RESEARCH ARTICLE

Prognostic value of inflammatory markers (notably cytokines and procalcitonin), nutritional assessment, and organ function in patients with sepsis

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ABSTRACT. *Objective*. Procalcitonin is useful for the diagnosis of sepsis, but its prognostic value regarding mortality is unclear. Our objective was to determine the prognostic value of procalcitonin determined at the onset of sepsis, and to compare it with other markers of inflammatory response, malnutrition and organ dysfunction data. *Methods*. We studied 253 hospitalized patients (146 men, 107 women) with a median age of 65 years. Sepsis was defined as infection, and at least two SIRS criteria. We assessed co-morbidities, nutritional status, bacteremia, procalcitonin and other inflammatory markers (PCR, TNF-α, IL6, TREM-1, IL-10, IL-1ra, CD14 and LBP), and organ function using the SOFA score. Mortality was assessed at 28 days after onset of sepsis. *Results*. At day 28, 49 (19%) patients had died. Inflammatory markers showed only moderate predictive value for mortality, with IL-10 and IL-6 being the best predictors. Mortality was mainly related to organ dysfunction indicators (SOFA and Glasgow scores), serum lactate, ferritin and LDH levels, and to nutritional data such as subjective assessment, handgrip strength and serum transferrin levels. The most frequent location of sepsis was the lung, with 140 cases (55%), which showed more comorbidity, worse nutritional status, less frequent bacteremia and lower inflammatory response. When the analysis was limited to patients with non-pulmonary sepsis, organ dysfunction, nutritional status and inflammatory markers showed the best prognostic value. Of the inflammatory markers, procalcitonin showed only moderate predictive value; however it showed the highest correlation with bacteremia and the ability to discriminate non-complicated sepsis from severe forms. *Conclusion*. Procalcitonin only showed moderate predictive value for sepsis-related mortality, being surpassed by organ dysfunction, nutritional status, IL-10 and IL-6. However, it proved useful to discriminate between non-complicated and severe forms of sepsis.

Keywords: procalcitonin, IL-6, IL-10, mortality, sepsis

Sepsis is defined as a systemic inflammatory response of the host to infection. Excessive immune response, with overproduction of inflammatory mediators, results in tissue damage and organ dysfunction [1, 2]. Sepsis presents a wide range of severity and is one of the major causes of death. Hence the need for precise staging and prognostic assessment based on clinical data and on easily available laboratory parameters [3-5].

Procalcitonin (PCT) and C-reactive protein (CRP) have proved useful in the diagnosis of bacterial infection and sepsis [6-9]. Like other mediators of the inflammatory response, PCT correlates with the severity of sepsis. PCT may also serve to discriminate between severe sepsis and less severe forms of infection [10, 11]. However, there is some controversy about its prognostic value for mortality compared with other inflammatory markers, organ dysfunction, and with disease severity scores such as Sequential Organ Failure Assessment (SOFA) [12-26].

The objective of this study was to analyse the prognostic value for short-term mortality of serum PCT levels, and of other mediators of the inflammatory response, such as CRP, pro-inflammatory (TNF-α and IL-6) and anti-inflammatory [IL-10 and IL-1 receptor antagonist (IL-1ra)] cytokines, lipopolysaccharide-binding protein (LBP), soluble receptor CD14 (sCD14), and triggering receptor expressed on myeloid cells-1 (TREM1) in patients hospitalized for sepsis. We also compared its prognostic value with those of nutritional status and organ dysfunction.

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PATIENTS AND METHODS

We prospectively studied 253 patients with sepsis (146 men and 107 women), with a median age of 65 years (range 19-94 years) throughout 2007. All patients were hospitalized at the Hospital Universitario de Canarias. Two hundred and three patients with community-acquired sepsis were included after evaluation at the emergency department, and 50 patients with nosocomial infection at the time of diagnosis.

Clinical evaluation

A complete clinical history was recorded and physical examination performed for all patients. Basic chemistry and blood tests, arterial blood gas determinations and chest radiography were performed. Hospital admission and type of care (conventional: 125 or critical: 128) was decided upon by the team on duty; microbiological studies were requested by the patient's physician. In addition to the 253 patients mentioned above, four other patients were excluded as a non-infectious diagnosis was established during follow-up.

