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Significance of Biomarkers in Early Diagnosis of Abdominal Sepsis

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Rezumat

Rolul markerilor serici în diagnosticarea precoce a sespsisului cu punct de plecare abdominal

Introducere/Obiective: Peritonita este una dintre cele mai importante surse de sepsis abdominal. Infecția intra-abdominală determină activarea răspunsului inflamator, astefl încât mediatorii inflamației ar putea fi utilizați ca markeri ai severității sepsisului, dar mai ales pentru a confirma sau a exclude diagnosticul de sepsis. Scopul acestui studiu a fost evaluarea sensibilității şi specificității markerilor serici ai inflamației - proteina C reactivă (PCR), procalcitonina (PCT) şi amiloidul A seric (AAS), la pacienții cu peritonită secundară generalizată.

Metode: Studiul este de tip prospectiv şi a fost realizat în Clinica de Chirurgie de Urgență a Centrului Clinic al Serbiei din Belgrad. Grupul de studiu a fost format din 100 de pacienți cu vârsta cuprinsă între 18 şi 70 de ani, cu semne de abdomen acut prin peritonită secundară generalizată.

Rezultate: PCR şi PCT sunt în prezent printre cei mai importanți markeri preoperatori cu ajutorul cărora se poate face diferența între sepsis şi SIRS. În prima zi postoperatorie, analiza relației dintre sensibilitate şi specificitate a indicat o acuratețe diagnostică mai ridicată şi o sensibilitate mai mare a AAS în comparație cu PCR şi PCT. În restul perioadei postoperatorii din acest studiu, curba ROC a coincis în cea mai mare parte cu linia diagonală, astfel încât diagnosticul sespsisului prin PCR, PCT şi AAS a avut o acuratețe mai redusă.

Concluzie: Rezultatele studiului nostru sugerează că identificarea unui marker specific pentru diagnosticarea sepsisului abdominal sau pentru diferențierea între SIRS şi sepsis, pre şi post-operator, ar fi foarte utilă.

Cuvinte cheie: SIRS, sepsis, proteină C reactivă, procalcitonină, amiloid A seric

Abstract

Introduction/Objective: Peritonitis is one of the most important sources of abdominal sepsis. Since intra-abdominal infection leads to the activation of the inflammatory response, this suggested that some of these mediators could be used as markers of the severity of newly formed sepsis, but primarily to identify or rule out new-onset sepsis. The aim of this study was to evaluate the sensitivity and specificity of serum markers of inflammation: C-reactive protein, procalcitonin and serum amyloid A in the serum of patients with diffuse secondary peritonitis.

Methods: The prospective cohort study was conducted at the Clinic for Emergency Surgery of the Clinical Center of Serbia in Belgrade. The study group consisted of 100 patients aged 18 to 70 years, with signs of acute abdomen due to diffuse secondary peritonitis.

Results: CRP and PCT are so far among the most valuable preoperative markers for distinguishing sepsis from SIRS. On the first postoperative day the analysis of the relationship between sensitivity and specificity at the different breakpoints used indicates a greater diagnostic accuracy and greater sensitivity of SAA compared to CRP and PCT. In the remaining postoperative period in our study, the ROC curve mostly coincided with the diagonal line, so CRP, PCT, and SAA had little diagnostic accuracy.

Conclusion: The results of our study suggest that finding a specific marker for the diagnosis of abdominal sepsis, a marker that would differentiate between SIRS and sepsis, pre- and post-operatively, would be very useful.

Key words: SIRS, sepsis, C-reactive protein, procalcitonin, serum amyloid A

Introduction

Peritonitis is one of the most important sources of abdominal sepsis, which, despite modern therapeutic approaches, is followed by a relatively high mortality rate (1). The most common causes of peritonitis are gram-negative, gram-positive as well as anaerobic bacteria including intestinal flora-Escherichia coli, Klebsiella pneumoniae, Strepotococcus spp., and Bacteroides fragilis (2).

Since intra-abdominal infection leads to the activation of an inflammatory response that generates numerous mediators responsible for peritonitis, it is suggested that some of these mediators may be used as markers of severity of sepsis, but primarily to identify or rule it out (3). Identification of patients who have developed sepsis is very important primarily from the aspect of applied therapeutic measures as well as monitoring the results of treatment. Gustot et al. noted that of many markers used in sepsis, none have sufficient specificity and sensitivity to be routinely used in clinical practice. Also, given the complexity of the inflammatory response in sepsis, it is unlikely that an ideal biomarker will be found, and a combination of several markers would be more effective in identifying sepsis (4).

