ORIGINAL ARTICLE

# Daily monitoring of biomarkers of sepsis in complicated long-term ICU-patients: can it support treatment decisions?

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## ABSTRACT

**Background.** Diagnosis/grading of infection and the systemic response to infection may be difficult on admission to the intensive care unit, but it is even more complicated for severely ill patients with long intensive care stays. The ACCP-SCCM criteria are difficult to apply for such patients, and objective, validated biomarkers would be of great use in this setting.

**Methods.** Long-term (>6 days) critically ill patients in the general ICU of University Hospital were prospectively enrolled in the study. All patients were assessed daily by the attending physician using the ACCP-SCCM classification. C-reactive protein (CRP, mg/dL), procalcitonin (PCT, ng/mL), and interleukin-6 (IL-6, pg/mL) of daily stored sera were measured after each patient's discharge. After discharge, an independent, overall clinical evaluation and an a posteriori ACCP-SCCM classification were chosen as the reference standard for all comparisons. The assessor was aware of the patient's clinical course but was blinded to levels of biomarkers.

**Results.** We studied clinical variables and biomarkers of 26 patients over a total of 592 patient days. The day-by-day ACCP-SCCM classification of the attending physician overestimated the severity of the inflammatory response to infection. The diagnostic discriminative ability of severe-sepsis/septic-shock for PCT was high (ROC area 0.952 [0.931-0.973]) and had a best threshold value of 1.58 (83.7% sensitivity, 94.6 % specificity). IL-6 had better discriminative ability than CRP, but both were worse than PCT.

**Conclusion.** PCT > 0.43 ng/mL could add to the clinical propensity for sepsis vs. SIRS not related to infection. Values higher than 1.58 ng/mL may support the bedside clinical diagnosis of severe-sepsis. PCT between 0.5 and 1.0 suggest tight daily monitoring of clinical conditions and re-evaluation of PCT. *(Minerva Anestesiol 2010;76:814-23)*

**Key words:** Sepsis - C-reactive protein - Interleukin-6 - Intensive care

Diagnosis of sepsis is a subjective decision that is made using clinical variables predictive of systemic inflammatory response to stress (SIRS),[1] together with nonspecific markers, such as white blood cell count (WBC), in the presence of a documented or even suspected infection. The severity of sepsis is graded according to the concomitant perfusion abnormalities and organ dysfunction.[1-3]

The application of these criteria for the diagnosis of sepsis, severe sepsis, or septic shock is quite easy in patients admitted to the intensive care unit (ICU) with a very serious disease. However, it is certainly more difficult or even impossible in patients who stay for weeks in the ICU, as their clinical course is marked by improvement without definite recovery and may be followed by several relapses. Body temperature, pulse, respirato-

ry rate and WBC, as well as organ function and tissue perfusion, are frequently abnormal, and cultures confirm infection in only a minority of cases when bacteria are found in the blood or in the cerebro-spinal fluid.[4-7] In most ICU patients with slowly evolving disease, bacterial colonization in multiple sites is the rule, and some degree of inflammation is nearly unavoidable. In such patients, the daily dilemma of deciding whether there is a new infection *versus* persisting inflammation and of whether to start a new course of antimicrobials *versus* waiting and observing is well-known to clinicians. Appropriate and timely treatment with antimicrobials could save lives, whereas excessive and prolonged treatment favors the emergence of multi-resistant strains. The available diagnostic tools are of little help. Clinical signs and laboratory parameters are even less specific than in the initial phase, infection always has to be suspected, and microbiology, seldom specific, requires time to give results.

