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A comparison of miRNAs with proinflammatory cytokines and procalcitonin of sepsis in the intensive care unit

A comparison of miRNAs with proinflammatory cytokines and procalcitonin

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Abstract

Aim: The development of sepsis, the low efficacy of antibiotics used, long-term antibiotic use, and the development of resistance to antibiotics are significant problems in patients in intensive care units. The use of biological markers is promising for the diagnosis and treatment of sepsis. In this study, proinflammatory cytokines, procalcitonin and four miRNA expressions were analyzed in a time-dependent manner in a patient group and control group, and the correlations between them were examined.

Material and Methods: The study included 30 patients in the intensive care unit who were diagnosed with sepsis and applied with SOFA and APACHE-2 scoring and 30 control subjects. Serum samples were taken at 24 hours, 72 hours, and on the 7th day. Analyses according to time were made for interleukin-1 beta, interleukin-6, interleukin-10, TNF-alpha, procalcitonin and four miRNAs (miR-122, miR-146a, miR-150, and miR-223) in the collected samples and comparisons were made between the patients and the control group.

Results: At 24 hours, there was a decrease in the miRNA-146a, miRNA-150, and miRNA-122 values and an increase in the miRNA-223 values in the sepsis group compared to the control group. At 72 hours, a decrease was observed in the miRNA-146a, miRNA-150, miRNA-122, and miRNA-223 values in the sepsis group compared to the control group.

Discussion: When procalcitonin and inflammatory cytokines were compared with selected miRNAs in the diagnosis, treatment follow-up, and prognosis of sepsis in the intensive care unit, a correlation between procalcitonin levels, proinflammatory cytokines and miRNA-150, miRNA-146a, and miRNA-223 was found.

Keywords

Gene Expression, miRNA, Procalcitonin, Sepsis, qRT-PCR

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Introduction

The development of sepsis, low efficacy of antibiotics used, long-term antibiotic use, and the development of resistance to antibiotics are significant problems in patients in ICUs.

The beginning of the pathophysiology of sepsis is the formation of the natural immune response to an infectious pathogen. Cytokines are important in ensuring that the infection remains localized and preventing systemic spread [1].

In addition to the relationship of procalcitonin with bacterial infections, elevated levels may also be seen in conditions other than infection, such as trauma, acute pancreatitis. Therefore, procalcitonin is an insufficient biomarker in the diagnosis of sepsis [2].

Different studies related to sepsis have determined that miRNA 150, miRNA 146a, miRNA 223, and miRNA 122 expressions show significant differences [3-7]. Determination of the serum levels of these candidate miRNAs has the potential to be a guide to diagnosis and treatment.

The aim of this study was to compare procalcitonin and proinflammatory cytokines with selected miRNAs in the diagnosis, treatment and prognosis of sepsis in ICU patients.

Material and Methods

Approval for the study was granted by the Clinical Research Ethics Committee of Suleyman Demirel University School of Medıcıne (decision no:31, dated:2016). The study included patients aged >18 years who were diagnosed with sepsis in the Intensive Care Unit (ICU) and not taking antibiotics. Patients who were admitted to the ICU with a diagnosis of sepsis or were taking antibiotics for any infection before the diagnosis of sepsis was made in the ICU were excluded from the study even if they were diagnosed with sepsis.

Of the patients in the ICU throughout the study period, a total of 30 were included for analysis, including 16 females and 14 males. A control group was formed of 30 patients without a diagnosis of sepsis matched to the study group. Serum samples were taken from both groups.

To evaluate the efficacy of antibiotic treatment started within 24 hours of the sepsis diagnosis, blood samples were taken at 72 hours and on the 7th day after starting antibiotics. Preparation of the serum samples

For the miRNA assays, 10cc blood samples were taken into sterile tubes not containing anticoagulant (DNase-RNase free) at room temperature from both the patient and control groups. RNA Extraction

RNA extraction was performed from the serum using Trizol® LS (ThermoFisherScientific), which is used for total RNA isolation from tissue fluids. To determine the amplification efficacy of the miRNA extraction performance, 100 fmol synthetic Caenorhabditiselegans miRNA (cel-miR-39) prepared in water not containing nuclease was added to the serum (spike-in) and the extraction procedures were applied according to the manufacturer's protocol.

