

## SERUM LEVEL OF HMGB1 PROTEIN AND INFLAMMATORY MARKERS IN PATIENTS WITH SECONDARY PERITONITIS: TIME COURSE AND THE ASSOCIATION WITH CLINICAL STATUS

SERUMSKI NIVO PROTEINA HMGB1 I INFLAMATORNIH MARKERA KOD PAČIJENATA SA SEKUNDARNIM PERITONITISOM: VREMENSKI TOK I POVEZANOST SA KLINIČKOM SLIKOM

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### Summary

**Background:** Intra-abdominal infection in secondary peritonitis drives as excessive production of inflammatory mediators and the development of systemic inflammatory response syndrome (SIRS) or sepsis. Finding a specific marker to distinguish SIRS from sepsis would be of immense clinical importance for the therapeutic approach. It is assumed that high-mobility group box 1 protein (HMGB1) could be such a marker. In this study, we examined the time course changes in the blood levels of HMGB1, C-reactive protein (CRP), procalcitonin (PCT) and serum amyloid A (SAA) in patients with secondary peritonitis who developed SIRS or sepsis.

**Methods:** In our study, we evaluated 100 patients with diffuse secondary peritonitis who developed SIRS or sepsis (SIRS and SEPSIS group) and 30 patients with inguinal hernia as a control group. Serum levels of HMGB1, CRP, PCT, and SAA were determined on admission in all the patients, and monitored daily in patients with peritonitis until discharge from hospital.

### Kratak sadržaj

**Uvod:** Intraabdominalna infekcija u sekundarnom peritonitisu dovodi do prekomernog stvaranja inflamatornih medijatora i razvoja sistemskog inflamatornog odgovora (SIRS) ili sepse. Pronalaženje specifičnog markera za razlikovanje SIRS-a od sepse bilo bi od ogromnog kliničkog značaja za terapijski pristup. Pretpostavlja se da bi *high-mobility group box 1 protein* (HMGB1) mogao da bude takav marker. U ovoj studiji smo ispitivali promenu nivoa u krvi HMGB1, C-reaktivnog proteina (CRP), procalcitonina (PCT) i serumskog proteina amiloid A (SAA) kroz vreme kod pacijenata sa sekundarnim peritonitisom koji su razvili SIRS ili sepsu.

**Metode:** U ovoj studiji smo ispitivali 100 pacijenata sa difuznim sekundarnim peritonitisom koji su razvili SIRS ili sepsu (grupa SIRS i grupa SEPSA) i 30 pacijenata sa preponskim kilama koji su bili kontrolna grupa. Svim pacijentima su određivane vrednosti HMGB1, CRP, PCT, SAA u serumu na prijemu u bolnicu, dok je u grupi sa peritonitisom nastavljeno postoperativno merenje ovih parametara svakog dana do otpusta iz bolnice.

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List of abbreviations: Systemic inflammatory response syndrome (SIRS), high-mobility group box 1 protein (HMGB1), C-reactive protein (CRP), procalcitonin (PCT), serum amyloid A (SAA), postoperative (p.o.).

**Results:** Preoperative HMGB1, CRP, PCT and SAA levels were statistically highly significantly increased in patients with peritonitis compared to patients with inguinal hernia, and significantly higher in patients with sepsis compared to those with SIRS. All four inflammatory markers changed significantly during the follow-up. It is interesting that the patterns of change of HMGB1 and SAA over time were distinctive for SIRS and SEPSIS groups.

**Conclusions:** HMGB1 and SAA temporal patterns might be useful in distinguishing sepsis from noninfectious SIRS in secondary peritonitis.

**Keywords:** HMGB1, inflammation markers, secondary peritonitis, sepsis, systemic inflammatory response syndrome

## Introduction

Abdominal sepsis is a medical problem that is associated with significant morbidity and mortality (1). Peritonitis is one of the most important sources of abdominal sepsis (2). The outcome of peritonitis depends on systemic and peritoneal response, along with the quantity, nature and duration of the presence of pathogens in the peritoneal cavity (3). In a systemic bacterial infection, the intense reaction results in excessive formation of inflammatory mediators and development of the systemic inflammatory response syndrome (SIRS) (4). Secondary peritonitis is a surgical condition that requires immediate treatment. Sepsis remains a major problem in the postoperative period. Re-intervention in proven abdominal septic foci significantly contributes to increased survival rate (2). However, repeated surgical procedures are an additional risk factor for patients. Hence, there is great interest in finding optimal diagnostic markers for early, noninvasive and reliable diagnosis of abdominal infections and sepsis (2).