More specific markers of infection would be of particular value in this context, and much research concerning the diagnostic role of inflammatory cytokines and reactant proteins, including interleukin (IL-6; detectable for 18-24 hours), procalcitonin (PCT; starts to rise within 4 hours, remaining at a plateau for 8 to 24 hours) and C-reactive protein (CRP; rises slowly and peaks at 36 hours [8]) have been performed. Measurements of these inflammatory markers at ICU admission or even in the emergency room are thought to distinguish between inflammation without infection and to delineate the various degrees of inflammatory response to infection.[9-15] In our opinion, in critically ill patients, it is quite reasonable to focus on the evolution of inflammation/infection throughout the patient's entire ICU stay (LOS). Treatment decisions must be adapted daily to the changing clinical severity of the patient, and objective criteria for doing this are often lacking.

In this study, we compared the day-by-day measurements of the biomarkers CRP, PCT, and IL-6, either singly or in combination, with our best clinical *a posteriori* evaluation of the infectious state.

The aim of this study was to assess which marker, if any, could mirror changes in the patient's infectious state and at which value such a marker could add diagnostic information that would enhance clinical assessment.[7] This aim was pursued in the difficult context of long-term ICU patients, who, even in small numbers, consume a large proportion of available resources.

## Materials and methods

From February 1, 2008, and August 31, 2008, an observational cohort study was designed to investigate the time course of inflammatory markers in adults with a long-term critical illness admitted to the general ICU of a university hospital. Patients were enrolled consecutively in a prospective manner. Patients considered at high-risk [16] for ICU admission were eligible. Exclusion criteria included the following: severe immunocompromised patients, autoimmune diseases, chemotherapy, or chronic steroid therapies.

Patients' characteristics, diagnosis, and SAPS-II [17] score were recorded at ICU admission. We kept a daily record of all clinical variables and quantified organ failure with the Sequential Organ Failure Assessment (SOFA) score.[18]

Based on standard clinical laboratory data and the available microbiological data, each day was classified by the attending physicians as a day without or with SIRS, proven or suspected infection without or with SIRS (colonization[7] or sepsis, respectively), severe sepsis, or septic shock. These assessments were quantified based on the American College of Chest Physicians/Society of Critical Care Medicine (ACCP-SCCM) classification.[1-3] Based on this classification, they determined the overall course of treatment and when to begin, continue, or stop antimicrobials. LOS and vital signs at ICU/hospital discharge were also recorded.

An aliquot of daily sera from eligible patients was stored at -70 °C until ICU discharge. For high-risk patients who spent more than six days in the ICU, this sera was subsequently analyzed for CRP and PCT. IL-6 serum levels were determined for patients enrolled in the last four months of the study. The hospital ethics committee approved the protocol, and patients or next-of-kin gave informed consent.

After the patient's discharge, we (G.I. S.M.) reviewed the clinical sheets for the entire ICU stay and re-classified each ICU day using the ACCP-

SCCM classification, with the advantage of knowing the patient's entire clinical course, the results of the microbiological samples collected that day, and the overall clinical evolution of infection and organ failure. The revision was blind to CRP, PCT and IL-6 measurements. This *a posteriori* day-by-day infectious state classification is artificial and unfeasible in a clinical setting. However, in a research setting where there is no clear gold standard,[4] it is the best available standard reference for determining the diagnostic value of CRP, PCT and IL-6. In a sensitivity analysis, we compared the day-by-day and the *a posteriori* classifications.

*Statistical analysis*

CRP was measured by an immunoturbidimetric assay on a Roche/Hitachi automated clinical Chemistry Analyzer (Roche Diagnostics S.p.A., Milan, Italy). The reference range was less than 5 mg/dL. The within-run coefficients of variation (CVs; N=21) were respectively 2.76%, 1.77% and 0.76% at 3.4, 51 and 150 mg/dL; the between-run CVs (N.=21) were 4.61%, 1.8% and 1.6% at 3.8, 50 and 153 mg/dL; the detection limit of the test was 0.425 mg/dL. The calibration curve was linear over the range of 1-258 mg/dL.

