Serge Masson
Pietro Caironi
Caterina Fanizza
Ralf Thomae
Roberto Bernasconi
Andrea Noto
Roberto Oggioni
Giovanni Stefano Pasetti
Marilena Romero
Gianni Tognoni
Roberto Latini
Luciano Gattinoni

Circulating presepsin (soluble CD14 subtype) as a marker of host response in patients with severe sepsis or septic shock: data from the multicenter, randomized ALBIOS trial

S. Masson (✉) · R. Bernasconi · R. Latini
Department of Cardiovascular Research, IRCCS–Istituto di Ricerche Farmacologiche "Mario Negri", Via Privata Giuseppe La Masa 19, 20156 Milan, Italy
e-mail: serge.masson@marionegri.it
Tel.: +39 0239014615

P. Caironi · L. Gattinoni
Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico, Università degli Studi di Milano, Milan, Italy

P. Caironi · L. Gattinoni
Dipartimento di Anestesia, Rianimazione, ed Emergenza Urgenza, Fondazione IRCCS Ca' Granda–Ospedale Maggiore Policlinico, Milan, Italy

C. Fanizza · M. Romero · G. Tognoni
Fondazione Mario Negri Sud, Santa Maria Imbaro, Italy

R. Thomae
Mitsubishi Chemical Europe GmbH, Duesseldorf, Germany

A. Noto
Azienda Ospedaliera San Paolo, Milan, Italy

R. Oggioni
Ospedale del Mugello, Borgo San Lorenzo, Italy

G. S. Pasetti
Ospedale Civile S. Giovanni di Dio, Orbetello Scalo, Italy

Abstract *Purpose:* Presepsin is a soluble fragment of the cluster-of-differentiation marker protein 14 (CD14) involved in pathogen recognition by innate immunity. We evaluated the relation between its circulating concentration, host response, appropriateness of antibiotic therapy, and mortality in patients with severe sepsis. *Methods:* Plasma presepsin was measured 1, 2, and 7 days after enrollment of 997 patients with severe sepsis or septic shock in the multicenter Albumin Italian Outcome Sepsis (ALBIOS) trial. They were randomized to albumin or crystalloids. We tested with univariate and adjusted models the association of single measurements of presepsin or changes over time with clinical events, organ dysfunctions, appropriateness of antibiotic therapy, and ICU or 90-day mortality. *Results:* Presepsin concentration at baseline (946 [492–1,887] ng/L) increased with the SOFA score, the number of prevalent organ dysfunctions or failures, and the incidence of new failures of the respiratory, coagulation, liver, and kidney systems. The concentration decreased in ICU over 7 days in patients with negative blood cultures, and in those with positive blood cultures and appropriate antibiotic therapy; it increased with inappropriate antibiotic therapy ($p = 0.0009$). Baseline presepsin was independently associated with, and correctly reclassified, the risk of ICU and 90-day mortality. Increasing concentrations of presepsin from day 1 to day 2 predicted higher ICU and 90-day mortality (adjusted $p < 0.0001$ and 0.01, respectively). Albumin had no

effect on presepsin concentration. *Conclusions:* Presepsin is an early predictor of host response and mortality in septic patients. Changes in concentrations over time seem to reflect the appropriateness of antibiotic therapy.

Introduction

Despite recent improvements, the incidence of sepsis in hospitalized patients increases steadily and mortality rates remain unacceptably high [1]. Recent failures with new therapeutic strategies may stem from an incomplete understanding of the underlying biology of sepsis [2]. Considering the variety of clinical phenotypes of severe sepsis and septic shock, we need to understand more accurately the pathophysiology of the disease to propose more personalized therapies. In this context, circulating biomarkers may improve early recognition, optimal selection of appropriate therapies, and early risk stratification [3–5].

Recognition of pathogen-associated molecular patterns (PAMPs) from infecting microorganisms is mediated, within the innate immune system, by proteins of the Toll-like receptor (TLR) family [6]. The mechanism for the recognition of lipopolysaccharide (LPS), a major PAMP from the outer membrane of most Gram-negative bacteria, involves accessory molecules that facilitate the presentation of LPS to the TLR4–MD2 complexes. CD14 is one of these accessory proteins, expressed on the surface of myelomonocytic cells and as a soluble molecule in the circulation. CD14 is a broad-spectrum affinity component of the innate immunity and detects a variety of ligands associated also with Gram-positive bacteria, fungi, and viruses [7, 8]. Acute inflammation may furthermore contribute to CD14 up-regulation by tissues other than monocytes [9]. During recognition of PAMPs by soluble CD14, a 13-kDa fragment is formed, named sCD14 subtype (sCD14-ST) or presepsin [10]. Presepsin is emerging as a novel circulating marker of sepsis [10–15]. However, like other candidates in sepsis, this biomarker needs to be validated in large, multicenter studies with representative populations [3].

