Diagnostic Microbiology and Infectious Disease 93 (2019) 219–226

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Calprotectin and calgranulin C serum levels in bacterial sepsis☆

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ARTICLE INFO

Article history:
Received 26 March 2018
Received in revised form 2 October 2018
Accepted 10 October 2018
Available online 17 October 2018

Keywords:
Sepsis
Calprotectin
Calgranulin C
Neutrophil-lymphocyte count ratio
Procalcitonin
White blood cell count

ABSTRACT

The aim of this study was to evaluate the serum levels of calprotectin and calgranulin C and routine biomarkers in patients with bacterial sepsis (BS). The initial serum concentrations of calprotectin and calgranulin C were significantly higher in patients with BS ($n = 66$) than in those with viral infections ($n = 24$) and the healthy controls ($n = 26$); the level of calprotectin was found to be the best predictor of BS, followed by the neutrophil-lymphocyte count ratio (NLCR) and the level of procalcitonin (PCT). The white blood cell (WBC) count and the NLCR rapidly returned to normal levels, whereas PCT levels normalized later and the increased levels of calprotectin, calgranulin C, and C-reactive protein persisted until the end of follow-up. Our results suggest that the serum levels of calprotectin are a reliable biomarker of BS and that the WBC count and the NLCR are rapid predictors of the efficacy of antimicrobial therapy.

1. Introduction

Calprotectin and calgranulin C are calcium-binding proteins that belong to a group of danger-associated molecular patterns (DAMPs) known as alarmins. Calprotectin and calgranulin C are stored in most human cells, including neutrophils, monocytes, epithelial cells, and keratinocytes. Calprotectin is a highly abundant protein in neutrophils, accounting for approximately 45% of the cytosolic content (Edgeworth et al., 1991). Calprotectin consists of a complex of 2 intracellular proteins, namely, calgranulin A (also known as S100A8) and calgranulin B (also known as S100A9); this complex is translocated as a heterodimer from the cytosol to the neutrophil cell membrane following calcium mobilization (Roth et al., 1993). Calgranulin C (also known as S100A12) is also located predominantly in the cytosol of neutrophils, regulates the interaction between the cell membrane and the cytoskeleton, and performs Ca^{2+}-dependent chaperone and antichaperone functions (Donato et al., 2013). The functions of both proteins include calcium regulation, cell motility, and intracellular signaling that leads to the production of cytokines (Meijer et al., 2012).

Calprotectin and calgranulin have been implicated in acute conditions such as sepsis, acute lung injury, and asthma as well as in chronic conditions including arthritis, gout, atherosclerosis, cystic fibrosis, Crohn's disease, inflammatory muscle diseases, psoriasis, and chronic skin wounds (Chan et al., 2012). Recently, it was demonstrated that calgranulin A and calgranulin C are upregulated after elective gastrointestinal surgery (Máca et al., 2017).

Although the above-mentioned studies have suggested that there is a clear association of these proteins with infectious and noninfectious inflammatory states, their regulation and pathophysiological roles are still unclear. Nevertheless, the proposed functions of calprotectin and calgranulin C include myeloid cell differentiation, neutrophil activation, and the induction of oxidative stress, chemotaxis, and antibacterial activity (Meijer et al., 2012; Passey et al., 1999). It seems obvious that calprotectin and calgranulin C, when secreted extracellularly, contribute to the innate immune response. This extracellular secretion and participation in the innate immune response suggest that these proteins might be used as potential biomarkers of infectious diseases, including sepsis. To date, elevated serum levels of calgranulin C and calgranulin B have been found in children with acute otitis media (AOM) and adults with pulmonary tuberculosis (TB), postoperative sepsis, and posttraumatic secondary infections (Huang et al., 2016; Liu and Pichichero,

☆ Declarations of interest: none.

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https://doi.org/10.1016/j.diagmicrobio.2018.10.006
0732-8893/

2012; Xu et al., 2015). Taken together, these findings indicate that both proteins are potential biomarkers of community- and hospital-acquired infections, including sepsis.

Thus, the first aim of our study was to evaluate the serum levels of calprotectin and calgranulin C in adult patients with bacterial sepsis (BS), adult patients with acute viral infections, and healthy controls and to test the diagnostic accuracy for BS of both proteins in comparison to routinely used biomarkers. The second aim was to analyze the kinetics of calprotectin and calgranulin C serum levels over the course of BS and to compare it with those of routine biomarkers.

2. Materials and methods

2.1. Study subjects

This prospective observational single-center study was performed in the Department of Infectious Diseases of the Military University Hospital, Prague, Czech Republic. The study was approved by the local ethics committee, and all study subjects signed an informed consent form. Data were collected in the period between March 2015 and May 2017.

Only adult subjects were included in our study, and they were divided into 3 groups as follows: 1) the BS group with patients who met the sepsis criteria valid at the time of the beginning of the study (Levy et al., 2003), 2) the viral infection group with confirmed viral etiology of disease, and 3) the control group with healthy volunteers. The patients were enrolled by the infectious disease specialists participating in the study immediately after the patients' admission or first examination in the acute clinic of the department. The patients had characteristic clinical findings of localized bacterial infection and elevated serum levels of C-reactive protein (CRP), and their condition required antibiotics. The patients in the viral infection group demonstrated characteristic findings of viral infectious diseases, and they had low CRP levels. Healthy volunteers were recruited in the vaccination clinic, and they were enrolled before receiving their scheduled vaccinations. The exclusion criteria consisted of the presence of chronic inflammatory diseases (COPD, rheumatoid arthritis), malignancy, pregnancy, and severe immunodeficiencies including HIV infection.

