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Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: A case for procalcitonin

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Summary *Objectives:* Fever suggests the presence of microbial infection in critically ill patients. The aim was to compare the role of old and new biomarkers in predicting absence or presence of microbial infection, its invasiveness and severity in critically ill patients with new onset fever.
Methods: We prospectively studied 101 patients in the intensive care unit with new onset fever (>38.3 °C). Routine infection parameters, lactate, procalcitonin (PCT), midregional pro-adrenomedullin (MR proADM), midregional pro-atrial natriuretic peptide (MR proANP) and copeptin (COP) were measured daily for three days after inclusion. Likelihood, invasiveness (by bloodstream infection, BSI) and severity of microbial infection were assessed by cultures, imaging techniques and clinical courses.
Results: All patients had systemic inflammatory response syndrome; 45% had a probable or proven local infection and 12% a BSI, with 20 and 33% mortality in the ICU, respectively. Only peak PCT (cutoff 0.65 ng/mL at minimum) was of predictive value for all endpoints studied, i.e. BSI, septic shock and mortality (high risk infection) and infection without BSI, shock and mortality (low risk infection), at areas under the receiver operating characteristic curves varying between 0.67 ($P = 0.003$) and 0.72 ($P < 0.001$). In multivariable analysis, the combination of C-reactive protein and lactate best predicted high risk infection, followed by PCT. For low risk infection, PCT was the single best predictor.
Conclusions: In critically ill patients with new onset fever, plasma PCT as a single variable, among old and new biomarkers, best helps, to some extent, to predict ICU-acquired, high risk microbial infection when peaking above 0.65 ng/mL and low risk infection when peaking below 0.65 ng/mL.

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Introduction

New onset fever in the critically ill raises the suspicion of microbial infection that may lead to sepsis and other harmful sequelae.[1,2] Fear of undertreatment contributes to ordering tests and prescribing antibiotics, before results of cultures become available, while overtreatment carries the risk of bacterial selection and overgrowth by induction of resistance.[3] The systemic inflammatory response syndrome (SIRS) criteria, including elevated white blood cell counts (WBC) may not accurately predict microbial infection and the common use of C-reactive protein (CRP) to predict infection, severity and outcome in critically ill patients is controversial.[4–11] Even minimally elevated lactate levels may predict a dismal outcome of infection during critical illness, relatively independent of sepsis and shock.[12–14]

The use of procalcitonin (PCT) for predicting microbial infection and its severity in the critically ill patient, rather than the general hospitalized patient,[15,16] is fraught with difficulty, because of varying sensitivity and specificity, even if potentially higher than of C-reactive protein (CRP).[1,5–7,9–11,17–21] This might relate to varying study populations and endpoints. PCT has been used to predict sepsis, i.e. the host response to either proven or suspected microbial infection and its severity, and rarely of microbiologically proven infection or bacteraemia[5,9–11,22–24] whereas the latter would be more helpful when considering potentially life-saving antibiotics in the critically ill.[25,26] Studies on PCT in critically ill patients were either small, up to 50 patients,[1,5] confined to medical[5–7] or surgical patients, which may differ in infection and biomarker profiles,[9,11,18,21,27] and only rarely included both.[10,17,22,28] They mostly included patients at admission to the intensive care unit (ICU)[4–7,18,21,22] and were mostly not designed, with exceptions,[1,10,21] to predict ICU-acquired microbial infection and associated risks. Studies evaluated PCT as an isolated biomarker[28,29] or compared it with a variety of others.[1,5–7,9–11,17,18,21,22,27] Single, but different,[1,5,9,11] or multiple endpoints as organ failures and mortality have been studied.[6,7,10,18,22,28] The heterogeneity among studies may explain, in part, why meta-analyses may contradict each other.[15,19,20] In any case, a low or decreasing PCT in the ICU has been used to help deciding on antibiotics, thereby reducing potentially harmful antibiotic exposure.[30,31] In the critically ill, however, an association of a low or decreasing PCT with a low risk has not been documented, the cutoff PCT values used have not been validated, and a small increase in mortality by PCT-guided antibiotics cannot be excluded.[30,31]

Novel biomarker prohormones for sepsis and its severity include midregional (MR) pro-adrenomedullin (proADM), MR pro-atrial natriuretic peptide (proANP) and copeptin (COP), precursors of adrenomedullin, atrial natriuretic peptide and vasopressin, respectively. They are secreted by vascular endothelium, heart and pituitary, respectively, and are involved in circulatory homeostasis, also during microbial infection.[33–35] They could particularly predict the sequelae of severe microbial infection, i.e. the development of septic shock and associated mortality, but evaluation in the critically ill is scarce and inconclusive up till now.[33–36]

In the hypothesis that ICU-acquired infection differently increases circulating biomarkers, depending on invasiveness and severity of disease, we compared predictive values for microbial infection, bloodstream infection (BSI), septic shock or mortality, i.e. high risk infection, and in predicting infection without these complications (low risk infection), in patients with new onset fever in a mixed medical/surgical ICU. The goal was to find the ideal, single biomarker for prediction of high and low risk infection and to determine the associated cutoff value, for future studies on biomarker-guided antibiotics in the ICU.

