LETOPIS NAUČNIH RADOVA / ANNALS OF AGRONOMY
Vol. 45, No 2, 143-152
UDK: 616.151:599.324.2

Poljoprivredni fakultet
Univerzitet u Novom Sadu

Principal component and correlation analysis of blood routine parameters in experimental sepsis in rats

Dragica Stojanović[a]*, Marko Cincović[a], Filip Štrbac[a], Kosta Petrović[a], Romeo Teodor Cristina[b], Zorana Kovačević[a]

[a]University of Novi Sad, Faculty of Agriculture, Department of Veterinary medicine, Novi Sad, Serbia
[b] Banat's University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Faculty of veterinary medicine, Timișoara, Romania
*Autor za kontakt: dragicas@polj.edu.rs

Abstract
Sepsis can be defined as a multisystem response of the organism on bacterial infection agents. The aim of this study is to provides an extensive analysis of associations between numerous routine biochemical and hematological markers in function of type of sepsis caused by experimental Gram-positive (G+, *Staphylococcus aureus*), Gram-negative (G-, *Escherichia coli)* and mixed (MIX) microorganism in laboratory rats model. In G- and G+ sepsis higher values of erythrocytes, total protein, albumin, creatinine, sodium and plasma osmolarity were noted and lower triglycerides values compared to MIX sepsis. Existence of statistically significant interaction between time and type of sepsis was showed that values of erythrocytes, neutrophiles, LUC, glucose, total protein, urea, Na, Cl, osmolarity and LDH were changed during time in function of sepsis type. Distinguish of animals with G+, G- or MIX sepsis is not possible because principal component analysis (PCA) demonstrated similar expression of blood hematological and metabolic parameters in animals regardless type of experimental sepsis (G+, G- or MIX), with minimal deviation of animal with MIX sepsis from animals with sepsis caused by pure culture (G+ or G-). Correlation coefficient analysis showed 507 significant correlations between routine blood parameters (207 in G-, 186 in G+ and 114 in MIX) and some of them are in function of type of sepsis. Distinguish of type of sepsis in function of blood parameters is not possible. After extraction of two components they load by blood parameters was similar in all type of sepsis (positive load with different type of white blood cells, PLT, glucose, amylase, Cl, urea and osmolarity, and negative load with albumin, total protein, cholesterol, creatinine and ALT). Although our results demonstrated difference in values and correlation coefficients for routine blood biochemistry and hematology parameters, multiparametric statistic showed they not be a useful tool for distinguishing type of sepsis according bacterial causative.

Key words: sepsis, blood parameters, experimental model, rats

Introduction

Cecal ligation and punction in laboratory rats represents standard model for sepsis examination (Fink, 2014; Coletta et al., 2014). In this method hematologic, hemodynamic and metabolic changes occur and they be compared with sepsis in humans. So this method is often used in different researches of sepsis pathophysiology and therapy (Liu et al., 2017; Zhai et al., 2018). Parrillo et al. (1990) showed that Gram- negative (G-) bacteria are the most common cause of sepsis. Contrary, Martin et al. (2003) have shown that Gram-positive (G+) bacteria are the most significant factor in sepsis development.

Sepsis can be defined as a multisystem response of the organism on bacterial infection agents and it is manifested with hyperthermia or hypothermia, tachypnea, tachy- or bradicardia, leucocytosis of leucopenia, higher concentration of CRP, changes in values of pro-inflammatory cytokine, arterial hypotension with positive fluid balance, oliguria with higher concentrations of urea and creatinine in the blood, change of coagulation cascade and reduced tissue perfusion with lactate accumulation (Iskander et al., 2013; Li et al., 2018). Sepsis has a pro-inflammatory and compensatory anti-inflammatory phase in which specific biomarkers are produced (Faix, 2013). Pierrakos et al. (2020) in their meta-analysis identified 258 biomarkers related to sepsis, but there are not included blood hematology and biochemistry biomarkers. These biomarkers are important part of laboratory evaluation of organ dysfunction, which fallow severe sepsis after infection and SIRS (Wang et al., 2007; Ulevitch and Tobias, 1999). Our previous studies show that, in sepsis caused by a pure or mixed culture, there are similar but not identical hematologic and metabolic responses, without multivariate analysis of dynamical change and expression of metabolic parameters in function of type of sepsis (Stojanović et al., 2012, 2013). There is no many data about influence of type of sepsis on blood parameters. Alexandraki and Palacio (2010) found a much higher concentration of C-reactive

protein and IL-6 in G- than in G + sepsis. Also, Bilgili et al. (2018) concluded that procaltitonin value may be a useful tool for distinguishing causative pathogen, G+ from G-, in sepsis. The use of advanced statistical multi-parameter analysis can change the perception of the clinical significance of certain parameters in sepsis. It is known based on the pathophysiology of sepsis that cytokines levels could be used as sepsis markers. Lvovschi et al. (2011) found that cytokine profiles in sepsis have limited relevance for stratifying patients when applied multi-parametric statistic methods.

The aim of this study is to determine association between type of sepsis and blood hematologic and biochemical parameters in laboratory rats by multi-parameter data analysis (correlation, principal component analysis and agglomerative hierarchical clustering).

