Original article

Diagnostic Agreement of Presepsin, Procalcitonin, C-Reactive Protein and White Blood Cell Count in Patients with Suspected Sepsis

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SUMMARY

Intoduction/Aim. Agreement or disagreement of inflammatory parameters becomes important for making diagnosis when disparate values are encountered in a patient with suspected sepsis. The aim of our study was to test the agreement between the four commonly used tests for diagnosing systemic infection: white blood cell count (WBC), serum levels of C-reactive protein (CRP), procalcitonin and presepsin.

Methods. This cross-sectional study included 479 adult patients hospitalized in the Clinical Center Kragujevac during 2019, who were suspected to have systemic infection and whose microbiological analyses were positive.

Results. In a sample of hospital patients with isolated bacteria from the sites of suspected infection, the parameters of inflammation showed low agreement when used for diagnosing systemic infection. Only presepsin serum levels showed significant level of agreement with CRP and procalcitonin (Cohen's kappa = 0,257, p = 0,000Cohen's kappa = 0,169, p = 0,000, respectively, but also with low kappa values, while the agreement between CRP and procalcitonin was insignificant, as well as between the white cell count and the remaining three parameters.

Conclusions. When disparate values of parameters of inflammation are encountered in a patient with suspected sepsis, a decision about antibiotic therapy should be based on either of the two pairs of parameters, presepsin/C-reactive protein or presepsin/procalcitonin.

Keywords: sepsis, presepsin, procalcitonin, C-reactive protein, white blood cell count

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INTRODUCTION

Systemic infections are a big challenge for health care professionals all over the world, especially if they reach high severity level classified as sepsis. According to the latest international consensus, sepsis is defined as "life-threatening organ dysfunction caused by a dysregulated host response to infection", and its presence is associated with significant morbidity and mortality (1). The quickSOFA score (quick Sequential Organ Failure Assessment) (qSOFA), introduced by the Sepsis-3 group in February 2016, functions as an initial tool to recognize patients with a heightened risk of unfavorable outcomes in the context of infection. If a patient exhibits two or more qSOFA points early in the course of infection, it indicates an increased likelihood of having sepsis and increased risk of mortality. A patient has high probability of having sepsis if at least two of the following clinical criteria are met: respiratory rate of 22/min or greater, altered mentation, or systolic blood pressure of 100 mm Hg or less (1, 2). The most common sites of infections that can cause sepsis are the respiratory tract – especially lungs, followed by the abdomen, bloodstream and genitourinary tract (3). Global epidemiology of sepsis is difficult to ascertain. It is estimated that more than about 50 million people worldwide are affected by sepsis annually, including 11 million deaths (4). The incidence rate of sepsis has been increasing steadily over the past three decades, whereby elderly population was increasingly affected (5).

Important practical issue regarding sepsis is to differentiate it from non-infectious systemic inflammatory response syndrome (SIRS), which can be present in patients with other diseases. SIRS refers to an overactive defensive reaction by the body against a harmful stressor (such as infection, trauma, surgery, acute inflammation, ischemia or reperfusion, malignancy, and more) the purpose of which is to identify and eliminate the internal or external cause of the insult (6). SIRS is characterized by meeting at least two of the following criteria: body temperature exceeding 38° C or falling below 36° C, heart rate surpassing 90 beats/minute, respiratory rate exceeding 20 breaths/minute or partial pressure of CO_2 dropping below 32 mmHg, and leukocyte count exceeding 12,000 or falling below 4,000/μL, or having over 10% immature forms or bands (7).

Outcome of sepsis depends on timely treatment following accurate diagnosis. Mortality of septic shock increases by 7.6% with every hour of delay with antibiotic treatment (7). Positive cultures are usually essential for establishing the diagnosis of infection, however, there are several problems - it takes a couple of days to obtain microbiological results, they are negative in up to one-half of the patients, especially in cases when antibiotics have been administrated before taking specimens for microbiological analysis, and sometimes isolates are not causative agents but rather contaminants (8, 9). Biomarkers are used to diagnose infectious diseases early, to give prognosis, as well as to help in deciding about antibiotic treatment. The white blood cell count was traditionally used when an infection had been suspected, because it usually increases with bacterial infection; however, there are also other medical conditions unrelated to infections associated with abnormalities of the white blood cell count. The most commonly used biomarkers for sepsis are C-reactive protein (CRP), procalcitonin (PCT) and, in recent years, presepsin.