cDNA Synthesis and qRT-PCR

Using the TaqMan® Advanced MiRNAcDNA Synthesis kit (Thermo Fisher) for the miRNA assays, cDNA synthesis was applied from total RNA. For the assays of the cDNAs synthesised with TaqMan® Fast Advanced Master mix and the related miRNAs (hsa-miR-150, hsa-miR-146a, hsa-miR-223, hsa-miR-122, and cel-miR-39), the TaqMan® Advanced miRNA test systems were used. Reactions were made with the StepOne RT-PCR system (Applied Biosystem) and Relative Quantification (RQ) values were obtained.

ELISA analyses of the cytokines and procalcitonin amounts

In the measurements of serum interleukin-1beta (IL-1β), IL-6, IL-10, and TNF-α levels, the eBioscience IL-1β High Sensitivity Human ELISA Kit was used. For the measurement of serum procalcitonin levels, the Biovendor Human Procalcitonin ELISA Kit was used.

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS vn 23.0 software (Statistical Package for Social Sciences, IBM, Chicago, IL, USA). Conformity of the data to normal distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Repeated measurement variance analysis was applied to data showing a normal distribution and the Friedman test to data not showing a normal distribution. When a significant difference was determined as a result of the tests, multiple comparison tests were applied to determine which time measurement had created the difference.

When a difference emerged in the measurements in the repeated measurement variance analysis, Bonferroni multiple comparison tests were used for data with normal distribution. For data not showing normal distribution, when a difference emerged in the Friedman test, the Dunn test was used to determine the level of significance.

The Mann Whitney U-test was applied in comparisons between the study group and the control group, and the Wilcoxon Signed Ranks test was used in the comparisons of time-related changes. A value of $p<0.05$ was accepted as statistically significant.

Results

A total of 120 serum samples were examined: 30 samples from the control group and 90 from the study group, 30 at each of three different time points (24 hours, 72 hours, 7 days). Analyses of proinflammatory cytokines (TNF-α, IL-1β, IL-6, IL-10), procalcitonin, and 4 miRNAs (miR-122, miR-146a, miR-150, miR-223) were performed. Comparisons were made between the study group and the control group, and between the time measurement points in the study group.

In the analysis of miRNA-146a expression, a significant decrease was determined in the study group compared to the control group and according to time (Figure 1A-1B). In the time related changes of the miRNA results, a statistically significant difference between the values at 24 hours and 72 hours ($p=0.005$) and between 24 hours and 7 days ($p=0.0001$) (Figure 1B) was determined.

In the analysis of miRNA-150 expression, a significant decrease was determined in the study group compared to the control group at 72 hrs and 7 days (Figure 1C). In the time related changes of the miRNA-150 results of the study group, a statistically significant difference between the values at 24 hours and 72 hours ($p=0.013$) and between 24 hours and 7 days ($p=0.002$) (Figure 1D) was determined.

In the analysis of miRNA-223 expression, a significant decrease was determined in the study group compared to the control