Material and methods

Escherichia coli (ATCC 25922) and Staphylococcus aureus (ATCC 25923) strains were used in experiment. Given suspension of standard strains (inoculums) are taken by sterile syringe and applied in ceacum of rat. 1 ml of inoculums contained about 10^9 bacteria. Ceacal ligation and punction sepsis model (CLP) was reproduced with use of explanation technique and characteristics of Wichterman model with some modifications. Surgical intervention was conducted on anesthetized animals. For anesthesia thiopentobarbitol in dose of 50 mg/kg was applied intraperitoneally. Abdominal incision was done in medial line, 2 cm bellow ceacum and after that two ceacum punctions were done by the 18 diameter needle. Rats were divided in the four groups. Three of them contained 28 animals and one 20-control group. All three groups were operated. Abdominal wall was opened and cecum was ligated (1/3 bellow ileocecal valvulae) in experimental groups, excluding control group (C) that haven't had cecal ligation. Control group (20 animals) had only abdominal incision (fake surgery). Three experimental groups were inoculated by Escherichia coli (EC, G-), Staphylococcus aureus (SA, G+) or mixed culture (MIX, G- and G+).

In order to follow sepsis development animals were examined and sacrificed 12, 24, 74 and 120 hours after surgical interventions. Clinical signs of sepsis (reduced movement, acorned hair, lethargy, tachypnoae, and diarrhea) were noted and completed with measurements of rectal temperature. In noted hours animals were sacrificed. Blood samples were taken by punction of a. abdominalis and it is used for determining hematologic and biochemical parameters.

Blood samples were processed with automatic analyzers of American producer Technicon. EDTA (ethylene diamino tetra acetate) blood samples were used to determination of blood hematologic parameters with differential leucocytes formula and are processed with hematologic analyzer ,,H-1'' Technicom. Extruded serum was used for determination of biochemistry parameters concentrations: total proteins, albumin, urea, creatinine, glucose, triglycerides, cholesterol, electrolytes (Na, Cl) and enzymes (ALT, AST, LDH). All named parameters were determined at automatic biochemical analyzer.

For determination of influence of sepsis type, time after sepsis induction and interaction of sepsis type×time on values of hematologic and biochemical parameters we used ANOVA analysis. Pearson's correlation was used for examination of linear correlation between hematologic and biochemical parameters of blood samples in all times in all types of sepsis. Multi-parameters analysis visualization as heat maps and cluster analysis will be presented in function of type of sepsis. Principal component analysis include exclusion of two component and graphic representation of factor load by parameters for each type of sepsis. Statistic software SPSS (IBM, USA) and online platform (http://www.heatmapper.ca/ and https://biit.cs.ut.ee/clustvis/, visit in July 2020) were used for this purposes.

Results and discussion

Two factor ANOVA results showed significant influence of type of sepsis, time and their interaction on value of routine blood biochemistry and hematology parameters (Figure 1). Higher values of erythrocytes, total protein, albumin, creatinine, sodium and osmolarity, like lower value of triglycerides were founded in SA in EC sepsis compared to MIX sepsis. Existence of statistically significant interaction between time and type of sepsis was showed that during time values of some parameters were changed in function of sepsis type. This interaction was statistically significant for the following parameters: erythrocytes, neutrophiles, LUC, glucose, total protein, urea, Na, Cl, osmolarity and LDH. Glucose value was significantly lower 12 and 24h after inoculation and 72h in MIX sepsis after which showed increase to values of control group. Total protein value was reduced compared to control group in MIX sepsis but their value increased in first 24h in EC and SA sepsis and after that showed

decrease. In all three types of sepsis total protein concentration was higher compared to control group 120h after inoculation. Urea concentration was significantly decreased 12 hours after inoculation after that started to increase to reach the values of the control group. Higher values of urea were founded 72 hours after inoculation in SA sepsis. Decreased concentrations of Na values were showed 24h after inoculation but 48 h after inoculation Na concentration was still reduced in MIX sepsis and it is increased in SA and EC sepsis compared to values of control group. In 72 and 120h they values were bellow control group values. 12h after inoculation chloride level was reduced and in 48h the lowest concentration was measured after which increase was noted. The greatest reduction was noted in SA sepsis and the greatest increase at the end was noted in MIX and SA sepsis. Osmolarity have showed biphasic nature and significant influence has interaction type x time. Osmolarity was bellow control group values in all three type of sepsis 12h after inoculation after which significant increase in EC and SA sepsis was noted but in MIX sepsis was still in reduction 48h after inoculation. From 72 to 120h plasma osmolarity in MIX sepsis showed increase but in EC and SA reduction was noted until bellow control values. LDH value was significantly higher 12 and 24 hours compared to decrease value in EC and SA sepsis. In MIX sepsis LDH value was decreased bellow the values of the control group and after which started to increase.

Animal clustering shows that at the beginning of sepsis there is a higher expression of erythrocytes, hemoglobin, cholesterol, ALT and osmolarity, but with the development of sepsis over time the expression of WBC, amylase, glucose, urea, Cl and platelets increases (Figure 2). Distinguish of animals with G+, G- or MIX sepsis is not possible because PCA analysis demonstrated that animals with EC and SA sepsis are similar and MIX sepsis showed minimal deviation in relation to mentioned sepsis caused with pure bacterial culture (Figure 3). Distinguish between time after bacterial inoculation is possible, so that one group includes animals in early period after sepsis (12 and 24h), and second group include animals in late period (72 and 120h).

