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Genetic variability of ANG2 -35G>C gene as a predictor factor in sepsis

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Abstract

To date it is unknown if there is a predisposition to sepsis. In this respect, genetic studies have been conducted with the aim to find gene variants which can point out a higher predisposition to developing sepsis. The primary objective of this study is to highlight whether the genetic polymorphism of Angiopoietin-2 gene (ANG2 -35G>C) is present mainly in septic patients. As secondary objectives we aimed to evaluate if there are any associations between ANG2 -35G>C polymorphism and the severity scores Acute Physiology and Chronic Health Evaluation II (APACHE II) and Simplified Acute Physiology Score (SAPS) as well as routine tests in septic patients such as C reactive protein (CRP), procalcitonin (PCT). We enrolled adult patients admitted to the Intensive Care Unit (ICU). After admission to the ICU and the diagnosis of sepsis, blood samples were collected and the severity scores: APACHE II, SAPS were calculated on the first day of ICU admission. We recorded the following from the blood samples: CRP, PCT, angiopoietine2 (Ang-2). We performed several one-way ANOVA tests to determine any significant mean difference of the analyzed variables. We observed that variant genotypes of ANG2 -35G>C gene polymorphism are significantly related to CRP, aspect which increases this biomarker credibility compared with others (i.e., PCT), in septic patients. ANG2 -35G>C gene polymorphism is associated with severity scores, APACHE II, and SAPS in sepsis.

Keywords: *Angiopietine-2, genetic variability, sepsis, severity scores, prognosis*

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Introduction

Sepsis has been a leading cause of death in Intensive Care Unit (ICU) patients for a few decades (1).

The latest definition of sepsis, devised by the Sepsis 3.0 consensus in 2016, is "life threatening organ dysfunction caused by dysregulated host response to infection" (2). This dysfunction is clinically diagnosed by Sequential Organ

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Failure Assessment (SOFA) score calculation, a value over or equal to 2 points being suggestive for sepsis. To date it is not known if there exists a predisposition to sepsis (2). This is the reason for which, over time, several markers have been studied and based on the conclusions of the studiess, medical conduct in ICU patients is guided (3, 4).

Although the aforementioned findings are helpful, they can only be useful when sepsis is already present. Currently, we do not have certain indicators which can be used as predictors for a patient's predisposition to sepsis.

In this respect, genetic studies have been conducted with the aim to find modifications which can point to a higher predisposition for developing sepsis or which can relate to this entity in such manner that detection of such modification can help modify the outcome of the septic patient.

Response to sepsis is different for each individual and it is influenced by several variables: gender, age, race, medical history (5).

In the ICU environment, finding quick answers is lifesaving, because the patients admitted to ICU need immediate care for their health issues due to the life threatening characteristics. Genetic studies are less performed in ICU patients because they do not offer immediate results and are not immediate problem solvers, although the literature presents studies which have concluded that death caused by infection is fivefold more inherent than the same outcome determined by cancer (6).

The innate immune response, which is in charge of the modifications which normally appear in bacterial infections, is found to be associated with an increased number of gene alterations (7). Any of these changes, once studied, can have a predictor potential of sepsis development and outcome.

Angiopoietines

In sepsis, a link between the severity of the injury at the endothelial level, which is being regulated by the system comprised in the endothelial specific angiopoietin (*Ang*) – tyrosine kinase and immunoglobulin-like loop of the epidermal growth factor domain (*Tie*) ligand-receptor, is known to exist (8).

The first time *Ang-1 and -2* were mentioned was in studies regarding embryonic vasculogenesis and angiogenesis (9, 10). *Ang-1* and *Ang-2* have opposed binding actions on extracellular domains of the Tie-2 receptor, which has been discovered to be almost exclusively in endothelial cells (8).

Among the known roles of *Ang-1* are: endothelium stability, it inhibits vascular leakage and disables inflammatory and coagulation gene expression by creating a bond with *Tie-2* (8, 11), *Ang-2* has the ability to promote the loss of barrier integrity, it mediates the proinflammatory and proangiogenic effects which have, as an end point, vascular leakage and organ dysfunction (8, 12).