PCT was measured by an automated immunoluminometric assay (Brahms) based on two specific monoclonal antibodies using a Liaison analyzer (Dia Sorin). The reference range was less than 0.5 ng/mL. The within-run CVs (N=30) were 3.6%, 2.9% and 2.5% at 0.8, 12.5 and 160 ng/mL, and the between-run CVs (N=30) were 5.5%, 3.5% and 2.9% at 0.3, 16 and 101 ng/mL; the detection limit of the test was 0.04 ng/mL. The calibration curve was linear over the range of 0.1-500 ng/mL.

Il-6 was measured by a solid phase enzyme immunometric assay (ELISA) in a microplate format (Milenia). The reference range was less than 41 pg/mL. The within-run CVs (N=20) were 3.6%, 2.9% and 2.2% at 25, 45 and 210 pg/mL, and the between-run CVs were (N=20) 4.5%, 3.6% and 2.9% at 30, 50 and 180 pg/mL; the detection limit of the test was 1.2 pg/mL. The assay has a calibration range up to 1,000 pg/mL; samples were diluted and re-assayed when IL-6 exceeded that concentration.

All data are reported as mean ± standard deviation (SD), or median and quartiles when appropriate. Statistical significance was tested with Student's t-test and the analysis of variance (ANOVA); the Scheffe's multiple comparison test was used if variables were normally distributed, while nonparametric tests were used for data that were not.

To assess the relationships between biochemical markers and their time courses, we used a general linear mixed model (GLM) for repeated measures based on each single patient. The model took into account the effect of time as a within-subject factor, with patients fitted as random, so we could correct for differences in the size of the treatment effect.

We compared the performance of the day-by-day infection classifications (test) with the *a posteriori* classification (standard) using a 2x2 contingency table. We tested the ability of each biochemical marker to detect on a daily basis either a severe sepsis-septic shock state vs. a less severe infectious state or sepsis *vs.* SIRS not-related to infection, colonization or the lack of SIRS by building a univariate logistic mixed model for repeated measures. Then, we built a multivariate mixed model using every marker as a covariate. We assessed how well each model predicted the dependent variable (severe sepsis-septic shock or sepsis) by generating and comparing the receiver operating characteristic (ROC) curves. The best threshold value with 95% confidence intervals for each marker was defined as the one that maximized the sum of sensitivity and specificity and the percentage of correctly classified patients.

We considered p-values less than 0.05 to be statistically significant. Analysis was done using Stata Statistical Software, release 9.2 (Stata Corporation, College Station, TX, USA).

## Results

During the study period, 276 patients were admitted. Of these, 30 fulfilled the admission criteria, and 26 had a LOS longer than six days (range 7-58 with overall 592 ICU days); the stored sera of these patients were analyzed for biomarkers. Table I summarizes their clinical characteristics. Eighteen patients were admitted with proven bacterial infection, one with acute

TABLE I.—*Demographics, clinical characteristics and profile of biochemical markers of the case-mix.*