The diagnosis of BS was determined clinically in accordance with the sepsis criteria and the laboratory finding of CRP serum levels above 60 mg/L. The site of the infection was confirmed by specific clinical, laboratory, and radiological findings. All patients enrolled in the BS group were hospitalized and received intravenous antibiotics immediately upon their admission to the hospital. The microbial etiology of BS was determined by the cultivation of the pathogenic bacteria from specimens, antigen detection tests, polymerase chain reaction (PCR), and/or positive serologic tests. The diagnosis of acute viral infection was also determined by the presence of characteristic clinical signs and laboratory findings, and it was confirmed in all patients enrolled in the viral infection group of this cohort by serologic tests, PCR, and/or antigen detection. Patients with acute viral infections were either hospitalized or treated as outpatients, and they did not receive antibiotics. The control group consisted of age- and sex-matched healthy volunteers who were recruited from the general population.

2.2. Blood sample collection and measurement

Blood was collected from the patients in the BS group within 24 h after their admission to the department and then again on days 3, 5, and 7 of their hospital stays. The blood collected from the patients in the viral infection group and the control group was limited to one specimen that was collected at the time of the patient's admission or at the time of their first examination in the outpatient clinic.

The samples of peripheral venous blood taken from all study subjects were collected into BD Vacutainer® tubes (Becton Dickinson, Plymouth, England) for serum preparation and analysis. Immediately after collection, the blood sample was allowed to clot for 30 min, and then the supernatant was centrifuged at 2500*g* for 10 min at 20 °C. The serum samples were frozen and stored at −80 °C until further analyses were conducted.

2.3. Analysis of calprotectin (S100A8/A9) and calgranulin C (S100A12) levels

Calprotectin and calgranulin C levels were measured using the EVOLIS™ Microplate system (Stratec Biomedical Systems AG, Munich, Germany). We performed the analysis with commercially available enzyme-linked immunosorbent assay kits (BioVendor-Laboratorni Medicina, Brno, Czech Republic). The parameters in the specimens from the study subjects were measured according to the manufacturer's protocols.

2.4. White blood cell count, differential count, and neutrophil-lymphocyte count ratio

White blood cell (WBC) counts and differential counts were routinely measured immediately after blood collection using the Analyzer XT-2000i system (Sysmex, Kobe, Japan). The neutrophil-lymphocyte count ratios (NLCRs) were calculated from the differential WBC count. The values were obtained from the medical records.

2.5. CRP levels

Serum CRP levels were routinely analyzed immediately after sample collection by a commercially available immunoturbidimetric assay (Roche Diagnostics GmbH, Mannheim, Germany) using the Cobas® 8000 modular analyzer. We obtained the values from the medical records.

2.6. Procalcitonin levels

The serum levels of procalcitonin (PCT) were not routinely measured in all patients. We analyzed the serum levels of PCT from stored, frozen samples from all study subjects after the end of the study. We used a commercially available kit (Roche Diagnostics GmBH, Mannheim, Germany), and the analysis was performed according to the manufacturer's protocol using the modular analyzer Cobas® 8000 (Roche Diagnostics GmBH, Mannheim, Germany).

2.7. Statistical analysis

Due to their nature, the observed variables were subjected to nonparametric statistical methods. Comparisons among experimental groups were performed using a Kruskal–Wallis test accompanied by pairwise post hoc Wilcoxon tests, with *P* values adjusted for multiple comparisons by Holm's method. Receiver operating curves depicting the predictive values of the observed variables were compared using DeLong's test. For univariate comparisons, a Wilcoxon test was used. Relations between metric variables were studied by Spearman's correlation coefficient and a test of its significance. The analyses were conducted using the R statistical package, version 3.4.2, R Core Team (2017). The statistical analyses were performed by the certified biostatistician Dr. Václav Čapek.

3. Results

3.1. Characteristics of the study subjects

Overall, we recruited 116 study subjects as follows: 1) the BS group consisted of 66 patients, 2) the viral infection group included 24 patients, and 3) the control group comprised of 26 healthy volunteers.

The patients with BS demonstrated on admission the following vital sign abnormalities: 44 (66.7%) had tachycardia (heart rate > 90 beats/min), 43 (65.2%) had fever (body temperature > 38.3 °C), 12 (18.2%) had tachypnea (respiration rate > 20 breaths/min), and 5 (7.6%) had hypotension (systolic blood pressure < 90 mmHg). Next, 36 (54.5%) of the patients had the following comorbidities: arterial hypertension ($n = 27$), type 2 diabetes mellitus ($n = 10$), dyslipidemia ($n = 6$), ischemic heart disease ($n = 6$), atrial fibrillation ($n = 3$), benign prostatic hyperplasia ($n = 3$), chronic liver disease ($n = 2$), chronic renal insufficiency ($n = 2$), bronchial asthma ($n = 2$), psychiatric disease ($n = 1$), and chronic venous insufficiency ($n = 1$). In addition, 2 patients reported sepsis in their personal history. Altogether, 62 (97%) patients were treated in standard wards, while 2 (3%) patients required intensive care units, and all patients had uneventful recoveries. The microbial etiology in the BS group was confirmed in 40 patients (60.6%) who were treated with directed antibiotic therapy. A group of 26 patients (39.4%) classified as probable BS (i.e., clinically documented but with negative or nonobtainable cultures) received empirical antibiotic therapy. All patients with BS improved during the antibiotic therapy. The most frequently cultivated pathogens were *Escherichia coli* (17× in urine, 8× in urine and blood, and 2× in blood), *Staphylococcus aureus* (2× in blood, 1× in sputum), *Campylobacter jejuni* (2× in stool), and *Streptococcus pyogenes* (2× from wound swabs). The following pathogens were each cultivated once: *Streptococcus pneumoniae* (blood), *Streptococcus epidermidis* (wound swab), group C beta-hemolytic *Streptococcus* (throat swab), *Klebsiella pneumoniae* (urine), *Proteus mirabilis* (blood), and *Salmonella enteritidis* (stool). *Staphylococcus aureus* was confirmed as the etiologic agent of septic arthritis in 1 patient by PCR in the synovial fluid obtained by shoulder joint arthrocentesis. *Legionella pneumophila* infection was confirmed by the detection of the relevant urinary antigens in 1 case. One case of systemic Salmonella infection was confirmed by a positive Widal serological test. *Clostridium difficile* antigens and toxins were detected in 1 case of gastrointestinal infection.

