ORIGINAL ARTICLE

THE CORRELATION OF CONCENTRATION OF C-REACTIVE PROTEIN AND PROCALCITONIN WITH WHITE BLOOD CELLS COUNT IN ACUTE INFECTION AND SEPSIS

KORELACIJA KONCENTRACIJE C-REAKTIVNOG PROTEINA I PROKALCITONINA SA BROJEM LEUKOCITA U AKUTNOJ INFEKCIJI I SEPSI

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Abstract

Introduction: Infection is a specific response to an active or passive pathogen penetration and/or its reproduction in the biological system. Unlike the infection, sepsis is characterized by a systemic inflammation response of the host. The most commonly used biomarkers for the diagnosis of infections and sepsis are C-reactive protein (CRP) and procalcitonin (PCT).

Aim: The aim of the study was to examine the correlation of CRP and PCT with white blood cells (WBC) count in patients with acute infection and sepsis.

Material and methods: This retrospective study included 69 patients who underwent a blood test at the Central Laboratory of the University Clinical Center of Kragujevac to determine the presence of infection/sepsis during hospitalization. Patients were divided into two groups: patients with local bacterial infections of different localization and patients with sepsis. In the group of patients with local infection, four subgroups were distinguished: patients with respiratory tract infections, urinary tract infections, gastrointestinal and hepatobiliary infections, and skin infections. The control group comprised 40 healthy subjects. The study analyzed data about WBC count, neutrophil and lymphocytes count as well as the concentration of CRP and PCT.

Results: The analysis of mentioned parameters indicated that patients with infection/sepsis had significantly higher values of WBC ($p < 0.001$), neutrophils ($p < 0.001$), lymphocytes ($p = 0.007$), CRP ($p < 0.001$) and PCT ($p < 0.001$) concerning control subjects. There was a statistically significant difference in PCT between the examined groups of patients ($p = 0.029$), so the highest values have been recorded in septic patients. In patients with sepsis, there was a significant positive correlation between the concentration of CRP and WBC counts ($r = 0.538$, $p = 0.008$).

Conclusion: There is a significant increase in the concentration of CRP and PCTin patients with local infections and sepsis.The concentration of CRP is positively correlated with WBC counts in patients with sepsis.

Sažetak

Uvod: Infekcija je specifičan odgovor organizma na prodiranje i razmnožavanje mikroorganizma u biološki sistem. Za razliku od infekcije, sepsu karakteriše sistemski inflamatorni odgovor organizma. Najčešće korišćeni biomarkeri za postavljanje dijagnoze infekcije i sepse su C-reaktivni protein (CRP) i prokalcitonin (PCT).

Cilj: Osnovni cilj istraživanja bio je da se ispita korelacija koncentracije CRP i PCT sa brojem leukocita kod pacijenata sa akutnom infekcijom i sepsom.

Materijal i metode: Ovom retrospektivnom studijom je obuhvaćeno 69 pacijenata kod kojih je, radi utvrđivanja prisustva infekcije tokom hospitalizacije, urađena analiza krvi u Centralnoj laboratoriji Univerzitetskog kliničkog centra u Kragujevcu. Pacijenti su podeljeni u dve grupe: pacijenti sa lokalnom bakterijskom infekcijom različite lokalizacije i pacijenti sa sepsom. U grupi pacijenata sa lokalnom infekcijom izdvojene su četiri podgrupe: pacijenti sa infekcijama respiratornog trakta, urinarnog trakta, gastrointestinalnog i hepatobilijarnog trakta, i grupa pacijenata sa infekcijama kože. Kontrolna grupa bila je sastavljena od 40 zdravih ispitanika. U studiji su analizirani podaci o parametrima zapaljenja: broj leukocita, neutrofila i limfocita, CRP i PCT.

Rezultati: Analiza pomenutih parametara ukazala je da pacijenti sa infekcijom/sepsom imaju statistički značajno veće vrednosti ukupnog broja leukocita (p < 0,001), neutrofila (p < 0,001), limfocita (p = 0,007), CRP (p < 0,001) i PCT (p < 0,001) u odnosu na kontrolne ispitanike. Utvrđena je statistički značajna razlika u koncentraciji PCT između ispitivanih grupa pacijenata (p = 0,029), tako da su najviše vrednosti zabeležene kod pacijenata sa sepsom. Kod pacijenata sa sepsom postoji značajna pozitivna korelacija koncentracije CRP i ukupnog broja leukocita (r = 0,538, p = 0,008).