| Patients | Overall (N.=26) | Never SS-SH (N.=7) | SS-SH recovered (N.=14) | SS-SH not recovered (N.=5) | P value |
|---|---|---|---|---|---|
| At ICU admission | | | | | |
| Age (years) | 61.0±13.9 | 66.3±8.0 | 57.2±15.2 | 64.4±15.7 | 0.3207[a] |
| Male sex | 12 (46.2%) | 4 (57.1%) | 4 (28.6%) | 4 (80%) | 0.1120[b] |
| Admission from | | | | | 0.407[b] |
| Other hospital | 3 (11.5%) | 0 | 3 (21.4%) | 0 | |
| Surgical ward | 7 (26.9%) | 1 (14.3%) | 5 (35.7%) | 1 (20%) | |
| Medical ward | 10 (38.5%) | 3 (42.9%) | 4 (28.6%) | 3 (60%) | |
| Emergency | 6 (23.1%) | 3 (42.8%) | 2 (14.3%) | 1 (20%) | |
| *Diagnosis:* | | | | | 0.180[b] |
| Pulmonary infection | 13 (50%) | 1 (14.3%) | 8 (57.1%) | 4 (80%) | |
| Peritonitis | 2 (7.7%) | 1 (14.3%) | 1 (7.1%) | 0 (0%) | |
| Pyelonephritis | 1 (3.8%) | 0 (0%) | 1 (7.1%) | 0 (0%) | |
| Septic gangrene | 1 (3.8%) | 0 (0%) | 1 (7.1%) | 0 (0%) | |
| Mediastinitis | 1 (3.8%) | 0 (0%) | 1 (7.1%) | 0 (0%) | |
| Acute pancreatitis | 3 (11.5%) | 0 (0%) | 2 (14.3%) | 1 (20%) | |
| Myocardial infarction | 3 (11.5%) | 3 (42.9%) | 0 (0%) | 0 (0%) | |
| Thoracic trauma | 1 (3.8%) | 1 (14.3%) | 0 (0%) | 0 (0%) | |
| ARDSd | 1 (3.8%) | 1 (14.3%) | 0 (0%) | 0 (0%) | |
| Admission in SS/SH | 17 (65.4%) | 0 (0%) | 12 (85.7%) | 5 (100%) | |
| Admission type: | | | | | 0.538[b] |
| Medical | 20 (76.9%) | 6 (85.7%) | 10 (71.4%) | 4 (80%) | |
| Surgical Unscheduled | 6 (23.1%) | 1 (14.3%) | 4 (28.6%) | 1 (20%) | |
| SAPS II (points) | 51.3±16.9 | 46.4±17.0 | 49.5±16.2 | 60.8±17.2 | 0.2772[a] |
| SOFA (points) | 6.2±2.6 | 4.1±1.5 | 6.6±2.0 | 8.0±3.9 | 0.0236[a] |
| WBC (n/mm3) | 17.4±11.3 | 15.4±4.6 | 17.3±13.5 | 20.6±12.4 | 0.7421[a] |
| PLT (n/mm3) | 192.6±148.9 | 216.8±62.2 | 197.9±189.6 | 153.6±63.7 | 0.7966[a] |
| CRP (mg/dL) | 134.7 (22.2-215.4) | 127.8 (15.3-178.1) | 210.8 (42.4-235.8) | 89.6 (22.2-179.7) | 0.4088[c] |
| PCT (ng/mL) | 2.3 (1.1-12.3) | 2.2 (0.4-3.2) | 9.1 (1.6-19.1) | 2.0 (0.1-3.4) | 0.3938[c] |
| IL-6[e] (pg/mL) | 369.5 (207.0-816.5) | 185.3 (134.8-210.4) | 507.8 (310.4-816.5) | 847.3 (304.4-943.1) | 0.0482[c] |
| Ventilatory support | 26 (100%) | 7 (100%) | 12 (100%) | 5 (100%) | 1[b] |
| Vasoactive drugs | 15 (57.7%) | 3 (42.8%) | 8 (57.1%) | 5 (100%) | 0.430[b] |
| ICU STAY – days | 592 | 163 | 340 | 89 | |
| LOS (days) | 26.2±16.8 | 29.7±19.6 | 27.1±16.5 | 18.6±14.3 | 0.5225[a] |
| cLOS (days) | 20.4±14.6 | 22.3±15.0 | 20.1±15.5 | 18.6±14.3 | 0.0955[a] |
| Worst SOFA (points) | 8.8±4.2 | 5.8±2.7 | 7.9±2.1 | 13.9±4.1 | <0.0001[a] |
| ICU mortality | 8 | 1[d] | 2[f] | 5[g] | |
| Hospital mortality | 9 | 1 | 3 | 5 | |

SAPS II: Simplified Acute Physiology Score II; SOFA: Sequential Organ Failure Assessment; WBC: White Blood Cell count; PLT: platelets count; PCT: procalcitonin; IL-6: interleukin-6; CRP: C-reactive protein; ICU: Intensive Care Unit; LOS: length of stay; cLOS: critical LOS i.e. days with invasive organs support; SS-SH: Severe sepsis or septic shock; ARDS, Acute Respiratory Distress Syndrome. Differences among the three groups of patients [a] ANOVA; [b] Chi square; [c] Kruskal-Wallis. [d] At autopsy: neoplastic alveolar infiltration. [e] measured on 16 patients. [f] ICU death after recover from SS-SH: 1 haemoptysis and 1 bronchopleural fistula. [g] ICU death for septic related organ failure.