Materials and methods

Patients and protocol

In this prospective study, approved by the ethical committee of the VU University Medical Center, Amsterdam, 101 consecutive patients presenting with new onset fever in the 24-bed mixed medical/surgical ICU were included. The department has about 1200 admissions annually. Limited by available research staff and office hours, we included 21 patients from August–December 2003, 33 patients from January–December 2004, 23 patients January–December 2005, 10 patients from January–December 2006 and 14 patients from January–August 2007. We did not perform a power analysis since we could not estimate the expected results and relatively arbitrarily stopped inclusion after reaching 100 patients. The Consort diagram details eligible and included patients (Fig. S1 electronic supplementary material, esm). All included patients or closest relatives gave informed consent prior to the study. Inclusion criteria were as follows. New onset fever was defined as a body temperature of $\geq$38.3 °C, measured rectally while in the ICU, preceded by a period of at least 24 h in the absence of fever ($<$37.5 °C). Exclusion criteria were absence of informed consent, pregnancy and a life expectancy of less than 24 h. Enrolment in the study followed within 12 h after inclusion criteria were met. At inclusion (Day 0), demographic and historical variables were recorded, such as age, gender, prior use of antibiotics, including selective decontamination of the digestive tract (SDD), steroids, immune status (active malignancy or other causes of an immunocompromised state) and reasons of admission. SDD was introduced for routine use on July 17 2006 and consisted of 4*x* daily administration of an oral paste and of a suspension via the nasogastric tube, containing the non-absorbable antibiotics tobramycin, amphotericin-B and colistin, in patients longer than 48 h on mechanical ventilation and 72 h in the ICU. Assessment of disease severity on ICU admission was done according to the Simplified Acute Physiology Score II (SAPS II). Parameters to calculate the Sequential Organ Failure Assessment Score (SOFA) were collected on the study days, at inclusion (Day 0) and on Days 1 and 2. From Day 0–2 clinical data were recorded, such as temperature, heart rate, respiratory parameters taken from the ventilator, white blood cell counts (WBC) using a Sysmex SE-9000 analyzer (Toa Medical Instruments, Kobe, Japan), C-reactive protein (CRP, Immunoturbidimetric assay, Modular analytics <P> Roche diagnostics, Mannheim, Germany) and lactate (Enzymatic method, Modular analytics <P> Roche diagnostics, Mannheim, Germany).

Blood samples were obtained on Days 0 (at time of inclusion) and daily at 7:00 h AM of each of the following Days 1 and 2. Samples were collected from an arterial catheter in standard Vacutainer tubes (Becton, Dickinson and Company, Erembodegem, Belgium) with ethylenediaminetetraacetic acid (EDTA), benzamidine and soybean trypsin inhibitor added. After tubes were centrifuged for 10 min at 1300 g, the plasma was aliquoted and stored at -80 °C until further handling. Chest and sinus radiographs were obtained on Day 0, but other imaging was at the discretion of treating physicians. Blood was taken from routinely placed arterial catheters and collected in delayed vial entry bottles for aerobic and anaerobic cultures and processed with help of the BACTEC FX automatic analyzers (Becton, Dickinson and Company, Erembodegem, Belgium). Bottles were incubated for a maximum of five days. If the analyzers showed growth, Gram stains were prepared and identification and sensitivity cultures were processed. Other local specimens for microbial investigation were collected and sent to the microbiological laboratory, depending on the clinical infection, as judged by treating clinicians not involved in the study and unaware of PCT and novel biomarker results. Further investigations on infection e.g. fungal, viral or Chlamydia cultures, were left at the treating clinicians discretion. All collected specimens were handled using standardized procedures. All culture and staining results from specimens collected from Day 0—2 were evaluated. Only positive cultures that were not considered to reflect colonization were used for analysis. For example blood cultures containing coagulase-negative staphylococci were considered contaminated if only one bottle revealed growth. The microbial agents isolated during microbiological evaluation were grouped into major species classes. Patients were followed until discharge from ICU or hospital or death.