Zaključak: Kod pacijenata sa lokalnom infekcijom i sepsom postoji značajan porast koncentracije C-reaktivnog protein i prokalcitonina. Koncentracija C-reaktivnog protein pozitivno korelira sa stepenom leukocitoze u ispitivanoj populaciji.

Ključne reči:
C-reaktivni protein,
infekcija,
prokalcitonin,
leukociti,
sepsa

Introduction

Infection is a specific response of the host to an active or passive pathogen penetration and/or its reproduction in the biological system, which depends on pathogenic properties (pathogenicity, virulence and invasiveness), as well as the host's defense mechanisms. It can be acute, subacute or chronic infection, while it is characterized as subclinical, latent or manifest according to clinical presentation, respectively (1).

A dysregulated host response to infection leads to life-threatening organ dysfunction, known as sepsis (2). Systemic inflammatory response to infection is a major component in the pathogenesis of sepsis (3). Thus, sepsis is a serious medical condition that causes extensive damage to the tissues and organs, which degree could vary from mild impaired function to complete, irreversible organ failure (4). Accordingly, it is considered the leading cause of death in the units of intensive care worldwide (5).

In the complex interaction of the host and pathogens, regardless of whether it is local or systemic, certain molecules are being released. Their concentration might be significant in the early detection of infection, i.e. sepsis. The importance of laboratory measurement of biomarkers' concentration is not only in confirming the diagnosis of infection but also in the identification of the type of inflammatory process. They also indicate the course and prognosis of the disease and could help in therapeutic decisions (6). The two most widely used biomarkers as diagnostic and prognostic "tools" for infection and sepsis are C-reactive protein (CRP) and procalcitonin (PCT) (7).

The CRP is an acute-phase protein, primarily synthesized in the liver (8), whose levels increase up to 1.000-foldduring inflammatory disorders (9). Considering the kinetic of its blood concentration changes, it is assumed to be a suitable marker of sepsis onset (10). Although previously conducted studies have shown that CRP levels increase during inflammatory diseases, the precise role of CRP in their course and progression remains unknown (11, 12).

Similarly, procalcitonin, a precursor of mature calcitonin is a part of the host's systemic response to inflammation. All PCT formed in parafollicular cells of the thyroid gland is converted into calcitonin, so its blood concentration in healthy subjects is very low. Conversely, during inflammation, there is an increase in PCT concentration, especially in the cases of bacterial infection (13). It has been found that PCT is more sensitive and specific than CRP. In such a context,it may be helpful in the prognosis of critically ill patients (14).

Keeping in mind that the results of previous research on the relationship between certain biomarkers of inflammation are quite uneven, the study aimed to examine the correlation of the concentration of CRP and PCT with white blood cell (WBC) count in patients with acute infection and sepsis.

Material and methods

Study population

The investigation was conducted as a retrospective, cross-sectional study that involved 69 patients of both genders who underwent blood test examination at the Central Laboratory of the University Clinical Center of Kragujevac to determine the presence of infection/sepsis during 2019. There were 47 patients with local bacterial infections of different localization: 22 patients with respiratory tract infection, 8 patients with urinary tract infection, 10 patients with gastrointestinal and hepatobiliary tract infection and 7 patients with the infection of the skin. Also, the study included 22 patients with a confirmed diagnosis of sepsis according to Sepsis-3 criteria (2).The study did not include: (1) patients under the age of 18, (2) patients who received antibiotic therapy and/or immunosuppressive therapy, (3) patients with immune disorders, (4) patients with malignant diseases, (5) patients with chronic inflammatory diseases, (5) patients suffering from autoimmune diseases, (6) pregnant women, and (7) patients who did not give written consent to participate in the study. The control group was comprised of 20 healthy volunteers without clinical evidence of acute inflammatory diseases, or some other conditions that could affect the investigated parameters.

The study was performed according to the Declaration of Helsinki, and it was approved by the Ethical committee of the University Clinical Center Kragujevac (number 01/19-1216). Written informed consent for participation in the study was obtained from all patients and control subjects.