non-pneumonia respiratory insufficiency, three with acute pancreatitis, three with cardiogenic shock and one with thoracic trauma. Patients significantly differed in SOFA scores and IL-6 at admission. During the ICU stay, seven patients had sepsis but never severe sepsis or septic shock; five were admitted and died with sepsis, while the remaining fourteen recovered from their severe sepsis or septic shock. Later, two patients died from pulmonary complications. The ICU days stratified according to the re-evaluated SIRS classification are displayed in Table II.

The discriminative ability of the infection diagnosis for the classification made day-by-day by the attending physician (test) compared with the *a posteriori* overall clinical classification at the patient's discharge (standard) for 592 patient days is shown in Table III. The infectious state assessed *a posteriori* resulted in a 73% decrease in days where the patient was judged to be without inflammation

TABLE II.—*Medians and interquartile ranges of C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6) of days stratified "a posteriori "in groups of systemic inflammatory response.*

| | Days 592 (100%) | CRP (<5 mg/dL) | PCT (<0.5 ng/mL) | IL-6[b] (<41 pg/mL) |
|---|---|---|---|---|
| No-SIRS | 36 (6.1%) | 70.1 (2.2-100.3) | 0.25 (0.14-0.46) | 32.8 (1.9-131.7) |
| Colonized | 235 (39.7%) | 62.9 (41.7-97.2) | 0.13 (0.08-0.25) | 80.0 (55.1-106.1) |
| SIRS | 14 (2.4%) | 49.5 (20.4-118.1) | 0.47 (0.26-0.90) | 105.1 (79.8-114.6) |
| Sepsis | 163 (27.5%) | 102.6 (44.0-184.6) | 0.46 (0.20-0.91) | 134.7 (77.9-197.7) |
| Severe sepsis | 97 (16.4%) | 163.1(72.0-239.7) | 3.66 (1.79-10.03) | 203.9 (105.6-289.3) |
| Septic shock | 47 (7.9%) | 88.5 (44.0-182.9) | 4.33 (2.52-15.72) | 352.7 (239.2-685.6) |
| P value[a] | | 0.0001 | 0.001 | 0.001 |
| SIRS/colonized/No-SIRS | 285 (48.1%) | 63.4 (39.9-99.9) | 0.14 (0.09-0.34) | 79.9 (38,9-108.7) |
| Sepsis | 163 (27.5%) | 102.6 (44.0-184.6) | 0.46 (0.20-0.91) | 134.7 (77.9-197.7) |
| Severe sepsis septic shock | 144 (24.3%) | 137.3 (66.4-227.4) | 4.10 (1.86-11.9) | 235.2 (123.7-363.7) |
| P value[a] | | <0.001 | <0.001 | <0.001 |

SIRS: systemic inflammatory response syndrome; CRP: C-reactive protein; PCT: procalcitonin; IL-6: interleukin-6, with reference range values. [a] Kruskal-Wallis test; [b] Measured on 332 days: 30 No SIRS, 141 Colonized, 11 SIRS, 58 sepsis, 71 severe sepsis and 21 septic shock from the last 16 out of 26 patients.