Definitions

The SIRS criteria according to the ACCP/SCCM consensus conference criteria of: a body temperature >38 °C; a heart rate of >90 beats/min; a respiratory rate of >20 breaths/min or mechanical ventilation or white cell count (WBC) of $<4.0 \times 10^9$/L or $>12.0 \times 10^9$/L, were used. When SIRS and a probable/proven infection or BSI was present, patients were classified as having sepsis. On the basis of the collected data two investigators, blinded to the study results (SHH, ABJG), decided after completion of the study whether a possible, probable or proven infection was present from Day 0—2 after inclusion. In case of disagreement a third party was consulted. Source and likelihood of infection were based on criteria defined at the International Sepsis Forum Consensus Conference.[37] Patients were divided into groups of increasing likelihood of infection and invasiveness of associated micro-organisms, suggestive of increasing severity: Group 1 without infection or with possible infection but negative cultures, Group 2 with probable or proven local infection without BSI and Group 3 with BSI irrespective of local infection. Shock was defined by a systolic arterial pressure <90 mmHg or mean arterial pressure (MAP) < 70 mmHg for at least 1 h despite adequate fluid resuscitation or requirement of vasopressor support to maintain MAP, from Day 0—7. In the presence of sepsis, shock was considered septic shock. All-cause mortality refers to Day 28 (within ICU or hospital) mortality after inclusion and ICU mortality (also beyond 28 days). While presence of either BSI, septic shock or mortality after inclusion in the disease course was considered indicative of high risk microbial infection, a low risk was defined by a probable or proven infection without BSI Day 0—2 and septic shock Day 0—7 and with survival up to 28 days. We evaluated a change of antibiotics during Day 0—7 and defined a change as starting or discontinuing one or more antibiotics of a different class. Tracheobronchitis was defined by fever with purulent sputum, acquired by tracheobronchial aspiration and yielding a positive culture of a potential pathogen, but no indication of infiltrates on chest imaging.

Biomarker assays

Biomarkers were measured using the Kryptor^R compact system (Brahms Diagnostica, Henningsdorf, Germany) which uses Time Resolved Amplified Cryptate Emission (TRACE) technology. Assays were performed according to manufacturer's instructions. PCT was measured by use of the PCT sensitive, the lower detection limit being 0.02 ng/mL while the upper limit in healthy subjects is 0.05 ng/mL. The functional assay sensitivity (FAS) of the test is 0.06 ng/mL, with an intra-assay coefficient of variation (CV) and inter-assay CV of $<6\%$ in samples containing >0.3 ng/mL. Test specifics of MR proADM: lower detection limit 0.05 nmol/L, upper limit of normal 0.55 nmol/L. The FAS is 0.25 nmol/L, with an intra-assay CV of $<4\%$ and an inter-assay CV of $<11\%$ in samples containing 0.5—2.0 nmol/L. Test specifics of MR proANP: lower detection limit 2.1 pmol/L, upper limit in healthy controls 85.2 pmol/L. A FAS of 10 pmol/L and an intra-assay CV of $<2.5\%$ and inter-assay CV of $<6.5\%$ in samples containing 20 pmol/L. Test specifics for COP: lower detection limit 4.8 pmol/L, upper limit in healthy subjects 17.4 pmol/L. The FAS is <12 pmol/L, with an intra-assay CV of $<12\%$ and an inter-assay CV of $<13\%$ in samples containing >20 pmol/L.

Statistical analysis

This was performed using SPSS version 15 (SPSS inc., Chicago, Ill., USA). Data are expressed as median (range) or as number of patients (percentage) where appropriate, with median $\pm$ interquartile range (IQR) in figures. All test were two-sided and a $P < 0.05$ was considered statistically significant. Exact P's above 0.001 are given. Group differences were evaluated by use of the Kruskal—Wallis test or X^2 test, where appropriate. Non-Gaussian distributed data were logarithmically transformed, when appropriate, and generalized estimating equations (GEE) were done to evaluate group effects on variables, taking repeated measures in the same patients into account. Areas under the receiver operating characteristic curves (AUC ROC) were calculated to evaluate predictive values. We evaluated predictive values of Day 0 and peak levels (Day 0—2). Since reporting microbiological results takes at least 1—2 days after sending specimens for culture, it is hypothesised that the highest biomarker level reached within D0-2 would precede culture results and is therefore appropriate for predicting likelihood of infection. The peak value is the highest biomarker

value measured on either D 0,1 or 2 for each individual patient. The optimum cutoff value was calculated on the basis of the highest sensitivity and specificity combined. Positive and negative predictive values and likelihood ratios were calculated. Backward multiple logistic regression was done, including all logarithmic transformed biomarker levels and selecting on the basis of the likelihood ratio, to find the smallest set of best predictors for high and low risk microbial infection. To this end, high risk infection was defined by either BSI, septic shock or 28-day mortality. The Hosmer Lemeshow test was done to evaluate goodness of fit.

Results

Patient characteristics are shown in Table 1, grouped according to likelihood and invasiveness of microbial infection. All patients had SIRS either at inclusion (99%) or on day 1 (1%), so that the 73 patients with probable/proven microbial infection (Groups 2 and 3) had sepsis. Females had higher risk for BSI than males, possibly explained by higher frequency of immunodepression in females than males (females 5 (83%) vs. males 1 (17%), $P = 0.005$). Table S1 (esm) shows the sources of microbial infection and the organisms involved. The mortality rates did not differ between patients without or with BSI (22 (25%) vs. 4 (33%), $P = 0.52$), nor did they differ between patients with or without septic shock (16 (24%) vs. 10 (29%), $P = 0.55$), even though BSI predisposed to septic shock (Table 1).