Determination of laboratory parameters

Investigated laboratory parameters were obtained by using standard accepted methods in the Central Laboratory of the University Clinical Center of Kragujevac. Total WBC count (range 3.70 – 10.0 x 10^9/L), as well as the absolute counts of leucocytes´ subtypes (range 2.10 – 6.50 x 10^9/L for neutrophils and range 1.20 – 3.40 x 10^9/L for lymphocytes) were assessed by the automated DxH 800 Hematology Analyzer (Beckman Coulter, Inc. Brea, USA). Based on the mentioned measurement, it was calculated neutrophil-to-lymphocyte ratio (NLR).

Oly AU 680 (Beckman Coulter Inc. Brea, USA) was applied for the measurement of CRP concentration, while Cobas e 411 chemical analyzer (Roche Diagnostics GmbH, Mannheim, Germany) was utilized for the determination of PCT concentration. The reference ranges were as follows: CRP < 5 mg/L; PCT < 0.5 ng/mL.

Statistical analysis

The commercial SPSS version 20.0 for Windows was used for statistical analysis. All data were expressed as mean ± standard deviation. For statistical analysis of independent samples t-test was used. In the case of the non-normal distribution of variables, the Mann-Whitney test was utilized for testing the difference among investigated groups. The relationship between the concentration of CRP and PCT with WBC count was obtained by the Bivariate correlation test (with determination of Pearson/Spearman coefficient). The p value less than 0.05 was considered statistically significant.

Results

The results of the study are presented in **tables 1–4** and **figure 1**.

The study included 69 patients, 28 (40.6%) females of mean age 55.1 ± 23.4 years, and 41 (59.4%) males of mean age 61.7 ± 16.5years. The control group comprised 40 healthy subjects, 20 (50%) females of mean age 51.45 ± 19.8 years and 20 (50%) males of mean age 50 ± 22.9 years.

Analysis of laboratory parameters revealed that patients with infection/sepsis had significantly higher values of WBC ($p < 0.001$), neutrophils ($p < 0.001$), lymphocytes ($p = 0.007$), CRP ($p < 0.001$) and PCT ($p < 0.001$) concerning control participants (**table 1**).

Concerning the analyzed parameters among the investigated groups of patients, a statistically significant difference in PCT concentration was shown ($p = 0.029$), so the highest values were demonstrated in the group of patients with sepsis and then among those with respiratory tract infection (**table 2**).

In the current study, the relationship of CRP and PCT with leukocytes number has shown a statistically significant positive correlation between the concentration of CRP and with total WBC count in each subgroup of patients) (**table 3**). Similarly, our study showed a significant positive correlation of CRP with total WBC count in the patients with sepsis ($r = 0.538$, $p < 0.008$). Contrarily, one cannot observe such results in patients with local infections (**table 4**).

Additionally, analysis of NLR ratio in all participants of our study indicated that NLR value was significantly higher in patients with acute infection and sepsis compared to control subjects) ($p < 0.001$) (**figure 1**). There was a significant positive relationship between NLR and the concentration of CRP both in patients ($r = 0.269$, $p = 0.043$) and control subjects ($r = 0.542$, $p < 0.001$).

Discussion

It was shown that total leukocytes count, as well as the counts of neutrophils and lymphocytes were significantly higher in the patients with infection/sepsis compared to the control values ($p_{WBC} < 0.001$, $p_{Neutrophils} < 0.001$, $p_{Lymphocytes} = 0.007$, **table 1**). Also, the patients with infection/sepsis had statistically higher concentrations of CRP and PCT ($p_{CRP} < 0.001$, $p_{PCT} < 0.001$, **table 1**). A significant difference in the concentration of PCT was registered among certain groups of patients. Namely, the patients with sepsis had the most pronounced PCT values ($p = 0.029$, **table 2**).

Bearing in mind that sepsis is the leading cause of mortality worldwide (15), it is a permanent focus of scientific research. However, the pathogenesis of sepsis is still

Table 1. The values of laboratory parameters in patients and control subjects.

