Gene polymorphisms in surgical patients with diffuse secondary peritonitis - genetic predisposition for sepsis

Kristina Doklesić, Nela Maksimović, Bojan Jovanović, Jelena Veličković, Vesna Bumbasirević
1 Faculty of Medicine, University of Belgrade, Serbia. Clinic for Emergency Surgery of Serbia, Clinical Center of Serbia, Belgrade, Serbia
2 Faculty of Medicine, University of Belgrade, Institute of Human Genetics
3 Faculty of Medicine, University of Belgrade, Serbia. Department for Anaesthesiology, Clinical Center of Serbia, Belgrade, Serbia

Despite the great progress that has been made in recent decades in the intensive treatment of critically ill patients with intra-abdominal infections, antibiotic therapy and modern surgical techniques, secondary peritonitis is still accompanied by significant morbidity and mortality. The response to infection can be variable between different individuals. Scientific evidence supports the hypothesis that this inter-patient variability can be at least partially explained by genetic influence which may have an important role in the development of sepsis, as well as in severity of complications and death.

An infection triggers complex immune response of the host and affects the balance between coagulation and fibrinolysis. This imbalance can lead to hypercoagulability but also to the microcirculation disturbance which can lead to multiple organ dysfunction syndrome (MODS) and septic shock. Common polymorphisms of genes which encode proteins with important role in coagulation and fibrinolysis could be of great importance in susceptibility for sepsis, possible complications and clinical outcome. Early genetic information may become very useful for identification of patients with high risk of developing severe sepsis and multiple organ dysfunctions, in order to design better, more personalized therapy with less severe adverse effects.

Key words: intra-abdominal infections, secondary peritonitis, sepsis, genetic polymorphisms

INTRODUCTION

Despite the remarkable progress in surgical treatment and modern intensive care unit, secondary peritonitis in surgical patients with acute abdomen is still accompanied by a high morbidity and mortality rate. Secondary peritonitis represents peritoneal inflammation due to lesions of intra-abdominal organs. As a result there is bacterial contamination of the abdominal cavity which leads to an infection that triggers a complex immune response of the host and affects the balance between coagulation and fibrinolysis. This can lead to hypercoagulability and also to microcirculation disturbance which can lead to Multiple Organ Dysfunction Syndrome (MODS) and septic shock. The fact is that patients with similar clinical presentation and severity of infection, can have very different outcome. Great interest arises from the idea that individual differences in clinical and pathophysiological manifestations of the disease, can be influenced by genetic predisposition for the development and outcome of sepsis.

In 1988 Sorensen et al. changed our understanding of infectious diseases by pointing out that there is a significant genetic influence on death from an infectious cause. It became obvious that clinical presentation of the septic patient is not determined just by the type of microorganism, its response to antibiotic treatment or location of the infection but also by the response of the host. Today we know that genetic variations, mostly single nucleotide polymorphisms (SNPs), can influence the response of the host from the moment of the microbial invasion to the organ failure and finaly death. There are more and more studies which investigate the association of different gene polymorphisms and susceptibility for sepsis or adverse outcomes in critically ill patients.

INTRA ABDOMINAL INFECTIONS (IAI) AND SEPSIS

SECONDARY PERITONITIS

Intra-abdominal infections (IAI) are frequent and 80-90% of them are secondary peritoneal infections. Generalized secondary peritonitis resulting from the hollow abdominal organ perforation, is one of the most common conditions that require emergency surgery. Organ
Sepsis is a complex clinical syndrome which occurs as a result of amplified and dysregulation host response to microbial pathogens. In secondary peritonitis and subsequent sepsis, it is necessary to implement resuscitation following the guidelines for management of severe sepsis, bearing in mind that the treatment of sepsis requires state of the art intensive care. Resuscitation should provide hemodynamic stability, satisfactory tissue perfusion and oxygenation of cells. Resuscitation implemented urgently in the first 6 hours of the onset of sepsis would have a positive effect on survival. Peritonitis is an abdominal infection and early administration of antibiotics is necessary. Community acquired secondary peritonitis is mixed aerobic/anaerobic bacterial infection and antibiotic therapy should be initiated pre-operatively or intra-operatively.