C-reactive protein (CRP) is synthesized in hepatocytes. A key role in inflammation and host response to infection mediated by CRP is related to the complement system, apoptosis, phagocytosis, NO release, and cytokine production (5,6). In infectious conditions, the level of CRP increases in the first 6-8 hours

and peaks from 350 to 400 mg/l, after approximately 48 hours. After inflammation or tissue destruction, CRP levels decline rapidly with an elimination half-life estimated at 4-9 hours. This rapid anti-inflammatory decline makes it a useful marker of disease activity (5-8).

Serum amyloid A (SAA) is a protein of acute phase of inflammation, produced in hepatocytes and macrophages/monocytes thanks to inflammatory mediators TNF, IL-1ß, and IF (9,10). During inflammation, SAA becomes part of high density lipoprotein (HDL), where it replaces apolipoprotein A1 (APOA1) (11). The human SAA family consists of the most common SAA1 and other isoforms - SAA, SAA2α, SAA2β, and SAA3 (12). During human endotoxemia, the level of SAA increases within 14-16 hours as a result of de novo expression of early cytokines that are inducers of its subsequent synthesis and secretion (13).

Parenchymal cells (adipocytes) are a source of high concentrations of procalcitonin (PCT) in conditions of severe bacterial infection and sepsis. Release of PCT is a result of activation of adipocytes during their interaction with monocytes through inflammatory mediators (IL-1ß and TNF) (14). Cytokines (IL-2 and IL-6) and TNF induce elevated procalcitonin levels even in the absence of infection (15).

Depending on the stimulus by toxin, plasma procalcitonin levels increase after 2-6 hours (16).

The aim of this study was to evaluate the sensitivity and specificity of serum markers of inflammation: CRP, PCT, and SAA in the serum of patients with diffuse secondary peritonitis.

Material and Methods

The prospective cohort study was conducted at the Clinic for Emergency Surgery of the Emergency Center of the Clinical Center of Serbia in Belgrade. The study group consisted of 100 patients aged 18 to 70 years, with signs of acute abdomen due to diffuse secondary peritonitis of the following etiology: pathological perforations: stomach and duodenum (longer than 12 hours), gallbladder, appendix, small and large intestine; complications arising after previous elective or emergency surgery; progression of inflammatory diseases of the gastrointestinal tract; vascular diseases of the intestine. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Belgrade (No 29/V-5).

The diagnosis of sepsis/SIRS was made based on the presence of at least two of the following clinical criteria: body temperature >38 or <36 ° C; heart rate >90/min; number of respirations >20/min or PaCO2 <4.3 kPa; leukocyte count >12000 or <4000, or the presence of >10% of immature neutrophils. The criterion for defining abdominal sepsis was a defined abdominal source of infection with a positive microbiological culture from the peritoneal cavity.

Within the study group, two groups of subjects were formed: the group designated as the SIRS group and the group designated as the SEPSA group, depending on the microbiological findings postoperatively.

Exclusion criteria in this study were: pregnancy, puerperium, diabetes mellitus, malignancies, patients with signs of acute pancreatitis, patients receiving cytostatics or on immunosuppressive therapy, patients infected with hepatitis B and C virus as well as HIV, patients with psychosis. Patients with suspected extra-abdominal origin of the infection were also excluded from the study.

Diffuse peritonitis was defined by the presence of toxic contents in at least three quadrants of the abdominal cavity.

The American Society of Anesthesiologist (ASA) score was calculated preoperatively for all patients, while the Acute Physiology And Chronic Health Evaluation II (APACHE II) score was determined on the first postoperative day.

All patients of the study group were sampled the contents of the abdominal cavity, which were subjected to standard microbiological analysis in the Microbiological Laboratory of the Emergency Center of the Clinical Center of Serbia in Belgrade.

