

Calprotectin as an early biomarker of bacterial infections in critically ill patients: an exploratory cohort assessment

Niklas Jonsson, Tom Nilsen, Patrik Gille-Johnson, Max Bell, Claes-Roland Martling, Anders Larsson and Johan Mårtensson

The diagnosis of bacterial infections in critically ill patients is challenging. Clinical manifestations such as fever, tachycardia and, in severe cases, hypotension and shock are non-specific. Moreover, results from microbial cultures may be delayed for several days and are often negative, especially when obtained after commencement of empirical antimicrobial therapy. Early diagnosis of bacterial infections is critical because delayed antimicrobial therapy is associated with increased mortality.¹ In addition, unnecessary treatment with broad-spectrum antibiotics contributes to the emerging global problem of multiresistant bacteria.

To facilitate early diagnosis of severe bacterial infections, the use of a multitude of sepsis biomarkers have been suggested. Among such biomarkers white blood cell count (WBC), C-reactive protein (CRP) and procalcitonin (PCT) are the most widely used, despite limitations.²⁻⁴

Inflammation biomarkers react to the inflammatory response but do not distinguish between bacterial infections and other types of inflammatory response. The performances of WBC, CRP and PCT for differentiating sepsis from non-infectious inflammatory response have been questioned.⁵⁻⁷ In the 2016 Surviving Sepsis campaign guidelines, their use is recommended only as adjuncts to clinical assessment of infection resolution.⁸ They are not included in the third international consensus definitions for sepsis and septic shock (Sepsis-3).⁹

Neutrophil degranulation is a major response to bacterial infections. Among granular proteins, calprotectin is the most abundant protein in the cytosolic fraction of neutrophils.¹⁰ It is released into the circulation when neutrophils are activated or consumed. It has previously been shown, in a porcine septic shock model, that after an endotoxin infusion, there was an initial decrease in circulating WBC and a limited increase in interleukin-6.¹¹ The synthesis of CRP and PCT have been attributed to increases in interleukin-6 levels.^{12,13} This animal study raised the question of whether calprotectin, which is degranulated early from the neutrophils through an IL-6 independent pathway,¹⁴ would perform better as a marker of infection than WBC, CRP and PCT. The value of calprotectin to facilitate early detection of bacterial infections in critically ill patients has not been systematically explored before.

We conducted a prospective exploratory investigation of plasma calprotectin levels in critically ill patients with and

ABSTRACT

Background: Calprotectin is the most abundant protein in the cytosolic fraction of neutrophils, and neutrophil degranulation is a major response to bacterial infections.

Objectives: To assess the value of plasma calprotectin as an early marker of bacterial infections in critically ill patients and compare it with the corresponding values for procalcitonin (PCT), C-reactive protein (CRP) and white blood cell count (WBC).

Methods: We measured daily plasma calprotectin levels in 110 intensive care unit patients using a newly developed turbidimetric assay run on clinical chemistry analysers. The likelihood of infection was determined according to the International Sepsis Forum criteria.

Results: Overall, 58 patients (52.7%) developed a suspected or confirmed bacterial infection. Plasma calprotectin predicted such infections within 24 hours with an area under the receiver operating characteristics curve (ROC area) of 0.78 (95% CI, 0.68–0.89). The ROC area for calprotectin was significantly greater than the corresponding ROC areas for WBC ($P < 0.001$) and PCT ($P = 0.02$) but only marginally better than the ROC area for CRP (0.71; 95% CI, 0.68–0.89).

Conclusion: Plasma calprotectin appears to be a useful early marker of bacterial infections in critically ill patients, with better predictive characteristics than WBC and PCT.

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without evidence of bacterial infection. We hypothesised that calprotectin levels would be higher in patients with microbiological and/or clinical evidence of a bacterial infection than in critically ill patients without suspected infection. In addition, we hypothesised that the predictive and diagnostic accuracy of plasma calprotectin would be superior to that of WBC, CRP and PCT.

Methods

The regional ethics review board in Stockholm approved our study and we obtained written informed consent from patients or their next of kin.

Patient selection

Patients admitted to the general intensive care unit at the Karolinska University Hospital, Solna, Sweden, between August 2007 and November 2013 were assessed for eligibility. The original purpose of collecting data from these patients was to assess the predictive values of acute kidney injury biomarkers. Hence, only patients without acute or chronic kidney disease (estimated glomerular filtration rate [eGFR], > 60 mL/min/1.73 m²) on ICU admission, who were expected to stay beyond the next calendar day, were included. For the purpose of our study, aiming to assess biomarkers of early infections, we excluded patients who were receiving ongoing antibiotic therapy at ICU admission and patients without a study sample obtained before or on the day when antibiotic treatment was started in the ICU.