In a pilot study, we previously showed that presepsin has a better prognostic performance than procalcitonin in patients with severe sepsis [16]. Here, we extend our investigations on the clinical role of presepsin assay for monitoring host response and appropriateness of antibiotic therapy to a larger subpopulation of 997 patients enrolled in a multicenter randomized clinical trial that assessed the efficacy of albumin replacement in severe sepsis and septic shock [17]. In particular, we aimed to elucidate (1) the characteristics associated with increased presepsin levels, (2) the clinical value of presepsin monitoring during the disease, both in relation to the development of complications and to the treatment applied, and (3) prognostic accuracy of presepsin for early and long-term outcomes.

Materials and methods

Study design

The Albumin Italian Outcome Sepsis (ALBIOS) study was a multicenter, open-label trial that enrolled 1,818 patients with severe sepsis admitted to 100 intensive care units (ICUs). The patients were randomly assigned to receive either 20 % albumin and crystalloids or crystalloids alone, from randomization until day 28 or discharge from the ICU, whichever came first. Study design, inclusion and exclusion criteria, and main results were published elsewhere [17] and are summarized in the Supplementary Appendix.

The study was compliant with the 1975 Declaration of Helsinki as revised in 2008, and approved first by the Institutional Review Board of the Fondazione IRCCS Ca' Granda-Ospedale Maggiore Policlinico, Milan, Italy (coordinating center), and subsequently by the appropriate institutional review boards of all the other participating centers. Written informed consent was obtained for all participants.

Sample collection and circulating presepsin measurement

In a subset of 997 patients recruited in 40 centers that participated in a predefined biomarkers substudy, venous blood samples were serially collected 1, 2, and 7 days after enrollment (or at ICU discharge, whichever came first), centrifuged, and plasma was shipped on dry ice to a central repository and stored at -70 °C until being assayed. Presepsin was measured with a chemiluminescent enzyme immunoassay (PATHFAST Presepsin, Mitsubishi Chemical) by personnel blinded to patients' characteristics. Intra-assay and inter-assay coefficients were 4.4 % at 445 ng/L, and the limit of detection was 20 ng/L. Samples from a subset of 100 patients have already been analyzed with the same method and included in a preliminary publication [16].

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Definitions

Severe sepsis was defined as the presence of a proved or suspected infection in at least one site, two or more signs of systemic inflammatory reaction [18], and at least one severe and acute sepsis-related organ dysfunction. Shock at the time of randomization was defined as a score of 3 or 4 for the cardiovascular component of the Sequential Organ Failure Assessment (SOFA) score [19]. New organ failures were defined as a modification of each component of the SOFA score during the study period to 3 or 4 from a baseline of 0, 1, or 2. Acute kidney injury was defined according to the risk, injury, failure, loss, and end-stage kidney injury (RIFLE) criteria [20], based on the daily incremental increases in serum creatinine from baseline. Clinical resolution of the primary infection, if any, was established by the treating physician according to standard clinical signs. Acquired secondary infections were defined as new infections other than the primary infection responsible for the development of severe sepsis, arising during the study, based upon microbiological and clinical assessments, and evaluated by the treating physician.

Appropriateness of antibiotic therapy was based on all the pathogenic microorganisms isolated in either the site or blood cultures. We considered the first-line empirical antibiotic therapy as appropriate when all the microorganisms isolated and reported as responsible for the primary infection were susceptible to at least one antibiotic administered. For this purpose, we considered antibiotic therapy empirically ongoing on day 1, i.e., about 24 h from enrollment. Antibiotic therapy was considered inappropriate if applied after the first day of enrollment, if it did not cover all the pathogens isolated, or when pathogenic microorganisms were resistant. Microorganism susceptibility to antibiotics was defined by a local microbiologist who was unaware of the allocation group or treatment applied.

Statistical methods

Categorical variables are presented as proportions, and continuous variables as means (standard deviation) or medians (Q1–Q3), based on the normality of distribution by Kolmogorov–Smirnov test. Differences in clinical characteristics according to tertiles of presepsin concentration were analyzed with the Chi-square test or Fisher's exact test for categorical variables, and with the analysis of variance or non-parametric Kruskal–Wallis test for continuous variables.

We performed multivariable linear regression analysis with backward elimination to identify the clinical determinants independently associated with a high log-transformed concentration of presepsin on day 1 (see additional statistical methods in the Supplementary Appendix). The concentrations of presepsin in clinical subgroups were compared with the Kruskal–Wallis test and trends across tertiles of presepsin concentration were assessed with the Cochran–Armitage test or one-way analysis of variance for categorical and continuous variables, respectively.

Changes in presepsin concentrations over time according to survival status, treatment, and septic shock at baseline were analyzed with two-way analysis of variance for repeated measurements using log-transformed data. Moreover, to account for the hierarchical nature of the data (repeated measurements within patients) and to control simultaneously for the possible confounding effects of the different variables, multivariate multilevel linear regression models were performed [21] including as covariates SOFA score, time, appropriateness of antibiotic therapy, and an interaction term between time and appropriateness of antibiotic therapy. All available measurements at each time point for each patient were considered.