Blood samples were collected before surgery and every day after surgery, with Vacutainers (BD Vacutainer Systems, Franklin Lakes, New

Jersey). Samples for the determination of C-reactive protein, Serum amyloid A and Procalcitonin were left at room temperature for 30 minutes to coagulate and then centrifuged at 2680 g for 10 minutes to obtain serum samples. C-reactive protein and Procalcitonin were determined using a Roche Cobas 6000 automated analyzer (Roche Diagnostics, Mannheim, Germany). Serum amyloid A values were determined using an enhanced immunophelometric test (Siemens Healthcare GmbH, Germany) on BNII System (Siemens Healthcare GmbH, Germany). The parameters on the basis of which the patients were scored were also determined - APACHE II score.

From the obtained data, a database was formed in which the numerical values of the monitored features within the observed groups were input.

The normality of the distribution was assessed by the Kolmogorov-Smirnov test. Normally distributed data are presented as mean values for the group with standard deviation (SD), and the rest as the median with interquartile range (IQR). Statistical significance of initial differences in patients with hernia, sepsis, and SIRS was evaluated by the Hi-square test, the Fischer test, the t-test, and the Wilcoxon test for independent samples. Sensitivity and specificity are shown using the ROC curve so that values closer to unity have a higher diagnostic utility compared to values of 0.5 which indicate that the test has no diagnostic utility. Statistical analysis and graphical representations were done in R 3.1.0. (17).

Results

The prospective cohort study included 100 patients with secondary peritonitis, divided into two groups: in the SIRS group there were 45 patients, while in the SEPSA group there were 55 patients. The mean age in the SIRS group was 45.91±13.1, in the SEPSA group it was 48.8±13.3, while in the hernia group it was 46.23±15.7. In both study groups, two-thirds were male patients. The average treatment of both examined groups lasted about seven days. No mortality was recorded in the study group.

The ASA score in both examined groups was 2. APACHE II score calculated on the first postoperative day was 7 in the SIRS group, while in the SEPSA group it was 8.

In comparison between patients in the study group, PCT and CRP also had statistically significant higher values in patients in the SEPSA group compared to patients in the SIRS group.

Preoperative ratio of sensitivity and specificity in different used breakpoints, where the ROC curve is closer to the left corner and indicates a higher degree of test usefulness, i.e., higher diagnostic accuracy and higher sensitivity of CRP and PCT in distinguishing SIRS syndrome from sepsis while the ROC curve generally coincides with the diagonal line, indicates a small degree of usefulness of the test, i.e., lower diagnostic accuracy of SAA in distinguishing SIRS syndrome from sepsis (*Fig. 1*).

Therefore, CRP and PCT are so far among the most valuable preoperative markers for

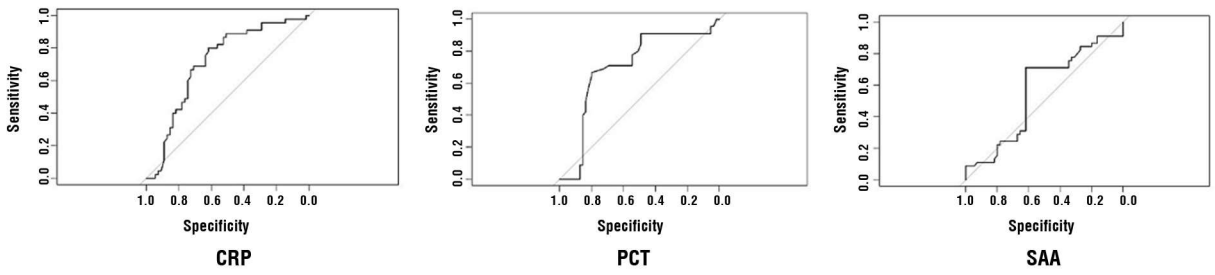


Figure 1. Sensitivity and specificity of CRP and PCT and SAA values preoperatively in distinguishing sepsis syndrome from SIRS (ROC curve)

distinguishing sepsis from SIRS (among them, in terms of AUC there is no statistically significant difference, p = 0.9125), while SAA is in this sense a very weak marker, having an area value below the curve very close to 0.5, which would correspond to a "random guess" (*Table 1*).

On the first postoperative day, the situation is different, the analysis of the relationship between sensitivity and specificity at the different breakpoints used, where the ROC curve is closer to the left corner, indicates a higher degree of usefulness of the test, i.e., greater diagnostic accuracy and greater sensitivity of SAA in distinguishing SIRS syndrome from sepsis compared to CRP and PCT (*Fig. 2*).