Data collection and operational definitions

Microbial culture results and timing of antibiotic therapy initiation were recorded. An infectious disease specialist blinded to the calprotectin results determined the likelihood of infection as no infection, possible infection, probable infection or confirmed infection, according to the international sepsis forum criteria (see Appendix Table S1, online at cicm.org.au/Resources/Publications/Journal).¹⁵

We classified patients as having sepsis if they fulfilled three or more criteria for the systemic inflammatory response syndrome (SIRS) and had a possible, probable or confirmed infection.^{16,17} Sepsis was further stratified into “severe sepsis” if it was associated with organ dysfunction, and “septic shock” if it was associated with arterial systolic blood pressure < 90 mmHg or mean arterial pressure < 70 mmHg for at least 1 hour, despite adequate fluid resuscitation, adequate intravascular volume status or the use of vasopressors in an attempt to maintain these pressures (Appendix Table S2).¹⁸ We defined onset of infection as the time when the clinician prescribed antibiotic therapy.

To compare biomarker levels obtained from patients at the onset of a possible, probable or confirmed infection with biomarker levels obtained from patients without infection, we used the following approach:

- First, we calculated the median time from ICU admission to antibiotic therapy initiation in patients with possible, probable or confirmed infection.
- Second, we identified all biomarker levels obtained at the equivalent time from admission (equivalent ICU day) in non-infected patients.
- Third, we compared biomarker levels obtained on the day of antibiotic initiation in patients with possible, probable or confirmed infection with all available biomarker levels from non-infected patients at the equivalent ICU day. A similar approach was used to compare biomarker levels obtained on the day preceding antibiotic initiation.

To facilitate a sensitivity analysis, we performed 1:1 greedy matching on the ICU day when antibiotic therapy was initiated.¹⁹ For each patient with possible, probable or confirmed infection, we randomly selected one control patient (without replacement) with available biomarker data for the corresponding ICU day. The process was repeated until the list of treated patients for whom a matched control could be found was exhausted. The same approach was used to match biomarker levels obtained on the day before initiation of antibiotic therapy.

We recorded patient demographics, comorbidities, Acute Physiology and Chronic Health Evaluation (APACHE) II scores, ICU admission diagnoses and ICU and 30-day mortality.

Sampling and biochemical analyses

We collected EDTA blood as soon as possible after arrival in the ICU and daily thereafter until ICU discharge or initiation of renal replacement therapy. Calprotectin was analysed for up to 7 consecutive days. When two daily blood tests were available, we used the mean value. After centrifugation at 2000 rpm at 4°C for 10 minutes, the supernatant plasma was stored at -80°C. Study samples were analysed at the Department of Clinical Chemistry, Uppsala University Hospital, Uppsala, Sweden. Calprotectin results were not available to the clinicians, but CRP and PCT were used on a regular basis in the ICU. Laboratory personnel were blinded to clinical patient data.

Calprotectin was analysed on a Mindray BS-380 (Mindray Medical International) with reagents from Gentian. The instrument settings for the method were: sample volume, 3 µL; R1 volume, 200 µL; R2 volume, 30 µL. The detection wavelength was 605 nm.

The calprotectin assay is a particle-enhanced turbidimetric immunoassay using affinity-purified avian antibodies bound to particles. The antibody-coated particles bind to calprotectin present in the samples, leading to an aggregation of the particles and an increased turbidity. The increase in turbidity is proportional to the amount of calprotectin in the samples.²⁰

We analysed PCT using an enzyme-linked immunosorbent assay (coefficient of variation [CV], 6% at 0.25 ng/mL; CV, 3% at 10.4 ng/mL) on the Cobas EE (Roche Diagnostics). The expected normal PCT level was < 0.05 ng/mL. We analysed CRP by turbidimetry (CV, 4%) on the Architect Ci8200 (Abbott Laboratories). The expected normal CRP level was < 5 mg/L.

Statistical analysis

We analysed data using Stata, version 12 (StataCorp). Continuous variables are expressed as medians with interquartile ranges (IQRs) and compared using the Mann–

Whitney U test. Categorical variables are summarised as frequencies with percentages and assessed using the χ^2 test or the Fisher exact test. Changes over time for biomarker levels were tested by repeated-measures analysis of variance (RM-ANOVA), treating ICU day as the repeated-measures variable. For comparison of change over time between groups, an interaction variable (between group and time) was introduced in the RM-ANOVA model.