The patients who presented with acute viral infection at enrollment had the following vital sign abnormalities: 7 (29.2%) had tachycardia, 5 (20.8%) had fever, and 2 (8.3%) had tachypnea. In addition, 5 (20.8%) patients had the following comorbidities: type 2 diabetes mellitus ($n = 2$), arterial hypertension ($n = 2$), ischemic heart disease ($n = 1$), hypothyroidism ($n = 1$), bronchial asthma ($n = 1$), and benign prostatic hyperplasia ($n = 1$). All patients were treated in standard wards or outpatient clinics and had uneventful recoveries. The diagnosis of viral infection was confirmed by the laboratory finding of specific IgM antibodies in the blood of patients with mumps, Dengue fever, primary cytomegalovirus, and Epstein–Barr virus infections as well as in those with acute hepatitis A and B. Next, PCR revealed the DNA of the varicella-zoster virus (VZV) in the cerebrospinal fluid collected from all patients with VZV meningitis, the diagnosis of influenza B was based on the PCR detection of specific RNA in pharyngeal swab samples, and the etiology of rotavirus infection was established by antigen detection in stool samples with the immune-chromatography test. The baseline and clinical characteristics of all the study subjects are shown in Table 1.

Table 1
Baseline characteristics of the study groups.

Bacterial sepsis group characteristics	$n = 66$
Age in years, mean ± SD	52 ± 17.2*
Sex, male	51.5%
Length of hospital stay in days, mean ± SD	9 ± 6.5*
Vital signs on admission	
Body temperature (°C), median (IQR)	38.3 (1.7)*
Heart rate (beats/min)	97.5 (18)*
Systolic blood pressure (mmHg)	127 (26.5)
Respiratory rate (breath/min)	15 (4.0)
Charlson comorbidity score (points), median (IQR)	1 (2)
Etiology	
Gram-negative bacteria	34
Escherichia coli	27
Salmonella spp.	2
Campylobacter jejuni	2
Proteus mirabilis	1
Klebsiella pneumoniae	1
Legionella pneumophila	1
Gram-positive bacteria	10
Staphylococcus aureus	4
Streptococcus pyogenes	2
Streptococcus pneumoniae	1
Streptococcus epidermidis	1
Streptococcus β-haemolyticus group C	1
Clostridium difficile	1
Not identified	22
Site of infection	
Urogenital tract	30
Respiratory tract	16
Skin and soft tissue	9
Gastrointestinal tract	4
Other or not identified	7
Patients with blood culture tested	61
Positive blood culture result	15
– Evaluated as contamination	2
Viral infection group characteristics	$n = 24$
Age in years, mean ± SD	39 ± 18.5
Sex, male	70.8%
Inpatient care	21
Length of hospital stay in days, mean ± SD	6 ± 4.6
Outpatient care	3
Vital signs on admission	
Body temperature (°C), median (IQR)	36.7 (0.8)
Heart rate (beats/min)	82 (14)
Systolic blood pressure (mmHg)	122 (22)
Respiratory rate (breath/min)	15 (2.0)
Charlson comorbidity score (points), median (IQR)	0 (2)
Etiology	
Mumps	10
Varicella zoster meningitis	4
Primary cytomegalovirus infection	2
Dengue fever	2
Hepatitis A	2
Acute hepatitis B	1
Rotavirus enteritis	1
Influenza B	1
Primary EBV infection	1
Control group	$n = 26$
Age in years, mean ± SD	51 ± 12.6
Sex, male	57.7%

SD = standard deviation; IQR = interquartile range; EBV, Epstein–Barr virus.
* $P < 0.05$ for bacterial vs. viral infection groups.

3.2. Calprotectin and calgranulin C serum levels in BS patients, viral infection patients, and healthy controls

At the time of admission, the calprotectin serum levels were significantly higher in the BS group than in the viral infection group and the healthy controls. The serum concentrations of calgranulin C were also significantly elevated in the BS group compared to the viral infection group and the healthy controls. There were also significant differences in the serum levels of both proteins between the viral infection group and the healthy controls. The results are shown in Table 2 and Fig. 1.

3.3. Routine biomarkers in BS and viral infections

The serum concentration of PCT, the WBC count, the NLCR, and the CRP level were also measured at the time of admission. All 4 parameters were significantly higher in the BS group than in the viral infection group. In addition, the serum PCT levels were significantly lower in the healthy controls than in the BS or viral infection groups. The results are shown in Table 2.

Table 2
Comparison of laboratory results among all study groups.

Parameters	Bacterial sepsis	Viral infection	Healthy controls
	$n = 66$	$n = 24$	$n = 26$
Calprotectin (ng/mL)	7441.15 (4028.50–13,405.00)	1801.15 (665.70–2886.25)	847.45 (484.80–1376.00)
Calgranulin C (ng/mL)	57.88 (31.56–120.60)	21.95 (12.71–31.27)	12.26 (5.77–17.20)
WBC ($\times 10^9$/L)	12.63 (9.56–16.24)	6.73 (4.90–8.50)	ND
NLCR	9.42 (5.56–13.00)	1.65 (0.89–3.09)	ND
Procalcitonin (μg/L)	0.62 (0.22–1.89)	0.06 (0.02–0.18)	0.020 (0.020–0.022)
CRP (mg/L)	200.60 (150.00–276.00)	7.40 (2.10–14.60)	ND

Data are presented as medians (interquartile range). ND = not determined.