Expressions of correlation between blood parameters in function of type of sepsis are presented on Figure 4. Correlation coefficient analysis showed 507 significant correlations between routine blood parameters (207 in EC, 186 in SA and 114 in MIX) and some of them are in function of type of sepsis. Correlation coefficient analysis showed some specific relation between hematology and biochemistry parameters in function of type of sepsis. Correlation of ALT with some of the hematologic parameters was showed in EC and MIX but not in SA sepsis. No significant correlation was showed between AST and any hematologic parameters in all three type of sepsis. Correlation of LDH and erythrocyte number was showed in SA and MIX sepsis. Correlation of urea and some parameters was noted in EC and SA sepsis but not in MIX sepsis. Significant linear correlation was noted between glucose with some hematologic parameters in SA and EC sepsis but not in MIX sepsis. Significant correlation was noted between creatinine and hematologic parameters in EC sepsis but not in MIX and SA sepsis. Linear correlation was noted between Na and only one blood parameter in SA and MIX sepsis but none in EC type. Significant correlation was noted between chloride and some of hematologic parameters in EC and SA sepsis but not in MIX sepsis. Statistically significant correlation of albumin and total leukocytes and neutrophiles was noted in all three types of sepsis. Total proteins have showed correlations in EC and SA sepsis but not in MIX sepsis. Amylase has showed positive correlation with total leucocytes and neutrophiles in all three types of sepsis. Osmolarity showed correlation with some parameters in MIX sepsis, but not in EC and SA sepsis. No statistically significant correlation was noted between CK and TGC in any of sepsis types. Significant correlation was noted between cholesterol in EC and SA type, but not in MIX type of sepsis.

Comparison between sepsis types in function of expression of blood parameters was analyzed trough PCA. For all types of sepsis PCA were given components explain 55-61% of all original routine blood parameters variance (Figure 5). Loadings close to -1 or 1 indicate that the variable strongly influences the component. After extraction of two components they load by blood parameters was similar in all type of sepsis, including positive load with different type of white blood cells, PLT, glucose, amylase, Cl, urea and osmolarity, and negative load with albumin, total protein, cholesterol, creatinine and ALT.

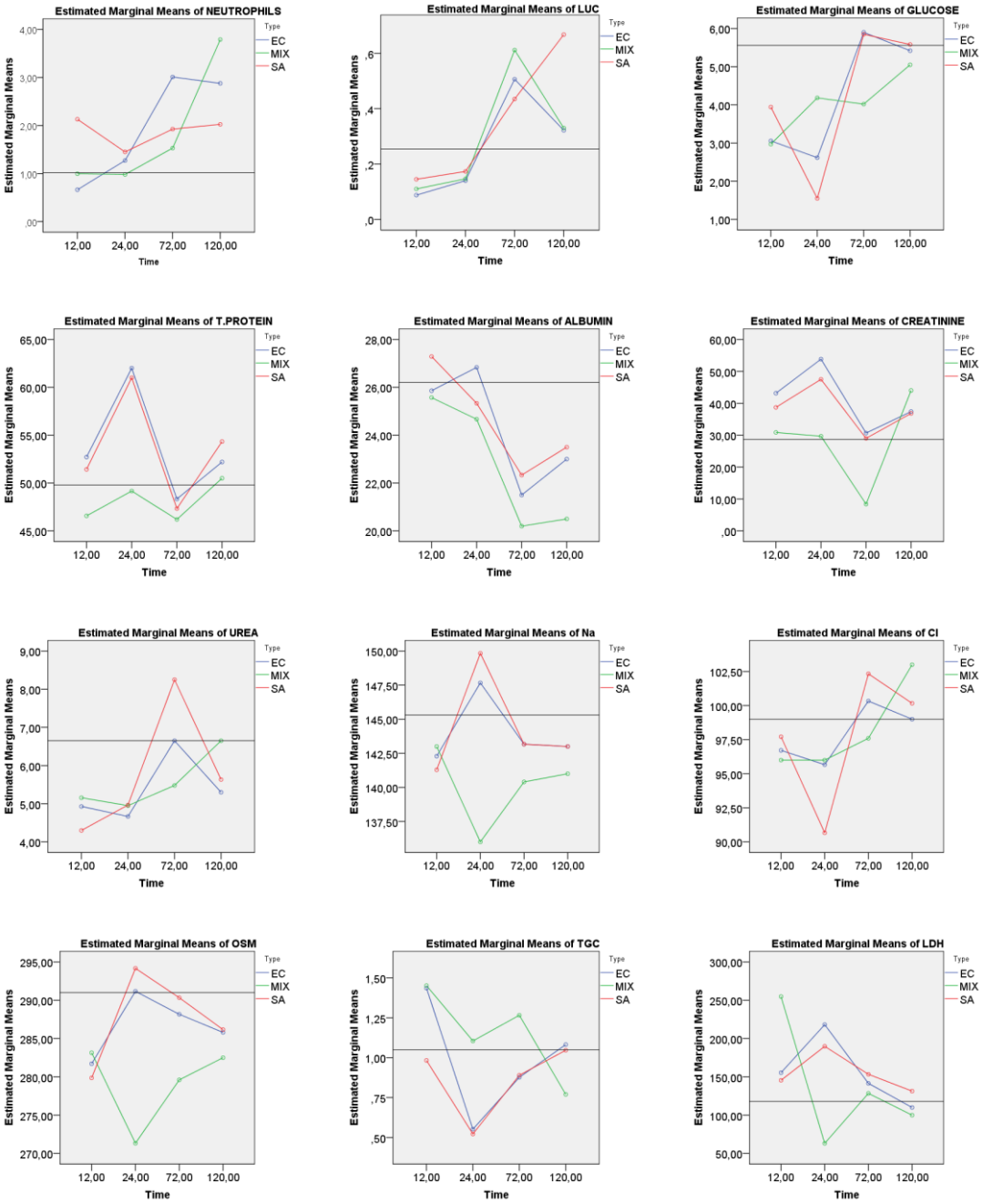


Figure 1. Blood parameters with significant effect of sepsis type and sepsis×time interaction (horizontal line represents mean value of control group).
Slika 1. Krvni parametri sa statistički značajnim uticajem vrste sepse i interakcije sepsa×vreme (horizontalne linije pretstavljaju srednju vrednost kontrolne grupe).