We used multivariable logistic regression analysis to assess the independent association between calprotectin levels and infection. Variables were included in the multivariable model if they were statistically significant at $P < 0.10$ in the univariable analyses. We assessed predictive and diagnostic values by calculating the area under the receiver-operating characteristics (ROC) curve. We tested equality of ROC areas by using the non-parametric approach, developed by De Long et al.²¹ Optimal cut-off levels were determined using Youden index calculations together with the ROC curve analysis.²² A two-sided $P < 0.05$ was considered statistically significant.

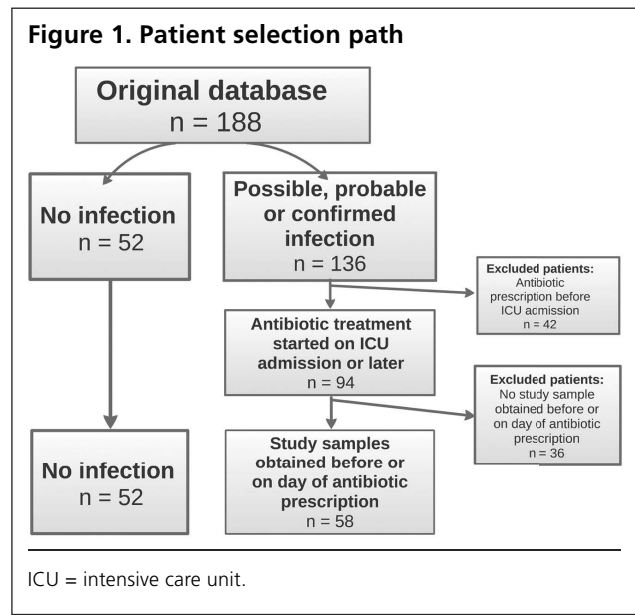
Results

Patient selection

We enrolled 188 patients with eGFR > 60 mL/min/1.73 m² and an expected length of stay > 24 hours. We excluded 42 patients for whom antibiotic therapy had been initiated before ICU admission, and 36 patients without a study sample obtained before or on the day of antibiotic treatment initiation. Therefore, we included 110 patients for analysis. Overall, 52 patients (47.3%) did not have an infection during their ICU admission. In contrast, 58 patients (52.7%) had antibiotic therapy initiated after a median of 2.6 days (IQR, 1.0–4.0 days) due to possible, probable or confirmed infection (Figure 1).

Patient characteristics

Baseline characteristics and outcomes for non-infected patients and patients with possible, probable or confirmed infection are summarised in Table 1. Compared with non-infected patients, patients with possible, probable or confirmed infection had higher APACHE II and Sequential Organ Failure Assessment scores, and higher baseline creatinine levels. On admission, 36 non-infected patients (71%) and 51 patients with possible, probable or confirmed infection (88%) fulfilled at least three SIRS criteria ($P = 0.02$). Most patients were admitted after multi-trauma (about two-thirds). The median length of stay in the ICU was 3 days (IQR, 3–5 days) in the non-infected group and 7 days (IQR, 4–12 days) in the group of patients with possible, probable or confirmed infection ($P < 0.001$). By 30 days, four of the non-infected patients (8%) and 10 of the patients with



possible, probable or confirmed infection (17%) had died ($P = 0.14$).

Characteristics and severity of infection

Among the 58 patients with possible, probable or confirmed infection, 38 (66%) had a confirmed infection, 13 (22%) had a probable infection and seven (12%) had a possible infection. Pneumonia was the primary infection in 44 patients (76%). In six patients (10%), no primary infection could be identified. Blood cultures were taken before initiation of antibiotic therapy for 56 patients (97%) and were positive in eight patients (14%), with *Staphylococcus aureus* being the most common pathogen (Appendix Table S3). Overall, 24 patients (41%) developed severe sepsis and 25 patients (43%) developed septic shock in the ICU (Appendix Table S4).

Biomarker levels

We analysed 1643 calprotectin measurements obtained within a median of 8.8 hours (IQR, 3.3–19 hours) of ICU admission and for a maximum of 7 consecutive days in ICU.

Admission, peak and mean calprotectin levels were higher in patients with possible, probable or confirmed infection than in non-infected patients (Table 2), and remained higher during the first week in the ICU ($P < 0.001$) (Figure 2). In addition, calprotectin levels were significantly higher at the time of antibiotic therapy initiation (median, ICU Day 3 [IQR, Day 1–4]) and on the day before initiation of such therapy (median, ICU Day 2 [IQR, Day 0–3]) than calprotectin levels obtained on the corresponding ICU day for non-infected patients.