Biomarkers of infection and its invasiveness

Fig. S2 (esm) shows plasma levels in the course of time, according to likelihood and invasiveness of infection. Peak levels occurred at Day 0 in 61% of patients for PCT, 60% for COP, 55% for MR proADM, 48% for MR proANP, 46% for WBC, 40% for lactate and 35% for CRP, so that PCT was the first to peak. At Day 0, patients with BSI (Group 3) had higher CRP (in BSI 208 (52–421) vs. without BSI 113 (5–440) mg/L, $P = 0.043$), higher lactate levels (1.6 (0.8–3.7) vs. 1.15 (0.4–2.2) mmol/L, $P = 0.018$), and higher PCT values than patients without BSI (2.40 (0.84–73.2) vs. 0.60 (0.07–37.1) ng/mL, $P = 0.030$). Peak levels are shown in Table 2. Peak CRP in BSI measured 231 (71–436) and without BSI 158 (5–454) mg/L ($P = 0.008$). Lactate was higher in BSI with 1.9 (1.1–3.9) vs. 1.4 (0.5–13.1) mmol/L without BSI ($P = 0.006$). Peak PCT was higher in BSI with 2.92 (0.09–75.29) than with 0.65 (0.08–37.14) ng/mL in the absence of BSI ($P = 0.021$). Peak MR proADM was higher in BSI with 3.60 (0.82–18.57) vs. 1.60 (0.37–9.96) nmol/L in the absence of BSI ($P = 0.012$).

Septic shock

Day 0 CRP values were raised in patients with vs. without septic shock (174 (5–440) mg/L vs. 101 (5–279) mg/L, respectively, $P = 0.001$). Day 0 PCT values were higher in septic shock (1.09 (0.07–73.2) ng/mL than without septic shock (0.35 (0.08–37.1) ng/mL ($P < 0.001$). The Fig. S3 (esm) shows the course in time and Table 2 the peak values.

Mortality

Non-survivors had higher MR proADM and MR proANP on Day 0 (2.93 (0.63–12.62) nmol/L and 396 (58–1684) pmol/L) than survivors (1.33 (0.05–9.47) nmol/L, $P < 0.001$, and 202 (22–1613) pmol/L, $P = 0.001$, respectively). PCT on Day 0 was also increased in non-survivors vs. survivors (0.87 (0.14–73.18) ng/mL vs. 0.56 (0.07–45.06) ng/mL, $P = 0.040$). Fig. S4 (esm) shows the course in time and Table 2 the peak values.

Low risk infection

A change of antibiotics was as frequent in patients with a low risk (67%) as in patients with high risk infection (53%, $P = 0.62$). Fig. 1 shows the course in time and Table 2 the peak values. Day 0 CRP was lower (87 (5–279) vs. 125 (5–440)) in low risk infection ($P = 0.02$); the same applied for PCT (0.32 (0.10–37.1) vs. 0.69 (0.07–73.2), $P = 0.008$) and MR proADM (1.26 (0.05–2.41) vs. 1.72 (0.05–12.62), $P = 0.008$).

Predictive values

Statistically significant predictions by peak values are depicted in Table 3. At a cutoff of 0.65 ng/mL, peak PCT carried sensitivities, specificities, positive and negative predictive values for BSI, septic shock and mortality of 67–77, 51–57, 14–44 and 78–92%, respectively. This indicates high negative predictive values for all four endpoints studied. Plasma PCT as a single variable best helps to predict ICU-acquired, high risk microbial infection when peaking above 0.65 ng/mL ands low risk infection when peaking below 0.65 ng/mL. At Day 0, the predictive value for BSI was highest for lactate and PCT (AUC 0.72 and 0.69, $P = 0.03$ or less, respectively), for septic shock highest for CRP and PCT (AUC 0.72 and 0.68, $P = 0.002$ or lower, respectively), and for 28-day mortality highest for MR proADM and PCT (AUC 0.74 and 0.64, $P = 0.04$ or lower, respectively). At Day 0, PCT was most predictive for low risk infection, followed by MR proADM (AUC 0.77 and 0.70, $P = 0.001$, respectively) and CRP (AUC 0.69, $P = 0.003$). Otherwise, SAPS II score at admission predicted at an AUC of 0.63 ($P = 0.044$) and SOFA score at Day 0 at 0.73 ($P = 0.001$).