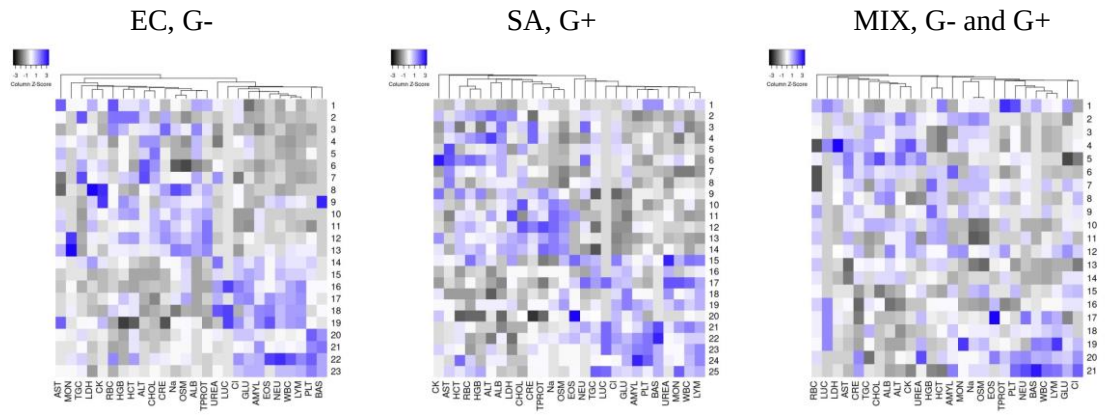


Figure 2. Animal clustering and heat map of expression of blood parameters during the time after bacterial inoculation (12, 24, 72 and 120h) in EC (left), SA (central) and MIX (right) sepsis.
Slika 2. Klasterovanje životinja i toplotna mapa ekspresije krvnih parametara sa protokom vremena posle bakterijske inokulacije (12, 24, 72 i 120h) kod EC (levo), SA (centralno) i MIX (desno) sepse.

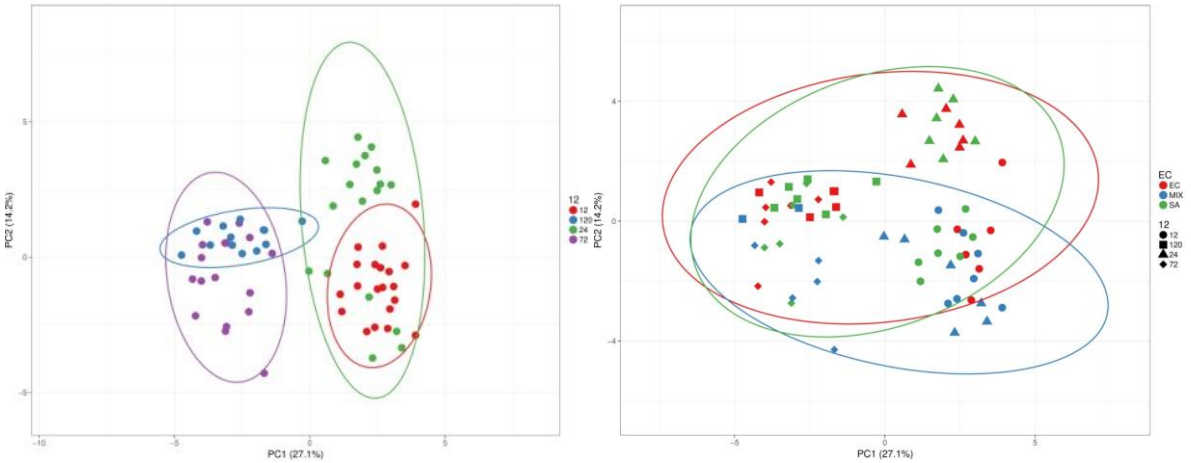


Figure 3. Animal classification and principal component in function of time after bacterial inoculation and sepsis×time interaction.
Slika 3. Klasifikacija životinja i glavne komponente u funkciji protoka vremena od bakterijske inokulacije i interakcije vrsta sepse×vreme.