Table 1. Patient characteristics and outcomes

Variable	No infection (<i>n</i> = 52)	Possible, probable or confirmed infection (<i>n</i> = 58)	<i>P</i>
Median age, years (IQR)	38 (26–64)	48 (28–66)	0.30
Female, <i>n</i> (%)	18 (35%)	9 (16%)	0.02
Median APACHE II score (IQR)	13 (9–19)	17 (13–24)	0.001
Median SOFA score (IQR)	5 (4–8)	8 (6–11)	< 0.0001
SIRS, <i>n</i> (%)	36 (71%)	51 (88%)	0.02
Median bodyweight, kg (IQR)	76 (70–90)	81 (70–91)	0.13
Median baseline creatinine level, µmol/L, with MDRD (IQR)	78 (68–88)	88 (76–94)	0.01
Median baseline creatinine level, µmol/L, without MDRD (IQR)	73 (62–83)	83 (69–91)	0.05
True baseline creatinine level available, <i>n</i> (%)	35 (67%)	37 (64%)	0.70
Median time admission to enrolment, hours (IQR)	9 (3–19)	8 (3–15)	0.76
Median time admission to antibiotic therapy, days (IQR)	–	2.6 (1–4)	–
Co-morbidity, <i>n</i> (%)			
Diabetes	5 (10%)	3 (5%)	0.37
Cardiovascular disease	13 (25%)	16 (28%)	0.76
COPD or asthma	2 (4%)	5 (9%)	0.31
Gastrointestinal or liver disease	2 (4%)	8 (14%)	0.07
Any malignancy	3 (6%)	8 (14%)	0.16
Admission diagnosis, <i>n</i> (%)			
Neurological	3 (6%)	3 (5%)	0.89
Respiratory	4 (8%)	10 (17%)	0.13
Cardiovascular	5 (10%)	3 (5%)	0.37
Trauma	36 (69%)	37 (64%)	0.55
Gastrointestinal	4 (8%)	2 (3%)	0.33
Sepsis	0	3 (5%)	0.14
Outcomes			
Renal replacement therapy, <i>n</i> (%)	0	0	–
Median ICU length of stay, days (IQR)	3 (3–5)	7 (4–12)	< 0.0001
ICU mortality, <i>n</i> (%)	3 (6%)	4 (7%)	0.81
30-day mortality, <i>n</i> (%)	4 (8%)	10 (17%)	0.14

IQR = interquartile range. APACHE = Acute Physiology and Chronic Health Evaluation. SOFA = Sequential Organ Failure Assessment. SIRS = systemic inflammatory response syndrome. MDRD = modification of diet in renal disease. COPD = chronic obstructive pulmonary disease.

Admission, peak and mean CRP and PCT levels were higher in patients with possible, probable or confirmed infection than in non-infected patients. CRP levels were significantly higher at the time of antibiotic therapy initiation and on the day before. However, PCT levels obtained at the time of antibiotic therapy initiation or on the previous day did not differ significantly from PCT levels obtained on the corresponding ICU days in non-infected patients. WBC levels did not differ significantly between the two groups (Table 2).

Peak calprotectin levels were higher in patients with SIRS than in patients without SIRS and increased further with increasing sepsis severity ($P < 0.001$) (Appendix Figure S1).

Biomarker performance at onset of possible, probable or confirmed infection

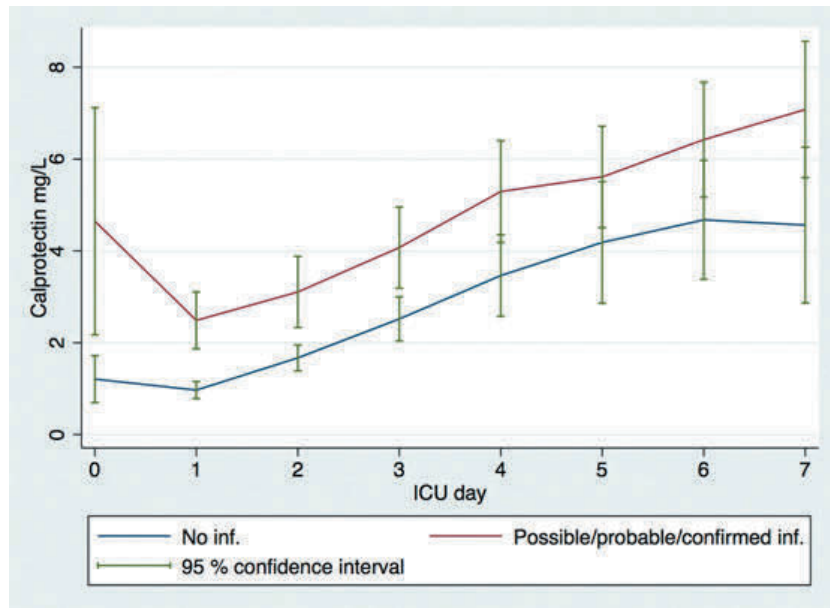
In the multivariable logistic regression analysis, calprotectin on the day of antibiotic prescription was independently associated with possible, probable or confirmed infection (odds ratio [OR] 2.0; 95% CI, 1.32–3.14) (Appendix Table S5). Calprotectin showed a higher ROC area than CRP, WBC and PCT (Table 3). ROC area for calprotectin was 0.76 (95% CI, 0.65–0.86). Using a cut-off for calprotectin of 3.4 mg/L rendered a sensitivity of 56% and a specificity of 92%. PCT had an ROC area of 0.63 (95% CI, 0.49–0.77). Using a cut-off for PCT of 0.66 µg/L rendered a sensitivity of 70% and a specificity of 58%. CRP had an ROC area of 0.69 (95% CI,