Parameter	Patients (n = 69)	Controls (n = 40)	Significance
WBC count (x 10^9/L)	15.56 ± 9.6	6.8 ± 1.8	p < 0.001*
Neutrophils (x 10^9/L)	12.8 ± 8.7	4.2 ± 1.4	p < 0.001*
Lymphocytes (x 10^9/L)	1.6 ± 1.9	1.7 ± 0.8	p = 0.007*
CRP (mg/L)	179.2 ± 107.3	2.2 ± 1.6	p < 0.001*
PCT (ng/mL)	90.6 ± 390.2	0.11 ± 0.10	p < 0.001*

*statistically significant difference

Table 2. The values of laboratory parameters among investigated groups of patients.

Parameter	Respiratory infections (n =22)	Urinary infections (n =8)	Gastrointestinal infections (n =10)	Skin infections (n =7)	Sepsis (n =22)	Significance
WBC count (x 10^9/L)	16.2 ± 10.5	16.3 ± 10.6	12.5 ± 3.5	14.1 ± 7.8	14.6 ± 8.9	p = 0.982
Neutrophils (x 10^9/L)	13.2 ± 9.4	13.4 ± 8.1	10.6 ± 3.2	12.5 ± 7.0	8.9 ± 5.3	p = 0.289
Lymphocytes (x 10^9/L)	1.7 ± 2.2	2.3 ± 2.6	1.6 ± 0.5	1.0 ± 0.5	2.8 ± 6.3	p = 0.321
CRP (mg/L)	189.2 ± 99.2	137.0 ± 9.6	178 ± 111.8	158.9 ± 86.7	156.4 ± 113.8	p = 0.716
PCT (ng/mL)	120.1 ± 453.6	1.58 ± 1.4	6.57 ± 10.1	54.4 ± 103.7	5575 ± 23565	p = 0.029*

*statistically significant difference

Table 3. The correlation of CRP and PCT concentration with white blood cell count (WBC) in patients and control subjects.

	White blood cell count (WBC)	
	Patients	Control
CRP	r = 0.450, p < 0.001*	r = 0.255, p = 0,112
PCT	r = 0.139, p = 0.295	r = 0.056, p = 0.730

*statistically significant difference

Tabela 4. The correlation of CRP and PCT concentration with white blood cell count (WBC) in the subgroups of patients.

	White blood cell count (WBC)				
	Respiratory infections	Urinary infections	Gastrointestinal infections	Skin infection	Sepsis
CRP	r = 0.164, p = 0.455	r = 0.600, p = 0.208	r = 0.309, p = 0.385	r = 0.543, p = 0.266	r =0.538, p = 0.008*
PCT	r = 0.213, p = 0.381	r = 0.029, p = 0.957	r = 0.214, p = 0.610	r = 0.600, p = 0.400	r = 0.018, p = 0.938