There are two known types of *Ang-2*: exogenous, which promotes phosphorylation of *Tie-2* cultured endothelial cells (13) and the endogenous type of *Ang-2*, which activates *Tie-2* receptor (14).

The aim of this study is to highlight whether the *ANG2* -35G>C *gene* polymorphism is present mainly in septic patients. Moreover, we evaluate if there are any associations between *ANG2* -35G>C polymorphism and the severity scores APACHE II (Acute Physiologic Assessment and Chronic Health Evaluation) score and SAPS (Simplified Acute Physiology Score) and also to some of the routinely performed tests in septic patients – C reactive protein (CRP), procalcitonin (PCT).

Material and method

We enrolled adult patients admitted to the ICU of the Emergency Clinical County Hospital of Tîrgu Mures, Romania. The study obtained the

approval from the hospital Ethical Committee. Written informed consent was obtained from all participants or their relatives, before inclusion.

Inclusion criteria were: all patients admitted to the ICU with sepsis or without the sepsis criteria, with no regard to the site of infection and no surgical intervention in the last 72 hours. The diagnosis of sepsis was made after the diagnosis criteria of the third sepsis consensus (15). The exclusion criteria were patients with cardiac arrest and those who had had surgery in the last 72 hours.

Study protocol: after admission in the ICU and the diagnosis of sepsis, we took blood samples on the first day of ICU admission and, also, we calculated the severity scores: SOFA, APACHE II, SAPS. We recorded the following from the blood samples: CRP, PCT, *Ang-2.*

The blood samples were collected in the first 12 hours after ICU admission in tubes with no anticoagulant, centrifuged, and serum was kept at -70 Celsius degrees for further processing.

Enzyme Linked Immuno Sorbent Assay (ELISA) test (R&D Systems, Minneapolis, USA) was used to determine the Ang-2 serum expression as it was previously reported (16).

CRP and PCT values were determined using the immunoturbidimetry method (Cobas 6000, Roche Diagnostics, Germany).

As previously mentioned, we investigated *ANG2* -35G>C gene polymorphism. Serum was used for DNA isolation using the Quick-gDNA Mini-Prep kit from Zymo Research, USA. For genotyping the isolated DNA was amplified using the primers and Polymerase Chain Reaction (PCR) protocol described by Bányász et al (17). PCR amplicons were digested by FastDigest HindIII restriction enzyme from ThermoFisher Scientific, USA and were visualized in 2.5% agarose gel stained with ethidium bromide.

Statistical analysis was performed using Microsoft Excel (Microsoft, Washington, USA) and SPSS 17 for Windows (IBM, NY, USA).

Data series normality was tested using Kolmogorov-Smirnov test. The data recorded for Ang-2 and PCT variables failed this test, and they were further analyzed using non-parametric tests (Kruskal-Wallis test).

We used the following statistical tests for data analysis: one way ANOVA with Tukey post-hoc test, Kruskal-Wallis test, and a logistic regression model. The initial regression model included *Ang-2*, APACHE, PCT, CRP, SOFA, SAPS, and the genotypes as predictors. After testing for multicollinearity, several variables were excluded from the model, leaving only *Ang-2,* APACHE, CRP, and the genotypes of *ANG2* -35G>C.

Results

We enrolled 69 patients, divided into two groups - the sepsis group (S) = 38 patients and non-sepsis group (NS) = 31 patients. We found no significant difference between the groups regarding the demographic data of the enrolled patients.

The *ANG2* -35G>C genotypes in the entire studied group were as follows: GG the wild type genotype in 53 cases (76.8%), GC the heterozygous genotype in 13 cases (18.8%) while CC, the homozygous genotype with the variant allele, observed in 3 patients (4.3%).

When we compared the genotypes distribution between the groups (with sepsis and without sepsis), we found no statistically significant difference on their distribution (p=0.17).

The frequency of the GG genotype was 87.1% (27 cases), 9.7% (3 cases) of GC genotype, and 3.2% (1 case) of CC genotype in the group of patients without sepsis. In the sepsis group the genotype frequencies were as follow: GG in 68.4% (26 patients), GC in 26.3% (10 patients), and CC in 5.3% (2 patients).