In secondary peritonitis bacterial counts change depending on the site of perforation: in gastro-duodenal perforations bacterial counts are usually low (< 10^7/ml), while biliary perforations or jejunum perforation have intermediate bacterial counts (10^7 - 10^8). The highest bacterial counts (> 10^9) will be found in colon perforation. At first the empirical antibiotic treatment of IAI will be applied, followed by antibiotics administered according to the results of susceptibility testing and others factors (patients co-morbidities, immunosuppression, level of antibiotic toxicity and costs). Studies have shown that in secondary peritonitis every 30-min delay with antibiotics therapy increases mortality. To assess the severity of IAI, prognosis and outcome, different scoring systems are now available: the Acute Physiological and Chronic Health Evaluation score (APACHE II), the Mannheim Peritonitis Index (MPI), the Peritonitis Index Altona (PIA), the Sepsis Score, the Physiological and Operative Severity Score for Enumeration of Mortality and Morbidity (POSSUM). The Acute Physiology and Chronic Health Evaluation (APACHE) II score is most accepted for assessment of the severity of peritonitis. Mild peritonitis will be defined as an APACHE II score of ≤ 10 and severe peritonitis as a score APACHE II score of > 10. In providing a prognosis and risk of mortality in patients with secondary peritonitis APACHE-II score is effective in the range between 11 and 20. Sepsis is triggered by infection, leads to the systemic inflammatory response syndrome (SIRS) with evolves in clinical presentation from infection to sepsis, severe sepsis, and septic shock. Mortality in sepsis results primarily from sequential organ failure defined as a Sequential Organ Failure Assessment (SOFA) score of 3 or higher.

The presence of pathogen triggers the activation of the immunological signal transduction pathways that result in the host response and the systemic production of inflammatory cytokines counterbalanced by the production of anti-inflammatory cytokines. Cytokines initiate further sequence of events by activating various cellular and humoral defense systems. Dysregulation, inadequate response and the over inflammatory response, result in excessive production of mediators leading to an imbalance between pro-inflammatory and anti-inflammatory cytokines with development of the severe sepsis. In sepsis the causing agent and associated inflammatory response of the host lead to fibrin formation and deposition by following mechanisms: up – regulation of procoagulant pathway caused by increased expression of tissue factor (TF) in activated monocyte/macrophage cells; down – regulation of physiological anticoagulants which includes increasing of thrombomodulin (TM) and endothelial protein C receptor (EPCR) levels and decreasing levels of protein S (PS), protein C (PC) and tissue factor pathway inhibitor (TFPI) and suppression of fibrinolytic pathways by plasminogen activator inhibitor type I (PAI-1) and thrombin activatable fibrinolysis inhibitor (TAFI).
Research of the complex mechanisms of sepsis have shown that there is a tendency to different host response to infection. More recently, research of the human genome has shown that common variations in our genome can at least partially explain differences between patients in their sepsis susceptibility as well as severity of the disease and clinical outcome.

In the recent years there is an increased interest for the disease-gene association studies which can provide new informations about the role of certain genes and their variations in sepsis and hence provide better understanding of sepsis pathophysiology. Results of the studies which confirmed the association between common polymorphisms of genes encoding proteins of importance for coagulation and fibrinolysis and susceptibility to sepsis or clinical outcome have been summarized in table 1. Mostly these polymorphisms have been investigated in the field of cardiovascular and thrombotic diseases but there is more and more evidence about their influence in sepsis.

**TABLE 1**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Patients (n)</th>
<th>Gene, polymorphism</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>Sepsis ans severe sepsis, 577+476</td>
<td>TF haplotype-603A/G, -1322C/T, -1812C/t</td>
<td>GTT haplotype associated with lower mortality at 30 days</td>
</tr>
<tr>
<td>33</td>
<td>Severe sepsis, 53 patients and 70 healthy controls</td>
<td>Protein Z, G79A</td>
<td>AA genotype, risk factor for severe sepsis and septic shock</td>
</tr>
<tr>
<td>37</td>
<td>Sepsis, 631</td>
<td>Fibrinogen beta, -854G/A, 900G/A haplotype</td>
<td>GAA haplotype associated with lower mortality and/or severity of organ dysfunction</td>
</tr>
<tr>
<td>38</td>
<td>Children with meningococcal infection, 288</td>
<td>Protein C promoter polymorphisms C1654T, A1641G</td>
<td>TA-TA genotype has protective role for the development of sepsis</td>
</tr>
<tr>
<td>39</td>
<td>Severe sepsis, 464</td>
<td>Protein C, A1641G</td>
<td>AA genotype associated with decreases survival, more organ dysfunction, more systemic inflammation</td>
</tr>
<tr>
<td>40</td>
<td>Critically ill patients, 389</td>
<td>EPCR, H1, H2, H3 haplotypes</td>
<td>H1,H3 haplotypes at reduced risk of developing severe sepsis and/or septic shock</td>
</tr>
<tr>
<td>44</td>
<td>Severe sepsis, 3894</td>
<td>FactorV, Leiden variant</td>
<td>TAFI thr325Ile, Ile/Ile overrepresented in patients with DIC</td>
</tr>
<tr>
<td>46</td>
<td>Children with severe meningococcal sepsis, 112</td>
<td>TAFI Thr325Ile</td>
<td>TAFI thr325Ile, Ile/Ile overrepresented in patients with DIC</td>
</tr>
<tr>
<td>50</td>
<td>Severe sepsis, 260</td>
<td>PAI-1, 5G/4G</td>
<td>4G/4G higher mortality at 30 days and at 6 months</td>
</tr>
</tbody>
</table>