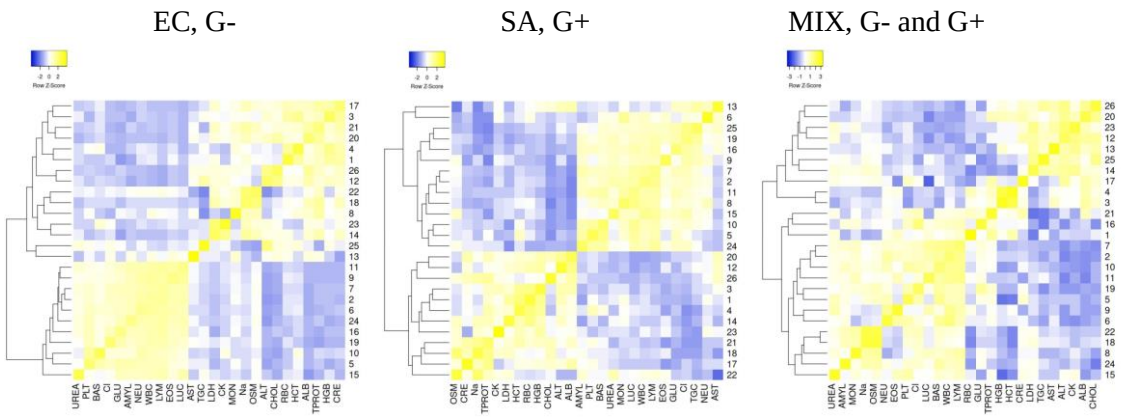
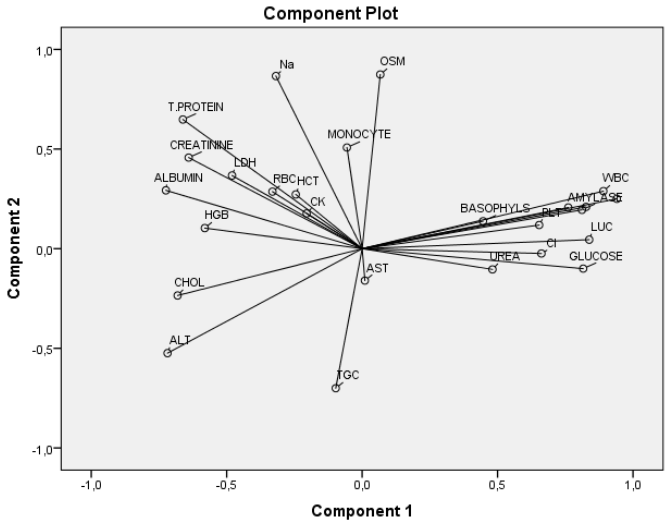
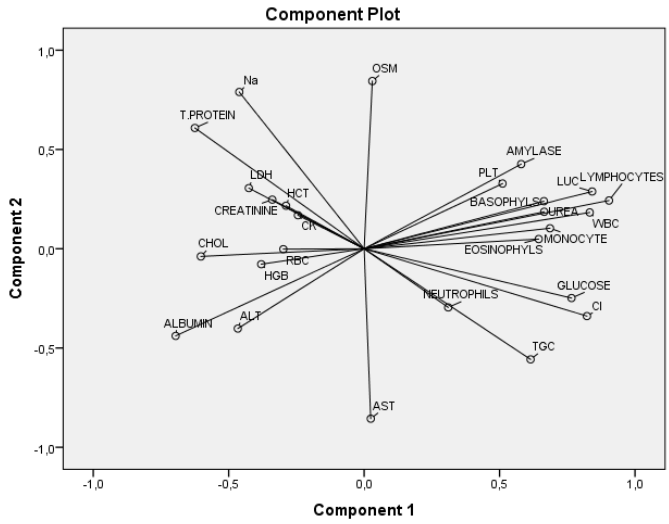


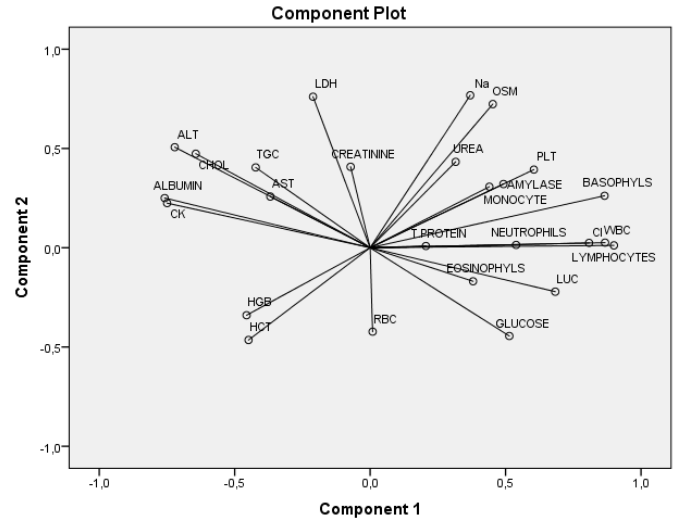
Figure 4. Heat map for expression of coefficient correlation between blood parameters in different of type of sepsis (EC-left, SA-in the middle and MIX- right).
Slika 4. Toplotna mapa i ekspresija vrednosti koeficijenata korelacije između krvnih parametara u različitim vrstama sepse (EC-levo, SA-u sredini i MIX- desno).



EC, G-



SA, G+



MIX, G- and G+

Figure 5. Principal component analysis and component load with blood parameters in EC (up), SA (in the middle) and MIX (down) sepsis.
Slika 5. Analiza glavnih komponenata i njihovo opterećenje pojedinačnim krvnim parametrima kod EC (gore). SA (u sredini) i MIX(dole) sepse.