Multivariable analysis

For high risk infection, the combination of peak CRP and lactate predicted best ($P = 0.033$ and 0.001, respectively; Hosmer Lemeshow X^2 8.3, df8, $P = 0.40$), followed by PCT as the best single predictive variable ($P = 0.017$; Hosmer Lemeshow X^2 5.1, df 8, $P = 0.75$). For low risk infection, PCT was the single best predictive variable ($P = 0.015$; Hosmer Lemeshow X^2 9.8, df8, $P = 0.28$).

Discussion

This study suggests that old and new biomarkers in critically ill patients with new onset fever differ in their predictive

Table 1 Patient characteristics.

	Group 1 $n = 44$	Group 2 $n = 45$	Group 3 $n = 12$	P
Age (year)	63 (22–77)	61 (19–81)	67 (19–81)	0.69
Gender (male)	32 (73)	34 (76)	3 (25)	0.003
SAPS II on admission	47 (19–85)	46 (23–78)	54 (21–78)	0.10
SOFA at Day 0	8 (2–13)	7 (2–14)	10 (3–13)	0.47
Days from admission to Day 0	7 (1–78)	7 (1–77)	6 (1–45)	0.91
Temperature, °C D0	38.9 (38.4–40.0)	38.9 (38.4–40.8)	40.8 (39.3–40.3)	0.06
D1	38.4 (36.3–40.0)	38.1 (35.9–40.4)	38.5 (37.1–40.1)	0.68
D2	38.1 (36.4–39.9)	38.2 (36.6–39.5)	38.1 (36.3–39.4)	0.01
SIRS D0	44 (100)	45 (100)	11 (92)	0.02
D1	41 (93)	43 (96)	11 (92)	0.83
D2	36 (86)	38 (56)	11 (100)	0.42
Septic shock D0	0	20 (44)	8 (67)	<0.001
D1	0	20 (44)	8 (67)	<0.001
D2	0	19 (43)	8 (67)	<0.001
D7	0	25 (56)	9 (75)	<0.001
ICU length of stay, days	29 (5–126)	23 (4–95)	35 (11–77)	0.38
28-day mortality	13 (30)	9 (20)	4 (33)	0.48
ICU mortality	13 (30)	9 (20)	4 (33)	0.48
Immunocompetence				
Active malignancies	2 (5)	4 (9)	3 (25)	0.09
Immunocompromised state	2 (5)	2 (4)	2 (17)	0.25
Admission category				
General surgical	24 (55)	26 (58)	4 (33)	0.32
Trauma	6 (14)	6 (13)	1 (8)	0.88
Cardiac	4 (9)	2 (4)	0	0.42
Vascular	6 (14)	4 (9)	0	0.36
Respiratory insufficiency	17 (39)	20 (44)	8 (67)	0.22
Sepsis	9 (21)	16 (36)	6 (50)	0.09
Shock	7 (16)	8 (18)	5 (42)	0.13
Post-CPR	5 (11)	3 (7)	2 (17)	0.54
Neurological	5 (11)	8 (18)	1 (8)	0.57
Other	3 (7)	5 (11)	1 (8)	0.78
Treatment up to 7 days prior to inclusion				
Antibiotics	37 (84)	40 (89)	10 (83)	0.77
Steroids	20 (46)	19 (42)	6 (50)	0.34
SDD	21 (48)	10 (22)	5 (42)	0.04
Treatment during study Day 0–7				
Therapeutic hypothermia	4 (9)	2 (4)	2 (17)	0.23
Antibiotics	41 (93)	42 (93)	12 (100)	0.65
Change in antibiotics	24 (55)	29 (64)	9 (75)	0.37
Steroids	19 (43)	23 (51)	8 (67)	0.88
SDD	23 (52)	9 (20)	5 (42)	0.006
Mechanical ventilation	43 (98)	40 (89)	12 (100)	0.14
Duration, days	24 (3–123)	17 (3–82)	29 (7–77)	0.09
Inotropic/vasopressor	29 (67)	24 (55)	9 (82)	0.18
Renal replacement	7 (16)	0	1 (8)	0.02
Surgery	9 (21)	7 (16)	1 (8)	0.58

Median (range), or number (percentage), where appropriate; CPR = cardiopulmonary resuscitation. SAPS = simplified acute physiology score; SOFA = sequential organ failure assessment score; ICU = intensive care unit; SDD = selective decontamination of the digestive tract. Group 1 = no or possible infection; Group 2 = probable or proven infection; Group 3 = bloodstream infection.

value according to invasiveness and severity of microbial infection, and that PCT may be of value as a single predictor of both high and low risk ICU-acquired infection.

We found that 57 of 101 (56%) febrile, critically ill patients had a probable/proven local infection or BSI, which agrees with the literature on comparable patient populations, in spite of female preponderance of patients with BSI's in our study.[2,21,26] Sources, micro-organisms and mortality rates are also in agreement with other studies, including those on ICU-acquired bacteraemia.[2,10,25,26]

Table 2 Peak values of biomarkers in the groups.