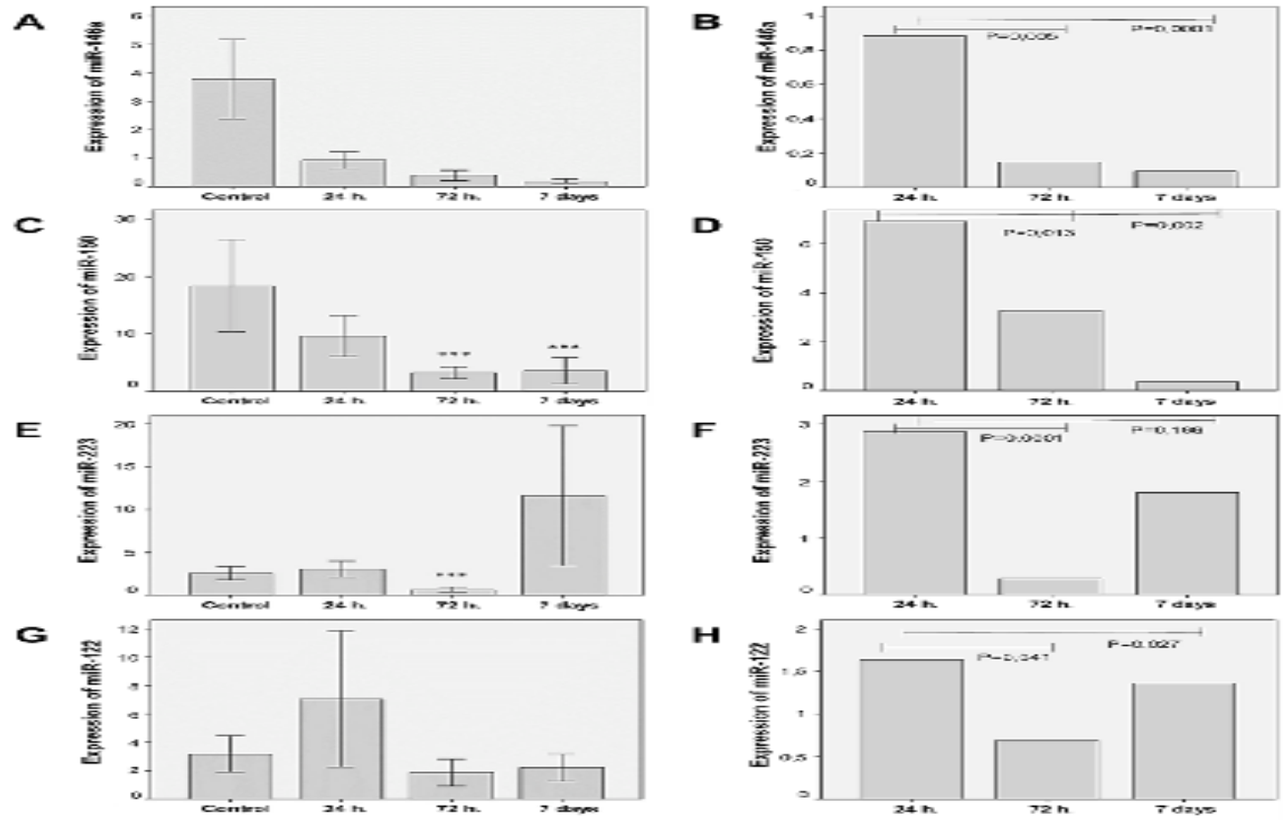


Figure 1. miRNA expression change. A- miR-146a significantly reduced at 24 hours, 72 hours and 7th day. B-miR-146a significantly reduced after treatment. C-miR-155 significantly reduced at 72 hours and 7th day. D- miR-155 significantly reduced after treatment. E- miR-223 significantly reduced at 72 hours. F- miR-223 significantly reduced at 72 hours. G- miR-122 expressions were not changed *P<0.05, **P<0.01, ***P<0.001