Here we see that the postoperative situation is just the opposite, SAA turns out to be a very valuable discriminator between sepsis and SIRS cases on the first postoperative day. The prognostic value of SAA markers in terms of the distinction between SIRS and sepsis is highly statistically significant, higher than the prognostic value of CRP (p=0.00186) (*Table 2*).

Also, in contrast to the SAA values in the study group patients on the first postoperative day, the analysis of the ratio between sensitivity and specificity at different breakpoints used, where the ROC curve generally coincides with the diagonal line, indicates a small degree of test usefulness, i.e., lower diagnostic accuracy of CRP and PCT in distinguishing SIRS syndrome from sepsis on the first postoperative day.

Table 1. AUC, 95% confidence interval, optimal breakpoint sensitivity and specificity of inflammatory markers in the optimal breakdown preoperatively

	AUC	95% CI	OBV	Sensitivity	Specificity
CRP	0.71	0.61-0.81	28.1	0.8	0.6
PCT	0.7	0.59-0.81	1.545	0.24	0.85
SAA	0.56	0.44-0.68	523.5	0.71	0.62

OBV, optimal breaking value

Table 2. AUC, 95% confidence interval, optimal breakpoint and sensitivity and specificity of inflammatory markers in the optimal breakdown, first postoperative day

	AUC	95% CI	OBV	Sensitivity	Specificity
CRP	0.64	0.53-0.75	201.55	0.8	0.51
PCT	0.46	0.34-0.58	5.64	0.67	0.53
SAA	0.85	0.76-0.95	1009.5	0.92	0.89

OBV, optimal breaking value

In the remaining postoperative period in our study, the ROC curve mostly coincided with the diagonal line, so CRP, PCT, and SAA had little diagnostic accuracy in distinguishing SIRS syndrome from sepsis.

On the second or third postoperative day, no marker can be said to have any statistically significant discriminant value in distinguishing between SIRS and sepsis (even CRP has a 95% confidence interval spanning 0.5).

Discussion

In our study, we considered the sensitivity and

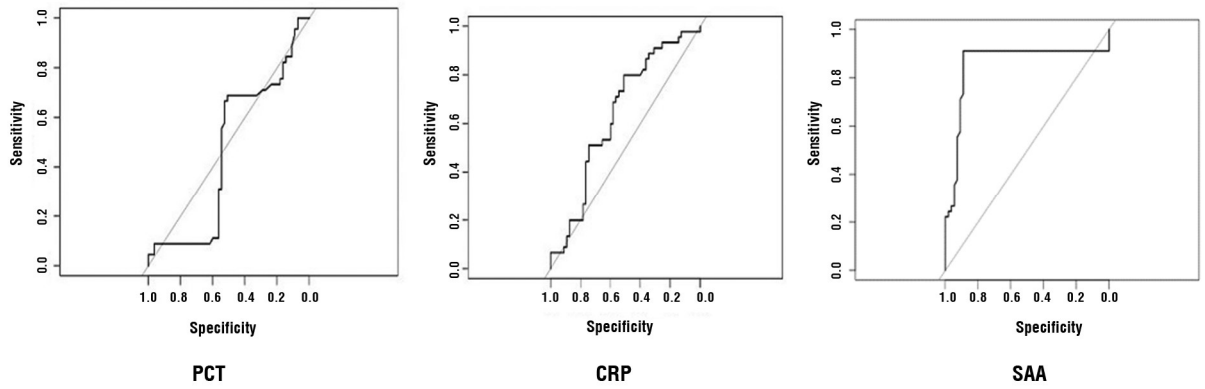


Figure 2. Sensitivity and specificity of CRP, PCT, and SAA values on the first postoperative day in distinguishing sepsis syndrome from SIRS (ROC curve).

specificity of acute markers of sepsis such as CRP, PCT, and SAA in patients with peritonitis. The values were increased in regard to the etiology of the disease, which significantly contributed to the diagnosis. Further analysis of our results concluded that the CRP values in our study were significantly higher in the SEPSA group compared to the SIRS group, which we explained by more intense inflammatory response in the SEPSA group.