3.4. Sensitivity and specificity of biomarkers in differentiating between BS and viral infections

The receiver operating characteristic (ROC) analysis showed that increased levels of calprotectin, the NLCR, PCT, and the WBC count had high sensitivity and specificity for the diagnosis of BS. The area under the curve (AUC) values of all 4 parameters were statistically significant and demonstrated their acceptable ability to discriminate between BS and viral infections. The AUC values were 90.97 for calprotectin levels, 90.19 for the NLCR, 88.19 for PCT, 83.88 for the WBC count, and 80.68 for calgranulin C levels. The ROC analysis is shown in Fig. 2.

3.5. Kinetics of calprotectin, calgranulin C, and routine biomarkers in BS

The serum levels of calprotectin and calgranulin C decreased during the 7 days of the study period. Specifically, the calprotectin serum levels in BS patients were significantly ($P < 0.01$) lower on days 3, 5, and 7 than the level on day 1, and calgranulin C levels in BS patients on day 3 were significantly lower than the level on day 1. Nevertheless, the serum levels of both proteins had not decreased to the levels observed in the healthy controls by the end of the study period. Similarly, the serum CRP and PCT levels were still higher than their reference ranges on day 7 and day 5, respectively. In contrast, the WBC count and the NLCR decreased rapidly and reached normal values by day 3 of the study. The calprotectin, calgranulin C, and routine biomarkers kinetics and graphs are shown in Table 3.

3.6. Correlations of calprotectin and calgranulin C with other parameters

Spearman's correlations showed no significant associations between calprotectin or calgranulin C levels and age, the length of hospital stay, or the levels of routine biomarkers in patients with BS.

4. Discussion

In this study, we found significantly higher serum levels of calprotectin and calgranulin C in patients with BS than in patients with viral infections and healthy controls. Moreover, the initial serum calprotectin levels demonstrated better diagnostic accuracy for the bacterial etiology of sepsis than the routine biomarkers. Although the levels of both proteins significantly decreased over the study period, the NLCR and the WBC count normalized more rapidly.

Elevated calprotectin concentrations in the blood had already been found in adults with different bacterial infections including typhoid

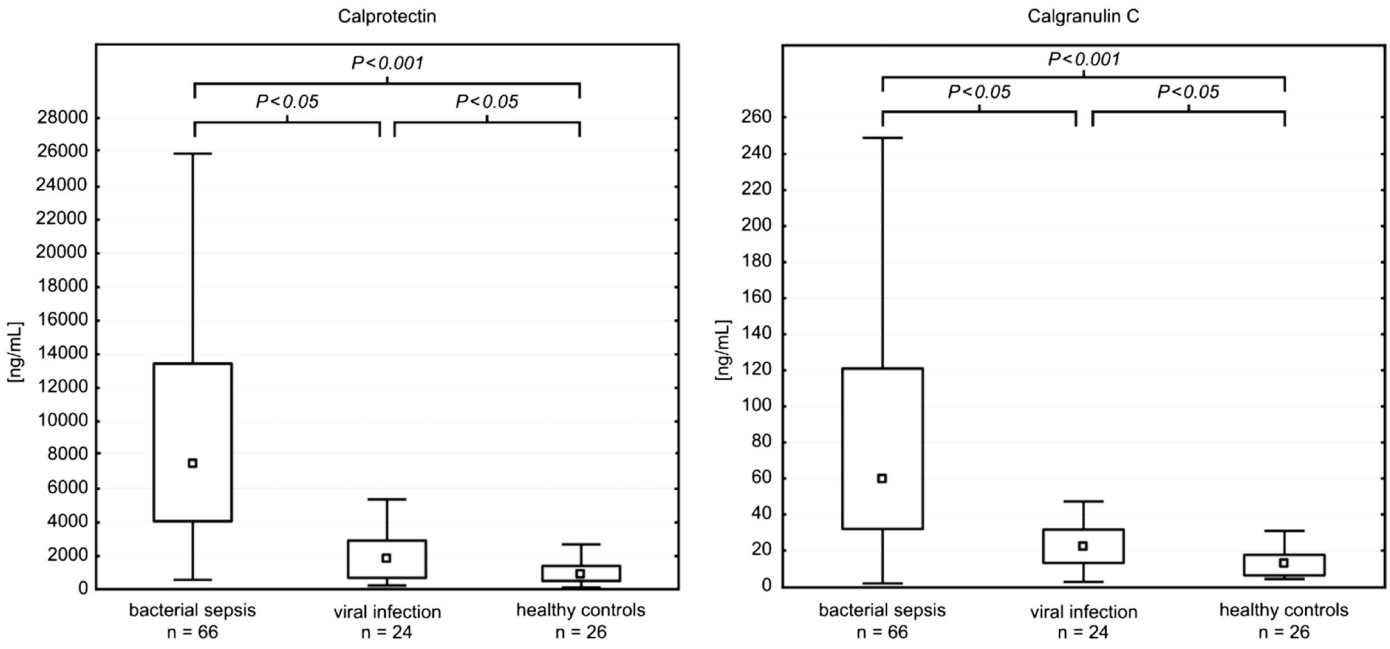


Fig. 1. Comparison of serum calprotectin and calgranulin C concentrations among all study groups.