The most frequently determined serological marker among the acute phase proteins important in the diagnosis of systemic response is C-reactive protein (CRP). CRP synthesis is stimulated by cytokines (5, 6). In an inflammatory disease, the level may be increased as soon as in 6–8 hours, reaching a peak after about 48 hours (5, 7, 8). Procalcitonin (PCT), a prohormone of calcitonin (9), was found to be increased in severe bacterial infections and sepsis (10). It is assumed that the activation of monocytes leads to the initial release of PCT. Subsequently, activated monocytes stimulate human adipocytes to release PCT (11). Depending on the toxic stimulus, an increase of the PCT concentrations in plasma occurs within 2–6 hours (12, 13). Serum amyloid A (SAA) is released from hepatocytes stimulated by proinflammatory mediators along with other acute phase proteins (14), but also from the resident macrophages (15). During endotoxemia in humans, SAA was consistently found to be one of the rapidly induced acute phase proteins, reaching a peak after 14–16 hours (16, 17).

**Rezultati:** Preoperativne vrednosti HMGB1, CRP, PCT, SAA bile su statistički značajno više kod pacijenata sa peritonitom u odnosu na pacijente sa inguinalnim kilama, i statistički značajno više kod pacijenata sa sepsom u odnosu na one sa SIRS-om. Sva četiri markera inflamacije su se značajno menjala tokom vremena praćenja. Zanimljivo je da su obrasci promena tokom vremena za HMGB1 i SAA bili karakteristični za grupe SIRS i SEPSA.

**Zaključak:** Vremenski obrasci nivoa HMGB1 i SAA mogli bi da budu korisni u razlikovanju sepse i SIRS-a bez infekcije u sekundarnom peritonitisu.

**Ključne reči:** HMGB1, inflamatorni markeri, sekundarni peritonitis, sepsa, sistemski inflamatorni odgovor

High-mobility group box 1 protein (HMGB1) is known as »alarmin« – a molecule released during cellular necrosis. Immune cells secrete it, and it is believed to be of importance in maintaining homeostasis as well (18). There are two mechanisms of release of this protein: passive release from the necrotic cells (initiating inflammation) and active secretion from macrophages (maintenance of inflammation) (19). It is important to highlight that the kinetics of HMGB1 release is biphasic (20). Excessive levels of HMGB1 result in an uncontrolled inflammatory response, tissue damage and organ dysfunction. Sepsis is a condition that is primarily characterized by the presence of high concentrations of serum HMGB1. HMGB1 occurs after 8–72 hours, and it is therefore designated as a »late« inflammatory mediator. Thus, it is assumed that HMGB1 might be a potent marker and a therapeutic target in sepsis.

The aim of this study was to assess the potential of HMGB1, CRP, PCT, and SAA to differentiate sepsis from noninfectious SIRS in secondary peritonitis.

## Materials and Methods

### Patients

The study was performed at the Clinic for Emergency Surgery of the Emergency Center, Clinical Center of Serbia in the period from September 2012 to September 2015. This prospective study included 130 patients. The study was approved by the Ethics Committee of the School of Medicine, University of Belgrade (No. 29/V-5).

In the study group, 100 patients (18 to 70 years old) with signs of acute abdomen due to peritonitis of different etiologies were included:

- pathological perforation of the stomach and duodenum (longer than 12 hours), gallbladder, appendectomy, small intestine and colon
- inflammatory diseases of the gastrointestinal tract
- vascular bowel disease.

The diagnosis of sepsis/SIRS was established by the presence of at least two of the following clinical manifestations: fever  $> 38$  or  $< 36$  °C; heart rate  $> 90$ /min; respiratory rate  $> 20$ /min or  $\text{PaCO}_2 < 4.3$  kPa; white blood cell count  $> 12,000$  or  $< 4,000$  per  $\mu\text{L}$ , or the presence of  $> 10\%$  of immature neutrophils. The criterion defining abdominal sepsis was the presence of an abdominal source of infection with positive microbiological culture from the peritoneal cavity. Patients with negative cultures were classified as SIRS, while those with proven abdominal infections were classified as SEPSIS group.

The control group included 30 patients (18 to 70 years old), operated for inguinal hernia without present infection and inflammation.

Exclusion criteria in this study were: pregnancy, puerperium, diabetes mellitus, malignant diseases, patients with signs of acute pancreatitis, patients on cytostatic agents or immunosuppressive therapy, patients infected with hepatitis B and C viruses and the HIV, and patients with psychosis. Patients with suspected extra-abdominal origin of infection were also excluded from the study.

ASA score was determined preoperatively and APACHE II score was determined on the first postoperative day in all the patients. ASA score ranged from 1 to 5 and APACHE II score ranged from 5 to 14.

#### *Surgical technique*

In the patients with secondary peritonitis, several types of surgery were undertaken. The most used was the suture of duodenum, small bowel and colon by single suture, stomach and sigmoid colon resection. Furthermore, stoma of small bowel or colon, cholecistectomy, appendectomy and total colectomy were performed. All patients were subjected to mid-line incision, peritoneal lavage with 3–4 L of saline with drain of both subphrenic space, subhepatic space and Douglas space. In all the patients, abdomen was closed by primary suture. Control group underwent Linchenstein hernioplasty of inguinal hernia.