Sepsis was defined as clinical evidence of infection, plus two or more of the following criteria for systemic inflammatory response syndrome (SIRS): 1) temperature > 38° or < 36°C; 2) heart rate > 90 beats/m; 3) respiratory rate > 20/m or $PaCO_2$ < 32 mmHg; and 4) WBC > 12.000 or < 4.000 cells/mm^3, or > 10% immature forms). Sepsis was considered severe when it was associated with hypoperfusion, or organ dysfunction such as metabolic acidosis, oliguria, or an acute alteration in mental status (otherwise, the diagnosis was non-complicated sepsis). Septic shock was diagnosed when hypotension (systolic blood pressure of < 90 mmHg or a reduction of ≥ 40 mmHg from baseline) persisted despite adequate fluid therapy or when vasoactive amines were needed [2]. Finally, the sequential organ failure assessment (SOFA) score was determined at inclusion, and organ failure was considered as a score of 10 points or more [27]. Seventy two cases were diagnosed with non-complicated sepsis, 115 severe sepsis, 43 septic shock and 23 with organ failure.

Comorbidity

Eighty three patients were diabetic, 37 had cancer, 46 were receiving steroids or immunosuppressive treatment, 28 had HIV infection, 41 cognitive impairment, 44 excessive ethanol intake, and 24 with cirrhoses. In 50 cases, the sepsis was nosocomial in origin, with 16 of them appearing after surgery.

Sepsis localization

One hundred and forty cases of sepsis were of pulmonary origin, 35 urinary, 11enteric, 10 biliary, 19 other abdominal and 17 cutaneous; there were four cases each of endocarditis and arthritis, three cases each of meningitis and of catheter-related sepsis, and seven cases of unknown origin.

Organ function was assessed by the SOFA score, which is based on six criteria: 1) respiratory function assessed by PaO_2/FiO_2; 2) coagulation by platelet count; 3) liver function by bilirubin levels; 4) cardiovascular function by the presence of hypotension and the need for dopamine, dobutamine or norepinephrine; 5) central nervous system function by the Glasgow coma scale; and 6) renal function by oliguria and serum creatinine levels [27, 28].

Nutritional assessment

Weight and height were recorded at admission, with further calculation of body mass index as weight/$height^2$. To assess visceral proteins, we determined serum prealbumin, albumin, transferrin, and IGF-1. Total lymphocyte and CD4 counts were determined to assess the T cell response. Handgrip strength was determined by dynamometry; it was only considered in cooperative patients with a Glasgow score no lower than 14.

Subjective nutritional evaluation (SNS) included examination of the muscle mass of the upper and lower limbs and of the temporal muscle, defining two degrees of atrophy (severe, moderate), and absence of atrophy; we assigned 2, 1 and 0 points to each category. Bichat's fat and subcutaneous fat atrophy, recorded by physical examination of the fat loss on the cheek and abdomen, respectively, were scored in the same way. Thus, we obtained a subjective nutritional score based on the sum of the assigned points. As previously reported, a score of 0 to 2 was considered normal, one of 3-4 points as mild malnutrition, and higher than 4 points as severe malnutrition [29].

Mediators of the inflammatory response

After inclusion, blood samples were taken and frozen at − 40°C for further determination, by chemiluminiscent enzyme immunometric assay (IMMULITE analyzer) of: IGF-1 sensitivity: 20 ng/mL, [Diagnostic Products Corporation (DPC), Los Angeles, USA]; TNF-α, sensitivity of 1.7 pg/ml (DPC, Los Angeles, USA); IL-6, sensitivity: 2 pg/mL (DPC, Los Angeles, USA); IL-10, sensitivity of 1 pg/mL (DPC, Los Angeles, USA); high sensitivity CRP, sensitivity of 0.02 mg/dL (DPC, Los Angeles, USA); and lipopolysaccharide binding protein (LBP), sensitivity of 0.2 μg/mL (DPC, Los Angeles, USA). We used enzyme immunoassay to determine IL-1ra, sensitivity of 4 g/mL (BioSource Europe SA, Nivelles. Belgium); sCD14, sensitivity of 0.24 ng/mL (Immuno-Biological Laboratories, Hamburg, Germany); Leptin, sensitivity of 0.5 ng/mL (BioVendor Heidelberg, Germany); and Insulin, sensitivity of 1 μU/mL (Abbott Laboratories, Wiesbaden, Germany). Soluble triggering receptor expressed on myeloid cells-1(sTREM-1) was determined by a customised enzyme immunoassay, sensitivity 4.7 pg/mL (R & D InC, Minneapolis, USA). PCT was determined using a quantitative inmunoluminometric assay, sensitivity: 0.04 ng/mL (LIAISON BRAHMS PCT, Brahms Diagnostics, Berlin, Germay). Serum leptin and insulin were also determined in 53 healthy controls (30 men and 23 women, age range 26-86 years) obtained from healthy hospital workers and their relatives. The study was approved by the institutional review board of the hospital; informed consent was obtained from all patients.