Day 0–2 infection	Group 1 $n = 44$	Group 2 $n = 45$	Group 3 $n = 12$	P
WBC, x 10^9/L	13.2 (5.5–38.5)	12.8 (0.2–25.7)	20.6 (2.5–81.7)	0.078
CRP, mg/L	142 (27–440)	153 (5–484)	231 (71–436)	0.004
Lactate, mmol/L	1.3 (0.5–2.3)	1.4 (0.5–13.1)	1.9 (1.1–3.9)	0.017
PCT, ng/mL	0.72 (0.09–13.9)	0.56 (0.08–37.1)	2.92 (0.09–75.3)	0.058
MR proADM, nmol/L	1.93 (0.50–9.80)	1.52 (0.37–9.96)	3.60 (0.82–18.57)	0.025
MR proANP, pmol/L	293 (77–2037)	187 (23–823)	342 (47–874)	0.090
COP, pmol/L	34.4 (5.0–157.9)	27.3 (5.0–97.4)	33.7 (5.9–154.4)	0.437
Day 0–7	No septic shock $n = 67$	Septic shock $n = 34$		P
WBC, x 10^9/L	12.9 (4.8–38.5)	15.0 (0.2–81.7)		0.16
CRP, mg/L	146 (5–440)	243 (5–484)		<0.001
Lactate mmol/L	1.4 (0.5–2.5)	1.6 (0.8–13.1)		0.07
PCT, ng/mL	0.57 (0.09–37.1)	1.28 (0.08–75.3)		0.005
MR proADM, nmol/L	1.75 (0.37–9.80)	1.89 (0.39–18.57)		0.23
MR proANP, pmol/L	296 (47–2037)	210 (23–874)		0.28
COP, pmol/L	31.5 (5.0–157.9)	29.5 (5.0–154.4)		0.46
Day 0–28	Survivors $n = 75$	Non-survivors $n = 26$		P
WBC, x 10^9/L	12.5 (2.5–27.5)	16.8 (0.2–81.7)		0.077
CRP, mg/L	177 (5–440)	201 (38–484)		0.303
Lactate, mmol/L	1.3 (0.5–3.5)	1.8 (0.9–13.1)		0.002
PCT, ng/mL	0.57 (0.08–45.1)	1.10 (0.23–75.3)		0.009
MR proADM, nmol/L	1.52 (0.37–9.47)	3.30 (0.63–18.57)		0.001
MR proANP, pmol/L	240 (23–1613)	385 (61–2037)		0.006
COP, pmol/L	27.3 (5.0–154.4)	38.1 (5.0–157.9)		0.042
Day 0–28 low risk infection	No $n = 84$	Yes $n = 17$		P
WBC, x 10^9/L	14.2 (0.2–81.7)	11.9 (4.8–21.6)		0.110
CRP, mg/L	198 (5–484)	155 (5–279)		0.120
Lactate, mmol/L	1.5 (0.5–13.1)	1.3 (0.5–2.1)		0.230
PCT, ng/mL	0.90 (0.08–75.3)	0.32 (0.11–37.14)		0.004
MR proADM, nmol/L	1.97 (0.39–18.6)	1.35 (0.37–2.98)		0.020
MR proANP, pmol/L	269.6 (23.2–2037.0)	194.4 (70.3–654.3)		0.220
COP, pmol/L	33.7 (5.0–157.9)	21.7 (5.0–73.4)		0.190

Median (range); Abbreviations: CRP = C-reactive protein, WBC = white blood cell count, PCT = procalcitonin, MR proADM = midregional pro-adrenomedullin, MR proANP = midregional pro-atrial natriuretic peptide, COP = copeptin. Group 1 = no or possible infection; Group 2 = probable or proven infection; Group 3 = bloodstream infection.

The associations between invasiveness of microbial infection and development of septic shock is not beyond expectations either. Since all patients had SIRS, the syndrome had no predictive value for infection and all patients with infection thus suffered from new onset sepsis in the ICU. Nevertheless, the (peak) WBC count had some predictive value for likelihood and invasiveness of microbial infection, in contrast to the literature,[8] but was of no predictive value for septic shock and mortality, in agreement with the literature.[4] Peak CRP predicted, to a certain extend, both BSI and septic shock but not mortality in our patients, the latter again in agreement with the literature.[4,6–9,18] The value of these two commonly applied surrogate indicators of microbial infection and its severity in the critically ill is thus limited. In contrast, minimally elevated lactate levels were of some predictive value for BSI and ICU mortality, in line with previous studies for the latter.[12–14] The predictive value of lactate for BSI is a novel finding. Early lactate production even before onset of shock in bacteremic patients might be explained by an increase in cellular Na+-K+ ATPase activity, among others.[38]