The relation between presepsin concentration and mortality was first assessed with univariate logistic models entering log-transformed concentration of the biomarker as a continuous variable. A backward selection method was applied to select the final model.

Prognostic discrimination of presepsin was evaluated from the area under the receiver-operating characteristic (ROC) curves (AUC), comparing multivariable models with or without presepsin. The increased discriminative value of presepsin for ICU and 90-day mortality was assessed using the category-free net reclassification improvement (cfNRI) and the integrated discrimination improvement (IDI) index [22]. Changes in presepsin concentrations over time were expressed as the difference in log-transformed concentrations. Kaplan–Meier survival curves were shown by tertiles of relative changes and compared with the log-rank test. Both unadjusted and covariate-adjusted logistic regression models were generated to assess potential associations between changes in presepsin concentrations over time and mortality. A two-sided p value of less than 0.05 was deemed statistically significant.

Results

Clinical characteristics related to the presepsin concentration

The concentration of presepsin on day 1 after randomization in the overall cohort of patients was 946 [492–1,887] ng/L (median [Q1–Q3], $n = 958$). Presepsin was associated with several demographic, clinical, hemodynamic, and laboratory variables (Table 1). Multiple linear regression analysis showed the strongest association of higher presepsin concentrations, by decreasing order of t value, with high serum creatinine

Table 1 Clinical characteristics according to baseline presepsin concentration

Characteristics	All	Presepsin			
		Tertile 1 (<597 ng/L)	Tertile 2 (597–1,397 ng/L)	Tertile 3 (>1,397 ng/L)	P across tertiles
n (%)	958 (100.0)	320 (33.4)	320 (33.4)	318 (33.2)	
Presepsin–median [Q1–Q3] [1,901–4,310]	946 [492–1,887] –	388 [293–493]	949 [753–1,124]	2609	
Age, years	66.6 ± 14.7	63.8 ± 15.9	66.8 ± 14.7	69.2 ± 13.0	0.0002
Women, n (%)	395 (41.2)	129 (40.3)	134 (41.9)	132 (41.5)	0.92
Body mass index, kg/m^2	26.4 ± 5.6	26.0 ± 5.4	26.8 ± 5.7	26.5 ± 5.6	0.19
Reason for admission to ICU, n (%)					
Medical	540 (56.4)	191 (59.7)	166 (51.9)	183 (57.6)	0.12
Elective surgery	67 (7.0)	19 (5.9)	29 (9.1)	19 (6.0)	0.21
Emergency surgery	351 (36.6)	110 (34.4)	125 (39.1)	116 (36.5)	0.47
Preexisting conditions, n (%)					
Liver disease	14 (1.5)	4 (1.3)	4 (1.3)	6 (1.9)	0.75
COPD	120 (12.5)	42 (13.1)	40 (12.5)	38 (12.0)	0.90
Chronic renal failure	42 (4.4)	3 (0.9)	7 (2.2)	32 (10.1)	<0.0001
Immunodeficiency	124 (12.9)	26 (8.1)	49 (15.3)	49 (15.4)	0.007
Congestive or ischemic heart disease	164 (17.1)	45 (14.1)	55 (17.2)	64 (20.1)	0.13
SAPS II score	46 [35–56]	38 [31–48]	46 [36–56]	53 [44–63]	<0.0001
Physiological/laboratory variables					
Heart rate, beats/min	103.8 ± 20.8	100.9 ± 20.2	105.6 ± 21.0	105.0 ± 20.9	0.0076
Mean arterial pressure, mmHg	74.6 ± 15.0	77.7 ± 14.8	74.9 ± 14.4	71.2 ± 14.9	<0.0001
Mean arterial pressure after 6 h, mmHg	78.3 ± 13.4	81.3 ± 12.5	79.3 ± 13.8	74.3 ± 13.0	<0.0001
Central venous pressure, mmHg	9.9 ± 4.6	9.5 ± 4.4	9.6 ± 4.6	10.8 ± 4.9	0.0003
PaO$_2$/FiO$_2$	2.14 ± 1.14	2.17 ± 1.11	2.11 ± 1.18	2.13 ± 1.12	0.51
Urine output, mL/h	75.8 ± 70.2	88.1 ± 70.8	83.1 ± 74.1	56.1 ± 60.8	<0.0001
Lactate, mmol/L	3.2 ± 2.7	2.5 ± 2.1	3.3 ± 2.9	3.8 ± 3.0	<0.0001
Serum albumin, g/L	24.4 ± 6.2	25.6 ± 6.5	24.0 ± 6.0	23.7 ± 6.1	0.0005
Hemoglobin, g/dL	10.9 ± 2.0	11.4 ± 2.1	10.9 ± 1.9	10.5 ± 1.8	<0.0001
Serum creatinine, mg/dL	1.97 ± 1.62	1.23 ± 0.87	1.82 ± 1.45	2.88 ± 1.91	<0.0001
White blood cells, 10^3/mm^3	13.2 ± 9.5	13.7 ± 8.4	12.1 ± 9.4	13.9 ± 10.6	0.0078
Central venous oxygen saturation, %	72.6 ± 9.8	73.1 ± 8.9	72.7 ± 10.0	72.0 ± 10.4	0.58
Serum bilirubin (mg/dL)	1.38 ± 1.96	0.96 ± 0.87	1.18 ± 1.07	2.00 ± 3.03	<0.0001
Platelet count (10^9/L)	188.1 ± 130.1	215.7 ± 131.5	181.4 ± 117.9	167.0 ± 135.6	<0.0001
SOFA score	7.0 [5.0–10.0]	5.0 [4.0–7.0]	8.0 [5.0–9.0]	9.0 [7.0–11.0]	<0.0001
Shock, n (%)	540 (56.4)	151 (47.2)	191 (59.7)	198 (62.3)	0.0002
Mechanical ventilation, n (%)	760 (79.3)	241 (75.3)	259 (80.9)	260 (81.8)	0.09
Randomized to albumin, n (%)	474 (49.5)	154 (48.1)	154 (48.1)	166 (52.2)	0.49
Positive blood culture, n (%)	271/852 (31.8)	62/276 (22.5)	88/290 (30.3)	121/286 (42.3)	<0.0001
Antibiotics at the time of randomization, n (%)	898 (93.7)	297 (92.8)	307 (95.9)	294 (92.5)	0.14
Antibiotics 6 h after randomization, n (%)	954 (99.6)	318 (99.4)	320 (100)	316 (99.4)	0.48
Appropriateness of antibiotic therapy on day 1 according to site culture, n (%)	422/558 (75.6)	119/156 (76.3)	151/199 (75.9)	152/203 (74.9)	0.95
Appropriateness of antibiotic therapy on day 1 according to blood culture, n (%)	211/259 (81.5)	43/58 (74.1)	75/85 (88.2)	93/116 (80.2)	0.09
ICU mortality, n (%)	253 (26.4)	42 (13.1)	74 (33.4)	137 (43.1)	<0.0001
90-day mortality, n (%)	369 (39.1)	78 (24.8)	107 (33.9)	184 (58.4)	<0.0001