For the entire group of patients enrolled in our study, we performed several one-way ANOVA

tests to determine any significant mean difference of the analyzed variables between the genotypes. The only statistically significant difference observed was for CRP, with a p value of 0.01. Tukey post-hoc test found a difference between wild type genotype (GG) and the heterozygous genotype (GC) (p=0.046) (Table 1, Figure 1).

The same significant difference was observed for CRP when we analyzed only the group of patients with sepsis, in this only case the difference was greater between CC and GG genotypes, although not statistically significant (p=0.150) (Table 2, Figure 2).

We constructed a logistic regression model to assess the influence of the studied parameters on

Table 1. Distribution of the analyzed variables according to the *ANG2* -35G>C genotypes, for entire group of patients.

ANG2 -35G>C	TIE2 (ng/ml) [1]	APACHE II [1]	SOFA [1]	SAPS [1]	CRP (mg/dl) [1]	ANG2 (ng/ml) [2]	PCT (ng/ml) [2]
CC	6.43 ± 1.33	25.00 ± 19.00	8.33 ± 2.08	42.66 ± 33.08	289.84 ± 212.93	5.82 [4.52]	0.34 [1.51]
GC	11.29 ± 5.99	27.15 ± 9.29	9.07 ± 4.27	50.76 ± 18.20	226.87 ± 155.18	8.23 [9.89]	1.50 [3.11]
GG	10.09 ± 5.07	26.56 ± 8.37	7.84 ± 3.42	46.69 ± 18.84	141.88 ± 93.92	5.85 [6.4]	0.35 [1.78]
p value	0.344	0.931	0.537	0.726	0.010	0.750	0.206

[1] mean ± SD; ANOVA analysis significance; [2] median [IQR]; Kruskal-Wallis test significance

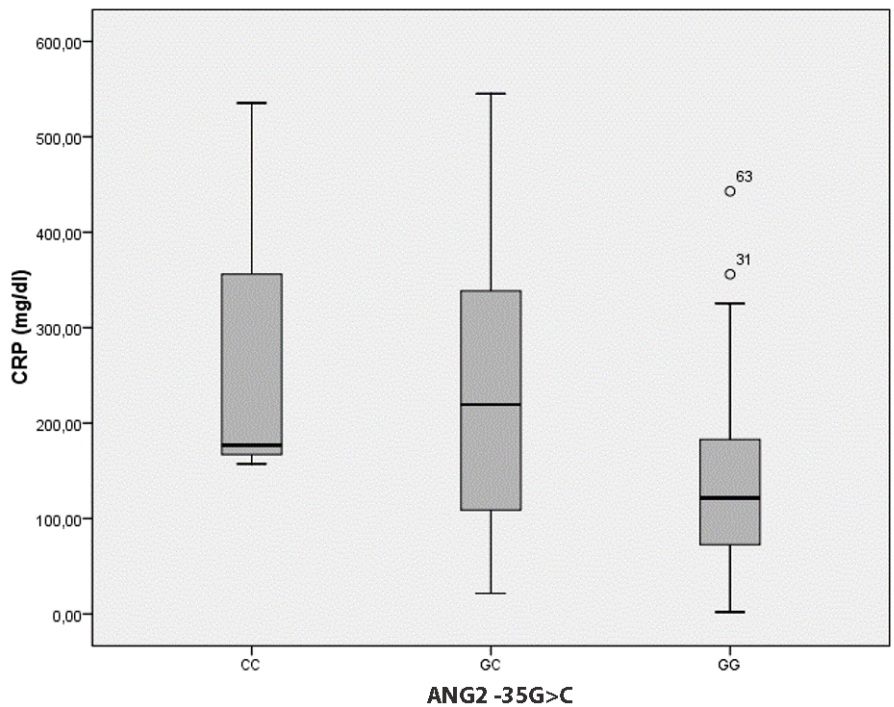

Fig. 1. The *ANG2* -35G>C gene polymorphism in association with CRP

Table 2. Distribution of the analyzed variables according to the *ANG2* -35G>C genotypes for the sepsis group