By analyzing the relationship between sensitivity and specificity, preoperative values of CRP and PCT indicate a higher degree of usefulness of the test in distinguishing SIRS syndrome from sepsis. However, on the first, second, and third postoperative day, we find that CRP and PCT lose sensitivity and specificity, and thus the degree of usefulness of the test is declining. The results of many studies regarding the sensitivity and specificity of CRP are contradictory (18-20).

In our study, there was a statistically significant difference between the values of PCT in the SIRS group and the SEPSA group in the preoperative period. We explain this by the significant synthesis of PCT in the SEPSA group, caused by the excessive release of the present bacterial endotoxins and exotoxins, which are potent and important for the synthesis of PCT. Based on the above, we conclude that the inflammatory response in both study groups was significant, but the fact that there was no difference between the groups, to some extent limits the use of PCT in distinguishing SIRS from sepsis. Research by Zheng Wao et al. showed that PCT is not an ideal biomarker in discriminating SIRS from sepsis (21). Also, Yang et al., in a meta-analysis that included 18 studies with a total of 1,827 patients, showed that sensitivity and specificity were relatively good. However, given the methodological limitations and significant heterogeneity in the groups, the general conclusion was that medical decisions should be made after summing the clinical findings and PCT levels. Also, PCT cannot be used alone as the gold standard for the diagnosis of peritonitis (22).

Conclusions of the studies by Niu et al. and Lai et al. were that the selection of optimal biomarkers should be done carefully. Individual detection of PCT, CRP, and SAA had some value in the diagnosis of infectious diseases, but there was evidence of misdiagnosis and missed diagnosis. However, the combined detection of these three biomarkers has improved the accuracy of diagnosis in a timely and efficient manner, especially for early detection of sepsis, as well as the rate of detection of infectious diseases and provided greater accuracy of diagnosis. Also, the medical context, demographics, sensitivity and specificity of biomarkers should be taken into account (19,23).

Analyzing our results and the published results of other studies, we conclude that it would be desirable to include in the research the measurement of SAA as an acute phase protein in secondary peritonitis.

Yuan et al. conclude that determining the level of SAA in sepsis detection is most useful in combination with other markers such as CRP and PCT as well as determining their correlation (24).

In our study, during the preoperative period, the analysis of the relationship between sensitivity and specificity found a low degree of usefulness of the SAA test in distinguishing SIRS syndrome from sepsis, while on the first postoperative day we found that sensitivity and specificity indicated a higher degree of usefulness of SAA test in distinguishing SIRS syndrome from sepsis compared to CRP and PCT. Arnon et al. showed that SAA had overall better diagnostic accuracy in predicting early sepsis compared to CRP (sensitivity 96% vs. 30%) and specificity (95% vs. 98%) (18). A meta-analysis conducted by Yuan H. et al. showed that the overall sensitivity for SAA was better compared to CRP (0.78 (95% CI 0.73-0.83) vs. 0.67 (95% CI 0.62-0.73)). Also, in the same study, the overall specificity for SAA was slightly lower than CRP and was 0.89 (95% CI 0.84-0.92) compared to 0.92 (95% CI 0.89-0.95), but their difference was not statistically significant ($p < 0.05$) (24).

Conclusion

Based on previous research, it can be said that finding a specific marker for the diagnosis of abdominal sepsis, a marker that would differentiate between SIRS and sepsis, would be very useful. This would enable a more successful therapeutic approach as well as a prognosis for the treatment of peritonitis which would contribute to a better outcome.

Conflict of Interest

The authors declare no conflicts of interests.