Our results show similarities but also minimal differences in the expression of hematological and metabolic changes during experimental sepsis. Similarities as related to generalized inflammation and tissue damage, but differences could be related to the different bacterial toxins that cause sepsis. *Staphylococcus aureus (S.aureus, SA)* and *Escherichia coli (E.coli, EC)* are the most common isolated bacterial cultures during septic state (Karzai et al., 2003). Ramachandaran (2014) summarizes some of G+ and G- bacterial toxins and their role in sepsis. Pathogenesis of immunology response showed certain differences between these two bacterial agents. EC poses structural endotoxin, respectively LPS that are located in membrane of the microorganism. LPS is recognized from the host via Toll-like receptors 4 (TLR-4). LPS induces immunologic cell to secrete proinflammatory cytokines (IL-8, IL-6, IL-1β, IL-1, IL-12, and IFNγ) and TNFα is crucial in development of endotoxic shock and tissue damage. The most potent toxin of SA for development of sepsis is super-antigen staphylococcal enterotoxin SE (A-E) and toxic shock syndrome toxin (TSST-1). These antigens directly stimulate numerous T-Cells by binding MHC-II molecules, Vβ-chain and CD28 receptors. Then intensive stimulation of Th-1 cells that produce cytokines including TNF, IFNγ, IL-2 causing inflammatory response and sepsis. Garrido and Figueiredo (2004) in their experiment aid the main characteristics of different models for sepsis research and have concluded that extraction of cecal content in experimental animals will cause synergistic action of all bacteria from bowels. Abe et al. (2010) found that gram-negative bacteremia induces greater magnitude of inflammatory response than Gram-positive bacteremia.

An in-depth study by Hoerr et al. (2012) offers interesting results for understanding the similarities and differences in metabolic response to G+ and G- types of sepsis. In both S. aureus and E. coli infections were found high cytokine and chemokine concentrations due to systemic infection, but magnitude of change is higher in E.coli infection. Main change in SA infection were related to fatty acid oxidation such as acetone, 3-hydroxybutyrate, and 2-hydroxybutyrate as well as isobutyrate and creatine, while the main changes in EC infection were in relation with amino acid blood level. Also, cytokines and chemokines negatively correlated with choline, glucose, as well as the TCA cycle parameters. Sepsis induces significant changes in glucose metabolism. In our research reduction of glucose concentration compared to control group is noticeable in first hours of research. Rittirsch et al. (2009) have been noted that during acute stress hypermetabolic response occurs followed by hyperinsulinemia which explains glucose reduction. Furthermore, it has been determined that hypoglycemia can be related with increased insulin sensitivity in early phase of sepsis (VanDerCrabben et al., 2009). Hypermetabolic response in first 12 hours can be related with decreased values of amylase after which increase has been noted in research of Nakajama (2016) that follows metabolic syndrome and obesity and all states where insulin secretion is disturbed. Development of hypoglycemia in septic patients was related with anorexia, kidney and liver insufficiency. Septic shock is much easier to occur in states of hypoglycemia (Rattarasarn et al., 1997). Other metabolic changes can be explained by changes dysfunctional status of liver and kidneys that are result of cecal ligations and punction and are in accordance with previous authors (Regueira et al. 2011; Yan et al., 2014). Given phenotype linear correlation between parameters of blood and metabolic profile showed interdependence of inflammatory and metabolic response. Diagnostic of sepsis implies use of different indicators and all-time purification of metabolic parameters in order to get parameters with the most important practical significance. In research of Liu et al. (2016) diagnostic use cytokines, acute phase proteins, blood elements have been determined. For evaluation of sepsis in clinical work Sequential Organ Assessement Score (SOFA) is used and includes platelets count, bilirubin concentrations, creatinine concentration, haemostatic parameters, partial pressure of oxygen and neurologic status evaluation (Marik and Taeb, 2017). Speaking of blood, diagnostic criteria for sepsis is WBC above 12,000 cells/mm^3 or <4000 cells/mm^3 or >10% immature (band) forms (Fan et al., 2016). During sepsis lymphocytes number decreases because of apoptosis causing reduction in total leucocytes count (Hotchkiss et al., 1997). Neutrophiles count is significant prognostic sign, so higher neutrophiles count means better survival rate in rats (Remick et al., 2002). Decrease of platelets count and changes in coagulation cascade have been determined in recent research (Li et al., 2018). Recent results by Li et al. (2018) show that phenotypes of multiple organ dysfunction were found in the CLP model, including increased liver alanine aminotransferase and aspartate transaminase; significantly reduced total protein, globulin, and serum albumin; increased blood urea nitrogen and creatinine; and decreased blood glucose. Our results are in line with the mentioned finding.

Conclusion

In conclusion, the obtained results about values and correlation coefficients for routine blood biochemistry and hematology parameters suggest the differences between G +, G- and MIX sepsis. Although our results demonstrated difference in values and correlation coefficients for routine blood biochemistry and hematology parameters, multiparametric statistic showed they not be a useful tool for distinguishing type of sepsis according bacterial causative.

Acknowledgement

This research is part of COST project CA18217 - European Network for Optimization of Veterinary Antimicrobial Treatment and project No451-03-68/2020-14/200117, Ministry of education, science and technological development, Serbia.

References

Abe, R., Oda, S., Sadahiro, T., Nakamura, M., Hirayama, Y., Tateishi, Y., Shinozaki, K., Hirasawa, H. 2010. Gram-negative bacteremia induces greater magnitude of inflammatory response than Gram-positive bacteremia. Critical Care 14:1-7.

Alexandraki, I., Palacio, C. 2010. Gram-negative versus Gram-positive bacteremia: what is more alarmin(g)? Critical Care 14: 161.

Bilgili, B., Haliloğlu, M., Aslan, M.S., Sayan, İ., Kasapoğlu, U.S., Cinel, I. 2018. Diagnostic accuracy of procalcitonin for differentiating bacteraemic Gram-negative sepsis from Gram-positive sepsis. Turk J Anaesthesiol Reanim 46:38.