We found PCT to be helpful in discriminating BSI from infections without BSI, as reported before.[1,7,10,24] though predictive values of PCT were somewhat lower than in other patient populations. In these studies, PCT on admission appeared helpful in predicting, irrespective of localisation, a suspected infectious cause of SIRS (sepsis) and its severity, as compared to non-infected, critically ill patients with SIRS.[5,6,9,18,21,22,28] This can be explained by our inclusion criteria of ICU-acquired fever in patients with prior infection, surgery or other conditions that may confound PCT. Few

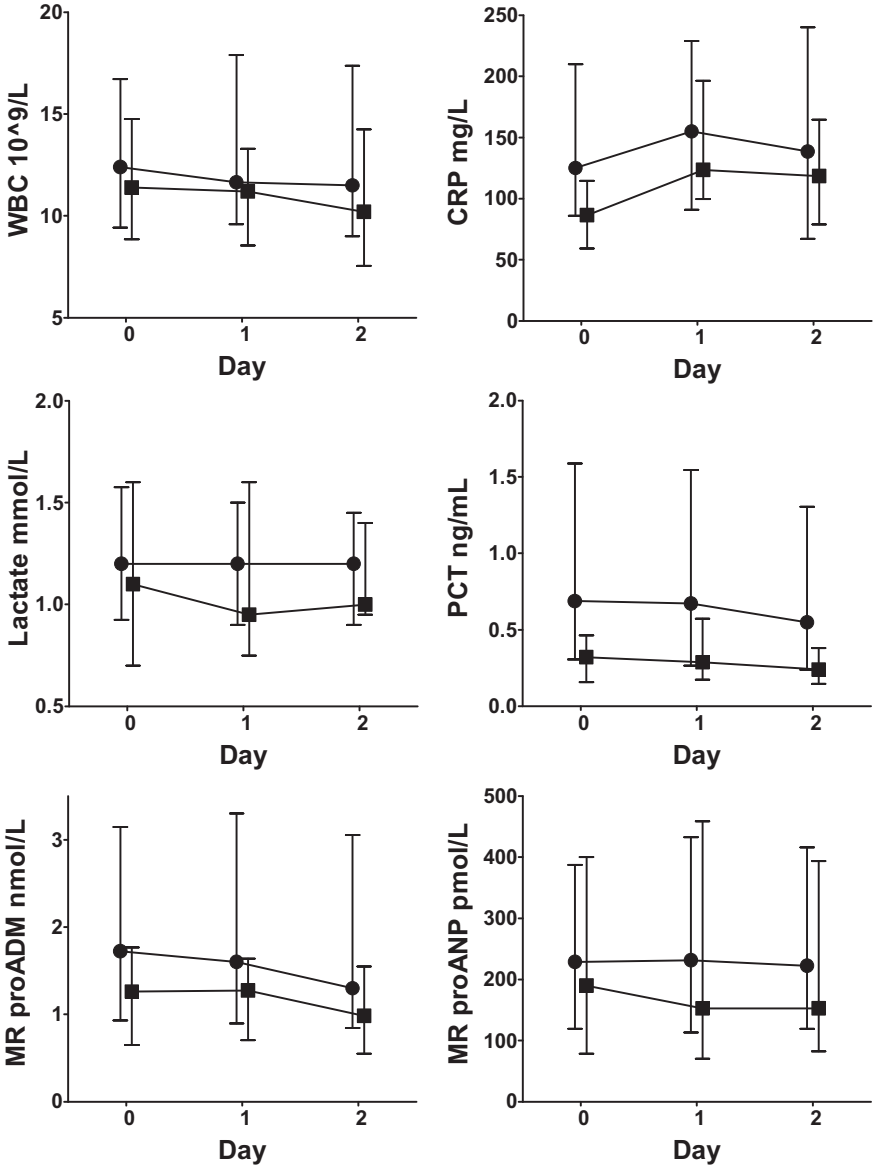

Figure 1 Course of plasma levels (median ± interquartile range (IQR)) in febrile critically ill patients, with low or high risk microbial infection. Symbols: ■ low risk infection (n=17) and • high risk infection (n=84). In generalized estimating equations (GEE): for PCT P=0.013 and MR proADM P=0.001.

studies that specifically addressed new onset fever in the ICU yielded highly variable results for PCT.[1,10,20,21] Nevertheless, for the 4 major endpoints, i.e. BSI, septic shock, mortality and low risk infection, PCT was superior to lactate and our results thus agree with improved prognostication in septic shock by PCT over lactate.[6,32] Combining biomarkers[23,32] has only rarely been done but may improve prediction.[16] In multivariable analysis of high risk infection in our critically ill patients with new onset fever, the combination of CRP and lactate proved superior, directly followed by PCT. For low risk infection also, PCT proved the single best predictive variable. Finally, PCT peaked earlier than the other markers. The superior predictive value of PCT over the other biomarkers for all endpoints studied can be explained, among others, by the kinetics in response to infection, in parallel with its invasiveness and severity.[39] In contrast to PCT, other prohormones may only transiently increase upon microbial products, whereas the response of WBC and CRP may be relatively slow.[39]