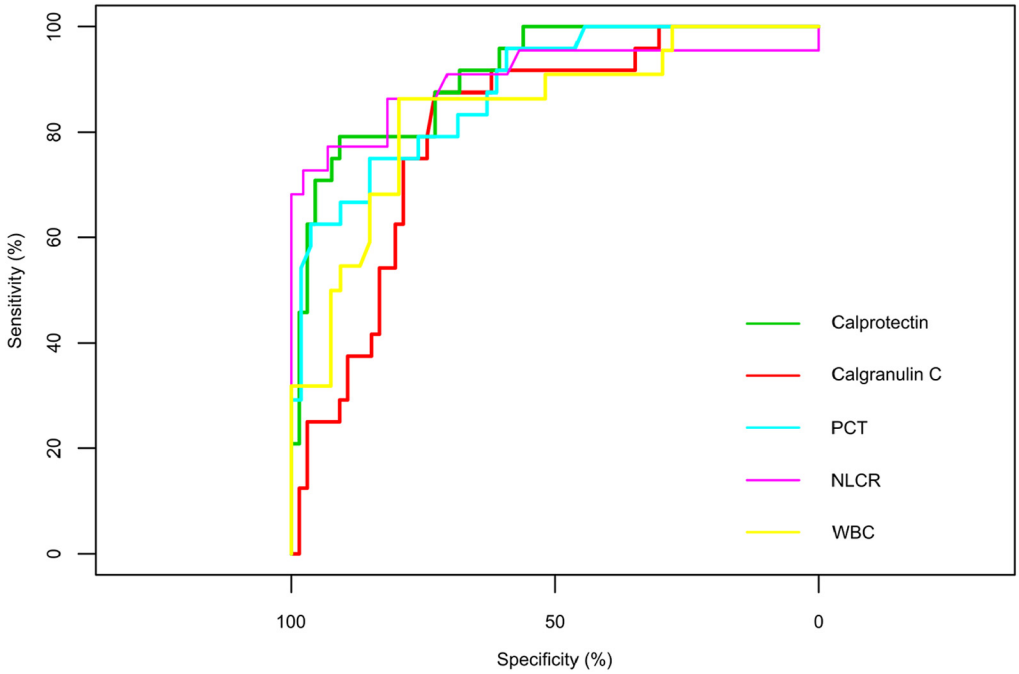


Fig. 2. Receiver operating characteristics analysis of biomarkers.

fever, TB, acute melioidosis, sepsis complicating surgery, and secondary bacterial infection after trauma (De Jong et al., 2015; Huang et al., 2016; Jonsson et al., 2017; Natesan et al., 2017; Pechkovsky et al., 2000). The reported median concentrations of calprotectin in those studies ranged from 373 ng/mL in mild TB to 5000 ng/mL in bacterial infections complicating trauma. Nevertheless, even the highest calprotectin serum levels found in the trauma patients were lower than those observed in our cohort with BS. Similarly, the control groups used for the comparisons with the typhoid fever and TB patients apparently had lower calprotectin concentrations (75 ng/mL and 145 ng/mL, respectively) than our healthy controls (847.5 ng/mL). Additionally, a study in HIV-positive patients demonstrated very high calprotectin serum levels (14,292 ng/mL) and high calprotectin concentrations in HIV-negative blood donors (3474 ng/mL) (Strasser et al., 1997). Taken together, these discrepancies could have been caused by the analytical methods utilized. In this regard, it is worth noting that a new turbidimetric calprotectin immunoassay with acceptable reproducibility is now available for automatized clinical analyzers (Nilsen et al., 2015).

The serum levels of calgranulin C were previously analyzed in children and young adults with severe systemic bacterial or viral infections in a study focused on juvenile rheumatoid arthritis (JRA) and fever of unknown origin (Wittkowski et al., 2008). In this study, the patients with severe systemic infections had a mean calgranulin C level of 470 ng/mL, which is almost 8-fold higher than the mean concentration found in BS patients in our study. A pediatric study on AOM demonstrated a mean serum calgranulin C concentration of 36.7 ng/mL in bacterial AOM, which was significantly higher than the mean concentration of 12.1 ng/mL detected before the onset of the infection (Liu and Pichichero, 2012). The authors also tested serum calgranulin C levels in children with viral upper respiratory tract infections, and the levels did not differ from the preinfection serum concentrations. These data are like our findings in patients with BS and the healthy controls, but different in patients with viral infections, in whom we observed significantly elevated calgranulin C levels in comparison to those of the healthy controls. Because there have been no other studies of calgranulin C blood levels in viral infections, we cannot compare our data with other findings. In addition, elevated serum calgranulin C concentrations with a median of 2.332 ng/mL were found in patients with pulmonary TB (Berrocal-Almanza et al., 2016). Since the control subjects in that study had very low calgranulin C concentrations (1.090 ng/mL), the results are not comparable to our data. In any case, our findings of elevated calgranulin C serum levels in patients with BS are like the results of a study with a large group of critically ill patients

Table 3
Kinetics of biomarkers in bacterial sepsis group during 7 days of hospital stay.

Parameter	Day 1	Day 3	Day 5	Day 7	Normal values
Calprotectin (ng/mL)	7441.15 (9376.50)*	5839.50 (6294.80)*	3989.00 (6031.20)*	3345.10 (3771.20)*	847.45 (891.20)
Calgranulin C (ng/mL)	57.88 (89.04)*	36.42 (45.59)*	30.27 (42.89)*	25.51 (37.74)*	12.26 (11.43)
WBC ($\times 10^9$/L)	12.63 (6.68)*	7.17 (3.24)	7.94 (3.03)	7.14 (4.16)	4.00–10.00
NLCR	9.42 (7.43)*	3.30 (4.90)	2.63 (2.49)	2.23 (2.47)	1.50–5.00
Procalcitonin (μg/L)	0.624 (1.667)*	0.306 (0.921)*	0.119 (0.416)*	0.097 (0.164)	< 0.1
CRP (mg/L)	200.60 (126.00)*	113.70 (118.30)*	45.90 (53.40)*	25.90 (36.35)*	< 8

Data are reported as median (interquartile range).
* $P < 0.01$ for bacterial sepsis vs. normal values.

that found a positive association between increased calgranulin C levels in blood and sepsis (Ingels et al., 2015).