Statistical analysis (SPSS15.0)

As serum cytokine and other inflammatory markers were not distributed normally, we used the non-parametric Kruskal-Wallis and Mann-Whitney U tests, Spearman's

correlation coefficient, Chi-2 and Fisher's exact test when necessary. Mortality was assessed mainly at day 28 (49 deaths), but also at day 7 after diagnosis of sepsis and at discharge to assess short-term prognosis. The predictive value of continuous variables for mortality was determined by receiver operating characteristics (ROC) curves with calculation of the area under the receiver operating characteristics curve (AUROC). Stepwise logistic regression was performed in order to discern which parameters yielded independent predictive value for survival. Fifty five patients died during hospitalization; the 198 patients who were discharged were followed up by telephone to assess long-term mortality at six months and one year after discharge. Data are presented as median, 25^{th} and 75^{th} percentiles.

RESULTS

Within 28 days of diagnosis of sepsis, 49 (19%) patients had died. Mortality was closely related to sepsis severity, with none of the 43 patients with non-complicated sepsis having died. Death was recorded in 13 out of 115 patients with severe sepsis (11.3%), 21 out of 72 with septic shock (29%) and 15 out of 23 with organ failure (55%), ($p < 0.001$). Increasing age was related with the mortality rate. Mortality rates per age group were: 6% in patients aged 35 years or less, 19% in those aged 36-85 years, and 40% in those aged > 85 years ($p = 0.048$). As shown in *table 1*, most of the inflammatory markers increased significantly with the severity of sepsis, except for sCD14, LBP and CRP.

As shown in *table 2*, the variables most closely related with 28-day mortality were organ dysfunction (SOFA and Glasgow coma scores), serum lactate, ferritin and LDH levels, and nutritional data such as SNS, handgrip strength and serum transferrin levels, whereas inflammatory markers showed only a moderate correlation. Among the inflammatory markers, elevated IL-10 and IL-6 showed a higher correlation with mortality than increased PCT, TNF, IL-1ra and TREM-1 and decreased LBP, whereas CRP and sCD14 were not related to mortality.

The relationship between PCT and mortality was weaker than those of the other inflammatory markers. Regarding the time course, the relationship between PCT and mortality was only significant at day 28, whereas TNF, TREM-1, IL-1ra, IL-6 and IL-10 were also related to mortality at day 7 and at the end of the hospital stay; IL-6 and IL-10 were also related to mortality after six months and one year. LBP, which increased with the severity of sepsis, was related to mortality at day 28, but, paradoxically, patients who died showed lower serum LBP levels.

Procalcitonin levels were significantly related to the severity of sepsis. Patients with a PCT > 0.5 ng/mL had a relative risk [RR (CI 95%)] of 4.1 (2.2-8.1) for either severe sepsis or worse; as PCT increased, the risk increased. For PCT > 3.3 ng/mL, the RR was 24.3 (3.3-180), whereas none of the patients whose PCT reached 10 ng/mL had non-complicated sepsis. Also, IL-6 and IL-10 were closely related to severity; IL-6 >170 pg/mL had a RR of 17.2 (2.3-127) and IL-10 > 25 pg/mL had a RR of developing severe sepsis, septic shock or organ failure of 14.6 (2-109).

Blood cultures were performed in 205 patients, and 36 of them (18%) were positive after excluding contaminants. Positive blood cultures were not directly related to mortality, but to severity of sepsis. Only 3% of patients with non-complicated sepsis showed positive blood cultures, whereas in those with severe sepsis, or a more advanced form, blood cultures were positive in 20.5% of cases. Positive blood cultures were found in 53% of patients with a SOFA score > 10 ($p = 0.001$). Patients with positive blood cultures showed increased levels of inflammatory markers. The best inflammatory marker for predicting positive blood culture was PCT, with an AUROC of 0.693 (0.598-0.787), followed by TNF-α, 0.677 (0.585-0.768) and IL-1ra, 0.664 (0.572-0.755). Only 6% of patients with a PCT < 0.5 ng/mL had positive blood cultures, whereas 35% of those with a PCT ≥ 10 ng/mL showed positive blood cultures ($p = 0.001$).

Regarding the origin of the sepsis, the most frequent focus was the lung, with 140 cases (55%). Pulmonary sepsis was more frequent in men (62%) than in women (46%) ($p = 0.013$). Patients with pulmonary sepsis had more predisposing factors, worse nutritional status and