(standardized beta coefficient = 0.34, $p < 0.0001$) and bilirubin (0.20, $p < 0.0001$), low hemoglobin (−0.13, $p < 0.0001$), older age (0.12, $p < 0.0001$), reduced urine output (−0.10, $p = 0.0005$), immunodeficiency (0.08, $p = 0.002$), and higher serum lactate (0.08, $p = 0.007$).

The baseline concentration of presepsin was higher in relation to the severity of organ dysfunctions, as denoted by the SOFA score, and the number of prevalent organ dysfunctions or failures (Supplementary Fig. 1). Presepsin concentration was significantly higher in patients with bacterial infection (according to the site or blood culture) than in those with negative culture or in those with no culture available (Supplementary Table 1). Patients with Gram-negative bacterial infections had higher levels of presepsin than those with Gram-positive infections ($p = 0.02$ for blood culture). Finally, patients with lung infections had lower baseline presepsin levels than patients with abdominal or urinary tract infections ($p < 0.0002$).

Time course of presepsin and clinical course during ICU stay

Higher presepsin on day 1 was associated with hemodynamic instability during the first 24 h of treatment:

patients with higher presepsin had a lower probability of mean arterial pressure of at least 65 mmHg or lactate lower than 2 mmol/L, and a greater probability of being under vasoactive drugs ($p < 0.0001$ for all, Supplementary Table 2). Higher presepsin on day 1 was closely associated with a higher incidence of subsequent new organ failures, as denoted by the respiratory, coagulation,
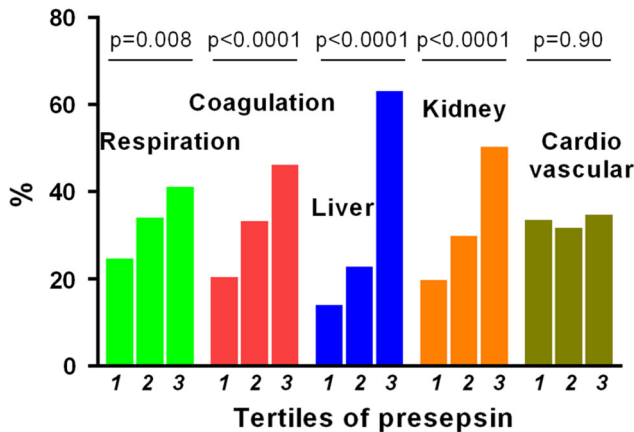