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**GENETIC PREDISPOSITION FOR SEPSIS - GENE POLYMORPHISMS IN GENES FOR COAGULATION AND FIBRINOLYSIS FACTORS**

Tissue factor (TF) and its endogenous inhibitor, tissue factor pathway inhibitor (TFPI), are the main regulators of the coagulation initiation. During sepsis, increased expression of TF from activated monocytes-macrophages has been observed. Dysfunction of TF and TFPI has been associated with severity and outcome from sepsis. In 2014 Shi et al. investigated the association of TF and TFPI polymorphisms with the susceptibility for sepsis and outcome for patients with severe sepsis. Their results have shown that plasma levels of TF are associated with the outcome of severe sepsis. Also they found the association of three polymorphisms in the promoter region of the TF gene: rs1361600 (-603A/G), rs3917615 (-1322C/T) and rs958587 (1812C/T) with the outcome of severe sepsis. Analyzed polymorphisms are in linkage disequilibrium and are defined as -603A/G. Patients with AG and GG genotype had significantly lower TF expression levels and lower mortality rate at 30 days. In this study association between analyzed polymorphisms in TF and TFPI genes and susceptibility for severe sepsis was not observed.

Sipahi et al. (2010) analyzed the association of protein Z, G79A polymorphism in the group of patients with severe sepsis. Protein Z is a vitamin K-dependent glycoprotein and a member of the coagulation cascade.
They came to the conclusion that 79AA genotype could be a risk factor for severe sepsis and septic shock.33

Another protein with the major role in coagulation and inflammation is fibrinogen. It has been shown that fibrinogen has an important role in the outcome of sepsis.34 Higher fibrinogen levels are associated with improved outcome while on the other hand, low fibrinogen levels are associated with increased organ failure and adverse outcome from sepsis.35,36 In 2006 Manocha et al. investigated the association of fibrinogen-beta gene haplotype (-854 G/A, -455 G/A and 9006 G/A) with organ dysfunction in a cohort of patients with sepsis. They concluded that GAA haplotype carriers have lower mortality and lower severity of organ dysfunction.37

Protein C acts as important regulator of thrombin activity. Two polymorphisms: C1654T and A1641G in the promoter region of this protein are described. It has been shown that they influence the level of protein C.38 Binder et al in 2007 have shown that TA-TA genotype has a protective role from development of sepsis in the group of children with meningococcal infection. Children with CG-CG genotype had lower systolic blood pressure and they were in the higher need for adrenergic support. In the study of Walley KR and Russell JA form 2007 patients with severe sepsis were genotyped for -1641 C/T polymorphism. Walley and Russell concluded that AA genotype is associated with increased survival, more organ dysfunction and more systemic inflammation in patients with severe sepsis.39

Endothelial protein C receptor (EPCR) which is involved in anticoagulant pathways of protein C was analyzed in the study of Vassillou et al. in 2013. In this study haplotypes in ECPR gene and their association with severe sepsis and/or septic shock development in critically ill patients were analyzed. Vassillou concluded that both the H1 and H3 haplotypes may be at reduced risk of developing severe sepsis and/or septic shock in the cohort of critically ill patients.40

One of the most widely analyzed factors of coagulation is factor V (FV).41 FV influences PC activation by promoting thrombin generation. It has been confirmed by number of studies that SNP Arg506Gln known as Leiden variant influences morbidity and mortality in severe sepsis. This polymorphism makes factor Va partially resistant to inactivation by APC which causes a prothrombotic state.42 FVL is a rare example of a balanced gene polymorphism since it may provide a survival advantage for heterozygous carriers with severe sepsis although it causes prothrombotic state. In a study of Kondaveeti et al. from 1999 association of FVL heterozygosity with increased incidence of purpura fulminans and trend to reduced mortality was observed in the group of children with meningococcal disease.43 Yan et al. in 2004 analyzed one of the largest cohorts of patients with severe sepsis. Their results have shown that Factor V Leiden heterozygous carriers may have a slightly decreased risk of developing severe sepsis from infection. However, no increase in mortality rate has been observed in this study.44