Table 1
Inflammatory markers in the diverse stages of sepsis

	Sepsis (n) median (25^{th}-75^{th})	Severe sepsis (n) median (25^{th}-75^{th})	Septic shock (n) median (25^{th}-75^{th})	Organ failure (n) median (25^{th}-75^{th})	KW	p
PCT ng/mL	(43) 0.33 (0.10-0.96)	(115) 0.7 (0.15-2.3)	(72) 3.1 (0.77-16.9)	(23) 9.5 (1.84-48.2)	51.1	0.000
CRP µg/mL	(42) 14.2 (7.2-23.4)	(114) 15.4 (8.2-26.5)	(72) 19.0 (9.7-31.2)	(23) 18.6 (15.8-28.4)	6.20	0.100
TNFα pg/mL	(43) 11.3 (6.5-18.5)	(115) 14.8 (9.2-25.2)	(72) 24.3 (13.1-58.2)	(23) 34.4 (22.5-81.9)	49.5	0.000
IL-6 pg/mL	(43) 31.2 (10.8-58.5)	(115) 44.6 (22.1-108)	(72) 114 (38.6-554)	(23) 231 (120-2010)	55.8	0.000
sTREM-1 pg/mL	(43) 47.4 (27-67.8)	(115) 55.1 (39.2-69.3)	(72) 56.7 (43.8-81.7)	(23) 66.9 (40.6-108)	8.07	0.045
IL-10 pg/mL	(43) 5 (5-8.6)	(114) 5.4 (5-15.5)	(72) 14.1 (6.5-72.5)	(23) 27.4 (11.8-103)	49.7	0.000
IL-1ra pg/mL	(43) 1010 (402-1690)	(115) 916 (534-2636)	(72) 2030 (1056-2860)	(23) 2860 (2073-2860)	38.3	0.000
CD14s ng/mL	(43) 10667 (8440-14282)	(115) 10271 (7732-13603)	(72) 11764 (8227-14994)	(23 10191 (7495-13127)	2.37	0.499
LBP µg/mL	(42) 119 (83-177)	(115) 117 (68-184)	(72) 135 (64-212)	(23) 130 (67-252)	1.48	0.688
Leptin ng/mL	(40) 7.87 (2.63-18.1)	(108) 13.4 (4.78-32.7)	(71) 4.72 (2.0-12.4)	(22) 3.54 (2.14-8.81)	21.6	0.000

KW: Kruskal-Wallis test; PCT: procalcitonin; CRP: C-reactive protein; TNF: tumor necrosis factor; IL: interleukin; IL-1ra IL-1 receptor antagonist sTREM-1: soluble triggering receptor expressed on myeloid cells-1; LBP: lipopolysaccharide binding protein.

Table 2
Prognostic value of inflammatory markers, nutritional assessment and organ function data in sepsis

	Alive at day 28 (n) median (25^{th}-75^{th})	Deceased (n) median (25^{th}-75^{th})	MW U		AUROC (95% CI)
			z	p	
PCT ng/mL	(204) 1.07 (0.25-3.64)	(49) 3.86 (0.46-11.3)	2.14	0.032	0.598 (0.506-0.691)
CRP µg/mL	(202) 17.2 (9.0-27.5)	(49) 18.6 (9.8-29.4)	0.40	0.687	0.519 (0.432-0.605)
TNFα pg/mL	(204) 15.8 (10-27.4)	(49) 22.5 (11.8-49.1)	2.51	0.012	0.615 (0.525-0.706)
IL-6 pg/mL	(204) 49.1 (23.1-133)	(49) 145 (49.3-422)	3.73	0.000	0.672 (0.586-0.758)
sTREM-1 pg/mL	(204) 52.5 (38-74.9)	(49) 60.1 (49.5-79.3)	2.13	0.033	0.598 (0.520-0.676)
IL-10 pg/mL	(203) 6.4 (5-16.3)	(49) 21.9 (6.3-90)	4.24	0.000	0.690 (0.604-0.776)
IL-1ra pg/mL	(204) 1171 (598-2860)	(49) 2860 (789-2860)	2.72	0.007	0.623 (0.533-0.714)
CD14s ng/mL	(204) 10838 (8360-14101)	(49) 9809 (7006-14137)	1.14	0.252	0.447 (0.349-0.545)
LBP µg/mL	(202) 122 (77-200)	(49) 88 (45-157)	2.00	0.045	0.592 (0.500-0.684)
SNS	(204) 3 (2-4)	(49) 4 (3-7)	4.70	0.000	0.714 (0.636-0.792)
Handgrip lb	(146) 12 (2-30)	(20) 0 (0-4)	3,97	0.000	0.770 (0.663-0.877)
Transferrin mg/dL	(202) 164 (130-205)	(41) 119 (91-132)	5.70	0.000	0.781 (0.707-0.855)
Cholesterol mg/dL	(203) 129 (99-167)	(41) 105 (73-141)	3.12	0.002	0.655 (0.561-0.748)
IGF1 ng/mL	(198) 68.8 (45.9-97.2)	(39) 51.4 (29.8-72)	3.26	0.001	0.665 (0.577-0.754)
Leptin ng/mL	(194) 8.77 (3.51-22.7)	(47) 4.29 (2.15-12.4)	2.39	0.017	0.612 (0.522-0.702)
Insulin µU/mL	(194) 12.3 (7.73-24.9)	(47) 8.4 (3.9-19.5)	2.95	0.003	0.639 (0.546-0.732)
Creatinine mg/dL	(204) 1 (0.7-1.7)	(49) 1.3 (0.85-2.3)	1.34	0.181	0.561 (0.470-0.653)
BUN mg/dL	(204) 23 (16-35)	(49) 27 (20.5-41)	1.77	0.078	0.581 (0.494-0.668)
LDH UI/L	(204) 440 (332-670)	(48) 995 (608-2279)	5.97	0.000	0.777 (0.694-0.860)
Ferritin ng/mL	(202) 339 (157-757)	(41) 1103 (385-2000)	4.87	0.000	0.741 (0.653-0.830)
Glasgow	(204) 14 (13-15)	(49) 13 (12-14)	4.69	0.000	0.707 (0.624-0.789)
PaO_2/fiO_2	(204) 309 (206-401)	(49) 266 (207-331)	3.48	0.001	0.660 (0.571-0.749)
Systolic BP mmHg	(204) 100 (80-120)	(49) 82 (70-100)	3.36	0.001	0.654 (0.567-0.740)
Lactate mmol/L	(204) 1.90 (1.30-2.98)	(49) 4.10 (2.30-7.70)	6.12	0.000	0.781 (0.707-0.855)
SOFA	(204) 4 (2-6)	(49) 8 (5-11)	6.24	0.000	0.786 (0.719-0.853)