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Reference

1. Blot S, Antonelli M, Arvaniti K, Blot K, Creagh-Brown B, de Lange D, et al. Epidemiology of intra-abdominal infection and sepsis in critically ill patients: "AbSeS", a multinational observational cohort study and ESICM Trials Group Project. Intensive Care Med. 2019;45(12):1703-1717.
2. Sartelli M, Coccolini F, Kluger Y, Agastra E, Abu-Zidan FM, Abbas A, et al. WSES/GAIS/SIS-E/WSIS/AAST global clinical pathways for patients with intra-abdominal infections. World J Emerg Surg. 2021;16(1):49.
3. Gotts JE, Matthay MA. Sepsis: pathophysiology and clinical management. BMJ. 2016;353:i1585.
4. Gustot T, Felleiter P, Pickkers P, Sakr Y, Rello J, Velissaris D, et al. Impact of infection on the prognosis of critically ill cirrhotic patients: results from a large worldwide study. Liver Int. 2014;34(10):1496-503.
5. Pathak A, Agrawal A. Evolution of C-Reactive Protein. Front Immunol. 2019; 10:943.
6. Sproston NR, Ashworth JJ. Role of C-Reactive Protein at Sites of Inflammation and Infection. Front Immunol. 2018;9:754.
7. Pepys MB. C-reactive protein fifty years on. Lancet. 1981;1(8221):653-7.
8. Young B, Gleeson M, Cripps AW. C-reactive protein: a critical review. Pathology. 1991;23(2):118-24.
9. Ramadori G, Sipe JD, Dinarello CA, Mizel SB, Colten HR. Pretranslational modulation of acute phase hepatic protein synthesis by murine recombinant interleukin 1 (IL-1) and purified human IL-1. J Exp Med. 1985;162(3):930-42.
10. Urieli-Shoval S, Meek RL, Hanson RH, Eriksen N, Benditt EP. Human serum amyloid A genes are expressed in monocyte/macrophage cell lines. Am J Pathol. 1994;145(3):650-60.
11. Coetzee GA, Strachan AF, van der Westhuyzen DR, Hoppe HC, Jeenah MS, de Beer FC. Serum amyloid A-containing human high density lipoprotein 3. Density, size, and apolipoprotein composition. J Biol Chem. 1986;261(21): 9644-51.
12. Sipe J. Revised nomenclature for serum amyloid A (SAA). Nomenclature Committee of the International Society of Amyloidosis. Part 2. Amyloid. 1999;6(1):67-70.
13. McAdam KP, Sipe JD. Murine model for human secondary amyloidosis: genetic variability of the acute-phase serum protein SAA response to endotoxins and casein. J Exp Med. 1976;144(4):1121-7.
14. Linscheid P, Seboek D, Schaer DJ, Zulewski H, Keller U, Muller B. Expression and secretion of procalcitonin and calcitonin gene-related peptide by adherent monocytes and by macrophage-activated adipocytes. Crit Care Med. 2004;32(8):1715-21.
15. Whang KT, Vath SD, Becker KL, Snider RH, Nylen ES, Muller B, et al. Procalcitonin and proinflammatory cytokine in interactions in sepsis. Shock. 1999;12(4):268-73.
16. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet. 1993;341(8844):515-8.
17. Braselmann H, Michna A, Hess J, Unger K. CFAssay: statistical analysis of the colony formation assay. Radiat Oncol 2015;10:223.
18. Ucar B, Yildiz B, Aksit MA, Yarar C, Colak O, Akbay Y, et al. Serum amyloid A, procalcitonin, tumor necrosis factor-alpha, and interleukin-1beta levels in neonatal late-onset sepsis. Mediators Inflamm 2008;2008:737141.
19. Niu T, Liu Y, Zhu F, Ma J, Gao J. Time-resolved fluorescent immunoassay-based combined detection of procalcitonin, C-reactive protein, heparin binding protein, and serum amyloid A1 to improve the diagnostic accuracy of early infection. J Clin Lab Anal. 2019;33(2):e22694.
20. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. Crit Care. 2010; 14(1):R15.
21. Xiao Z, Wilson C, Robertson HL, Roberts DJ, Ball CG, Jenne CN, et al. Inflammatory mediators in intra-abdominal sepsis or injury - a scoping review. Crit Care 2015;19:373.
22. Yang SK, Xiao L, Zhang H, Xu XX, Song PA, Liu FY, et al. Significance of serum procalcitonin as biomarker for detection of bacterial peritonitis: a systematic review and meta-analysis. BMC Infect Dis 2014;14:452.
23. Lai L, Lai Y, Wang H, Peng L, Zhou N, Tian Y, et al. Diagnostic Accuracy of Procalcitonin Compared to C-Reactive Protein and Interleukin 6 in Recognizing Gram-Negative Bloodstream Infection: A Meta-Analytic Study. Dis Markers 2020;2020:4873074.
24. Yuan H, Huang J, Lv B, Yan W, Hu G, Wang J, et al. Diagnosis value of the serum amyloid A test in neonatal sepsis: a meta-analysis. Biomed Res Int 2013;2013:520294.