#### *Sampling and measurements*

Blood samples were collected prior to surgery and every day afterwards into Vacutainer tubes (BD Vacutainer Systems, Franklin Lakes, New Jersey) with potassium EDTA as anticoagulant for determination of blood cell counts (Wbc, Rbc, Hgb, Hct, Plt), citrate EDTA as anticoagulant for determination of hemostasis parameters (PT, aPTT, fibrinogen, antithrombin III, plasminogen, D-dimer). Blood cell counts were determined with an HmX analyser (Beckman Coulter, USA) using commercial reagents. Commercial tests (Siemens Healthcare GmbH, Marburg, Germany) were used for determination of hemostasis param-

eters on BCS XP coagulation analyzers (Siemens Healthcare GmbH, Marburg, Germany). Blood samples for routine biochemical tests determination (glucose, urea, creatinine, bilirubin, total proteins, albumin,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$ , aspartate-aminotransferase, alanine-aminotransferase, alkaline phosphatase, alpha amylase), and C-reactive protein, SAA and PCT determination were allowed to coagulate at room temperature for 30 minutes and then centrifuged at 2680 g for 10 minutes to obtain serum samples (21). Serum routine biochemical parameters, C-reactive protein and PCT were measured using the commercial assay on a Roche Cobas 6000 automated analyzer (Roche Diagnostics, Mannheim, Germany). SAA concentration was measured by the particle-enhanced immunonephelometric test (Siemens Healthcare GmbH, Germany) on a BNII System (Siemens Healthcare GmbH, Germany).

#### *Microbiology cultures*

The patients' samples (peritoneal swab) that had been taken during surgical procedure were sent to a routine microbiology laboratory and were processed, cultivated and reported according to the standard protocols.

#### *Western blot*

Western blot to determine the HMGB1 in sera was done as previously described (22). Briefly, 1  $\mu\text{L}$  of serum proteins was separated by SDS-PAGE on 12% polyacrylamide gels. Upon protein transfer to a polyvinylidene difluoride membrane (Hybond-P, RPN303F, Amersham Pharmacia Biotech), the membranes were incubated with primary anti-HMGB1 antibody (K-12, Santa Cruz Biotechnology – dilution 1:750) and horse radish peroxidase-conjugated bovine anti-goat secondary antibody (sc-2379; Santa Cruz Biotechnology, Santa Cruz, CA, USA). Specific protein bands were visualized using Western Blotting Chemiluminescence Luminol Reagent (sc-2048, Santa Cruz Biotechnology) and Hyperfilm ECL (RPN 3103 K, Santa Cruz Biotechnology). HMGB1 quantification was performed in TotalLab v1.1 (Phoretix).

#### *Statistical analysis*

All the parameters were described in terms of mean and standard deviation, or median and IQR, where appropriate. The shape of variable distributions was evaluated by Kolmogorov-Smirnov test and parametric or nonparametric test were applied accordingly. The statistical significance of initial differences between HERNIA, SEPSIS and SIRS patients was evaluated with chi-square test, Fisher exact test, t-test or Wilcoxon test for unrelated samples. The temporal patterns of selected serum parameters were analyzed by linear mixed effect models. For the linear mixed

effect model, in order to satisfy the assumption of normality, the non-normal variables were log-transformed prior to entering. P values were adjusted for multiple comparisons according to Bonferroni, and p values less than 0.05 were considered significant. All the calculations and plotting were done in R 3.1.0. (23).

### Results

Demographic and clinical characteristics of the patients are summarized in Table I. There were no statistically significant differences between patients in SIRS and SEPSIS group. The major cause of secondary peritonitis in the SEPSIS group was perforative appendicitis, while in the SIRS group it was duodenal ulcer perforation, without a statistically significant differ-

ence in the distribution of causative conditions (Table I). On the other hand, distribution of operative interventions was significantly different (p=0.023) between these groups, with appendectomy being the most frequent procedure in the SEPSIS group and ulcer suture in the SIRS group (data not shown). All HERNIA patients underwent herniectomy with inguinal canal plastic. Most of the patients with sepsis had a polymicrobial infection (40%). The most frequent infectious agent in SEPSIS was *E. coli*, followed by *C. albicans* and anaerobic bacteria (Table I).

Leukocyte counts were significantly higher in patients with secondary peritonitis compared to those in the HERNIA group (p<0.001), without difference among SEPSIS and SIRS patients (Table II). Other basic hematological parameters were similar in all groups.