Fig. 1 Baseline presepsin concentration according to the incidence of new organ failures, defined as a change in each component during the study period to 3 or 4 from a value of 0, 1, or 2 at baseline. Number of patients with new organ-specific failures: respiration (238, 44.7 % of those without organ-specific failure at baseline), coagulation (186, 21.4 %), liver (57, 6.3 %), kidney (157, 21.8 %), cardiovascular (173, 44.0 %). *P* across categories by Kruskal–Wallis test. Number of patients without prevalent organ failure by organ and by presepsin tertiles (all; tertile 1, tertile 2, tertile 3): respiration (533; 188, 169, 176), coagulation (868; 303, 292, 273), liver (905; 320, 306, 289), kidney (720; 294, 255, 171), cardiovascular (393; 164, 120, 109)

liver, and kidney organ components of the SOFA score (Fig. 1).

There were no differences in circulating presepsin concentrations through the first 7 days after enrollment in patients randomized to albumin or to crystalloids (*p* for time *x* treatment interaction = 0.40). Consequently, relative changes in presepsin concentration from enrollment were not different in the two experimental arms on day 2 ($p = 0.28$) or on day 7 ($p = 0.53$), both in patients with or without shock. Although presepsin concentration was higher in patients with shock than in those without shock at baseline [1,017 (553–2,128) ng/L, $n = 540$ vs. 793 (426–1,606) ng/L, $n = 418$, $p < 0.0001$] and at subsequent time points, the time course was similar (*p* for time *x* shock interaction = 0.50).

Appropriateness of antibiotic therapy

Patients with increasing concentrations of presepsin over the first 7 days were less likely to have received early appropriate antibiotic therapy, based on the positivity of either site or blood cultures, than those with decreasing levels over time (Table 2). Presepsin concentration tended to decrease on day 7 in patients with negative blood cultures [−17 (−47 to +44) % vs. day 1, median (Q1–Q3)] and in those with positive blood cultures and appropriate antibiotic therapy [−18 (−53 to +38) %], whereas it increased in those with positive blood cultures and inappropriate antibiotic therapy [+24 % (−11 to +199 %), $p = 0.002$ by Kruskal–Wallis test, Supplementary Fig. 2a]. Similar trends were seen for site cultures [−15 (−49 to +41), −19 (−49 to +32), and +5

Table 2 Therapeutic procedures and changes in circulating presepsin concentrations over time

Therapeutic variables	Relative changes in presepsin concentration (%, day 7–day 1)			
	Tertile 1 (<−38 %)	Tertile 2 (−37 to +15 %)	Tertile 3 (>16 %)	*P* for trend across tertiles
Appropriateness of antibiotic therapy based on site cultures				
Appropriate therapy on day 1, *n* (%)	115/145 (79.3)	108/143 (75.5)	99/148 (66.9)	0.02
Total patient-days, *n* (%)	803/1,542 (52.1)	763/1,514 (50.4)	732/1,583 (46.2)	0.001
Appropriateness of antibiotic therapy based on blood cultures				
Appropriate therapy on day 1, *n* (%)	60/65 (92.3)	47/58 (81.0)	50/70 (71.4)	0.002
Total patient-days, *n* (%)	397/1,542 (25.7)	332/1,514 (21.9)	356/1,583 (22.5)	0.03
Activated protein C				
For at least 2 days, *n* (%)	4 (1.7)	3 (1.3)	5 (2.1)	0.73
Total patient-days, *n* (%)	19 (1.2)	17 (1.1)	24 (1.5)	0.48
Tight glycemic control				
For at least 3 days, *n* (%)	177 (74.4)	183 (76.6)	183 (76.6)	0.58
Total patient-days, *n* (%)	1,136 (73.7)	1,126 (74.4)	1,182 (74.7)	0.52
Corticosteroids				
For at least 3 days, *n* (%)	98 (41.2)	71 (29.7)	100 (41.8)	0.88
Total patient-days, *n* (%)	562 (36.4)	408 (26.9)	564 (35.6)	0.66
Extracorporeal hemofiltration for sepsis				
For at least 2 days, *n* (%)	8 (3.4)	5 (2.1)	8 (3.4)	0.99
Total patient-days, *n* (%)	34 (2.2)	23 (1.5)	32 (2.0)	0.72

(−40 to +83) %, respectively, $p = 0.02$, Supplementary Fig. 2b].

Presepsin and clinical outcomes

Higher presepsin levels on day 1 were associated with most of the tertiary outcomes assessed at post hoc analyses in the ALBIOS trial. Patients with higher presepsin on day 1 had a higher incidence of acute kidney injury and renal replacement therapy during the study, a longer ICU stay, longer mechanical ventilation and time to discontinuation of vasopressor or inotropic agents, as well as a longer duration and a lower degree of resolution of the primary infection (Supplementary Table 3).