Table 2. Biomarker levels

Biomarker (median [IQR])	n*	No infection	Possible, probable or confirmed infection	P
Calprotectin, mg/L				
On admission	52/58	0.78 (0.38–1.6)	1.5 (0.69–3.3)	0.042
Peak	52/58	1.9 (1.0–4.5)	5.0 (2.2–8.4)	< 0.001
Mean	52/58	1.6 (0.7–3.6)	3.5 (1.5–6.5)	< 0.001
On day antibiotics initiated	43/48	1.3 (0.77–2.3)	3.8 (1.4–6.3)	< 0.001
On day before antibiotics initiated	45/37	1.1 (0.67–2.0)	2.2 (0.82–3.8)	< 0.001
C-reactive protein, mg/L				
On admission	43/56	26 (8–43)	57 (8–122)	0.057
Peak	52/58	172 (107–252)	294 (212–369)	< 0.001
Mean	52/58	102 (38–180)	163 (97–260)	< 0.001
On day antibiotics initiated	49/52	129 (76–168)	202 (128–286)	0.003
On day before antibiotics initiated	48/49	114 (61–173)	135 (68–221)	0.007
Procalcitonin, µg/L				
On admission	51/58	0.4 (0.2–1.8)	0.9 (0.2–3.4)	0.02
Peak	51/58	0.7 (0.3–3.5)	2.7 (0.6–8.6)	0.016
Mean	51/58	0.4 (0.2–1.2)	0.9 (0.3–2.2)	< 0.001
On day antibiotics initiated	36/35	0.6 (0.3–2.4)	1.0 (0.4–8.6)	0.09
On day before antibiotics initiated	48/49	0.4 (0.2–1.6)	0.8 (0.1–1.3)	0.99
White blood cell count, x 10⁹/L				
On admission	51/58	11 (7.5–14)	9.3 (7.6–14)	0.89
Peak	52/58	14.8 (11.2–20.2)	16.3 (13.1–2)	0.26
Mean	52/58	10 (7.8–14)	10 (7.5–14)	0.17
On day antibiotics initiated	48/53	8.8 (7.1–11)	9.5 (7.0–13)	0.43
On day before antibiotics initiated	48/49	8.6 (7.1–11)	9 (6.6–13)	0.35

* Number of patients without infection/number of patients with possible, probable or confirmed infection.

Figure 2. Daily mean calprotectin levels for patients with no infection and patients with possible, probable or confirmed infection



ICU = intensive care unit. inf. = infection.

0.60–0.81). Using a cut-off for CRP of 133 mg/L rendered a sensitivity of 76% and a specificity of 54% (Table 3).

Predicting the development of possible, probable or confirmed infection

Calprotectin predicted possible, probable or confirmed infection within 24 hours before initiation of antibiotic therapy with an ROC area of 0.78 (95% CI, 0.68–0.89). The optimal cut-off value of 1.8 mg/L predicted possible, probable or confirmed infection with a sensitivity of 62% and a specificity of 88%. PCT had an ROC area of 0.50 (95% CI, 0.34–0.66). Using a cut-off for PCT of 0.78 µg/L rendered a sensitivity of 56% and a specificity of 58%. CRP had an ROC area of 0.71 (95% CI, 0.68–0.89). Using a cut-off for CRP of 130 mg/L rendered a sensitivity of 62% and a specificity of 94% (Table 3).