MW U: Mann-Whitney U test; AUROC: area under receiver operating characteristic curve; PCT: procalcitonin; CRP: C-reactive protein; TNFα: tumor necrosis factor; IL: interleukin; sTREM-1: soluble triggering receptor expressed on myeloid cells-1; IL-1ra IL-1 receptor antagonist; LBP: lipopolysaccharide binding protein. SNS: subjective nutritional score; IGF1: insulin growth factor 1; BUN: blood urea nitrogen; LDH: lactate dehydrogenase; PaO_2/fiO_2: O_2 arterial pressure/fraction of inspired oxygen; BP: blood pressure; SOFA: sequential organ failure assessment.

a blunted inflammatory response with lower serum cytokine levels *(table 3)*. Blood cultures were also less frequently positive (11% *versus* 24%) in pulmonary sepsis than in non-pulmonary sepsis ($p = 0.025$), whereas organ failure (SOFA) and death at 28 days (21% *versus* 18%) - or during the hospital stay (24% *versus* 19%) - were not significantly different. However, pulmonary sepsis was related to severe comorbidities such as dementia, cancer, liver cirrhosis and immunosuppressive therapy (including steroids). So, despite a similar age, pulmonary sepsis was related to higher, long-term mortality after six month or one year. Moreover, regarding the capacity to predict 28-day mortality in sepsis according to origin, inflammatory markers, nutritional assessment and organ function data had better predictive value in non-pulmonary than in pulmonary sepsis *(table 4)*. The following parameters showed a greater area under the ROC curve (non-pulmonary/pulmonary), PCT (0.679/0.534) IL-10 (0.792/0.626), IL-6 (0.774/0.619) serum transferrin (0.826/0.753), serum ferritin (0.817/0.673), lactate (0.837/0.756) and SOFA (0.854/0.737).

PCT only showed a moderate relationship to mortality. To determine whether it had independent prognostic value for mortality, we performed a logistic regression analysis including PCT, IL-6, IL-10 with SNS and SOFA. Procalcitonin was bettered by IL-10, SNS and SOFA.

We did not find increased serum leptin levels in patients with sepsis as compared with controls. There were no significant differences in these levels between non-complicated sepsis and severe sepsis; however, patients with septic shock and organ failure showed significantly decreased serum leptin levels when compared to patients with less severe forms of sepsis ($p < 0.001$). Serum leptin levels correlated directly to nutrition (BMI, SNS, serum cholesterol, albumin prealbumin and transferrin), and inversely with serum IL-6 levels. Patients who died showed lower serum leptin levels than those who survived.

DISCUSSION

In recent years, PCT has been considered as one of the main inflammatory markers for the diagnosis of sepsis. Moreover, it has been proposed as an additional criterion to improve the SIRS for sepsis diagnosis [3, 5]. It has proved useful to diagnose the more severe forms of sepsis [7, 10, 11, 21, 22], or to diagnose pneumonia in patients with lower respiratory tract infection [30, 31]. In our study, PCT was related to severity of infection and showed the best ability to discriminate between patients with non-complicated sepsis and good prognosis from more serious forms of sepsis. Moreover, positive blood