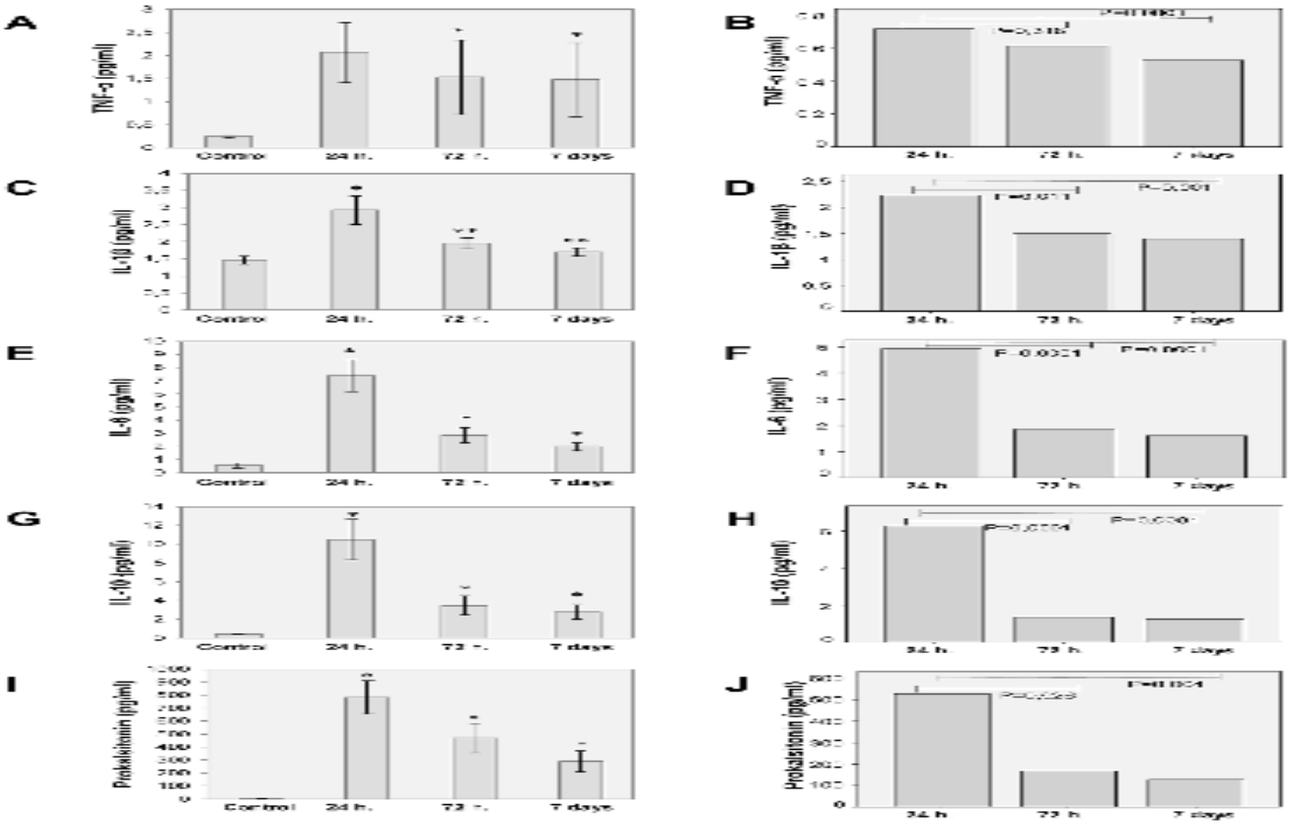


Figure 2. Inflammatory cytokines and procalcitonin levels change. A-C-E-G-I- inflammatory cytokines and procalcitonin levels were significantly higher than in the control group. B-TNF-α level significantly reduced on the 7th day. D-F-H-J- IL-1β, IL-6, IL-10 and procalcitonin levels significantly reduced in 72 hours and on the 7th day. *P<0.05, **P<0.01, ***P<0.001

group at 72 hrs (Figure 1E). In the time related changes of the miRNA-223 results in the study group, a statistically significant difference between the values at 24 hours and 72 hours (p=0.0001) and the difference between 24 hours and 7 days was not statistically significant (p=0.166) (Figure 1F).

In the analysis of miRNA-122 expression, no significant difference was determined between the study group and the control group (Figure 1G). In the time-related changes of the miRNA-122 results of the study group, a statistically significant difference between the values at 24 hours and 72 hours (p=0.041) and between 24 hours and 7 days (p=0.027) (Figure 1H) was determined.

The TNF-α results in the study group were found to be statistically significantly higher than in the control group at 72 hours and 7 days (Figure 2A). When the TNF-α results were evaluated according to time, no statistically significant difference was determined between the values at 24 hours and 72 hours (p=0.318), and a statistically significant decrease was observed between 24 hours and 7 days (p=0.049) (Figure 2B).

The IL-1β results in the study group were found to be statistically significantly higher than in the control group at 24 hours, 72 hours, and 7 days (Figure 2C). When IL-1β results were evaluated according to time, a statistically significant difference was determined between the values at 24 hours and 72 hours (p=0.011), and between 24 hours and 7 days (p=0.001) (Figure 2D).