A comparison of the diagnostic sensitivity and specificity of calprotectin and calgranulin C levels and routine biomarkers in discriminating between BS and viral infections has not been previously made. Thus, our study provides a novel insight into the potential clinical application of both proteins. Our data demonstrated an acceptable diagnostic value of calprotectin for BS. Similar findings were reported in a study in critically ill patients demonstrating the diagnostic accuracy of calprotectin for the discrimination between sepsis and noninfectious systemic inflammation, with an AUC of 0.901 (Gao et al., 2015). The diagnostic accuracy of calprotectin serum levels was also tested in neonatal sepsis in a cohort of 41 neonates with suspected or microbiologically proven sepsis (Decembrino et al., 2015). The AUC for calprotectin levels reported in that study was 0.61, which was slightly lower than the AUC for CRP levels but significantly higher than the AUC for WBCs or neutrophils. The diagnostic accuracy of the level of calprotectin for detecting severe systemic infections was also evaluated in patients with JRA (Wittkowski et al., 2008). For the discrimination between severe and nonsevere systemic infections, the authors reported an AUC of 0.881. Like those findings, the level of calprotectin had a high AUC in our study. Moreover, the AUC for the level of calprotectin was higher than those for the NLCR, the level of PCT, and the WBC count. This may be considered a surprising finding because these biomarkers are used in daily clinical practice due to their significant diagnostic accuracy. It may be possible that the AUC for PCT could have been decreased by the relatively high proportion of patients with probable BS. This was already demonstrated in bacteremic patients that had more frequently positive PCT than the patients with probable bacterial infection (Muñoz et al., 2004). On the other hand, the proven or suspected microbial etiology of BS probably did not influence our results because the only significant difference observed between the patients with proven and probable BS was that for NLCR and calgranulin C on day 3 (data not shown). Next, in our previous studies focused on biomarkers that could discriminate between bacterial and viral infections, an acceptable AUC of 0.799 was found for WBCs, and very high AUC values of 0.952 and 0.971 were found for the level of PCT and the NLCR, respectively (Chalupa et al., 2011; Holub et al., 2012). However, these data are not fully comparable to the results of the current study because the inclusion criteria of the previous studies were not specific for sepsis. In addition, we did not test the diagnostic accuracy of the level of CRP because it was used as an inclusion criterion. Nevertheless, a comprehensive review of biomarker studies demonstrates a wide range of AUC values for the level of CRP when distinguishing between bacterial and viral infections (Kapasi et al., 2016). Thus, the effort to find other biomarkers with acceptable sensitivities and specificities to supplement CRP levels seems justified.

The association between the kinetics of biomarkers and the efficacy of antimicrobial therapy has been extensively studied, mostly in critically ill intensive care unit (ICU) patients with sepsis. Among the evaluated biomarkers, PCT and CRP have received the most attention. The evolution of PCT and CRP levels during the antibiotic therapy of sepsis is a reliable predictor of the suppression of infection and the necessary duration of the antimicrobial treatment (Bréchot et al., 2015; Zilahi et al., 2016). Despite the wide use of these 2 biomarkers, they have certain limitations caused by their relatively long biological half-lives (Holub et al., 2008). Since the WBC count and the NLCR do not possess these limitations, these parameters may be more valuable in the assessment of the efficacy of antibiotics under certain conditions. Thus, the rapid normalization of the WBC count and the NLCR observed in our study, with normal values reached after only 3 days of antibiotic therapy, may support the use of both parameters as predictors of antibiotic effectiveness. This concept can be supported by the finding of increasing NLCR during the first 5 days of the hospital stays of septic shock patients with fatal outcomes (Riché et al., 2015). On the other hand, the decrease in the WBC count probably has a limited value because no association was observed between the evolution of the WBC count and the resolution of sepsis in critically ill patients (Póvoa et al., 2011). In addition, although the kinetics of calprotectin or calgranulin C levels in different pathologic conditions have been repeatedly reported, their association with the success of antibiotic therapy in BS is a novel observation.

This study had certain limitations. First, the significance of the data is limited by the monocentric character of the study, the use of convenience sampling, and the relatively high proportion of enrolled patients with probable BS. On the other hand, the study had an original design that directly compared BS and viral infections, and most enrolled cases received etiological diagnoses. Second, the patients with BS had more comorbidities and were significantly older than the patients with viral infections, which might be partly responsible for the observed differences. However, the presence of chronic diseases with previously established associations with elevated levels of calprotectin and calgranulin C was used as an exclusion criterion, and according to the current understanding, only age has some effect on the levels of these biomarkers. Third, there are no widely accepted assays for the analysis of the serum levels of calprotectin and calgranulin C, and therefore, our data are not comparable to the results of other studies. Nevertheless, our data strongly suggest that calprotectin is an important biomarker of BS and support the clinical use of the routine analytical method for calprotectin measurement that has already been developed. Fourth, the study was not designed for critically ill septic patients, and therefore, we could not compare the influence of the severity of sepsis on the levels of both evaluated proteins.