Prognostic value of presepsin

The presepsin concentrations remained high over 7 days in patients who died in the ICU but decreased in survivors ($p < 0.0001$ for interaction between time and survival, Supplementary Fig. 3).

Higher presepsin levels on day 1 were associated with subsequent mortality (Supplementary Fig. 4). Adjusting for all potential clinical risk factors, presepsin concentration on day 1 was significantly associated with increased mortality in ICU or at 90 days, in all patients, as well in the subgroup of patients with septic shock, whereas in the subgroup without shock it was significantly associated with increased ICU mortality only (Table 3). Addition of presepsin on day 1 to a clinical model that included all significant risk factors for mortality improved the prognostic accuracy (area under the ROC curve) in particular for patients with shock, as well as the metrics of reclassification (integrated discrimination improvement (IDI) or continuous category-free net reclassification improvement (NRI), Supplementary Table 4). This correct reclassification was mainly driven by the non-event component of the NRI. There was no significant interaction between presepsin concentration on day 1 and treatment allocation (albumin or crystalloids) on ICU or 90-day mortality (Supplementary Table 5).

Figure 2 shows the Kaplan–Meier survival curves for ICU or 90-day mortality by tertiles of relative changes of presepsin concentration from day 1 to day 2 or from day 1 to day 7. There was a graded increase in the risk of mortality across tertiles of changes (log rank test: $p \leq 0.0003$). In adjusted logistic models, increases in presepsin concentration over 2 or 7 days were independently associated with ICU or 90-day mortality, with the exception of 2-day changes and 90-day mortality in patients with shock (Supplementary Table 6). The same tendency was observed for patients with or without shock.

Discussion

In a preliminary study on a subgroup of 100 patients of the ALBIOS trial, we previously observed that presepsin had a better prognostic value than procalcitonin in patients with severe sepsis or septic shock [16]. Here, in a larger cohort of well-characterized contemporary patients, we found that early presepsin concentration was mainly influenced by advanced age, poor hepatic and renal functions, shock, and impaired tissue perfusion (low mean arterial pressure and hyperlactatemia). Moreover, high presepsin levels following the first hours of fluid resuscitation were associated with a greater hemodynamic instability, as indicated by a smaller proportion of patients with stable mean arterial pressure (≥ 65 mmHg), or a greater need for vasoactive drugs. These findings may explain the close relationship between presepsin and the number and degree of organ dysfunctions or failures, indicated by the SOFA scores in all its components. Presepsin concentrations were higher in patients with lower blood platelets, suggesting a possible relationship between this marker and coagulation disorders [23]. Even though presepsin was higher in patients with shock than in those with severe sepsis without shock, we observed only a weak relation with the cardiovascular component of the SOFA score, based on mean arterial pressure and on the doses of vasopressor drugs administered. Presepsin appears therefore to be a good marker of the host response in a variety of its clinical manifestations, encompassing several organ-specific alterations.

The exact mechanisms of membrane CD14 expression [24], presepsin release during sepsis, and its biological function are still largely unknown. CD14 mediates the response to a variety of PAMPs [7] with the simultaneous release of a soluble fragment [25]. We found slightly higher (ca. 25–30 %) levels of presepsin in patients with bacterial infection than in those with negative or unavailable culture. Soluble CD14 also recognizes bacterial patterns processed by the Toll-like receptor 2 complex (lipoteichoic acid and several lipoproteins), and hence is not restricted to the identification of Gram-negative endotoxins [8]. Accordingly, presepsin may reflect sepsis-induced immune host defense to microorganisms and its consequences on organ function. At the same time, it is worth noting that two of the strongest determinants of higher circulating presepsin concentration were renal and hepatic dysfunctions (high serum creatinine and bilirubin), suggesting that impaired plasmatic clearance may also contribute to the elevated levels of the marker.

Immunodeficiency was more frequent in patients with elevated presepsin levels. After adjustment for several clinical covariates (age, severity of the overall disease, number of organ dysfunctions) and for the presence of immunosuppression, patients with higher presepsin had a primary infection of longer duration, and a lower incidence of resolution, irrespective of the type and site of

infection. These results suggest that higher levels of presepsin, independently of the severity and type of infection per se, may indicate a loss of infection compartmentalization, or a state of immunoparalysis, leading to a spreading of the related inflammatory reaction, and of the innate immune host response, which may ultimately lead to multiple organ failure and death. Interestingly, $CD14^+$ monocytes have been recently considered responsible for the development of immunosuppressive activity through the production of interferon-gamma in human umbilical cord matrix stem cells [26] or through the IL-1β/prostaglandin E2 axis during pregnancy [27]. In our study, we also found that presepsin levels predicted the occurrence of new organ failures, thereby suggesting that it may be a marker of this process, as previously observed for soluble CD14 [28].