Table 3
Differences between pulmonary and non-pulmonary sepsis

	Pulmonary (n) median (25^{th}-75^{th})	Non-pulmonary (n) median (25^{th}-75^{th})	U MW	
			z	p
PCT ng/mL	(140) 0.98 (0.17-4.01)	(113) 1.39 (0.31-8.36)	1.75	0.080
CRP μg/mL	(138) 15.7 (8.36-27.2)	(113) 18.6 (12.3-28.3)	1.75	0.081
TNFα pg/mL	(140) 13.6 (9.10-26.1)	(113) 22.3 (13.1-40.4)	4.00	0.000
IL-6 pg/mL	(140) 48.2 (21.6-150)	(113) 75.1 (29.9-184)	2.40	0.016
sTREM-1 pg/mL	(140) 52.7 (39.7-67.8)	(113) 59.8 (39.2-85.5)	2.10	0.035
IL-10 pg/mL	(139) 6.6 (5-18.0)	(113) 9.3 (5-41.1)	2.33	0.020
IL-1ra pg/mL	(140) 1094 (535-2695)	(113) 1724 (1862-2860)	3.08	0.002
CD14s ng/mL	(140) 9836 (7637-13656)	(113) 11417 (8579-14740)	2.44	0.014
LBP μg/mL	(138) 116 (60.6-186)	(113) 133 (82.2-216)	2.20	0.028
SNS	(140) 4 (2-6)	(113) 3 (1-4)	4.04	0.000
BMI kg/m^2	(140) 24.4 (21.8-26.8)	(113) 26.2 (23.7-29.9)	3.97	0.000
Transferrin mg/dL	(133) 156 (126-200)	(110) 149 (112-186)	1.69	0.103
$CD4/mm^3$	(130) 342 (195-567)	(113) 483 (268-686)	2.62	0.009
Leptin ng/mL	(133) 5.59 (2.21-16.6)	(108) 10.5 (4.30-24.2)	2.37	0.003
Insulin μU/mL	(133) 10.9 (6.50-22.5)	(108) 12.0 (7.42-24.3)	0.72	0.472
Creatinine mg/dL	(140) 1 (0.7-1.5)	(113) 1.3 (0.9-2.1)	3.21	0.001
BUN mg/dL	(140) 23 (15-34.8)	(113) 26 (20-36)	2.15	0.031
LDH UI/L	(140) 472 (339-818)	(112) 478 (341-839)	0.43	0.667
Ferritin ng/mL	(133) 406 (174-1013)	(110) 401 (155-988)	0.23	0.820
Glasgow	(140) 14 (13-15)	(113) 14 (13-15)	0.26	0.792
PaO_2/fiO_2	(140) 281 (240-338)	(113) 344 (276-420)	0.11	0.912
Systolic BP mmHg	(140) 100 (80-120)	(113) 90 (80-117)	1.31	0.189
Lactate mmol/L	(140) 2 (1.4-3.0)	(113) 2.2 (1.5-3.6)	1.26	0.208
SOFA	(140) 5 (3-7)	(113) 5 (2-8)	0.365	0.715

MW U: Mann-Whitney U test; AUROC: area under receiver operating characteristic curve; PCT: procalcitonin; CRP: C-reactive protein; TNFα: tumor necrosis factor; IL: interleukin; sTREM-1: soluble triggering receptor expressed on myeloid cells-1; IL-1ra IL-1 receptor antagonist; LBP; lipopolysaccharide binding protein. SNS: subjective nutritional score; IGF1: insulin growth factor 1; BUN: blood urea nitrogen; LDH: lactate dehydrogenase; PaO_2/fiO_2: O_2 arterial pressure/fraction of inspired oxygen; BP: blood pressure; SOFA: sequential organ failure assessment.

cultures were related to severity of sepsis and to higher PCT levels. Only 3% of our patients with non-complicated sepsis showed positive blood cultures, whereas in those with severe sepsis, or worse, 20.5% showed positive blood cultures, and when SOFA was higher than 10, 53% had positive blood cultures. Patients with positive blood cultures showed elevated inflammatory markers, with PCT being the most increased. Many studies have reported raised PCT values in patients with bacteremia [13, 17, 18, 31-34].

However, there is some controversy about the prognostic value of PCT for mortality. While some authors [6, 19, 20, 22-24, 26, 35, 36] have found some predictive ability of PCT (determined at day 1 of the onset of sepsis), others have not [12, 34]. Yet others have reported that PCT determined at day 1 showed no prognostic value, but PCT determined later did correlate with mortality [13-16]. Dahaba *et al.* (2006), in a study of postoperative patients with severe sepsis, reported that the best discriminative value of PCT for mortality is reached at day 6 [15]. Regarding the possible independent predictive value of PCT, Phua *et al.* (2007) reported that IL-6, IL-10, lactate and SOFA were better outcome predictors than PCT determined on the first day of sepsis; Heper *et al.* (2006) reported that IL-10 and TNF were better predictors of mortality than PCT; Lee *et al.* (2008) found that the predictive value of PCT is surpassed by Mortality in Emergency Department Score (MEDS); and Ruiz-Alvarez *et al.* (2009) reported that, on multivariate analysis, PCT was displaced by CRP, and SOFA [16, 21, 25, 37]. However, Meng *et al.* (2009), reported that a PCT of over 10 ng/mL was a better predictor of outcome than APACHEII [36].