Sensitivity analyses

We performed our first sensitivity analysis after excluding patients with possible and probable infections. Calprotectin showed a higher ROC area than CRP, WBC and PCT. The ROC area for calprotectin was 0.70 (95% CI, 0.57–0.83). Using a cut-off for calprotectin of 3.5 mg/L rendered a sensitivity of 55% and a specificity of 90%. PCT had an ROC area of 0.65 (95% CI, 0.48–0.81). Using a cut-off for PCT of 0.66 µg/L rendered a sensitivity of 73% and a specificity of 58%. CRP had an ROC area of 0.71 (95% CI, 0.58–0.84). Using a cut-off for CRP of 133 mg/L rendered a sensitivity of 82% and a specificity of 54% (Appendix Table S6).

In the sensitivity analysis, calprotectin predicted confirmed infection 24 hours ahead of time, with an ROC area of 0.82 (95% CI, 0.70–0.94). The optimal cut-off value of 1.6 mg/L predicted confirmed infection with a sensitivity of 66% and a specificity of 93%. All the other biomarkers had lower ROC areas (Appendix Table S6).

We performed a second sensitivity analysis after greedy matching of patients to facilitate comparison of biomarker levels taken on the equivalent ICU day. The diagnostic values of calprotectin, CRP and PCT were similar but the diagnostic value of the WBC was significantly lower than the diagnostic value of calprotectin ($P = 0.003$). Calprotectin predicted possible, probable or confirmed infection 24 hours ahead of time with an ROC area of 0.68 (95% CI, 0.57–0.78). The predictive ROC areas for CRP, PCT and WBC did not differ significantly from 0.5 (Appendix Table S7).

Discussion

Key findings

We conducted an observational study to assess the predictive and diagnostic value of plasma calprotectin for bacterial infections in an exploratory cohort of critically ill patients. We found that, compared with patients without evidence of infection, calprotectin levels were significantly greater in patients with a possible, probable or confirmed infection 24 hours before the infection was suspected and antibiotic therapy was initiated. In addition, the predictive value of calprotectin was superior to PCT and WBC. Finally, we found that calprotectin levels increased with greater sepsis severity, and remained elevated during the first week in the ICU despite treatment.

Comparison with other studies

To our knowledge, ours is the first study to explore the performance of calprotectin as a predictor of bacterial infections in critically ill patients. However, a recent pilot study assessed the value of calprotectin to distinguish between infectious and non-infectious causes of SIRS. In that study, plasma calprotectin was analysed during the first

Table 3. Diagnostic and predictive performance of white blood cell count, procalcitonin, C-reactive protein, and calprotectin for possible, probable or confirmed bacterial infection

Accuracy	ROC area (95%CI)	Cut-off	Sensitivity	Specificity	<i>P</i> *
Diagnostic accuracy (same day as antibiotic prescription)					
White blood cell count	0.54 (0.43–0.65)	10.7	43%	74%	0.01
Procalcitonin	0.63 (0.49–0.77)	0.66	70%	58%	0.30
C-reactive protein	0.69 (0.60–0.81)	133	76%	54%	0.56
Calprotectin	0.76 (0.65–0.86)	3.4	56%	92%	–
Predictive accuracy (1 day before antibiotic prescription)					
White blood cell count	0.44 (0.33–0.56)	8.5	66%	41%	0.0003
Procalcitonin	0.50 (0.34–0.66)	0.78	56%	58%	0.02
C-reactive protein	0.71 (0.68–0.89)	130	62%	94%	0.41
Calprotectin	0.78 (0.68–0.89)	1.80	62%	88%	–

ROC = receiver operating characteristic. * *P* is for the test of equality between the ROC area of each biomarker v calprotectin.

3 days in the ICU in patients with sepsis ($n = 15$), in elective post-operative patients with SIRS ($n = 23$) and in patients without SIRS admitted to the ICU with severe intoxication ($n = 7$). No significant difference in calprotectin levels between septic and post-operative patients was found (the ROC area was 0.65 for discriminating between the two groups), which was inferior to the diagnostic accuracy of CRP (ROC area, 0.74). However, the likelihood of infection was not assessed in that study, and the timing of calprotectin measurement in relation to sepsis onset was not reported.