Though we did not design the present study to assess a role for presepsin in guiding antibiotic therapy, we evaluated its time course in relation to the appropriateness of first-line empirical antimicrobial treatment. We found that monitoring presepsin for the first week of treatment may be a good indicator of the adequacy of the antimicrobial therapy. Indeed, patients with decreasing levels of presepsin over 7 days in ICU were more likely to have received an early appropriate first-line empirical antibiotic therapy on day 1 than those with increasing levels (92 vs. 71 %). Changes in presepsin levels were also associated with the duration of appropriate antibiotic therapy, as estimated by blood or site culture. Interestingly, most of the inappropriate empirical antibiotic therapy was related to infections caused by multidrug-resistant Gram-negative bacteria. These findings suggest that measuring presepsin may help not only in risk stratification after the diagnosis of sepsis, but also in monitoring the efficacy of the treatment employed, and in guiding its appropriateness.

We also found that presepsin measured on day 1 predicted mortality, independently of age, reasons for ICU admission, SOFA score, hemodynamic and respiratory variables, laboratory markers, and appropriateness of antibiotic therapy. The association was stronger in patients with shock. Correct reclassification of the patients was mainly driven by non-events, suggesting that presepsin accurately lowered risk estimates for survivors. Therefore, early assessment of presepsin may help in identifying, among the most severe and fatal manifestations of sepsis, those states that are still reversible, facilitating a more effective second-line and highly intensive clinical treatments. Short-term increases in presepsin concentrations predicted mortality.

In the ALBIOS trial, patients were randomized to either albumin and crystalloids or crystalloids alone for volume replacement [17]. Albumin replacement did not improve survival at 28 and 90 days in the overall population, but gave small, significant hemodynamic advantages and was more effective in patients with septic shock. In the present study, presepsin concentrations were not significantly different in the two experimental arms, and there was no interaction between median presepsin concentration and randomized treatment on mortality. Thus, in our experimental conditions, we could not find any significant effect of correcting hypoalbuminemia on presepsin levels.

The present study has some limitations. First, it did not include all the patients enrolled in the ALBIOS trial, as only a subgroup of the participating centers were involved in the biomarker substudy. Nonetheless, these centers

Table 3 Presepsin concentration on day 1 and total mortality

Outcome	Patients	No. events/no. patients (%)	OR [95 % CI]	p
ICU mortality				
	All	253/958 (26.4)	1.77 [1.48–2.12]	<0.0001
	Severe sepsis	94/418 (22.5)	1.59 [1.23–2.05]	0.0004
	Shock	159/540 (29.4)	2.29 [1.80–2.92]	<0.0001
90-day mortality				
	All	369/945 (39.1)	1.77 [1.49–2.11]	<0.0001
	Severe sepsis	135/409 (33.0)	1.22 [0.92–1.63]	0.17
	Shock	234/536 (43.7)	2.17 [1.66–2.69]	<0.0001