**Table I** Description of SIRS, SEPSIS and HERNIA patients, causes of the secondary peritonitis and pathogens.

Demography and clinical evaluation parameters at admission	Peritonitis SEPSIS n=55	(n=100) SIRS n=45	SIRS vs. SEPSIS p value	HERNIA n=30	Peritonitis vs. HERNIA p value
Age (mean±SD)	48.8±13.3	45.9±13.1	nsa	46.2±15.7	nsa
Gender (% male)	69.1%	71.1%	nsb	100%	nsb
Days of hospitalization	7.1±1.9	7.2±1.8	nsa	2.0±0.0	<0.001a
Arterial hypertension	30.9%	28.9%	nsc	33.3%	nsc
ASA median (IQR)	2 (0)	2 (0)	nsc	1 (0)	nsc
APACHE II median (IQR)	8 (2.5)	7 (4)	nsd	4 (3)	nsd
Diagnosis n (%)					
Acute gangrenous perforative appendicitis	19 (34.5)	6 (13.3)	ns <sup>b</sup>		
Acute gangrenous perforative cholecystitis with calculosis	2 (3.6)	3 (6.7)			
Large intestine perforation	4 (7.3)	2 (4.4)			
Small intestine perforation	1 (1.8)	2 (4.4)			
Proximal perforation of the rectum	1 (1.8)	0 (0)			
Perforative duodenal bulbar ulcer	26 (47.3)	28 (62.2)			
Perforative ulcer of the pylorus and prepyloric region	2 (3.6)	4 (8.9)			
Pathogens isolated from SEPSIS patients (% of isolates)					
<i>Escherichia coli</i>	47.3%				
<i>Candida albicans</i>	40.0%				
<i>Anaerobic bacteria</i>	38.2%				
<i>Pseudomonas aeruginosa</i>	12.7%				
<i>Staphylococcus aureus</i>	5.5%				
Other pathogens (less than 5% each)	12.7%				

<sup>a</sup>t-test; <sup>b</sup>Fisher exact test; <sup>c</sup>Chi-square test; <sup>d</sup>Wilcoxon test for unrelated samples; <sup>e</sup>*Streptococcus spp.*, *Proteus mirabilis*, *Enterococcus spp.*, *Klebsiella spp.*, *Candida spp.*; ns, not significant; SD, standard deviation; IQR, interquartile range

**Table II** Preoperative hematologic, hemostasis, biochemical parameters with statistically significant differences between groups.

Parameter	Peritonitis (n=100)		SIRS vs. SEPSIS (p value)	HERNIA n=30	Peritonitis vs. HERNIA (p value)
	SEPSIS n=55	SIRS n=45			
Wbc (10 <sup>9</sup> /L)	13.9±5.5	14.5±8.2	nsa	6.5±1.43	<0.001 <sup>a</sup>
D-dimer (mg/L)	1.8±1.6	2.3±2.1	nsa	0.4±0.1	<0.001 <sup>a</sup>
Plasminogen (% of normal)	93.9±9.3	87.8±8.3	<0.01 <sup>a</sup>	106.3±6.87	<0.001 <sup>a</sup>
Glucose (mmol/L)	6.8 (2.6)	6.7 (2.8)	nsb	4.85 (0.775)	<0.001 <sup>b</sup>
Na <sup>+</sup> (mmol/L)	137.8±3.6	138.4±4.0	nsa	140.8±1.9	<0.001 <sup>a</sup>
Total proteins (g/L)	67.5±7.7	66.5±8.4	nsa	72.3±4.0	0.014
Albumin (g/L)	41.9±6.2	40.7±6.8	nsa	46.7±2.9	<0.001 <sup>a</sup>
CRP (mg/L)	46 (170.65)	9.2 (19.7)	0.018 <sup>b</sup>	0.8 (1.475)	<0.001 <sup>b</sup>
PCT (ng/L)	0.97 (0.94)	1.19 (0.55)	0.010 <sup>b</sup>	0.04 (0.02)	<0.001 <sup>b</sup>
SAA (mg/L)	623 (919)	451 (741)	nsb	2.95 (1.1)	<0.001 <sup>b</sup>
HMGB1 (band optical density)	161.8±27.9	137.2±26.6	<0.001 <sup>a</sup>	62.7±7.3	<0.001 <sup>a</sup>

Mean ± SD deviation or median (IQR) were presented; <sup>a</sup>t-test, <sup>b</sup>Wilcoxon test; ns, not significant.

**Table III** CRP, PCT, SAA and HMGB1 in SIRS and SEPSIS during hospitalization<sup>a</sup>

Parameter	SIRS vs. SEPSIS (p value)	Time (p value)	SIRS/SEPSIS×Time (p value)
CRP (mg/L)	0.004	<0.001	ns
PCT (μg/L)	ns	<0.001	ns
SAA (mg/L)	0.002	<0.001	<0.001
HMGB1 (band optical density)	<0.001	<0.001	0.035

<sup>a</sup>Linear mixed effect models, ns, not significant.

Considering hemostasis parameters, plasminogen and D-dimer were higher in SEPSIS compared to SIRS group, while D-dimer was higher ( $p<0.001$ ) in secondary peritonitis patients compared to control group (*Table II*). Other preoperative values of hemostasis parameters were similar in all groups.