Among neonates with suspected sepsis, serum calprotectin failed to distinguish blood culture-positive cases from culture-negative controls (ROC area, 0.61; 95% CI, 0.35–0.87). Calprotectin was inferior to both CRP and platelet count.²³

In a recent study including 75-year-old, healthy subjects, the reference interval for serum calprotectin was 0.3–2.6 mg/L.²⁴ In our study, the optimal cut-off for plasma calprotectin for predicting sepsis (1.8 mg/L) was within that range. However, the mean age in our study population was 46 years (SD, 21 years) (median age, 46.5 years [IQR, 28–66 years]). A reference interval for serum calprotectin for that age group has not yet been defined. Furthermore, our non-infected patients with a high degree of SIRS had a similar median admission calprotectin level (0.8 mg/L [IQR, 0.4–1.6 mg/L]) but a greater median peak level (1.9 mg/L [IQR, 1.0–4.5 mg/L] and mean (1.6 mg/L [IQR, 0.7–3.6 mg/L]) than the cohort of healthy 75-year-old individuals. This suggests that systemic inflammation triggers neutrophil degranulation of calprotectin to some extent, even in the absence of infection. This is further supported by the fact that plasma calprotectin concentrations are elevated in inflammatory conditions such as rheumatoid arthritis,²⁵ cystic fibrosis,²⁶ colorectal carcinoma,²⁷ multiple sclerosis²⁸ and after major surgery.²⁹

In our study, calprotectin showed an ROC area of 0.76 for the diagnosis of sepsis and an ROC area of 0.78 for the prediction of sepsis. This was despite a high SIRS prevalence in patients with possible, probable or confirmed infection and in non-infected patients. This finding contrasts with previous studies and suggest that bacterial infections trigger early neutrophil activation with concomitant release of calprotectin into the blood stream.

Significance of study findings

Bacterial infection is difficult to diagnose in the ICU. Patients often have concomitant acute and chronic conditions which may affect vital signs and attenuate the efficacy of traditional biomarkers used to detect bacterial infection, ie, CRP and PCT.^{12,30,31} Microbiological results come to hand late in the course and are often inconclusive. Methods for direct molecular detection of microbial DNA in blood are

now available.³² The minimum time for analysis is 8 hours but, in practice, in our ICU results are available only after 24–36 hours due to fixed-time analyses.

Our findings suggest that plasma calprotectin can be used as an early biomarker of severe bacterial infections in patients requiring intensive care.

Our methodology can be applied to all types of chemistry analysers in hospital laboratories, with a turnaround time of only 10 minutes for centrifugation and 10 minutes for the assay. The instrument platforms are usually available around the clock.

Rapid analysis time and high platform availability, in combination with an observed specificity of about 90% for possible, probable or confirmed bacterial infection, makes calprotectin an attractive biomarker to assist in decisions about antibiotic therapy in the ICU.

Early detection and initiation of antibiotic treatment in septic shock is associated with lower mortality.^{1,33} This therapeutic goal may be facilitated by using calprotectin as an additional tool in the decision-making process. In addition, calprotectin-guided therapy may prevent unnecessary use of antibiotics and the associated problem with multiresistant bacteria. Whether such calprotectin-guided therapy improves patient-centered outcomes needs to be tested in a randomised clinical trial.

Study strengths and limitations

Our study has several strengths. We collected data prospectively, thereby reducing the risk of bias. We performed daily calprotectin measurements during the entire stay in ICU or for up to 7 consecutive days, and showed significantly elevated calprotectin levels in patients with possible, probable or confirmed infection in this time frame. Treating clinicians, laboratory staff and infectious disease specialists were blinded to the results, which allowed an unbiased assessment of calprotectin as a predictor of bacterial infections.

Our study has some limitations. We excluded patients with renal dysfunction on admission. The predictive value of calprotectin can therefore not be extrapolated to patients with acute renal injury or chronic renal dysfunction present at admission. It was a single-centre study, but it was performed at a tertiary hospital, which permitted some external validity to similar centres in the developed world. The age of our study population was lower than a standard ICU population, therefore the generalisability of our study results to older ICU populations is limited.

We used a consensus classification to define infection. This involved the inclusion of patients with a lower likelihood of infection, ie, patients with possible or probable infection, thus carrying a risk of misclassification. However, our results remained after excluding such patients from the analysis.

Conclusions

In an exploratory cohort of critically ill patients with a high prevalence of SIRS, plasma calprotectin was an early and specific marker of bacterial infection, which reflects its role in host defence. In addition, our findings suggest that calprotectin may be more robust as a marker than conventional markers such as CRP, PCT and WBC. Our findings support further assessment of calprotectin as a diagnostic tool to guide initiation and de-escalation of antibiotic therapy in critically ill patients.