ANG2 -35G>C	APACHE II [1]	SOFA [1]	SAPS [1]	CRP (mg/dl) [1]	ANG2 (ng/ml) [2]	PCT (ng/ml) [2]
CC	35.50 ± 7.77	9.50 ± 0.70	61.50 ± 7.77	346.36 ± 267.42	7.69 [3.74]	1.68 [2.68]
GC	27.40 ± 9.78	9.30 ± 4.78	51.80 ± 19.17	266.58 ± 147.96	8.94 [12.22]	1.97 [4.78]
GG	29.03 ± 8.87	8.46 ± 8.90	52.34 ± 21.91	177.15 ± 98.14	6.82 [10.88]	1.52 [2.50]
p value	0.522	0.807	0.828	0.047	0.880	0.393

[1] mean ± SD; ANOVA analysis significance; [2] median [IQR]; Kruskal-Wallis test significance

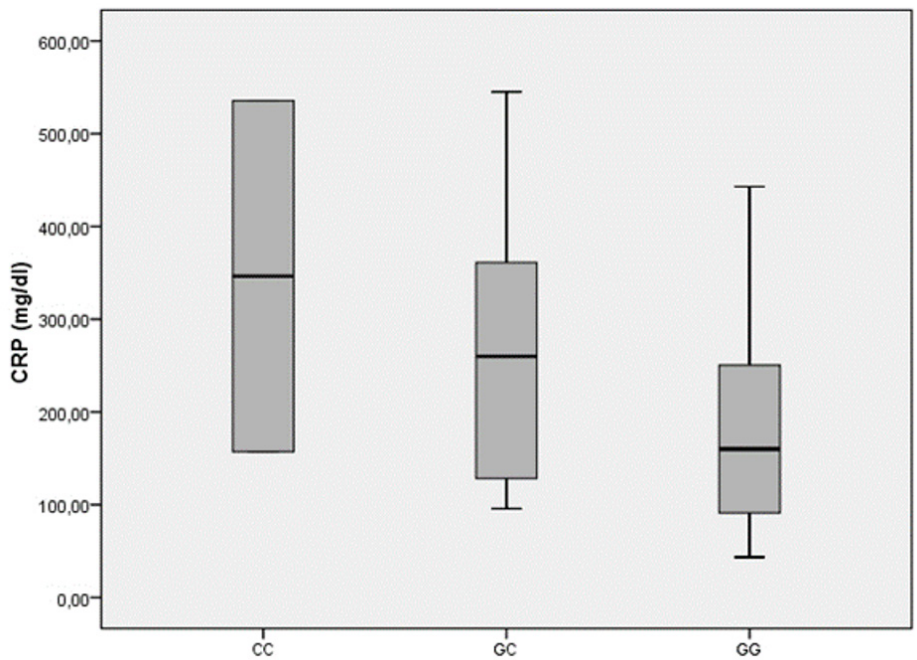

Fig. 2. The association between *ANG2* -35G>C gene polymorphism and CRP in sepsis group

sepsis development. The obtained coefficients are presented in Table 3. The model is valid with a correctly predicted value percentage of 75.4 % and a Nagelkerke R^2 of 0.423.

We performed one-way ANOVA statistic test for the non-sepsis group and found a significant association between the investigated gene polymorphism and the severity scores SAPS II (p=0.04) and APACHE (p=0.03). Figure 3 illustrates the variation of the mean values of the SAPS and APACHE II in the two groups (sepsis and non-sepsis) and also within the group.

Discussions

In sepsis, the genes which are involved in angiogenesis – such as *TIE, ANG1, and ANG2*- are downregulated and this adds up to the patho-

Table 3. The logistic regression results for assessing the influence of the studied parameters on sepsis development

	OR	95% CI for OR		p value
		Lower	Upper	
ANG2 (ng/ml) [1]	1.067	0.978	1.165	0.146
ANG2 -35G>C genotype CC	0.519	0.015	17.613	0.716
ANG2 -35G>C genotype GC	0.483	0.091	2.574	0.394
ANG2 -35G>C genotype GG[2]	1	N/A	N/A	N/A
APACHE score [1]	1.100	1.010	1.198	0.029
CRP (mg/dl) [1]	1.012	1.004	1.020	0.003