**FIBRINOLYSIS PATHWAY**

Thrombin activable fibrinolysis inhibitor (TAFI) represents an important link between coagulation and fibrinolysis processes.45 When activated by thrombin, TAFI potently attenuates fibrinolysis. Emonts et al, in 2008 investigated the functional polymorphism Thr325Ile, which has been associated with increased TAFIa stability and activity.46,47 Presence of Ile allele in TAFI gene increases the antifibrinolytic potential of the protein. This study found the association of TAFI gene polymorphism Thr325Ile with susceptibility to DIC in children with severe meningococcal sepsis.48 TAFI was also associated with severity and outcome of severe meningococcal infection in children.49 Another highly important protein involved in fibrinolysis is PAI-1. The primary role of PAI-1 is the inhibition of tissue- and urokinase-type plasminogen activators. During sepsis there is an increase in PAI-1 which causes elimination of tissue-type plasminogen activator and inhibition of fibrinolysis.50 PAI-1 Lorente et al. in 2015 analyzed 5G/4G insertion/deletion promoter polymorphism in PAI-1 gene in the group of severe septic patients. Their results have shown that septic patients, carriers of 4G/4G genotype have higher PAI-1 concentrations and higher risk of death in comparison to patients with 4G/5G and 5G/5G genotypes.50 Many more studies analyzing patients with severe trauma and children and adults with meningococcal disease had the same results.41 Detection of 5G/4G genotype in the future might enable detection of patients with 4G/4G genotype who would potentially have the largest benefit from anti-PAI-1 therapy.

**CONCLUSION**

Rapid surgical source control, modern intensive care and sepsis therapy may offer the chance for decreased morbidity and mortality of the intra-abdominal infections. Increasing evidence suggest the significant association between common polymorphisms in genes which encode proteins involved in coagulation and fibrinolysis and severe complications and mortality in critically ill patients. Huge progress in the field of molecular genetics provides techniques which enable fast detection of patients genotypes. In the near future genotyping will become important in clinical medicine. Early genetic information may become useful for identification of patients with high risk of developing severe sepsis and multiple organ dysfunctions, in order to design better and more personalized therapy with less severe adverse effects.51

**SUMMARY**

**GENSKI POLIMORFIZAM KOD HIRURŠKIH BOLESNIKA SA DIDUZNIM SEKUNDARNIM PERITONITISOM - GENETSKA PREDISPOZICIJA ZA SEPSU**

Uprkos velikom napretku koji je ostvaren poslednjih decenija u oblasti intenzivnog lečenja kritično oboljelih pacijenata sa abdominalnom infekcijom, uz antibiotsku
terapiju i moderne hirurške tehnike, sekundarni peritonitis i dalje prati značajna stopa komplikacija i smrtnosti. Odgovor na infekciju može biti drugačiji između različitih pojedinaca. Naučni dokazi podržavaju hipotezu da se različit odgovor na infekciju može delom objasniti genetskim uticajem, koji imaju važnu ulogu u razvoju sepspe, kao i u nastanku teških komplikacija i smrtnog ishoda. Infekcija izaziva kompleksan imunološki odgo

var domaćina i utiče na ravnateljice između koagulacije i fibrinolize. Ova neravnoteža može dovesti do hiperkoagulabilnosti, ali i do poremećaja na nivou mikrocirkulacije, koji mogu dovesti do sindroma multiple organske disfunkcije i sepičkog šoka. Uobičajeni polimorfizmi gena koji kodiraju proteine koji imaju važnu ulogu u koagulaciji i fibrinolizi, mogu biti od velikog značaja za osetljivost prema sepsi, kao moguće komplikacije i kliničkih ishod. Rane genetske informacije mogu biti veoma korisne u identifikaciji pacijenata sa visokim rizikom za razvoj teške sepspe i disfunkcije organsa, u cilju osmišljavanja bolje, individualne terapije, sa manje neželjenih efekata.

Ključne reči: intraabdominalna infekcija, sekundarni peritonitis, sepsa, genetski polimorfizam

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