Coletta, C., Módis, K., Oláh, G., Brunyánszki, A., Herzig, D.S., Sherwood, E.R., Ungvári, Z., Szabo, C. 2014. Endothelial dysfunction is a potential contributor to multiple organ failure and mortality in aged mice subjected to septic shock: preclinical studies in a murine model of cecal ligation and puncture. Critical care 18:511.

Faix JD. 2013. Biomarkers of sepsis. CRIT REV CL LAB SCI 50:23-36.

Fan, S.L., Miller, N.S., Lee, J., Remick, D.G. 2016. Diagnosing sepsis–The role of laboratory medicine. CLIN CHIM ACTA 460:203-10.

Fink, M.P. 2014. Animal models of sepsis. Virulence 5: 143–153.

Garrido, A.G., Figueiredo, L.F.P.D. 2004. Experimental models of sepsis and septic shock: an overview. Acta Cirurgica Brasileira 2004; 19(2): 82-88.

Hoerr, V., Zbytnuik, L., Leger, C., Tam, P.P., Kubes, P., Vogel, H.J. 2012. Gram-negative and Gram-positive bacterial infections give rise to a different metabolic response in a mouse model. J proteome res 11: 3231-45.

Hotchkiss, R.S., Swanson, P.E., Cobb, J.P., Jacobson, A., Buchman, T.G., Karl, I.E. 1997. Apoptosis in lymphoid and parenchymal cells during sepsis: findings in normal and T- and B-cell-deficient mice. Crit Care Med 25:1298–1307.

Iskander, K.N., Osuchowski, M.F., Stearns-Kurosawa, D.J., Kurosawa, S., Stepien, D., Valentine, C., Remick D.G. 2013. Sepsis: multiple abnormalities, heterogeneous responses, and evolving understanding. Physiol Rev 93:1247-88.

Karzai, W., Cui, X., Mehlhom, B., Straube, E., Hartung, T., Gerstenberger, E., Banks, S.M., Natanson, C., Reinhart, K., Eichacker, P.Q. 2003. Protection with antibody to tumor necrosis factor differs with similarly lethal Escherichia coli versus Staphylococcus aureus pneumonia in rats. Anesthesiology 99:81-89.

Li, J.L., Li, G., Jing, X.Z., Li, Y.F., Ye, Q.Y., Jia, H.H., Liu, S.H., Li, X.J., Li, H., Zhang, Y., Wang, H. 2018. Assessment of clinical sepsis-associated biomarkers in a septic mouse model. J Int Med Res 46(6):2410-2422.

Liu, C., Hu, J., Mao, Z., Kang, H., Liu, H., Fu, W., Lv, Y., Zhou, F. 2017. Acute kidney injury and inflammatory response of sepsis following cecal ligation and puncture in d-galactose-induced aging rats. Clin Interv Aging 12: 593-602.

Liu, Y., Hou, J.H., Li, Q., Chen, K.J., Wang, S.N., Wang, J.M. 2016. Biomarkers for diagnosis of sepsis in patients with systemic inflammatory response syndrome: a systematic review and meta-analysis. Springerplus 5: 2091.

Lvovschi, V., Arnaud, L., Parizot, C., Freund, Y., Juillien, G., Ghillani-Dalbin, P., Bouberima, M., Larsen, M., Riou, B., Gorochov, G., Hausfater, P. 2011. Cytokine profiles in sepsis have limited relevance for stratifying patients in the emergency department: a prospective observational study. PLoS One, 6: p.e28870.

Marik, P.E., Taeb, A.M. 2017. SIRS, qSOFA and new sepsis definition. J Thorac Dis 9:943-945.

Martin, G.S., Mannino, D.M., Eaton, S., Moss, M. 2003. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 348:1546–1554.

Opal, S.M., Garber, G.E., LaRosa, S.P., Maki, D.G., Freebairn, R.C., Kinasewitz, G.T., Dhainaut, J.F., Yan, S.B., Williams, M.D., Graham, D.E., Nelson, D.R., Levy, H., Bernard, G.R. 2003. Systemic host responses in severe sepsis analyzed by causative microorganism and treatment effects of drotrecogin alfa (activated). Clin Infect Dis 37:50–58.

Parrillo, J.E., Parker, M.M., Natanson, C., Suffredini, A.F., Danner, R.L., Cunnion, R.E., Ognibene, F.P. 1990. Septic shock in humans. Advances in the understanding of pathogenesis, cardiovascular dysfunction, and therapy. Ann Intern Med 113:227–242.