[1] OR = odds for sepsis development when predictor is increased by 1 unit; [2] Reference group

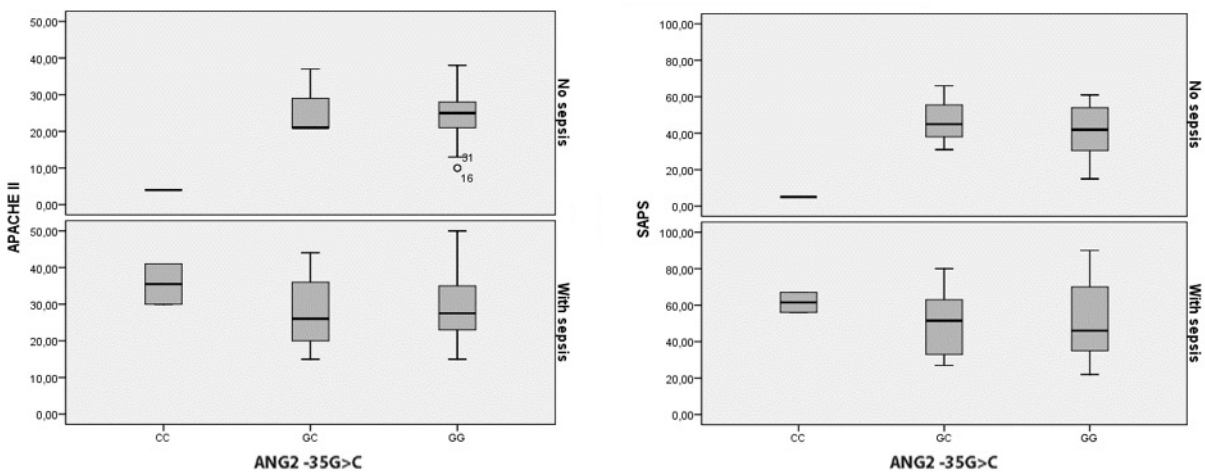

Fig. 3. The associations of the severity scores APACHE II and SAPS with *ANG2* -35G>C polymorphism in the groups

physiological process of microvascular leakage and end organ dysfunction (18). Numerous studies have focused predominantly on determining cytokine, such as interleukin 6 and its forms in septic patients, but the conclusions were that this biomarker cannot be used as a prognostic marker and the research is ongoing (19, 20). Endothelial cell dysfunction constitutes the principal mechanism of the related processes which occur in sepsis (21). *Ang-2* is a biomarker which is increased in septic patients and can be used for stratifying the severity of this condition (22). Literature describes associations between *Ang-2/Tie-2* ratio in septic patients, with no certain diagnostic value in septic patients as well as other biomarkers with potential prognostic value, such as soluble urokinase-type plasminogen activator receptor (SuPAR) (23, 16). This is the reason we chose to determine its polymorphism and to investigate if there are any associations between variant genotypes and the severity scores or other routinely used tests such as procalcitonin.

Polymorphism of ANG2 -35G>C and the severity scores

We did not find any significant association between the studied gene polymorphism and the

values of the biomarkers: *Ang-2, Tie-2*, CRP or PCT. Instead, we found significant associations with two of the severity scores we calculated, namely the APACHE II score and SAPS.

The APACHE II score has been proposed as the gold standard in the evaluation of the critically ill patients (24). APACHE II score identifies either low-risk patients or very-high-risk patients (25).

Here we found that the APACHE II is significantly correlated with the *ANG2* -35G>C. This means that it is likely to find the variant genotypes in patients with sepsis than in those without and based on the APCHE II score we can identify those patients with variant allele of *ANG2*, which has major impact in endothelial mechanisms of sepsis.

The SAPS score is used to predict mortality and it is significantly associated with the studied polymorphism.

To the best of our knowledge, this is one of the few studies aimed to investigate this gene polymorphism in sepsis and the literature is poor in offering comparable studies, except those which confirm that the future of understanding sepsis comprises genetic screening, genetic determination of biomarkers, and the new direction in medicine – personalized medicine (26-30).