CRP is one of acute phase reactants, produced in the liver within few hours after infection, inflammation or tissue injury. It serves as a marker of the present inflammation and its serum levels during acute inflammatory process can be 1000-fold. CRP is widely employed as sepsis biomarker because of wide availability and low cost (10). CRP levels correlate with the severity of infection, while changes in its levels between presentation and the day 4 were shown to be the spredictors of recovery (11, 12). Recent meta-analysis suggested that daily measurement of CRP levels was useful in the assessment of adequacy of antibiotic therapy. It was also suggested that CRP level on the day 4 alone could be used as a predictor of treatment outcomes (13).

Procalcitonin (PCT) is prohormone of calcitonin present in almost undetectable concentrations in healthy individuals, but its production and secretion increase in the presence of infection or systemic inflammation. It takes 8 to 24 hours to detect PCT after a triggering event and peak concentration is reached after 24 hours (14). Half-life of PCT is 24 - 35 hours, and that makes it suitable for serial monitoring (15). PCT levels correlate with the severity of infection or inflammation and can reach thousand-fold increase

in relation to normal values (16).

Presepsin, soluble N-terminal fragment of CD14 expressed on the membrane of monocytes and macrophages, mediates immune response to lipo-polysaccharides (17). Patients with sepsis have much higher levels of presepsin than healthy individuals and those with SIRS (18). It takes two hours to increase presepsin concentration after infection and three hours to reach the peak concentration, making it useful in early diagnosis of sepsis (19).

AIM

The aim of our study was to test the agreement between four commonly used tests for diagnosing a systemic infection: white cell count (WCC), serum levels of C-reactive protein (CRP), procalcitonin and presepsin.

PATIENTS AND METHODS

This cross-sectional study included adult patients hospitalized in the Clinical Center Kragujevac during 2019, with a suspicion of having systemic infection, and whose microbiological analyses were positive, i.e. bacteria were isolated from their samples. The patient's sample was consecutive, i.e. all patients who satisfied inclusion criteria during the study period were included in the study. Sixteen point twenty-four (16.24%) patients were with preserved general health and had come to the hospital for additional laboratory tests as a part of preparations for elective surgery. The following variables were collected from the patient files: age, sex, hospital ward, isolated bacteria, sampling site, white blood cell count (WBC) and serum levels of C-reactive protein (CRP), procalcitonin and presepsin. Bacteria were isolated in the hospital's Microbiology service (using Vitek 2 apparatus for biochemical isolation), and inflammatory parameters were measured in the hospital's Central Laboratory.

White blood cell count was measured by a Coulter LH780 (Beckman Coulter Inc., Miami, FL). The hsCRP concentrations were measured from serum samples using immunoturbidimetric method and carried out by analyzer Beckman Coulter AU680 (Beckman Coulter Inc.,Brea, CA, USA) with the re-ference range 0.0 - 5.0mg/L. PCT levels were measured from serum samples using electrochemiluminescence immunoassay (ECLIA) Elecsys BRAHMS PCT (Roche Diagnostics Mannheim,Germany), with a reference range of 0.0 - 0.05 ng/mL. The quantification of presepsin was measured in EDTA plasma and was performed by chemiluminescent enzyme immunoassay (Pathfast, Mitsubishi Chemical Medicine Corporation, Japan); the reference range for adults was below 300 pg/mL.

The data were described by mean and standard deviation if continuous in nature, and by rates and percentages, if categorical. Normality of the data distribution was tested by Kolmogorov-Smirnov test. The agreement between the four parameters of inflammation (WCC, CRP, procalcitonin and presepsin) was tested by Spearman's non-parametric correlation coefficient, and by McNemar and Cohen's Kappa, when they were turned to categorical variables after applying cut-off points for diagnosing systemic infection. Statistical significance was set to 0.05 probability of zero hypothesis. All calculations were performed with statistical software SPSS, version 18.

RESULTS

There were 479 participants in the study, whose characteristics are shown in Table 1.

White blood cells count, C-reactive protein in serum, procalcitonin in serum and presepsin in plasma were not normally distributed, so non-parametric correlation was tested among the study parameters of inflammation. Spearman's correlation analysis found statistically significant correlation between these two pairs of examined parameters: procalcitonin/presepsin and presepsin/CRP. The correlation matrix is shown in Table 2.

Diagnostic agreement for systemic infection between the four parameters of inflammation was tested after administration of the following cut-off points: 10×10^9/L for white cells count, 100 mg/L for C-reactive protein, 600 pg/ml for presepsin and 0,25 ng/ml for procalcitonin. Only presepsin serum levels exhibited a noteworthy level of agreement with CRP and procalcitonin, albeit with relatively low kappa values. The results of the McNemar and Cohen's kappa tests of agreement are shown in Table 3.

