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Full Length Article

Complement component consumption in sepsis correlates better with hemostatic system parameters than with inflammatory biomarkers

Dajana Lendak[a,b,*], Dunja Mihajlovic[c,d], Gorana Mitic[e,f], Milan Ubavic[g], Aleksandra Novakov-Mikic[h,i], Jasmina Boban[j], Snežana Brkic[a,b]

[a] Department for Infectious Diseases, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia
[b] Clinical Center of Vojvodina, Clinic for Infectious Diseases, Hajduk Veljkova 3, 21000 Novi Sad, Serbia
[c] Department for Anesthesiology and Perioperative Medicine, Faculty of Medicine, University of Novi Sad, Serbia
[d] Clinical Center of Vojvodina, Emergency Center, Hajduk Veljkova 3, 21000 Novi Sad, Serbia
[e] Department for Pathophysiology, Faculty of Medicine, University of Novi Sad, Serbia
[f] Laboratory Medicine Center, Department for Hematology, Hemostasis and Prevention of Thrombosis, Clinical Center of Vojvodina, Hajduk Veljkova 3, Novi Sad, Serbia
[g] Health Care Institution for Laboratory Diagnostics, Medlab, Ilije Ognjanovića 1, 21000 Novi Sad, Serbia
[h] Department for Gynecology and Obstetrics, Faculty of Medicine, University of Novi Sad, Serbia
[i] Clinical Center of Vojvodina, Clinic of Gynecology and Obstetrics, Hajduk Veljkova 1, 21000 Novi Sad, Serbia
[j] Department for Radiology, Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, Novi Sad, Vojvodina, Serbia

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ABSTRACT

Introduction: The aim of this study was to investigate the role of C3 and C4 complement components in prediction of sepsis outcome. The secondary aim was to determine relationship between complement components and other inflammatory parameters, and parameters of hemostasis.

Methods: One-hundred-thirty-seven patients with sepsis (Sepsis-3 criteria) were included in the study. Routine laboratory markers, predictive APACHEII and SOFA scores, concentrations of C3 and C4, activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT), fibrinogen, antithrombin (AT), protein C (PC), protein S (PS), endogenous thrombin potential (ETP), thrombomodulin, and D-dimer were available. Concentrations of C3 and C4 were correlated with the disease outcome, predictive scores, inflammatory markers and parameters of hemostasis. Statistical analysis was performed using the non-parametric approach and significance was set at $p < 0.05$.

Results: A significant depletion of the complement was observed in non-survivors ($AUCROC_{C3} = 0.692$, $p_{C3} < 0.001$,$AUCROC_{C4} = 0.672$, $p_{C4} = 0.001$). There was a significant negative correlation of C3and C4with APACHEII and SOFA (C3-APACHEII $\rho = -0.364$, $p = 0.011$, C3-SOFA $\rho = -0.460$, $p < 0.001$), aPTT ($\rho = -0.407$, $p < 0.001$), PT ($\rho = -0.408$, $p < 0.001$), and D-dimer ($\rho = -0.274$, $p = 0.001$). A significant positive correlation was observed with natural anticoagulants (C3-AT $\rho = 0.493$, $p < 0.001$; C3-PC $\rho = 0.450$, $p < 0.001$; C3-PS $\rho = 0.345$, $p < 0.001$), fibrinogen ($\rho = 0.481$, $p < 0.001$),and ETP ($\rho = 0.384$, $p < 0.001$). C3 and C4 correlated significantly only with CRP ($\rho = 0.207$, $p = 0.015$), while no significant correlations with procalcitonin and WBC were detected. Results were similar for C4 and C3, although C3 presented higher correlation coefficients.

Conclusion: In septic patients with poorer outcome, a significant depletion of the complement system was observed. Concentrations of complement components demonstrated stronger correlations with coagulation parameters than with inflammatory biomarkers.