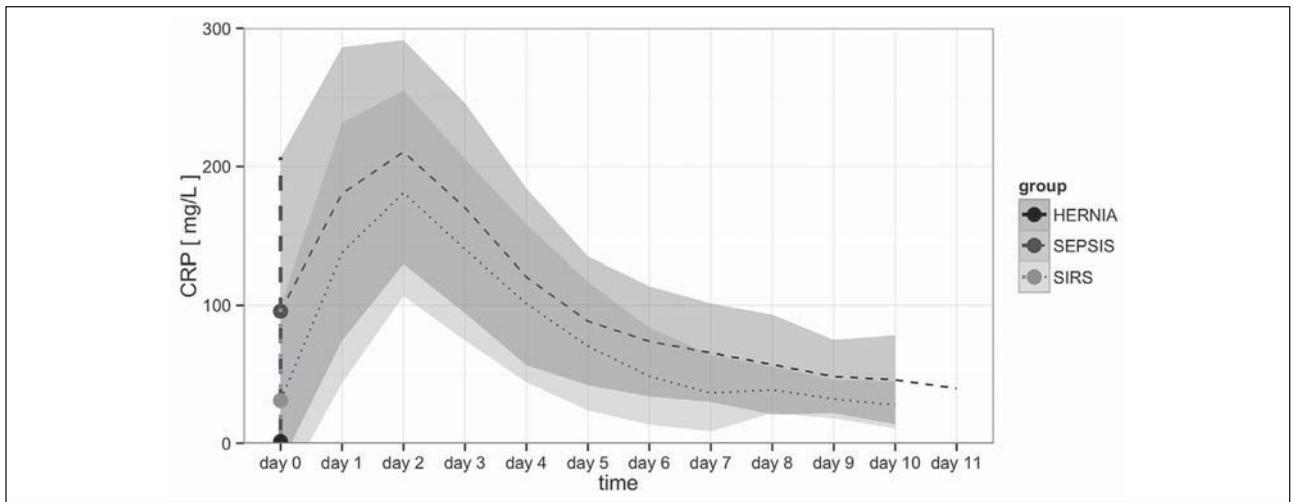
Blood biochemical parameters assessment showed that glucose levels in patients with secondary peritonitis were statistically highly significantly higher than in controls ( $p<0.001$ ), while preoperative concentration of sodium, total proteins and albumin were lower ( $p<0.001$ ,  $p=0.014$  and  $p<0.001$ , respectively, *Table II*). SIRS and SEPSIS group did not differ for any of the biochemical parameters.

Preoperative serum levels of CRP, PCT, SAA and HMGB1 were statistically highly increased ( $p<0.001$ ) in patients with peritonitis compared to patients with hernia (*Table II*). PCT, CRP and HMGB1 also had statistically significantly higher levels ( $p=0.018$ ,  $p=0.010$  and  $p<0.001$ , respectively) in septic patients than in the SIRS group (*Table II*).

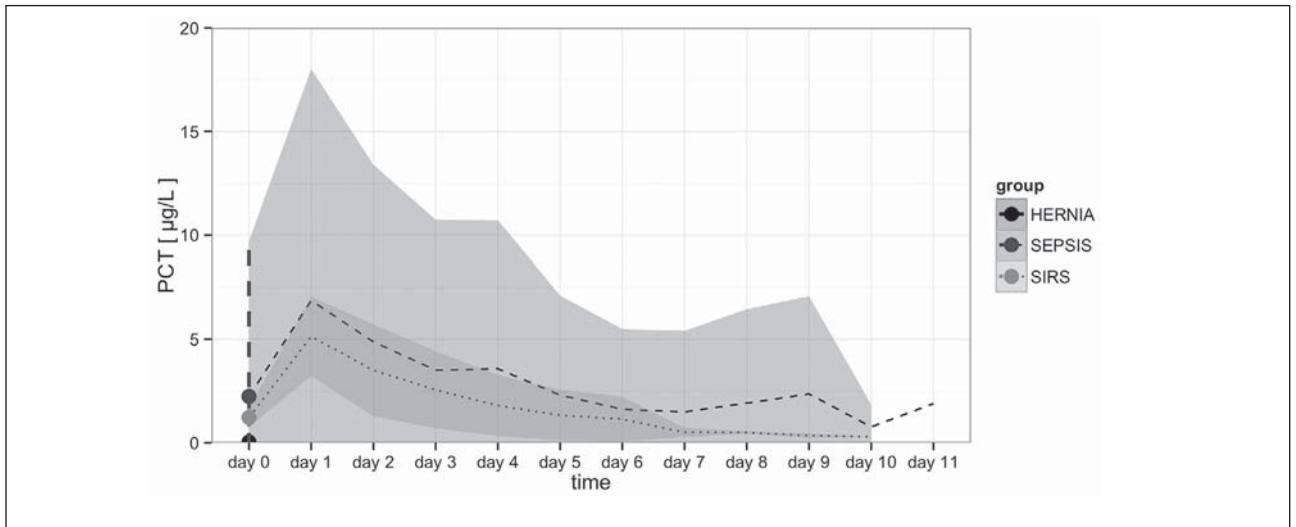
With the goal to distinguish SEPSIS from SIRS, we evaluated the temporal patterns of hematologic, hemostatic and biochemical parameters. A linear mixed model showed overall statistically significant differences between SIRS and SEPSIS groups for CRP, SAA and HMGB1 (*Table III*, column SIRS vs. SEPSIS). Specifically, it was found that all four inflammatory markers have shown significant changes over time in both groups (*Table III*, column Time). Furthermore, SAA and HMGB1 temporal patterns were highly significantly different between SIRS and SEPSIS patients (*Table III*, column SIRS/SEPSIS × time interaction). Although the concentrations of PCT showed a trend of higher values in the SEPSIS group, they failed to reach the level of statistical significance. Kinetics of CRP, PCT, SAA and HMGB1 are presented in *Figures 1–4*, respectively.