Polymorphism of ANG2 -35G>C and C reactive protein (CRP)

CRP is part of a superfamily of proteins - pentraxins - involved in acute immunological responses. It plays a role in pattern recognition receptors (PRRs). There are two types of pentraxins - the "short" and "long" pentraxins. The "short" category comprises serum amyloid P component (SAP) and CRP which is produced in the liver as a response to inflammatory reaction (31).

This is a biomarker which increases in the acute phase of inflammation and although it is a known factor of the inflammatory process, its role has not yet been well-established (32). It has a low specificity, but in spite of this, it is used for early detection of sepsis, in the first 24 hours, because it is considered to have a high sensitivity (33).

Our study identified a significant association between this biomarker and the *ANG2* -35G>C polymorphism, in the septic group. This finding makes the specificity of this biomarker to be stronger. The high levels of the biomarker associated with the presence of the C variant allele of *ANG2* -35G>C polymorphism, increase the accuracy of early detection of the septic patient.

Along with CRP, many studies compared capacity of this protein for sepsis detection with the one of PCT. At first, it was proved that PCT is more sensitive than CRP in detecting septic patients (34), but the debate is far from being over. A meta-analysis which comprised 49 studies, and compared CRP and PCT in septic patients, concluded that procalcitonin represents a good biological diagnostic marker for sepsis or septic shock. Another study concluded that PCT is superior to CRP (35, 36).

In the present study we did not find any significant association between this marker and the variant genotypes of *ANG2* -35G>C in septic patients. The validity test that we performed showed that CRP is better related to the studied polymorphism than PCT.

The scarcity of data in the literature on this matter settles this for further investigations in order to come up with a prompt conclusion on which is a better detector of sepsis: PCT or CRP.

Our study has limitations in terms of the small number of patients, and the fact that we could only determine the biomarkers serum level once, this being a drawback due to the progressive characteristic of sepsis.

Nevertheless, we can see that the future of diagnostics belongs to biomarkers, which are to become a master-key for an early and precise

diagnosis leading to a personalized treatment for septic patients (36). Yet, the enormous variety of septic patients, sepsis itself and the large array of the biomarkers that are identified or identifiable constitutes an obstacle in achieving a quick and precise sepsis diagnosis.

Conclusion

Variant genotypes of *ANG2* -35G>C gene polymorphism are significantly associated with CRP which increases this biomarkers credibility compared to others, such as PCT in septic patients.

Abbreviations list

ANG - Angiopoietine
APACHE - Acute Physiologic Assessment and Chronic Health Evaluation
CRP - C reactive protein
ICU - Intensive Care Unit
PCT - Procalcitonin
SAPS - Simplified Acute Physiology Score
SOFA - Sequential Organ Failure Assessment
SuPAR - soluble urokinase-type plasminogen activator receptor
Tie - receptor tyrosine kinase

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Authors' contributions

SJ - Conceptualization; formal analysis; methodology
LA - Data curation; Formal analysis; Writing – original draft, research design
MP - Methodology; Software
AH - Ang-2 serum expression, Data analysis
FT - Genotyping, Data Analysis
AMG - Resources; Funding acquisition; Resources design
AL - Writing – review & editing, Supervision; Validation

Conflict of Interest

The authors declare no conflict of interest.

References

1. Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T, Schlattmann P, et al. Assessment of Global Incidence and Mortality of Hospital-treated Sepsis. Current Estimates and Limitations, International Forum of Acute Care Trialists. Am J Respir Crit Care Med. 2016; 193(3):259-72. DOI: 10.1164/rccm.201504-0781OC
2. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016; 315(8): 801-10. DOI: 10.1001/jama.2016.0287
3. James D. Faix. Biomarkers of sepsis Crit Rev Clin Lab Sci, 2013; 50(1): 23-36. DOI: 10.3109/10408363.2013.764490
4. Christaki E, Anyfanti P, Opal SM. Immunomodulatory therapy for sepsis: an update. Expert Rev Anti Infect Ther 2011; 9:1013-33. DOI: 10.1586/eri.11.122
5. László I, Trásy D, Molnár Z. Sepsis: From Pathophysiology to Individualized Patient Care, J Immunol Res. 2015; 2015: 510436. DOI: 10.1155/2015/510436
6. Sørensen TI, Nielsen GG, Andersen PK, Teasdale TW. Genetic and environmental influences on premature death in adult adoptees. N Engl J Med 1988; 318:727-32. DOI: 10.1056/NEJM198803243181202
7. Matthew J, Delano, Peter A. The Immune System's Role in Sepsis Progression, Resolution and Long-Term Outcome. Immunol Rev. 2016; 274(1): 330-53. DOI: 10.1111/imr.12499
8. Fiedler U, Augustin HG: Angiopoietins: A link between angiogenesis and inflammation. Trends Immunol 2006; 27:552-58. DOI: 10.1016/j.it.2006.10.004
9. Maisonpierre PC, Suri C, Jones PF, Bartunkova S, Wiegand SJ, Radziejewski C, et al: Angiopoietin-2, a natural antagonist for Tie2 that disrupts in vivo angiogenesis. Science 1997; 277:55-60. DOI: 10.1126/science.277.5322.55