1. Introduction

The complement system is one of the major components of the human defense system against bacterial infection. After invasion of otherwise sterile tissues by microorganisms, a prompt reaction of the innate immunity occurs. Reaction of the complement system is pivotal,

* Corresponding author at: Hajduk Veljkova 3, 21000 Novi Sad, Serbia.
E-mail addresses: dajana.lendak@mf.uns.ac.rs (D. Lendak), dunja.mihajlovic@mf.uns.ac.rs (D. Mihajlovic), gorana.mitic@mf.uns.ac.rs (G. Mitic), milan.ubavic@faculty-pharmacy.com (M. Ubavic), aleksandra.novakov-mikic@mf.uns.ac.rs (A. Novakov-Mikic), jasmina.boban@mf.uns.ac.rs (J. Boban), snezana.brkic@mf.uns.ac.rs (S. Brkic).

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and occurs already in the first 4 h after microbial invasion [1]. The main biological roles of the complement system are to opsonize pathogens, to activate inflammatory response by recruiting white blood cells to the infection site, to assist in the removal of pathogen from circulation and injured tissues by initiating phagocytosis and lysis, as well as to activate the adaptive immunity (both humoral and cellular) [2]. However, an over-extensive activation of the complement system with induction of the excessive inflammatory response that may generate systemic inflammation occurs in a number of cases (depends both on genetic factors of the host and virulence of the pathogen) and leads to sepsis [2–4]. Recent studies showed the role of the complement in the innate and adaptive immunity interconnection, in maturation of synapses, clearance of immune complexes and regulation of angiogenesis [3,5].

Studies from the last decade performed in vitro or on animal models, showed that relationship between two basic pathophysiological mechanisms of sepsis - inflammation and coagulation, traditionally observed as separate– is far stronger than initially supposed. The complement system is now recognized as the main regulator of this connection [4,6,7]. To the best of our knowledge, there are only few clinical studies on human population that addressed this issue [8].

Activation of the complement system happens via three pathways: classical, alternative and lectin. Since component 3 (C3) represents the common component of all pathways, the determination of its concentration best depicts the complement status in the sepsis [8].

The primary aim of this study was to investigate the role of C3 and C4 complement components in the prediction of sepsis outcome. The secondary aim was to determine the relationship between complement components and other inflammatory parameters, and parameters of hemostasis.

2. Materials and methods

2.1. Patient selection

A total of 137 consecutive patients with sepsis, treated in the Intensive Care Unit and Clinic for Infectious Diseases in the one year period, were enrolled in this institutional ethical board-approved study. Inclusion criteria were: patients older than 18 years of age, diagnosed with sepsis (based on The Third International Consensus Definition for Sepsis and Septic Shock criteria: Sepsis-3) [9]. Exclusion criteria were: patients with malignant diseases, autoimmune disorders, patients receiving anticoagulant therapy, pregnancy, polytraumatized patients, patients with pancreatitis and burns. At the admission there were no participants with clinical signs or laboratory markers positive for disseminated intravascular coagulation (DIC). All participants (or their first relatives in cases when patients were unconscious) signed the informed written consent.

2.2. Laboratory testing

Routine laboratory testing and determination of predictive APACHE II (Acute Physiology Age Chronic Health Evaluation) and SOFA (Sequential Organ Failure Assessment) scores were performed in all participants within the first hour after admission. Additional 5 ml of the venous blood taken directly from the cubital vein underwent immediate centrifugation, with separation of plasma that was stored on −80 °C, until analyzed. Concentrations of C3 and C4 complement components, and concentrations of additional laboratory parameters were determined later from that sample. The additional parameters were: activated partial thromboplastin time (aPTT), prothrombin time (PT), fibrinogen, D-dimer, antithrombin (AT), protein C (PC), protein S (PS), thrombomodulin (TM) and endogenous thrombin potential (ETP).

Concentration of C-reactive protein (CRP) was determined using immunoturbidimetric assay (ABX Micros) and expressed in mg/l. Concentration of procalcitonin (PCT) was determined using automatic analyzer (mini Vidas), and the lowest limit of detection was set at 0.05 ng/l. Complement component concentrations were analyzed using turbidimetric method and commercially available reagents (Biosystems S.A., Barcelona, Spain) on Olympus AU 400 test unit. Platelet count ($nx10^9$/l) was determined using CELL-DYN Sapphire ABBOT analyzer (Abbot Park, Illinois, USA), with commercially available kits. PT, aPTT and fibrinogen concentrations were determined using coagulation method with reagents from Instrumentation Laboratory Milan, IT, on 9000 coagulation analyzers (ACL 9000; Milan, Italy). D-dimer level was determined by latex immunoassay methodology (using IL reagents) and

Table 1
Demographic features of the sample.