OR [95 % CI] for an increment of 1 SD unit in log-transformed concentration of presepsin. The covariates were introduced in the multivariable logistic models if they had a $p < 0.20$ at univariate analyses. The following covariates were retained in the final models after backward elimination. For ICU mortality, all patients: age, body mass index, chronic obstructive pulmonary disease, immunodeficiency, congestive or ischemic heart disease, reason for admission in ICU, SAPS II score, appropriate antibiotic therapy, PaO_2/FiO_2, platelet count, mechanical ventilation; for ICU mortality, severe sepsis: age, immunodeficiency, heart rate, PaO_2/FiO_2, platelet count, mechanical ventilation; for ICU mortality, septic shock: age, chronic obstructive pulmonary disease, body mass index, congestive or ischemic heart disease, PaO_2/FiO_2, platelet count, mechanical ventilation. For 90-day mortality, all patients: age, sex, body mass index, chronic obstructive pulmonary disease, immunodeficiency, congestive or ischemic heart disease, reason for admission in ICU, SAPS II score, appropriate antibiotic therapy, PaO_2/FiO_2, platelet count, mechanical ventilation; for 90-day mortality, severe sepsis: age, body mass index, mean arterial pressure at baseline or after 6 h, chronic obstructive pulmonary disease, chronic renal failure, immunodeficiency, SAPS II score, SOFA score at baseline, appropriate antibiotic therapy, PaO_2/FiO_2, platelet count, urine output; for 90-day mortality, septic shock: age, sex, body mass index, chronic obstructive pulmonary disease, congestive or ischemic heart disease, SAPS II score, appropriate antibiotic therapy, PaO_2/FiO_2, platelet count
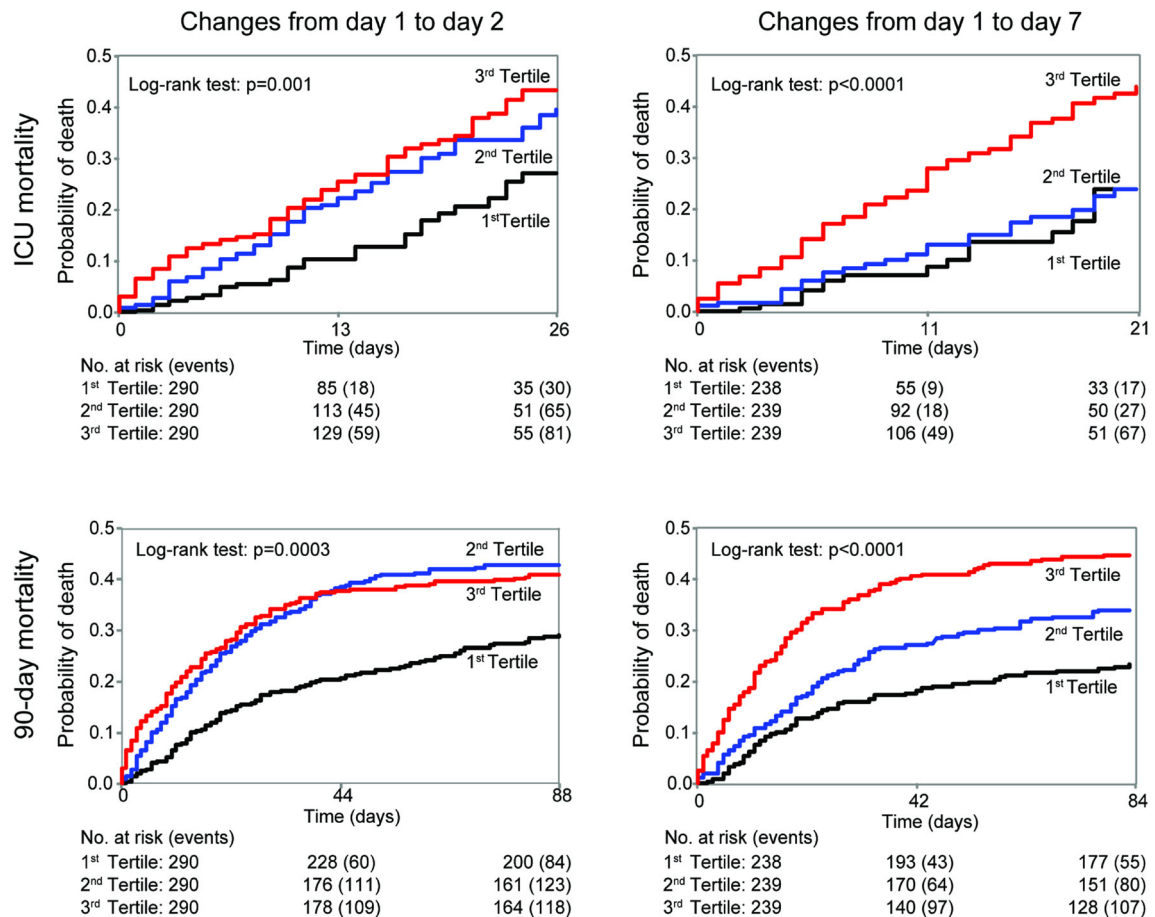
Fig. 2 Changes in presepsin concentrations over time and ICU or 90-day mortality. Kaplan–Meier survival curves for ICU or 90-day mortality by tertiles of changes in presepsin concentration from day 1 to day 2 or from day 1 to day 7. The number of patients at risk is shown below the curves. Tertiles of relative changes: from day 1 to day 2 (*1st tertile*, <-14.9 %; *2nd tertile*, -14.9 to 13.7 %; *3rd tertile*, >13.7 %), from day 1 to day 7 (*1st tertile*, <-38.4 %; *2nd tertile*, -38.4 to $+15.8$ %; *3rd tertile*, >15.8 %). *P* for log-rank test

enrolled the majority of the patients randomized in the ALBIOS trial and also in the biomarker substudy, therefore guaranteeing a representativeness of the disease investigated. Second, this analysis may have been underpowered. Third, as the ALBIOS was a pragmatic trial, the study protocol did not standardize the treatments employed (with the exception of fluid management), though they have been applied by the attending physicians according to the current guidelines for the treatment of severe sepsis and septic shock [29]. Fourth, the clinical value of presepsin has not been compared with other circulating biomarkers. Fifth, presepsin was not assessed on a daily basis over 1 week, precluding a full evaluation of its time course in the acute phase of sepsis.

Conclusions

The present study suggests that presepsin may be a good candidate for accurate and timely prediction of abnormal host response at an early stage before it becomes too severe and jeopardizes survival. It also gives preliminary indications that presepsin may be suited for monitoring appropriate antibiotic therapy. Although no single circulating marker is likely to be a panacea for the clinical management of severe sepsis in view of its complexity and rapid course, these findings are encouraging and should prompt further validation of presepsin in different clinical settings.

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