5. Conclusions

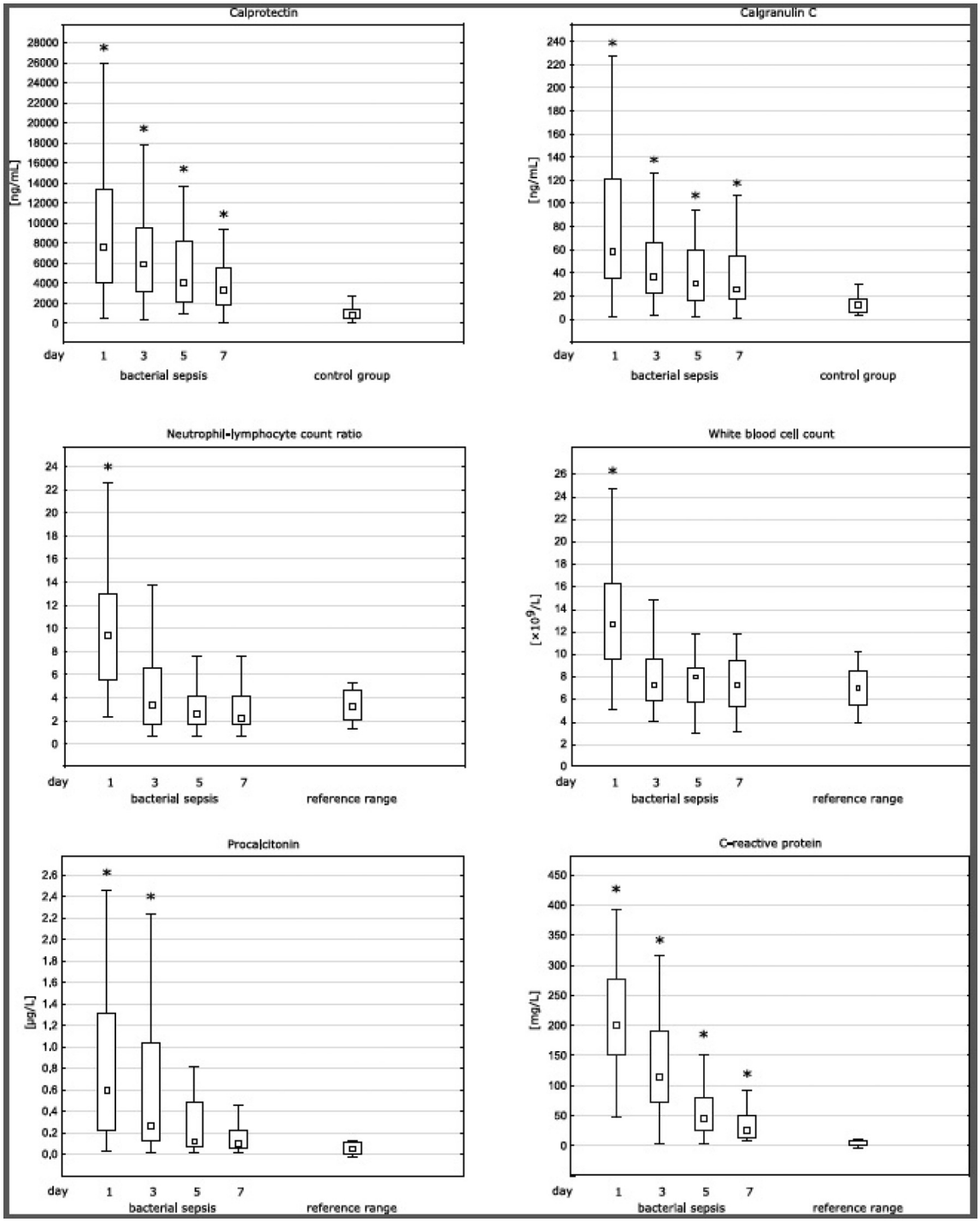
Our data clearly demonstrate that the serum calprotectin levels have significant diagnostic accuracy for the bacterial etiology of sepsis, supporting the implementation of the measurement of the serum calprotectin levels in routine diagnostic laboratory panels. Furthermore, the observed upregulation of calprotectin and calgranulin C suggests that both proteins play a role in the pathophysiology of sepsis, which should be further explored.

Acknowledgments

The study was supported by the Ministry of Health of the Czech Republic, Czech Republic (grant number 15-30186A), the Ministry of Defense of the Czech Republic, Czech Republic (project number MO1012), and Charles University, Czech Republic (project number SVV 260369).

Appendix A

Figure A1. Kinetics of calprotectin, calgranulin C, and routine biomarkers during antibiotic therapy



References

Berrocal-Almanza LC, Goyal S, Hussain A, Klassert TE, Driesch D, Grozdanovic Z, et al. S100A12 is up-regulated in pulmonary tuberculosis and predicts the extent of alveolar infiltration on chest radiography: an observational study. Sci Rep 2016;6(31798). https://doi.org/10.1038/srep31798.

Bréchot N, Hékimian G, Chastre J, Luyt CE. Procalcitonin to guide antibiotic therapy in th ICU. Int J Antimicrob Agents 2015;46:S19–24.

Chalupa P, Beran O, Herwald H, Kaspříková N, Holub M. Evaluation of potential biomarkers for the discrimination of bacterial and viral infections. Infection 2011;39:411–7. https://doi.org/10.1007/s15010-011-0126-4.

Chan JK, Roth J, Oppenheim JJ, Tracey KJ, Vogl T, Feldmann M, et al. Alarmins: awaiting a clinical response. J Clin Invest 2012;122:2711–9. https://doi.org/10.1172/JCI62423.

De Jong HK, Achouiti A, Koh GC, Parry CM, Baker S, Faiz MA, et al. Expression and function of S100A8/A9 (calprotectin) in human typhoid fever and the murine salmonella model. PLoS Negl Trop Dis 2015;9, e000366. https://doi.org/10.1371/journal.pntd.0003663.

Decembrino L, De Amici M, Pozzi M, De Silvestri A, Stronati M. Serum calprotectin: a potential biomarker for neonatal sepsis. J Immunol Res 2015;2015:147973. https://doi.org/10.1155/2015/147973.

Donato R, Cannon BR, Sorci G, Riuzzi F, Hsu K, Weber DJ, et al. Functions of S100 proteins. Cur Mol Med 2013;13:24–57.

Edgeworth J, Gorman M, Bennett R, Freemont P, Hogg N. Identification of p8,14 as a highly abundant heterodimeric calcium binding protein complex of myeloid cells. J Biol Chem 1991;266:7706–13.