Feature	Non-survivors	Survivors	Total	P
	(*n* = 46)	(*n* = 91)	(*n* = 137)	
Gender (m)	28/46 (60.9%)	54/91 (59.3%)	82/137 (59.9%)	0.116*
Age	65.0 (56.0–76.2)	63.0 (50.0–70.0)	64.0 (54.5–72.0)	0.506**
Comorbidity structure				
Hypertension	23 (35.4%)	42 (64.6%)	65 (47.4%)	0.403*
Serious CV event (CVI and/or MI)	5 (35.7%)	9 (64.3%)	14 (10.2%)	0.537*
DM	8 (36.4%)	14 (63.6%)	22 (16.1%)	0.470*
COPD	2 (22.2%)	7 (77.8%)	9 (6.6%)	0.365*
Psychiatric diseases	8 (50.0%)	8 (50.0%)	16 (11.7%)	0.117*
Sepsis source				
Abdomen	16 (34.8%)	19 (20.9%)	35 (25.5%)	0.001*
Lung	8 (17.4%)	11 (12.1%)	19 (13.9%)	
Urinary tract	2 (4.3%)	36 (39.6%)	38 (27.1%)	
CVK	0 (0%)	1 (0.7%)	1 (0.7%)	
Mediastinum	0 (0%)	2 (1.5%)	2 (1.5%)	
CNS	7 (15.2%)	8 (8.8%)	15 (10.9%)	
Soft tissue infection	4 (8.7%)	10 (11.0%)	14 (10.2%)	
Unknown	9 (19.6%)	4 (4.4%)	13 (9.5%)	
Bloodstream infection (positive hemoculture or Septifast)	19 (41.3%)	31 (34.1%)	50 (36.5%)	0.259*
Microbiologically confirmed bacteria from body fluid culture	27 (58.7%)	60 (65.9%)	87 (63.5%)	
G+	11 (57.9%)	8 (25.8%)	19 (38.0%)	0.036*
G−	8 (42.1%)	23 (74.2%)	31 (62.0%)	
S. aureus	3 (60.0%)	2 (40.0%)	5 (10.0%)	–
S. pneumoniae	5 (62.5%)	3 (37.5%)	8 (16.0%)	–
CONS	0 (0.0%)	1 (100%)	1 (2.0%%)	–
Enterococcus spp.	3 (50.0%)	3 (50.0%)	6 (12.0%)	–
Acinetobacter spp.	4 (50.0%)	4 (50.0%)	8 (16.0%)	–
N. meningitides	0 (0.0%)	4 (100%)	4 (8.0%)	–
E. coli	1 (14.3%)	6 (85.7%)	7 (14.0%)	–
Proteus mirabilis	0 (0.0%)	3 (100%)	3 (6.0%)	–
Pseudomonas aerug.	2 (66.7%)	1 (33.3%)	3 (6.0%)	–
Klebsiella pneumoniae	1 (25.0%)	3 (75.0%)	4 (8.0%)	–
Enterobacter spp.	3 (50.0%)	3 (50.0%)	6 (12.0%)	–
Hospital LOS	8.0 (2.0–14.2)	16.0 (13.0–23.0)	14.0 (8.0–21.0)	< 0.001**

Data are shown as number (%) for categorical and median (IQR) for continuous variables; Differences between groups were evaluated using χ^2 test for categorical variables and Mann-Whitney U test for continuous variables.
Abbreviations: CV – cardiovascular; CVI - cerebrovascular infarction; MI - myocardial infarction; DM - diabetes mellitus; COPD - chronic obstructive pulmonary disease; CVK – central venous catheter; CNS – central nervous system; CONS - coagulase negative *Staphylococcus*; LOS – length of stay.
* χ^2 test.
** Mann-Whitney *U* test.