## Discussion

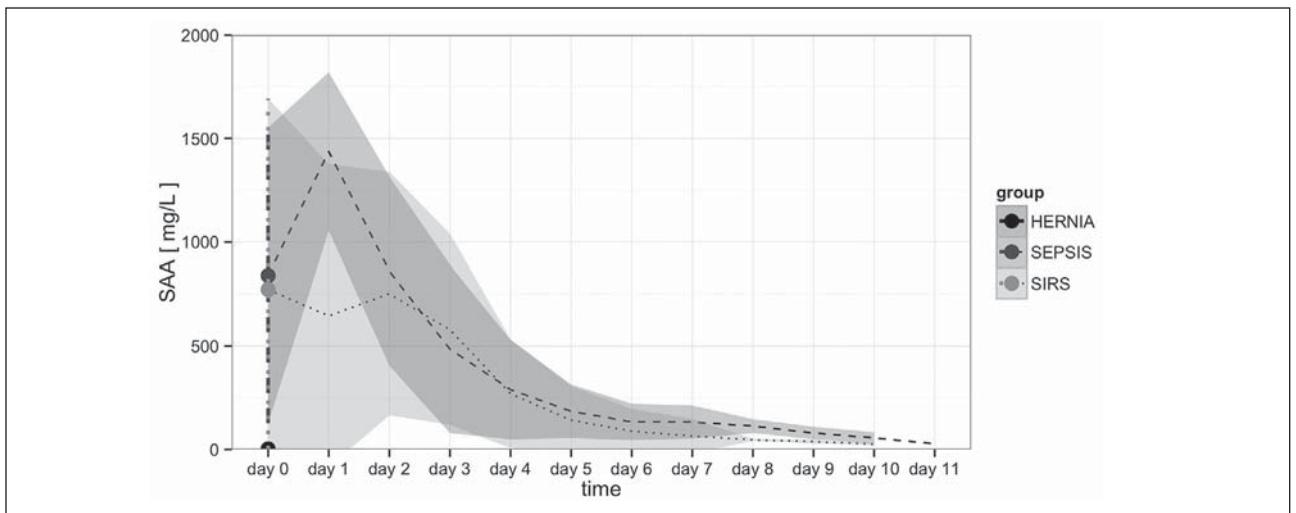
Difference in the therapeutic approach implies differentiation of sepsis from noninfectious SIRS.



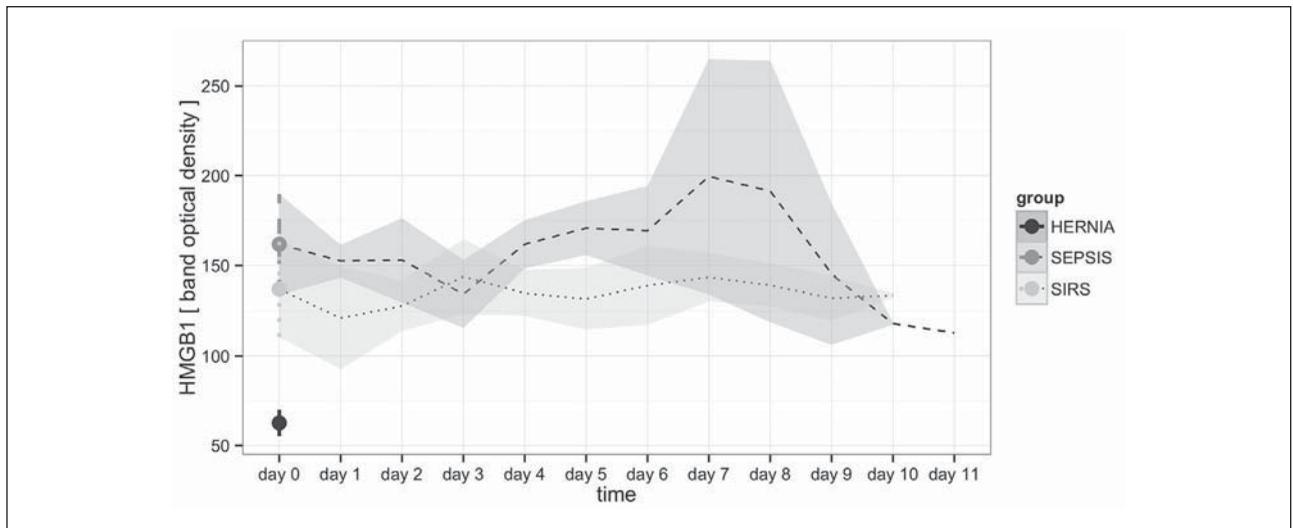
**Figure 1** CRP concentration during hospitalization. Line represents mean CRP concentration during hospitalization. Grey area represents SD.