Our results showed inflammatory markers to be moderately useful as predictors of 28-day mortality *(table 2)*, but the area under the receiver operating characteristics curve was less than 0.700 in all cases, whereas nutritional data (subjective nutritional score), handgrip or serum transferrin), and organ damage data (LDH, lactate, ferritin or SOFA), all showed an area under the receiver operating curve of over 0.700. According to our data and regarding mortality, PCT was not an ideal inflammatory marker, being surpassed by IL-6 and IL-10. Moreover, PCT showed no independent prognostic value, as on multivariate analysis it was displaced by SNS, SOFA and IL-10. The relationship between PCT and mortality was only significant at day 28; it was not significant at other times (day 7, at discharge, or after six months and one year from inclusion). Neither did the prognostic value of PCT improve when we considered PCT values over 10 ng/mL.

Pulmonary sepsis was observed in half of our patients with sepsis, and the differences with cases of non-pulmonary sepsis are noteworthy *(table 4)*. Patients with pulmonary sepsis showed worse nutrition, less frequent bacteremia and a blunted inflammatory response, with a less marked increase in inflammatory mediators. However, there were not many differences regarding organ

Table 4
Prognostic value of inflammatory markers in non-pulmonary sepsis

	Alive at day 28 (n) median (25^{th}-75^{th})	Deceased (n) median (25^{th}-75^{th})	U MW		AUROC (95% CI)
			z	p	
PCT ng/mL	(93) 1.20 (0.29-5.03)	(20) 7.77 (1.36-29)	2.75	0.006	0.697 (0.573-0.820)
CRP μg/mL	(93) 17.9 (12.3-27.6)	(20) 21.5 (11-31)	0.54	0.591	0.538 (0.397-0.679)
TNFα pg/mL	(93) 20.2 (12.3-34.5)	(20) 35.8 (22.5-81.3)	3.36	0.001	0.740 (0.631-0.849)
IL-6 pg/mL	(93) 58.5 (26.1-157)	(20) 251 (103-2229)	3.84	0.000	0.774 (0.664-0.885)
sTREM-1 pg/mL	(93) 54.7 (38.1-80.8)	(20) 72.4 (56.9-97.5)	2.11	0.035	0.651 (0.527-0.775)
IL-10 pg/mL	(93) 6.8 (5-16.6)	(20) 72.4 (18.4-1411)	4.15	0.000	0.792 (0.674-0.911)
IL-1ra pg/mL	(93) 1429 (855-2860)	(20) 2860 (1713-2860)	2.82	0.005	0.605 (0.563-0.828)
CD14s ng/mL	(93) 11417 (8782-14292)	(20) 11693 (7088-16192)	0.38	0.701	0.473 (0.302-0.643)
LBP μg/mL	(93) 133 (86.3-218)	(20) 138 (44.5-208)	0.88	0.379	0.563 (0.411-0.715)
SNS	(93) 2 (1-4)	(20) 5 (3-7)	3.87	0.000	0.772 (0.647-0.897)
Transferrin mg/dL	(92) 164 (122-196)	(18) 103 (77-124)	4.36	0.000	0.826 (0.729-0.922)
Cholesterol	(93) 127 (95-170)	(18) 101 (60-131)	2.41	0.016	0.680 (0.548-0.812)
IGF1 ng/mL	(91) 73 (44-100)	(17) 45.2 (29.3-73.3)	2.21	0.027	0.669 (0.538-0.800)
Leptin ng/mL	(90)13.9 (4.7-32.7)	(18) 6.05 (3.13-11.1)	2.37	0.018	0.677 (0.556-0.799)
Insulin μU/mL	(90) 12.4 (7.8-25.9)	(18) 7.5 (2-20)	2.32	0.021	0.673 (0.520-0.827)
Creatinine mg/dL	(93) 1.2 (0.85-1.75)	(20) 1.6 (1-2.3)	1.25	0.211	0.589 (0.450-0.728)
BUN mg/dL	(93) 24 (17.5-34.5)	(20) 31.5 (24.3-46.5)	2.29	0.022	0.663 (0.542-0.785)
LDH UI/L	(93) 452 (334-701)	(19) 1374 (624-2288)	4.22	0.000	0.808 (0.694-0.923)
Ferritin ng/mL	(92) 327 (142-678)	(18) 1364 (476-3417)	4.24	0.000	0.817 (0.705-0.929)
Glasgow	(93) 14 (13-15)	(20) 13 (12-14)	3.35	0.001	0.728 (0.608-0.849)
PaO_2/fiO_2	(93) 350 (290-422)	(20) 318 (177-371)	2.16	0.031	0.654 (0.513-0.795)
Systolic BP mmHg	(93) 97 (80-117)	(20) 80 (70-113)	1.91	0.056	0.635 (0.484-0.787)
Lactate mmol/L	(93) 2 (1.4-3.2)	(20) 5.10 (3.43-8.88)	4.72	0.000	0.837 (0.723-0.951)
SOFA	(93) 4 (2-6.5)	(20) 10 (7-13)	4.98	0.000	0.854 (0.769-0.939)