Pierrakos, C., Velissaris, D., Bisdorff, M., Marshall, J.C., Vincent, J.L. 2020. Biomarkers of sepsis: time for a reappraisal. Crit Care 24:1-15.
Ramachandran, G. 2014. Gram-positive and gram-negative bacterial toxins in sepsis: a brief review. Virulence 5:213-218.
Rattarasarn, C. 1997. Hypoglycemia in sepsis: risk factors and clinical characteristics. J Med Assoc Thai 80:760-766.
Regueira, T., Andresen, M., Mercado, M., Downey, P. 2011. Physiopathology of acute renal failure during sepsis. Med Intensiva 35: 424-432.
Remick, D.G., Bolgos, G.R., Siddiqui, J., Shin, J., Nemzek, J.A. 2002. Six at six: interleukin-6 measured 6 h after the initiation of sepsis predicts mortality over 3 days. Shock 17:463–467.
Rittirsch, D., Huber-Lang, M.S., Flierl, M.A., Ward, P.A. 2009. Immunodesign of experimental sepsis by cecal ligation and puncture. Nature Protocols 4:31–36.
Schulte, W., Bernhagen, J., Bucala, R. 2013. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets—an updated view. Mediators inflamm http://dx.doi.org/10.1155/2013/165974
Stojanović, D., Kovačević, Z., Žekić, M., Ašanin, J., Ratajac, R., Petrović, J., Petrović, T., Stojanov, I., Velhner, M. 2013. Examination of enzymes concentration in the rats blood with sepsid caused by mixed and pure bacterial cultures. Acta Vet Bg, 63: 609-620.
Stojanović, D., Velhner, M., Stojanov, I., Petrović, J., Ašanin, J., Kovačević, Z. 2012. Biochemical parameters in rats with an applved model of sepsis (cecal ligation and puncture) with pure and mixed bacterial culture. Acta Vet Bg, 62: 543-451.
Ulevitch, R.J., Tobias, P.S. 1999. Recognition of gram-negative bacteria and endotoxin by the innate immune system. Curr Opin Immunol 11:19–22.
Van Der Crabben, S.N., Blumer, R.M., Stegenga, M.E., Ackermans, M.T., Endert, E., Tanck, M.W., Serlie, M.J., van der Pool, T., Sauerwein, H.P. 2009. Early endotoxemia increases peripheral and hepatic insulin sensitivity in healthy humans. J Clin Endocrinol Metab 94:463-468.
Wang, H.E., Shapiro, N.I., Angus, D.C., Yealy, D.M. 2007. National estimates of severe sepsis in United States emergency departments. Crit Care Med 35:1928–1936.
Yan, J., Li, S., Li, S. 2014. The role of the liver in sepsis. INT REV IMMUNOL 33:498-510.
Zhai, X., Yang, Z., Zheng, G., Yu, T., Wang, P., Liu, X., Ling, Q., Jiang, L., Tang, W. 2018. Lactate as a potential biomarker of sepsis in a rat cecal ligation and puncture model. Mediators inflamm https://doi.org/10.1155/2018/8352727.

Analiza glavnih komponenti i korelacije rutinskih parametara krvi u eksperimentalnoj sepsi kod pacova

Dragica Stojanović[a]*, Marko Cincović[a], Filip Štrbac[a], Kosta Petrović[a], Romeo Teodor Cristina[b], Zorana Kovačević[a]

[a]Univerzitet u Novom Sadu, Poljoprivredni fakultet, Departman za veterinarsku medicinu, Novi Sad, Srbija
[b] Banatski univerzitet poljoprivrede i veterinarske medicine "King Michael I of Romania", Fakultet veterinarske medicine, Temišvar, Rumunija
*Autor za kontakt: dragicas@polj.edu.rs

SAŽETAK

Sepsa se može definisati kao multisistemski odgovor organizma na uzročnike bakterijske infekcije. Cilj ove studije je da pruži opsežnu analizu povezanosti brojnih rutinskih biohemijskih i hematoloških markera u funkciji tipa sepse izazvane eksperimentalnim Gram-pozitivnim (G+, *Staphylococcus aureus*), Gram-negativnim (G-, *Escherichia coli*) i mešani (MIX) mikroorganizam u modelu laboratorijskih pacova. U G- i G+ sepsi su zabeležene više vrednosti eritrocita, ukupnog proteina, albumina, kreatinina, natrijuma i osmolarnosti plazme i niže vrednosti triglicerida u odnosu na MIX sepsu. Utvrđeno je postojanje statistički značajne interakcije između vremena i tipa sepse da su se vrednosti eritrocita, neutrofila, LUC, glukoze, ukupnog proteina, uree, Na, Cl, osmolarnosti i LDH menjale tokom vremena u funkciji tipa sepse. Razlikovanje životinja sa G+, G- ili MIX sepsom nije moguće jer je analiza glavnih komponenti (PCA) pokazala sličnu ekspresiju hematoloških i metaboličkih parametara krvi kod životinja bez obzira na tip eksperimentalne sepse (G+, G- ili MIX), uz minimalno odstupanje od životinja sa MIX sepsom od životinja sa sepsom izazvanom čistom kulturom (G+ ili G-). Analiza koeficijenta korelacije pokazala je 507 značajnih korelacija između rutinskih parametara krvi (207 kod EC, 186 kod SA i 114 kod MIX sepse) i neke od njih su u funkciji tipa sepse. Nije moguće razlikovati vrstu sepse u funkciji parametara krvi. Nakon ekstrakcije dve komponente opterećenje po parametrima krvi bilo je slično kod svih vrsta sepse (pozitivno opterećenje različitim vrstama belih krvnih zrnaca, PLT, glukozom, amilazom, Cl, ureom i osmolarnošću, a negativno albuminom, ukupnim proteinom, holesterolom , kreatinin i ALT). Iako su naši rezultati pokazali razliku u vrednostima i koeficijentima korelacije za rutinske biohemijske i hematološke parametre krvi, multiparametarska statistika je pokazala da oni nisu korisno sredstvo za razlikovanje tipa sepse prema bakterijskom uzročniku.

KEY WORDS: sepsa, krvni parametri, eksperimetnalni model, pacovi

Primljen 31.10.2021.

Prihvaćen 23.11.2021.