The IL-6 results in the study group were found to be statistically significantly higher than in the control group at 24 hours, 72 hours, and 7 days (Figure 2E). When IL-6 results were evaluated according to time, a statistically significant difference was determined between the values at 24 hours and 72 hours (p=0.0001), and between 24 hours and 7 days (p=0.0001) (Figure 2F).

The IL-10 results in the study group were found to be statistically significantly higher than in the control group at 24 hours, 72 hours, and 7 days (Figure 2G). When IL-10 results were evaluated according to time, a statistically significant difference was determined between the values at 24 hours and 72 hours (p=0.0001), and between 24 hours and 7 days (p=0.0001) (Figure 2H).

The procalcitonin results in the study group were found to be statistically significantly higher than in the control group at 24 hours, 72 hours, and 7 days (Figure 2I). When the procalcitonin results were evaluated according to time, a statistically significant difference was determined between the values at 24 hours and 72 hours (p=0.026), and between 24 hours and 7 days (p=0.0001) (Figure 2J).

Discussion

Microbiological culture is the gold standard for the differentiation of sepsis from other non-infectious diseases. However, as this technique takes a long time, diagnosis and treatment are delayed. Therefore, several studies have been conducted to determine the ideal biomarker for sepsis.

Studies published in the literature that have examined procalcitonin and cytokine levels to evaluate the duration of antibiotic use in ICU patients with sepsis have not included patients using antibiotic treatment on admission to ICU [8-12]. Taking the above-mentioned literature and meta-analyses into consideration, the patients included in the current study were those being followed up in ICU who developed sepsis and who had not previously received antibiotic treatment, to be able to create a more homogenous standardized group. This study aimed to compare candidate miRNAs with procalcitonin and proinflammatory cytokines in the evaluation of the efficacy of antibiotic treatment in patients being followed up in ICU who were diagnosed with sepsis and started antibiotic treatment within the first 24 hours.

miRNAs directly target signal proteins and control NF-κB activity in immune cells, and are therefore defined as regenerators of the immune system [13]. A link between miRNA in particular and NF-κB has been shown in in-vitro studies.

It has been reported that miRNA-146a and miRNA-146b are expressed as a response to the microbial component and proinflammatory cytokines. In another study, Funahashi et al. showed that miRNA-146a has an anti-inflammatory role and reduced excessive inflammation by regulating IRAK-1 and TRAF6, thereby modulating NF-κB transcriptional activity. In an in-vitro environment, miRNA-146a has been shown to trigger severe sepsis in macrophages and to be correlated with sepsis severity at the cellular level [14].

In another study that analyzed chemokines and cytokines in sepsis patients, changes in miRNA-146 were shown to be correlated with polymorphonuclear cells in peripheral blood. IL-6, which is an important inflammatory mediator in sepsis, was reported to increase, while miRNA-146 decreased [15].

The IL-6 level increases more rapidly in sepsis and reaches a peak level at the end of the second hour. The elevated serum level lasts longer than for TNF-α and IL-1 [16]. Procalcitonin (PCT) is produced as a response to endotoxin or through mediators (IL-1, TNF-α, IL-6) expressed as a response to bacterial infections. It is correlated with the severity of bacterial infection [17].

In the current study, miRNA-146a was determined to be significantly reduced in the sepsis group compared to the control group at 24 hours, 72 hours, and 7 days of treatment, and a significant increase was determined in proinflammatory cytokine and procalcitonin levels, which was consistent with findings in the literature. In addition, a significant decrease was determined in the miRNA-146a levels in the sepsis group at 72 hours and 7 days compared to the values at 24 hours, and there was a significant decrease in proinflammatory cytokines and procalcitonin levels.

Roderburg C et al. compared 138 patients meeting sepsis criteria and 76 healthy control subjects, and reported that the serum miRNA-150 level was slightly reduced in the sepsis group, and was therefore not suitable as a marker for the diagnosis of sepsis. However, a correlation between serum miRNA levels and hepatic and renal function disorders, and low serum miRNA-150 levels were reported to be associated with a poor prognosis [18]. In a study of 120 sepsis patients and 50 healthy control subjects, Ma et al. showed that miRNA-150 was significantly decreased in the sepsis group compared to the control group, and there was a negative correlation between IL-6, TNF-α, and miRNA-150 values [19].

