola Hrytsai, MD, Prof.: mgrytsai02@gmail.com; https://orcid.org/0000-0003-1608-7879

✉ Litovka Iryna, MD, Prof.: litir@biph.kiev.ua; https://orcid.org/0000-0001-9163-3572

✉ Sabodash Vasyl: sabadoshv@gmail.com; https://orcid.org/0000-0002-3214-3298

✉ Natali Dekhterenko: natali.de@ukr.net; https://orcid.org/0000-0003-3330-533x

✉ Taisiia Kuzub: biohimii@ukr.net