Table 1. *Characteristics of the study sample*

Parameter	Value(s)
Age (years)	61.7 ± 16.5
Sex	311 (65%) females and 168 (35%) males
Hospital wards from which samples were sent to microbiology and biochemistry	Intensive care unit, 247 pts. (51.6%),
	Emergency center, 128 pts. (26.7%),
	Other departments, 104 pts. (21.7%).
Sites of an infection, from which samples were taken for analysis	Tracheal aspirate, 162 pts. 33.8%)
	Blood culture, 147 pts. (30.7%)
	Postoperative wound infection, 45 pts. (9.4%)
	Urine sample, 72 pts. (15%)
	Other samples, 53 pts. (11%)
Microorganisms isolated from the samples	Acinetobacter sp. 109 (22.8%)
	Klebsiella sp. 70 (14.6%)
	Escherichia coli 28 (5.8%)
	Proteus mirabilis 30 (6.3%)
	Pseudomonas aeruginosa 57 (11.9%)
	Enterococcus sp. 16 (3.3%)
	Staphylococcus sp. 122 (25.5%)
	Other 47 (9.8%)
Median white cell count (x 10⁹/L) and IQR*	12.9 [8.1]
Median C-reactive protein level in serum (mg/L) and IQR*	84.3 [99.6]
Median procalcitonin level in serum (ng/ml) and IQR*	2.0 [70]
Median presepsin level in serum (pg/ml) and IQR*	790.0 [977.0]

* IQR – interquartile range; pts. – patients

Table 2. *Correlation matrix of inflammatory parameters measured in the study sample. The numbers in the matrix relate to Spearman's correlation coefficient*

	White cell count	C-reactive protein	Procalcitonin	Presepsin
White cell count	1	r = 0.003; p = 0.948	r = 0.043; p = 0.348	r = 0.059; p = 0.199
C-reactive protein		1	r = 0.163; p = 0.076	r = 0.418; p = 0.000*
Procalcitonin			1	r = 0.299; p = 0.000*
Presepsin				1

* statistically significant

Table 3. *Agreement matrix of the four parameters of inflammation*

	White cell count	C-reactive protein	Procalcitonin	Presepsin
White cell count		McNemar = 73.894, p = 0.000	McNemar = 35.292, p = 0.000	McNemar = 5.286, p = 0.021
C-reactive protein	Cohen's kappa = 0.010, p = 0.804		McNemar = 172.481, p = 0.000	McNemar = 57.038, p = 0.000
Procalcitonin	Cohen's kappa = 0.014, p = 0.738	Cohen's kappa = 0.008, p = 0.758		McNemar = 76.056, p = 00000
Presepsin	Cohen's kappa = 0.042, p = 0.355	Cohen's kappa = 0.257, p = 0.000	Cohen's kappa = 0.169, p = 0.000	

DISCUSSION

Our study showed that in a sample of hospital patients with isolated bacteria from the sites of suspected infection, the parameters of inflammation had low agreement when used for diagnosing a systemic infection. Only presepsin serum levels showed a significant level of agreement with CRP and procalcitonin, respectively, however, with low kappa values, while the agreement between CRP and procalcitonin was insignificant as well as between the white cell count and the other three parameters.

Being the main cause of death in critically ill patients, sepsis needs to be early diagnosed in order to increase the chances of favorable outcome. Clinical presentation of sepsis often overlaps with SIRS of non-infectious origin, making them difficult to distinguish (20), therefore, an efficient biomarker could be of great help. There are lot of studies that confirmed the benefit of using biomarkers in order to diagnose and predict sepsis outcome (21, 22). The commonly studied markers include PCT, CRP, lipopolysaccharide-binding protein (LBP), interleukins, pro-vasopressin and myeloid cells expressing triggering receptor-1, but unfortunately, neither have been proven to be precise enough to differentiate between sepsis and SIRS (23 - 27). On the other hand, the only biomarker mentioned, although under low quality of evidence, in The Surviving Sepsis Campaign was procalcitonin, and its use was recommended to help deciding about stopping antimicrobial therapy (28). Another systematic review and meta-analysis revealed that presepsin, PCT and CRP have only moderate degree of diagnostic value – AUCs were 0.88, 0.85 and 0.77, respectively (29). Even more recent meta-analysis (22, 30) compared diagnostic accuracy in sepsis between presepsin, procalcitonin and CRP and showed that the probability of sepsis is three to four times more likely if presepsin result was positive and only one-fourth if negative. PCT is useful as a guide for antibiotic treatment, and the study shows that duration of antibiotic therapy is significantly shorter when treatment is PCT-guided - 6 days compared to 8 days in the group non PCT-monitored (31). Shorter antibiotic treatment is associated with reduced mortality, morbidity, shorter hospitalization and health-care costs (31). As early as 2004, presepsin was identified as a new sepsis marker, but its usefulness in the assessment of sepsis was reported in 2011 for the first time (32, 33). Main advantage of this marker over PCT was its early secretion after infection and reaching the peak level before PCT. Pooled sensitivity for presepsin calculated in meta-analysis from 2019 (30) was 0.83 and pooled specificity 0.69, resulting with certain rate of missed diagnosis (31%) and prognosis (17%).