In the current study, the miRNA-150 level was observed to be decreased in the sepsis patients compared to the control group,

but at 24 hours this decrease was not statistically significant. These differences from previously published results are most probably related to the size and characteristics of the patient groups analyzed in different studies. A statistically significant decrease was seen in the miRNA-150 levels of the sepsis group after 72 hours and 7 days of sepsis treatment compared to the control group. When the sepsis antibiotic treatment was evaluated over time, a significant decrease was determined in the miRNA-150 level of the sepsis group at 72 hours and 7 days compared to the value at 24 hours. The proinflammatory cytokines and procalcitonin values were seen to be significantly increased at 24 hours when sepsis was diagnosed, and significantly decreased after 72 hours and 7 days of treatment. Roderburg et al. determined no correlation between serum miRNA-150 levels and markers of bacterial infection and inflammation such as CRP or procalcitonin [18].

In addition to miRNA-122 being known to be a miRNA associated with the liver, it has also been stated to be a biomarker in the diagnosis and prognosis of sepsis [7]. In a study of 108 sepsis patients in the first 24 hours of admission to ICU and 20 control subjects, miRNA-122 expression was seen to be 40-fold higher in sepsis patients with a mortal course compared to the control group, and 6-fold higher in the patients with sepsis who survived [20].

In the current study, an increase was seen in miRNA-122 levels at 24 hours following the diagnosis of sepsis compared to the control group, and a decrease was determined after 72 hours and 7 days of treatment. Within the sepsis group, there was a significant decrease in the miRNA-122 levels at 72 hours and 7 days. Moreover, a parallel decrease was seen in proinflammatory cytokines and procalcitonin levels.

In a study by Wang et al of 166 patients diagnosed with sepsis and 24 control subjects, serum samples were taken from the sepsis patients within 24 hours. There was a diagnosis of severe sepsis and septic shock in 123 patients and mild sepsis in 43. The miRNA-223 expression level was reported to be significantly higher, and the miR-499-5p, miR-122, and miR-193b levels were significantly lower in mild sepsis, severe sepsis, and septic shock patients compared to the control group. In addition, the miRNA-223 expression level was statistically slightly elevated in the septic shock and severe sepsis group compared to the mild sepsis group [5].

In the current study, an increase, but not at a significant level, was seen in the miRNA-223 levels of the sepsis group compared to the control group, a statistically significant decrease was determined after 72 hours of sepsis treatment, and on the 7th day, an increase was seen, which was not statistically significant because of the increase in standard deviation.

Conclusion

In this study, which compared selected miRNAs with procalcitonin and inflammatory cytokines in the diagnosis, treatment follow-up, and prognosis of patients diagnosed with sepsis in ICU, a relationship was determined between miRNA-150, miRNA-146a, and miRNA-223, and proinflammatory cytokines and procalcitonin levels. Based on this study, by increasing the number of patients and with data obtained from comprehensive analysis systems such as all miRNA pool microarray or new-generation sequencing, new biomarkers will be able to be established for the diagnosis, treatment follow up, and prognosis of sepsis.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and human rights statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. No animal or human studies were carried out by the authors for this article.

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Conflict of interest

None of the authors received any type of financial support that could be considered potential conflict of interest regarding the manuscript or its submission.

References

1. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. Sepsis and septic shock. Nat Rev Dis Primers. 2016; 30(2):16045.

2 .Bréchot N, Hékimian G, Chastre J, Luyt CE. Procalcitonin to guide antibiotic therapy in the ICU. Int J Antimicrob Agent. 2015;46(1):19-24.

3. Wang JF, Yu ML, Yu G, Bian JJ, Deng XM, Wan XJ, et al. Serum miR-146a and miR-223 as potential new biomarkers for sepsis. Biochem Biophys Res Commun. 2010;394(1):184-8.

4. Vasilescu C, Rossi S, Shimizu M, Tudor S, Veronese A, Ferracin M, et al. MicroRNA finger prints identify miR-150 as a plasma prognostic marker in patients with sepsis. PLoSOne. 2009;4(10):e7405.