10. Suri C, Jones PF, Patan S, Bartunkova S, Maisonpierre PC, Davis S, et al: Requisite role of angiopoietin-1, a ligand for the TIE2 receptor, during embryonic angiogenesis. Cell 1996; 87:1171-80. DOI: 10.1016/S0092-8674(00)81813-9
11. Witzenbichler B, Westermann D, Knueppel S, Schultheiss HP, Tschope C: Protective role of angiopoietin-1 in endotoxic shock. Circulation 2005; 111: 97-105. DOI: 10.1161/01.CIR.0000151287.08202.8E
12. Parikh SM, Mammoto T, Schultz A, Yuan HT, Christiani D, Karumanchi SA et al: Excess circulating angiopoietin-2 may contribute to pulmonary vascular leak in sepsis in humans. PLoS Med 2006; 3:356-70. DOI: 10.1371/journal.pmed.0030046
13. Teichert-Kuliszewska K, Maisonpierre PC, Jones N, Campbell AI, Master Z, Bendeck MP et al. Biological action of angiopoietin-2 in a fibrin matrix model of angiogenesis is associated with activation of Tie2. Cardiovasc Res. 2001 Feb 16; 49(3):659-70. DOI: 10.1016/S0008-6363(00)00231-5
14. Daly C, Pasnikowski E, Burova E, Wong V, Aldrich TH, Griffiths J et al. Angiopoietin-2 functions as an autocrine protective factor in stressed endothelial cells. Proc Natl Acad Sci U S A. 2006 Oct 17; 103(42):15491-6. DOI: 10.1073/pnas.0607538103
15. MarikP.E, Taeb A.M. SIRS, qSOFA and new sepsis definition. J Thorac Dis. 2017; 9(4): 943-45. DOI: 10.21037/jtd.2017.03.125
16. Szederjesi J, Georgescu AM, Santini A, Almasy E, Lazar A, Hutanu A, et al. L. Ang-2, Tie-2 si Ang-2/Tie-2 ratio serum levels as diagnostic and prognostic biomarkers of sepsis in critically ill patients. Rev Romana Med Lab, 2016; 24:187-99. DOI: 10.1515/rrlm-2016-0007
17. Bányász I, Bokodi G, Vannay A, Szebeni B, Treszl A, Vásárhelyi B, et al. Genetic polymorphisms of vascular endothelial growth factor and angiopoietin 2 in retinopathy of prematurity. Curr Eye Res. 2006; 31(7-8):685-90. DOI: 10.1080/02713680600801123
18. Parikh SM. Dysregulation of the angiopoietin-Tie2 axis in sepsis and ARDS. Virulence. 2013; 4:517-24. DOI: 10.4161/viru.24906
19. Georgescu AM, Bănescu C, Badea I, Moldovan V, Huțanu A, Voidăzan S et al. IL-6 gene polymorphisms and sepsis in ICU adult Romanian patients: a prospective study. Rev Romana Med Lab, 2017; 25:75-89. DOI: 10.1515/rrlm-2016-0044
20. Tomescu DR, Dima OS, Ungureanu D, Popescu M, Tulbure D, Popescu I. First report of cytokine removal using CytoSorb® in severe noninfectious inflammatory syndrome after liver transplantation. Int J Artif Organs 2016; 16; 39(3):136-40. DOI: 10.5301/ijao.5000489
21. Bataille A, Galichon P, Ziliotis M, Iman S, Hertig A. Epigenetic changes during sepsis: on your marks! Crit Care. 2015; 19: 358. DOI: 10.1186/s13054-015-1068-5
22. Szederjesi J, Almasy E, Lazar A, Huțanu A, Georgescu A. The Role of Angiopoietine-2 in the Diagnosis and Prognosis of Sepsis. The Journal of Critical Care Medicine 2015; 1(1):18-23. DOI: 10.1515/jccm-2015-0004
23. Georgescu AM, Szederjesi J, Dobreanu M, Copotoiu SM, Voidăzan S, Huțanu A, et al. Soluble urokinase-type plasminogen activator receptor (suPAR) - a possible biomarker for bacteremia in sepsis. Rev Romana Med Lab, 2015; 23(1):59-73. DOI: 10.1515/rrlm-2015-0002
24. Corrêa TD, Sanches PR, de Morais LC, Scarin FC, Silva E, Barbas CS et al. Performance of noninvasive ventilation in acute respiratory failure in critically ill patients: a prospective, observational, cohort study. BMC Pulm Med. 2015; 15:144. DOI: 10.1186/s12890-015-0139-3
25. Vincent JL, Opal SM, Marshall JC. Ten reasons why we should NOT use severity scores as entry criteria for clinical trials or in our treatment decisions. Crit Care Med. 2010; 38:283-7. DOI: 10.1097/CCM.0b013e3181b785a2
26. Sutherland A, Thomas M, Brandon RA, Brandon RB, Lipman J, Tang B et al. Development and validation of a novel molecular biomarker diagnostic test for the early detection of sepsis. Crit. Care. 2011; 15:R149. DOI: 10.1186/cc10274
27. Tang BM, McLean AS, Dawes IW, Huang SJ, Lin RC. Gene-expression profiling of peripheral blood mononuclear cells in sepsis. Crit. Care Med. 2009; 37:882-8. DOI: 10.1097/CCM.0b013e31819b52fd
28. Werner JA, Schierding W, Dixon D, MacMillan S, Oppedal D, Muenzer J, et al. Preliminary evidence for leukocyte transcriptional signatures for pediatric ventilator-associated pneumonia. J. Intensive Care Med. 2012; 27(6):362-9. DOI: 10.1177/0885066611406835
29. Dobreanu M, Oprea OR. Laboratory medicine in the era of precision medicine - dream or reality?. Rev Romana Med Lab. 2019;27(2):115-24. DOI: 10.2478/rrlm-2019-0025