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Author contributions

Niklas Jonsson, Claes-Roland Martling and Johan Mårtensson designed and coordinated the study. Tom Nilsen developed the assay and he and Anders Larsson conducted biomarker analyses. Johan Mårtensson, Niklas Jonsson and Max Bell collected study data. Niklas Jonsson, Johan Mårtensson, Claes-Roland Martling and Patrik Gille-Johnson analysed and interpreted the data. Niklas Jonsson and Johan Mårtensson performed the statistical analysis. All authors participated in writing the manuscript. All authors read and approved the final manuscript.

Competing interests

Tom Nilsen is employed by Gentian, who developed the turbidimetric immunoassay used to analyse Calprotectin in our study.

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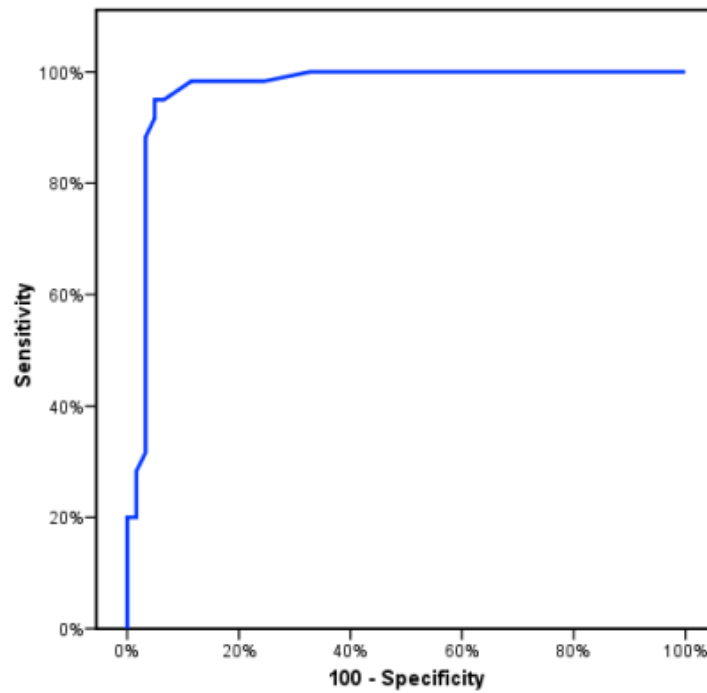
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Appendix

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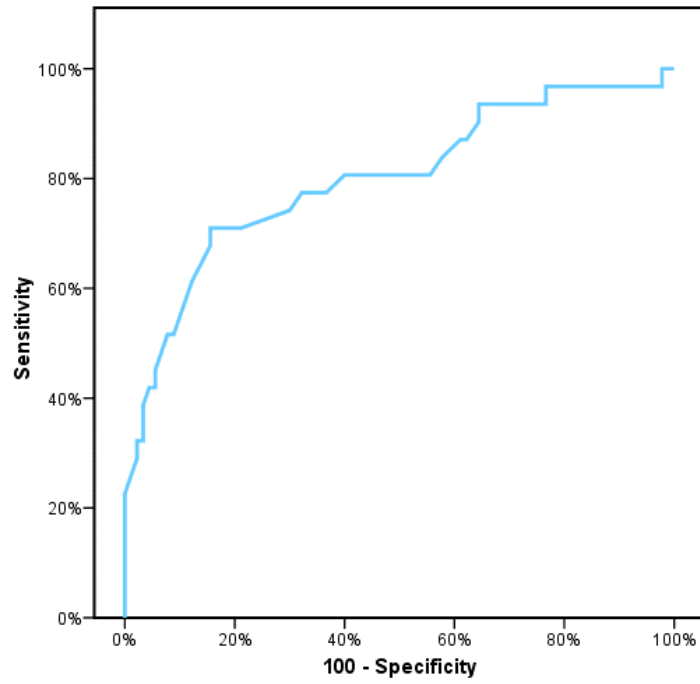
Figure7: ROC curve for USCOM SVV for predicting F responsiveness (compares SVV in SV>15% and not SV>15%)



	AUC	P	Cut off point	Sensitivit y	Specificit y	PPV	NPV	Accuracy
USCOM SVV	0.968	<0.001*	>21	95.0	95.1	95.0	95.1	95.1

Value of κ	Strength of agreement
< 0.20	Poor
0.21 - 0.40	Fair
0.41 - 0.60	Moderate
0.61 - 0.80	Good
0.81 - 1.00	Very good

Figure 8: ROC curve for SMII ability to predict cases with low cardiac output state (LVEF <35%).



	AUC	P	Cut off point	Sensitivity	Specificity	PPV	NPV	Accuracy
SMII	0.799 *	<0.001 *	≤1.16	69.97	84.44	61.1	89.4	80.99