Gao S, Yang Y, Fu Y, Guo W, Liu G. Diagnostic and prognostic value of myeloid-related protein complex 8/14 for sepsis. Am J Emerg Med 2015;33:1278–82. https://doi.org/10.1016/j.ajem.2015.06.025.

Holub M, Rozsypal H, Chalupa P. Procalcitonin: a reliable marker for the diagnosis and monitoring of the course of bacterial infection [in Czech]. Klin Mikrobiol Infekc Lek 2008;14:201–18.

Holub M, Beran O, Kaspříková N, Chalupa P. Neutrophil to lymphocyte count ratio as a biomarker of bacterial infections. Open Med 2012;7(25861). https://doi.org/10.2478/s11536-012-0002-3.

Huang L, Li J, Han Y, Zhao S, Zheng Y, Sui F, et al. Serum calprotectin expression as a diagnostic marker for sepsis in postoperative intensive care unit patients. J Interferon Cytokine Res 2016;36:607–16. https://doi.org/10.1089/jir.2016.0037.

Ingels C, Derese I, Wouters PJ, Van Den Berghe G, Vanhorebeek I. Soluble RAGE and the RAGE ligands HMGB1 and S100A12 in critical illness: impact of glycemic control with insulin and relation with clinical outcome. Shock 2015;43:109–16. https://doi.org/10.1097/SHK.0000000000000278.

Jonsson N, Nilsen T, Gille-Johnson P, Bell M, Martling CR, Larsson A, et al. Calprotectin as an early biomarker of bacterial infections in critically ill patients: an exploratory cohort assessment. Crit Care Resusc 2017;19:205–13.

Kapasi AJ, Dittrich S, González IJ, Rodwell TC. Host biomarkers for distinguishing bacterial from non-bacterial causes of acute febrile illness: a comprehensive review. PLoS One 2016;11, e0160278. https://doi.org/10.1371/journal.pone.0160278.

Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Crit Care Med 2003;31:1250–6. https://doi.org/10.1097/01.CCM.0000050454.01978.3B.

Liu K, Pichichero ME. Clinical significance of serum S100A12 in acute otitis media in young children. Pediatr Infect Dis J 2012;31:e56–8. https://doi.org/10.1097/INF.0b013e31824672cb.

Máca J, Burša F, Ševčík P, Sklienka P, Burda M, Holub M. Alarmins and clinical outcomes after major abdominal surgery — a prospective study. J Invest Surg 2017;30:152–61. https://doi.org/10.1080/08941939.2016.1231855.

Meijer B, Gearry RB, Day AS. The role of S100A12 as a systemic marker of inflammation. Int J Inflam 2012;2012:907078. https://doi.org/10.1155/2012/907078.

Muñoz P, Simarro N, Rivera M, Alonso R, Alcalá L, Bouza E. Evaluation of procalcitonin as a marker of infection in a nonselected sample of febrile hospitalized patients. Diagn Microbiol Infect Dis 2004;49(4):237–41.

Natesan M, Corea E, Krishnananthasivam S, Sathkumara HD, Dankmeyer JL, Dyas BK, et al. Calprotectin as a biomarker for melioidosis disease progression and management. J Clin Microbiol 2017;55:1205–10. https://doi.org/10.1128/JCM.02284-16.

Nilsen T, Sunde K, Larsson A. A new turbidimetric immunoassay for serum calprotectin for fully automatized clinical analysers. J Inflamm (Lond) 2015;12(45). https://doi.org/10.1186/s12950-015-0090-3.

Passey RJ, Xu K, Hume DA, Geczy CL. S100A8: emerging functions and regulation. J Leukoc Biol 1999;66:549–56.

Pechkovsky DV, Zalutskaya OM, Ivanov GI, Misuno NI. Calprotectin (MRP8/14 protein complex) release during mycobacterial infection in vitro and in vivo. FEMS Immunol Med Microbiol 2000;29:27–33.

Póvoa P, Teixeira-Pinto AM, Carneiro AH. C-reactive protein, an early marker of community-acquired sepsis resolution: a multi-center prospective observational study. Crit Care 2011;15:R169. https://doi.org/10.1186/cc10313.

Riché F, Gayat E, Barthélémy R, Le Dorze M, Matéo J, Payen D. Reversal of neutrophil-to-lymphocyte count ratio in early versus late death from septic shock. Crit Care 2015;19(439). https://doi.org/10.1186/s13054-015-1144-x.

Roth J, Burwinkel F, Van Den Bos C, Goebeler M, Vollmer E, Sorg C. MRP8 and MRP14, S-100-like proteins associated with myeloid differentiation, are translocated to plasma membrane and intermediate filaments in a calcium-dependent manner. Blood 1993;82:1875–83.

Strasser F, Gowland PL, Ruef C. Elevated serum macrophage inhibitory factor-related protein (MRP) 8/14 levels in advanced HIV infection and during disease exacerbation. J Acquir Immune Defic Syndr Hum Retrovirol 1997;16:230–8.

Wittkowski H, Frosch M, Wulffraat N, Goldbach-Mansky R, Kallinich T, Kuemmerle-Deschner J, et al. S100A12 is a novel molecular marker differentiating systemic-onset juvenile idiopathic arthritis from other causes of fever of unknown origin. Arthritis Rheum 2008;58:3924–31.

Xu D, Li Y, Li X, Wei LL, Pan Z, Jiang TT, et al. Serum protein S100A9, SOD3, and MMP9 as new diagnostic biomarkers for pulmonary tuberculosis by iTRAQ-coupled two-dimensional LC-MS/MS. Proteonomics 2015;15:58–67. https://doi.org/10.1002/pmic.201400366.

Zilahi G, McMahon MA, Povoa P, Martin-Loeches I. Duration of antibiotic therapy in the intensive care unit. J Thorac Dis 2016;8:3774–80. https://doi.org/10.21037/jtd.2016.12.89.