30. Lazăr A, Georgescu AM, Vitin A, Azamfirei L. Precision Medicine and its role in the treatment of sepsis: a personalised view. Journal of Critical Care Medicine, 2019; 5:90-6. DOI: 10.2478/jccm-2019-0017
31. Johnson SB, Lissauer M, Bochicchio GV Moore R, Cross AS, Scalea TM.. Gene expression profiles differentiate between sterile SIRS and early sepsis. Ann. Surg; 2007; 245:611-21. DOI: 10.1097/01.sla.0000251619.10648.32
32. Reinhart K, Bauer,M, Riedemann N.C, New approaches to sepsis: molecular diagnostics and Biomarkers. Clin Microbiol Rev. 2012; 25(4): 609-34. DOI: 10.1128/CMR.00016-12
33. Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. N Eng J Med. 1999; 340:448-54. DOI: 10.1056/NEJM199902113400607
34. Hofer N, Zacharias E, Muller W, Resch B. An update on the use of C-reactive protein in early-onset neonatal sepsis: current insights and new tasks. Neonatology. 2012; 102:25-36. DOI: 10.1159/000336629
35. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis. 2004; 39:206-17. DOI: 10.1086/421997
36. Szederjesi J, Almasy E, Lazăr A, Huțanu A, Badea I, Georgescu A. C. Biomarkers of Severe Sepsis. J Crit Care Med 2015;1(4): 147-53. DOI: 10.1515/jccm-2015-0022