MW U: Mann-Whitney U test; AUROC: area under receiver operating characteristic curve; PCT: procalcitonin; CRP: C-reactive protein; TNFα: tumor necrosis factor; IL: interleukin; sTREM-1: soluble triggering receptor expressed on myeloid cells-1; IL-1ra IL-1 receptor antagonist; LBP: lipopolysaccharide binding protein. SNS: subjective nutritional score; IGF1: insulin growth factor 1; BUN: blood urea nitrogen; LDH: lactate dehydrogenase; PaO_2/fiO_2: O_2 arterial pressure/fraction of inspired oxygen; BP: blood pressure; SOFA: sequential organ failure assessment.

function (decreased creatinine and BUN were rather related to impaired nutrition and decreased muscle mass). Early death (at day 7 and 28 or during the hospital stay), was similar in pulmonary and non-pulmonary sepsis patients. However, patients with pulmonary sepsis were more frequently affected by some of the severe predisposing factors such as cancer, dementia, cirrhosis, immunosuppression and malnutrition, so, despite similar age, pulmonary sepsis was related to higher, later mortality rates (after six months or one year).

These differences between pulmonary and non-pulmonary sepsis probably explain, in part, why the prognostic factors are more useful in non-pulmonary sepsis. Whereas outcome in pulmonary sepsis may be more related to severe predisposing conditions, such as cancer or dementia, in non-pulmonary sepsis, inflammatory mediators, malnutrition and data for impaired organ function were closely related to the prognosis. Regarding the capacity of inflammatory markers, nutritional assessment and organ dysfunction data to predict outcome after 28 days, the area under the ROC curve was greater in non-pulmonary sepsis. The differences observed between pulmonary and non-pulmonary sepsis indicate two different types of sepsis. One, pulmonary, in which severe predisposing factors are frequent, with impaired nutrition, less bacteremia, a less intense inflammatory response and higher long-term mortality, and another, non-pulmonary, with fewer underlying factors, better nutrition and a greater inflammatory response.

Two of the inflammatory markers, CRP and sCD14, did not show any relationship with severity of sepsis or with 28-day mortality. CRP is a widely used diagnostic marker of sepsis, but has not shown a strong relationship with mortality [17, 26, 35, 38]. CD14 receptor may be detached from the membrane and can be determined as soluble CD14. Landmann *et al.* (1995) and Burgmann *et al.* (1996), reported that patients who died from sepsis showed raised serum sCD14 levels [39, 40]. In accordance with other studies on pneumonia, we found no differences regarding survival [41].

Lipopolysaccharide binding protein (LBP) showed paradoxical behaviour. Whereas LBP levels increase in sepsis, especially in patients with bacteremia, and directly correlated with other inflammatory mediators [42], we found that decreased LBP related to poor prognosis: patients who died had lower values than patients who survived, as reported by Opal *et al.* (1999) [43]. However, these results have not been confirmed by other studies [41, 44].

There is some controversy about leptin in sepsis. Maruna *et al.* (2001) [45] consider it to be an acute phase reactant, as it increases in post-surgical sepsis. However, in our patients, serum leptin levels were related to nutrition and inversely correlated with IL-6 levels. Patients with septic shock and organ failure, and those who died, showed lower serum leptin levels than those who survived. These results are similar to those reported by Aleman *et al.* (2002) in lung cancer and Diez (2008) in

community-acquired pneumonia patients [46, 47]. So, we suggest that in patients with sepsis, leptin should be considered as a nutritional parameter rather than as an inflammatory marker.

In conclusion, the prognostic value of PCT and other inflammatory markers was only moderate, being surpassed by nutritional and organ function data. The best inflammatory markers related to mortality were IL-6 and IL-10, rather than PCT, which was only poorly related. However, among the inflammatory markers, PCT proved the best predictor of bacteremia and progression of sepsis, showing greater ability at discriminating between non-complicated and more severe forms of sepsis. The markers sCD14 and CRP showed no ability to predict outcome.

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