C-reactive protein is a commonly used marker in clinical practice, however, previous studies showed that it has lower diagnostic accuracy compared to PCT (34). On the other hand, meta-analysis comparing CRP with presepsin (22) revealed that there was no significant difference between these markers (AUC was 0.85 for both) in regard to diagnostic accuracy for sepsis, indicating similar, if not equal performance.

Although numerous studies were published which compared the diagnostic accuracy of CRP, PCT, presepsin and white cell count in patients with suspected sepsis, we are not aware of a single study investigating a diagnostic agreement of these inflammatory parameters. Yet, in clinical practice, one may often encounter patients with sepsis or without it, whose values of CRP, PCT, presepsin and white cell count are discordant (35). In such situations, clinicians could be confused what parameter to choose to base their decision to use antibiotic therapy. Our study suggested that in such situations, the best option is to focus on parameters with high agreement, like on either of the two pairs: presepsin + CRP or presepsin +PCT.

Our study has a few limitations that may introduce bias in the results. The first limitation was lack of gold standard of diagnosing sepsis, which precluded construction of receiver-operator curves for parameters of inflammation and calculation of their areas-under-the-curve, sensitivity and specificity. The second limitation was set by cross-sectional character of the study, so outcomes of the treatment could not have been followed, and prognostic value of inflammatory parameters and their combinations therefore could not have been determined. Neither smoking status nor the treatments employed were taken into account in the statistical analyses, and perhaps they could have influenced interrelations between the inflammatory parameters. Finally, some of biochemistry parameters were not measured due to shortages in supply, so severity of sepsis was not determined by the APACHE II score.

CONCLUSION

In conclusion, agreement or disagreement of inflammatory parameters becomes important for making diagnosis of sepsis when disparate values are encountered in a patient with suspected sepsis. Our study suggested that decision on antibiotic therapy in such cases should be based on either of two pairs of parameters, presepsin/C-reactive protein or presepsin/procalcitonin.

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Dijagnostičko usaglašavanje presepsina, prokalcitonina, C-reaktivnog proteina i broja leukocita kod bolesnika kod kojih postoji sumnja na sepsu

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SAŽETAK

Uvod/Cilj. Kada se kod bolesnika kod kojih postoji sumnja na sepsu naiđe na različite vrednosti inflamatornih parametara, važno je znati kakva je usaglašenost između njih kako bi se mogla postaviti dijagnoza. Cilj naše studije bio je da se proveri usaglašenost između četiri testa najčešće korišćena za dijagnostikovanje sistemske infekcije: broja belih krvnih zrnaca, serumskih nivoa C-reaktivnog proteina (CRP), prokalcitonina i presepsina.

Metode. Ovom studijom preseka obuhvaćeno je 479 odraslih bolesnika hospitalizovanih u Univerzitetskom kliničkom centru Kragujevac u toku 2022. godine za koje se sumnjalo da imaju sistemsku infekciju i čije su mikrobiološke analize bile pozitivne.

Rezultati. Na uzorku bolesnika sa bakterijama izolovanim sa mesta sumnje na infekciju, parametri inflamacije pokazali su nizak nivo usaglašenosti kada su korišćeni za dijagnostiku sistemske infekcije. Samo su serumski nivoi presepsina pokazali značajan nivo usaglašenosti sa CRP-om i prokalcitoninom (Koenova kapa = 0,257, p = 0,000; Koenova kapa = 0,169, p = 0,000, redom), ali sa niskim kapa-vrednostima. Usaglašenost CRP-a sa prokalcitoninom bila je beznačajna, kao i ona između broja belih ćelija i preostalih triju parametara.

Zaključak. Kada se kod bolesnika kod kojih postoji sumnja na sepsu uoče različite vrednosti parametara inflamacije, odluku o antibiotskoj terapiji treba zasnovati na bilo kom od dvaju parova parametara – presepsin/C-reaktivni protein ili presepsin/prokalcitonin.

Ključne reči: sepsa, presepsin, prokalcitonin, C-reaktivni protein, broj leukocita