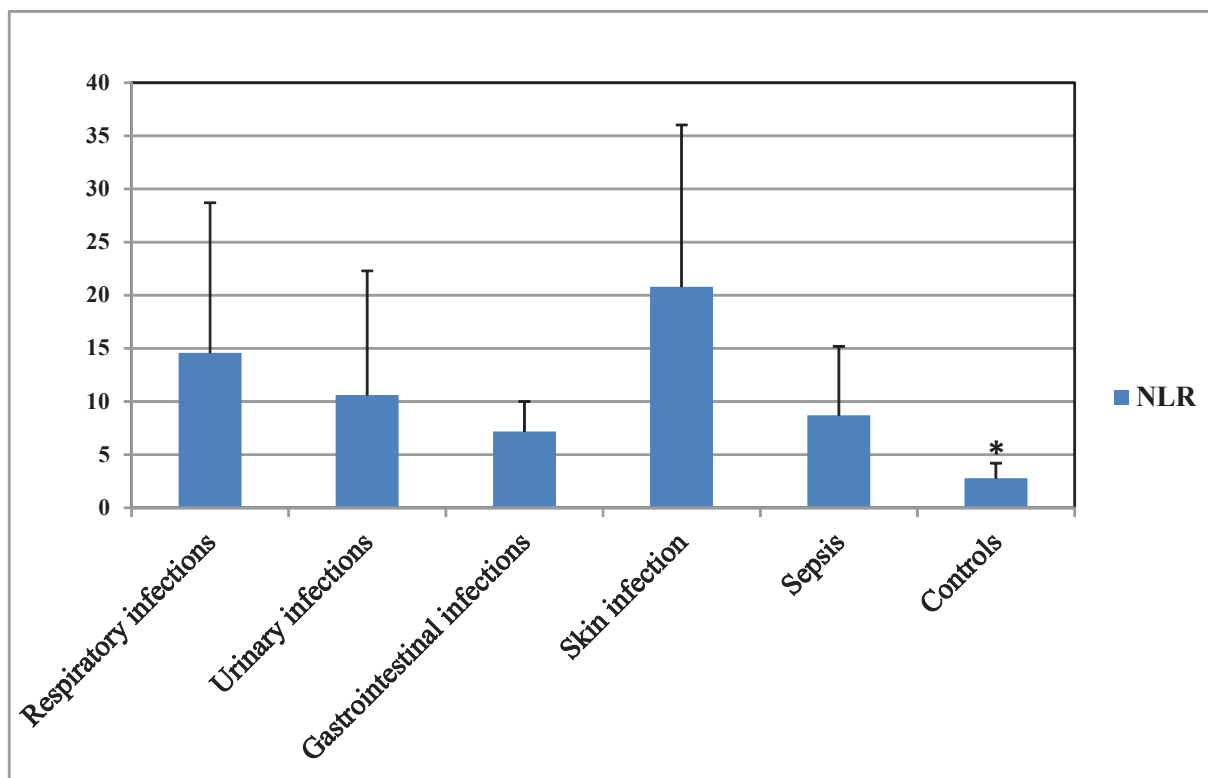*statistically significant difference

FIgure 1. The values of Neutrophile-Lymphocyte Ratio (NLR) in study participants. *all patients vs. controls, p < 0.001.

incompletely understood. According to available literature data, there is a global burden of sepsis with a high mortality rate (over 41% in Europe) (16). Timely diagnosis of infection or sepsis is crucial when it comes to healing. In such circumstances, inflammatory biomarkers play a key role.

The results of previous studies on the relationship between the concentration of biomarkers and leukocyte count in infection and sepsis are quite uneven. Some authors demonstrated a positive correlation between total leukocyte count and the concentration of PCT in infection and sepsis, without a significant correlation with the concentration of CRP (17). On the other hand, Xharra et al. found that the concentration of CRP directly reflects the severity of an infection and that it significantly correlates with leukocytosis (18). The results of our study estimated a statistically significant, positive relationship between the concentration of CRP and WBC count in all study patients (the patients with respiratory infections, urinary infections, gastrointestinal infections, and skin infections), as well as in the patients with sepsis (when they are analyzed separately) (p = 0.008, **table 4**).

The inconsistency of earlier research raises the question of the reliability of inflammatory biomarkers, and possibly greater sensitivity and specificity of PCT in the diagnosis of infection and sepsis (13). Among the various biomarkers that are currently used for the early confirmation of infection and sepsis, PCT is assumed to be more important because its concentration reaches the highest value in the first 6 - 24 hours and remains elevated for the next 7 days (19, 20). Results of our studyestimated that PCT is a more sensitive biomarker in sepsis than CRP, which better reflected the dynamics of cellular changes (**table 2**). Magrini and contributors also confirmed the greater

utility of PCT than CRP and WBC counts in the diagnosis of sepsis (17).

Additionally, the focus of the present study was the NLR ratio, too. Zahorec proposed the NLR ratio as an additional marker in the diagnosis of infection and sepsis (21). Thus, Huang and colleagues (22) have concluded that NLR is related to the prognosis of infection and sepsis since the higher values of this parameter indicated an unfavorable prognosis. In this study, the NLR index was statistically higher in all patients compared to control subjects (p < 0.001, **figure 1**), and it positively correlated with the concentration of CRP in both patients and healthy individuals.

Conclusion

There is a significant increase in the concentration of CRP and PCT, as well as a positive correlation of CRP concentration with the degree of leukocytosis in patients with infection and sepsis. The ratio of neutrophils and lymphocytes additionally reflects the dynamics of changes in the concentration of inflammatory biomarkers during infection and sepsis. Much progress has been made in addressing the predictive significance of inflammatory markers, such as CRP and PCT, in the diagnosis and monitoring of the course of infectious diseases and sepsis. However, further prospective studies with a greater number of participants are needed.

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