PCT values above 0.25—0.5 ng/mL have been used to guide starting or continuing antibiotics in the ICU[30,31] and our study suggests a higher cutoff at 0.65 ng/mL to discriminate between high and low risk microbial infection in ICU-acquired fever, for which empiric antibiotics could be instituted or withheld, respectively, in future studies.[25,26] Many patients were on antibiotics even when fever was unlikely associated with microbial infection, representing potential overtreatment. We could not identify an effect of changing antibiotics on outcome but did not evaluate appropriateness in the absence of uniformly accepted criteria.[26]

Whereas other investigators found a difference in MR proADM or COP between infected and non-infected and non-

Table 3 Prediction by peak values of biomarkers.

	WBC $\times 10^9$/L	CRP mg/L	Lactate mmol/L	PCT ng/mL	MR proADM nmol/L	MR proANP nmol/L	COP pmol/L
Bloodstream infection Day 0–2							
Cutoff	20.3	196	1.5	2.44	4.3	–	–
AUC	0.70	0.74	0.75	0.71	0.72	–	–
P	0.02	0.006	0.004	0.02	0.01	–	–
Sens	58	92	83	58	50	–	–
Spec	84	60	61	85	91	–	–
PPV	33	23	23	35	43	–	–
NPV	94	98	96	94	93	–	–
LHR	3.7	2.3	2.2	4.0	5.6	–	–
Septic shock Day 0–7							
Cutoff	–	208	–	1.98	–	–	–
AUC	–	0.75	–	0.67	–	–	–
P	–	<0.001	–	0.003	–	–	–
Sens	–	71	–	44	–	–	–
Spec	–	78	–	88	–	–	–
PPV	–	62	–	65	–	–	–
NPV	–	84	–	76	–	–	–
LHR	–	3.2	–	3.7	–	–	–
Mortality Day 0–28							
Cutoff	–	–	1.7	0.65	2.79	565	31.5
AUC	–	–	0.71	0.67	0.73	0.68	0.63
P	–	–	0.001	0.007	<0.001	0.005	0.04
Sens	–	–	60	77	62	46	69
Spec	–	–	75	57	81	93	57
PPV	–	–	44	39	53	71	36
NPV	–	–	85	88	86	83	84
LHR	–	–	2.4	1.8	3.3	6.9	1.6
Low risk infection Day 0–28							
Cutoff	–	–	–	<0.65	<1.91	–	–
AUC	–	–	–	0.72	0.67	–	–
P	–	–	–	<0.001	0.005	–	–
Sens	–	–	–	88	88	–	–
Spec	–	–	–	60	51	–	–
PPV	–	–	–	31	27	–	–
NPV	–	–	–	96	96	–	–
LHR	–	–	–	2.2	1.8	–	–

Abbreviations: D = day; WBC = white blood cell count; CRP = C-reactive protein, PCT = procalcitonin, MR proADM = midregional pro-adrenomedullin, MR proANP = midregional pro-atrial natriuretic peptide, COP = copeptin, AUC = area under the receiver operating characteristic curve, *P* = *p* value, Sens = sensitivity, Spec = specificity, PPV = positive predictive value, NPV = negative predictive value, LHR = likelihood ratio; non-significant data have been omitted.

surviving and surviving critically ill patients,[33–35] we found varying predictive values of the novel biomarker prohormones. However, MR proADM was predictive in 3 of 4 endpoints evaluated and thereby directly ranked behind PCT. The greater predictive value than that of PCT for outcome is in accordance with other studies.[36] Nonetheless, MR proADM levels did not supplement predictive values of PCT in multivariable analyses. We observed only minor predictive value of COP in our study in agreement with prior observations.[35]

Limitations of the study include the evaluation of new onset fever only, the heterogeneity of the study population and the persistently imperfect predictions by biomarkers of (severity of) infection. Heterogeneity could also be regarded as an advantage, however, concerning generalisibility of results. We separately studied medical and surgical patients and found no difference. The introduction of SDD did not change the predictive values of the biomarkers in this study, which may help in deciding on the use of biomarkers during SDD. That the percentage of fever from infectious vs non-infectious causes decreased after introduction of SDD is in line with expectations. Another advantage of our study is the rigid documentation and classification of infection as an endpoint.

Conclusion

In conclusion, our study in critically ill patients with new onset fever suggests that plasma PCT best serves as a single variable, among old and new biomarkers, to predict high and low risk microbial infection, although this prediction remains imperfect. The study may support evaluation of

clinical decision making on starting or postponing empiric antibiotics at a cutoff of 0.65 ng/mL for peak PCT in ICU-acquired fever, in future studies.

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Appendix. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jinf.2012.01.002.

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