5. Wang HJ, Zhang PJ, ChenWJ, Feng D, Jia YH, Xie LX. Four serum microRNAs identified as diagnostic biomarkers of sepsis. J Trauma Acute Care Surg. 2012;73(4): 850–4.

6. Benz F, Roy S, Trautwein C, Roderburg C, Luedde T. Circulating MicroRNAs as Biomarkers for Sepsis. Int J Mol Sci. 2016;17(78):1-17.

7. Wang HJ, Yu B, Deng J, Jin Y, Xie L. Serum miR-122 correlates with short-term mortality in sepsis patients. Crit Care. 2014;704(6):12-8.

8. Hochreiter M, Köhler T, Schweiger AM, Keck FS, Bein B, von Spiegel T, et al. Procalcitonin to guide duration of antibiotic therapy in intensive care patients: a randomized prospective controlled trial. Crit Care. 2009;13(3):R83.

9. Schroeder S, Hochreiter M, Koehler T,Schweiger AM, Bein B, Keck FS, et al. Procalcitonin (PCT)-guided algorithm reduces length of antibiotic treatment in surgical intensive care patients with severe sepsis: results of a prospective randomized study. Langenbecks Arch Surg. 2009;394(2):221-6.

10. Branche AR, Walsh EE, Vargas R, Hulbert B, Formica MA, Baran A, et al. Serum Procalcitonin Measurement and Viral Testingto Guide Antibiotic Use for Respiratory Infections in Hospitalized Adults: A Randomized Controlled Trial. J Infect Dis. 2015(11)212:1692-700.

11. Kopterides P, Siempos II, Tsangaris I, Tsantes A, Armaganidis A. Procalcitonin-guided algorithms of antibiotictherapy in theintensive care unit: a systematic review and meta-analysis of randomized controlled trials. Crit Care Med. 2010;38(11):2229-41.

12. Rhee C. Using Procalciton into Guide Antibiotic Therapy. Open Forum Infect Dis. 2016; 7;4(1)ofw249. DOI: 10.1093/ofid/ofw249.

13. Ma X, Becker BLE, Barker JR, Li Y. MicroRNAs in NF-kappa-B signaling. J Mol Cell Biol. 2011;3(3):159-66.

14. Funahashi Y, Kato N, Masuda T, Nishio F, Kitai H, Ishimoto T, et al. miR-146a targeted to splenic macrophages prevents sepsis-induced multiple organ injury. Lab Invest. 2019;99(8):1130–42.

15. Zhou J, Chaudhry H, Zhong Y, Ali MM, Perkins LA, Owens WB, et al. Dysregulation in microRNA expression in peripheral blood mono nuclear cells of sepsis patients is associated with immunopathology. Cytokine. 2015;71(1):89-100.

16. Tanaka T, Narazaki M, Kishimoto T. IL-6 in inflammation, immunity, and disease. Cold Spring Harb Perspect Biol. 2014;6(10):a016295

17. Brodská H, Malíčková K, Adámková V, Benáková H, Šťastná MM, Zima T. Significantly higher procalcitonin levels could differentiate Gram-negative sepsis from Gram-positive and fungal sepsis. Clin Exp Med. 2013;13(3):165–70.

18. Roderburg C, Luedde M, Cardenas DV, Vucur M, Scholten D, Frey N, et al. Circulating MicroRNA-150 Serum LevelsPredictSurvival in Patientswith Critical Illness and Sepsis. PLoS ONE.2013;8(1): e54612.

19. Ma Y, Liu Y, Hou H, Yao Y, Meng H. MiR-150 predicts survival in patients with sepsis and inhibits LPS-induced inflammatory factors and apoptosis by targeting NF-kB1 in human umbilical vein endothelial cells. Biochem Biophys Res Commun 2018;500(3):828-37.

20. Rahmel T, Schäfer ST, Frey UH, Adamzik M, Peters J. Increased circulating

microRNA-122 is a biomarker for discrimination and risk stratification in patients definedby sepsis-3 criteria. PLoS ONE. 2018;13(5):e0197637.

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