**Figure 2** PCT concentration during hospitalization. Line represents mean PCT concentration during hospitalization. Grey area represents SD.



**Figure 3** SAA concentration during hospitalization. Line represents mean SAA concentration during hospitalization. Grey area represents SD.



**Figure 4** HMGB1 level during hospitalization. Line represents mean HMGB1 band optical density. Grey area represents SD.

Here, we show that SAA and HMGB1 kinetics have the potential to make such a distinction in a group of patients with secondary peritonitis.

In our study, causes of diffuse peritonitis, demographic characteristics, ASA and APACHE II scores were similar in patients who developed sepsis and patients who did not. Furthermore, routine preoperative hematologic, hemostatic (except plasminogen) and biochemical analyses were not distinctive for SIRS and SEPSIS groups, although several of them were statistically significantly different compared to control group. Likewise, the temporal pattern of these parameters, measured daily throughout the follow-up period, could not differentiate SIRS from SEPSIS. Peritonitis is one of the most important sources of abdominal sepsis, and in spite of modern therapeutic approaches is accompanied by relatively high incidence and mortality (2). It was reported that ASA score correlated with the surgical characteristics of the procedures, length of stay in the hospital, postoperative infections, and the overall mortality of patients undergoing gastrointestinal tract surgery (24, 25). In our study, there were no deaths, possibly due to several facts: 1) exclusion criteria were restrictive (for example, patients with diabetes and malignancy that frequently develop complications following surgery were excluded); 2) preoperative ASA score in both groups was 2, indicating that our subjects had mild systemic disease and were relatively healthy; 3) APACHE II score on the first day p.o. in the SIRS and SEPSIS group was comparable and low, with low expected mortality (26); 4) the patients were relatively young. All these factors might explain the favorable outcome in patients with diffuse peritonitis. However, in our group of patients with sepsis, polymicrobial infection with the predominance of *E. coli* and anaerobic bacteria was the most frequent finding, similarly

to the findings of randomized studies summarized by the Expert Panel of the Surgical Infection Society and the Infectious Diseases Society of America (27). Therefore, we may exclude the option that the type of infectious agent contributed to the absence of mortality in our study.

We assessed the markers of inflammation such as CRP, PCT, SAA and HMGB1 preoperatively, and their kinetics during the hospitalization period. In our study, preoperative values of CRP were significantly higher in the group with secondary peritonitis compared to HERNIA group, while in SEPSIS group they were significantly higher than in SIRS group. Similarly, in a recent study conducted by Gucyetmez and Atalan (28), CRP values were higher in patients with sepsis compared to those with SIRS. Although CRP values higher than 2 SD over reference value were suggested to be included in the diagnostic criteria for sepsis (29), in our study this criterion could not discriminate SIRS from SEPSIS patients. Over time, the concentration of CRP changed significantly: there was an evident increase following surgical procedure reaching the maximum value on the second postoperative day, and then the values would fall continuously until the end of the hospitalization period. This pattern was present in both SIRS and SEPSIS groups and could be attributed to an uncomplicated recovery. Similarly, Berger et al. (30) showed that CRP reaches a maximum after 2 p.o. days, and decreases afterwards. Determining the CRP concentration is relatively cheap, easily feasible and therefore suitable in the daily routine work. However, its predictive value might be limited for predicting presence of infection in patients with SIRS due to the large overlap of values in these two groups.

Patients in SEPSIS group had higher PCT concentrations compared to those in SIRS group prior to

surgery. Infection readily induces PCT, which could explain the preoperative difference between the examined groups (10). However, other stimuli such as proinflammatory cytokines and tissue damage (31) that are certainly present in patients with secondary peritonitis may drive PCT synthesis and blunt the initial difference between patients with and without infection. In our group of patients with secondary peritonitis, PCT reached the maximum value on the first p.o. day, and steadily declined afterwards, which can be attributed to the rapid recovery of our patients. Baykut et al. showed that the value of PCT remained elevated during 4 days p.o. in patients with infection after cardiac surgery, with another jump between days 4 and 6 p.o. In patients who did not have an infection, elevated PCT levels begin to decrease on day 2 p.o. (32). In studies by Novotny et al. (33) and Jung et al. (34) on patients with peritonitis where the trend of plasma PCT was assessed, a decrease two days after surgical removal of the septic focus was reported.

Luzzano et al. (35) in their study involving 800 patients showed that PCT is a better marker of sepsis than CRP. CRP was also less precise in distinguishing SIRS from sepsis. In a meta-analysis of 33 published studies measuring CRP and PCT values, it was concluded that PCT is a good diagnostic biomarker for the diagnosis of these conditions and that it is superior in comparison to CRP (36). However, they also highlighted that there are studies without statistically significant differences between patients with and without sepsis. Yang et al. (37) included 18 studies in their meta-analysis with 1827 patients in total to determine the potential benefit of PCT levels in the diagnosis of bacterial peritonitis, showing relatively good sensitivity and specificity. However, given the methodological limitations and significant heterogeneity in groups, they suggested that medical decisions should be made after the summation of clinical findings and PCT levels and that PCT cannot be used as the gold standard for diagnosis of peritonitis, alone or with other markers (37).

Data on the temporal pattern of SAA concentration in adult patients with sepsis is scarce. We found that preoperative SAA concentration was significantly higher in patients with peritonitis compared to patients with hernia. There was no difference in preoperative SAA concentration between the groups SIRS and SEPSIS, but SAA levels were higher on day 1 p.o. in SEPSIS compared to SIRS, and then continuously declined until the end of hospitalization in both groups, possibly due to the initiation of antibiotic therapy and elimination of infective foci. There was a highly significant group  $\times$  time interaction, i.e. SIRS and SEPSIS had distinctive kinetics of SAA postoperatively. Temporal pattern of SAA in our SEPSIS group was similar to the one reported by Cicarelli et al. in a group of survivors from septic shock (38).

Preoperative HMGB1 levels in our group of patients with peritonitis were increased compared to patients with hernia, which could be explained by the tissue destruction that occurs in peritonitis. HMGB1 levels were significantly higher in SEPSIS than in SIRS group, probably due to an intensive inflammatory response caused by the presence of an infectious microorganism in the group SEPSIS. HMGB1 concentration was decreased in the following three days possibly due to abundant peritoneal lavage. In patients with sepsis, however, HMGB1 concentration rose again probably due to its release from mononuclear cells activated by bacterial products. Alternatively, a high concentration of HMGB1 may be the result of persistence of infective or necrotic foci, but since none of our patients required re-intervention, we cannot speculate on this matter. Interestingly, Van Zoelen et al. showed that HMGB1 release kinetics depend on the source of infection, but this did not affect the mortality and severity of the disease (39), while Gibot et al. (40) demonstrated an association of HMGB1 levels with the severity of septic shock.

Although HMGB1 and SAA were demonstrated to be significant parameters for the distinction of SIRS and sepsis, due to the patient number, this study should be considered somewhat preliminary. Determining them in a larger cohort would provide a definite conclusion about their utility in routine clinical practice. It is also worth noticing that the exclusion criteria lead to a study group predisposed to recover well. Such criteria were chosen to avoid the presumably complex influence of the excluded underlying conditions on the determined parameters. In order to broaden the potential application of these markers, however, they should be assessed in patients with a variety of comorbidities. Although in our study the SIRS and SEPSIS group did not differ in the frequency of particular diagnosis, they did differ in the frequency of a certain type of surgical management, due to several treatment modalities for each causative condition of secondary peritonitis and, at least to some extent, this cannot be excluded as the reason for differences in the inflammation markers kinetics. We are fully aware that a limitation of our study is the fact that the diagnosis of sepsis was based on positive intraperitoneal swabs as evidence of infection. Nevertheless, this definition of sepsis (SIRS with presence of infection – local or systemic) (41, 42) would most probably include a septic patients with falsely negative blood culture and some of those who actually were not septic. Keeping this in mind, the choice of a microbiological criterion would rather blunt the observed differences in inflammatory marker levels between the SIRS and SEPSIS group.

## Conclusions

Inflammatory markers HMGB1, CRP, PCT, and SAA were significantly elevated in secondary peritonitis compared to patients operated for inguinal hernia without signs of present infection and inflammation. Our study demonstrates higher HMGB1 release in patients with sepsis compared to those with SIRS. As a late marker of inflammation, HMGB1 may reflect release of bacterial products following antibiotic treatment. HMGB1 and SAA temporal patterns might be

useful in distinguishing sepsis from noninfectious SIRS in secondary peritonitis.

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## Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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