TABLE III.—*Discriminative ability for severe sepsis-septic shock, for sepsis and for SIRS/Colonized/No-SIRS of the classification made day-by-day by the attending physician (test) compared with the "a posteriori" classification at patient's discharge (standard) in 592 patient's days. Each status is compared with the remaining two.*

| | 592 study days | Sensitivity (95% CI) % | Specificity (95% CI) % | Correct classification (95% CI) % | Likelihood ratio + (95% CI) | Likelihood ratio (95% CI) |
|---|---|---|---|---|---|---|
| Severe sepsis-septic shock | 144 | 68.7 (62.2-75.1) | 98.0 (96.6-99.4) | 88.2 (85.6-90.8) | 33.8 (16.9-67.6) | 0.32 (0.26-0.39) |
| Sepsis | 163 | 57.1 (49.5-64.7) | 80.0 (76.2-83.8) | 73.7 (70.1-77.2) | 2.8 (2.3-3.6) | 0.54 (0.45-0.65) |
| SIRS/Colonized/No-SIRS | 285 | 69.1 (63.8-74.5) | 94.1 (91.5-96.8) | 82.1 (79.0-85.2) | 11.8 (7.5-18.6) | 0.33 (0.28-0.39) |

TABLE IV.—*A posteriori SIRS classification and courses of antibiotic treatment.*

| Antibiotic courses | Number | No-SIRS | Colonised | SIRS | Sepsis | Severe sepsis | Septic shock |
|---|---|---|---|---|---|---|---|
| 1st day of 1st course | 26 | 1 (3.9%) | 1 (3.9%) | 2 (7.7%) | 5 (19.2%) | 7 (26.8%) | 10 (38.5%) |
| Last day (alive patients) | 35 | 3 (8.6%) | 21 (60.0%) | 2 (5.8%) | 8 (22.8 %) | 1 (2.8%) | — |
| 1st day of new course | 16 | 1 (6.2%) | 7 (43.8%) | 1 (6.2%) | 5 (31.2%) | 2 (12.5 %) | — |

and infection, a 27% decrease in days where the patient was classified as having severe sepsis and septic shock, and a net 9% decrease in days where the patient was classified as having sepsis. However, we found a three-fold increase in the days with proven infection, but without SIRS, re-classified as "colonized". In short, several severe septic or septic shock days turned into days classified as sepsis, while several sepsis and No-SIRS or No-infection days were reclassified as colonization.

All patients received antibiotics. Of the 26 patients included in the study, 23 received antibiotics from ICU admission, and 42 antibiotic treatment courses were recorded and analyzed. A total of 16 patients received only one course of antibiotic therapy, six received two, and four received more than two. The mean length of antibiotic course was 10.5±5.7 days. The *a posteriori* classification at the start of the first antibiotic course, at the last day of the 35 antibiotic courses in patients who survived and at the first day of the 16 additional antibiotic courses are in Table IV.

TABLE V.—*Inflammatory markers and antibiotic treatment.*

| Treatment | Courses number | CRP (<5 mg/dL) | PCT (<0.5 ng/mL) | IL-6 (<41 pg/mL) |
|---|---|---|---|---|
| 1st day antibiotic | 42 | 135.0 (42.4-210.8) | 1.28 (0.20-3.40) | 164.9 (98.7-484.3) |
| Last day antibiotic | 42 | 53.4 (36.1-90.9) | 0.26 (0.09-0.48) | 70.4 (42.1-122.3) |
| P value[a] | | 0.016 | 0.0007 | 0.0008 |
| Antibiotic courses | 42 | 86.9 (49.4-166.8) | 0.47 (0.15-2.21) | 119.5 (77.1-232.5) |
| Between antibiotic courses | 16 | 56.6 (31.8-104.6) | 0.17 (0.09-0.62) | 74.3 (1.9-94.6) |
| P value[a] | | < 0.001 | < 0.001 | < 0.001 |
| Lowest inter-course value | 16 | 42.5(30.0-58.0) | 0.11(0.08-0.34) | 60.2(51.4-81.5) |
| 1st day recycle | 16 | 105.4 (30.0-159.9) | 0.34 (0.21-0.81) | 108.72(94.6-137.6) |
| P value[a] | | 0.0059 | 0.0039 | 0.0251 |

CRP: C-reactive protein; PCT: procalcitonin; IL-6: interleukin-6 with their reference ranges. [a] Wilcoxon rank sum test.

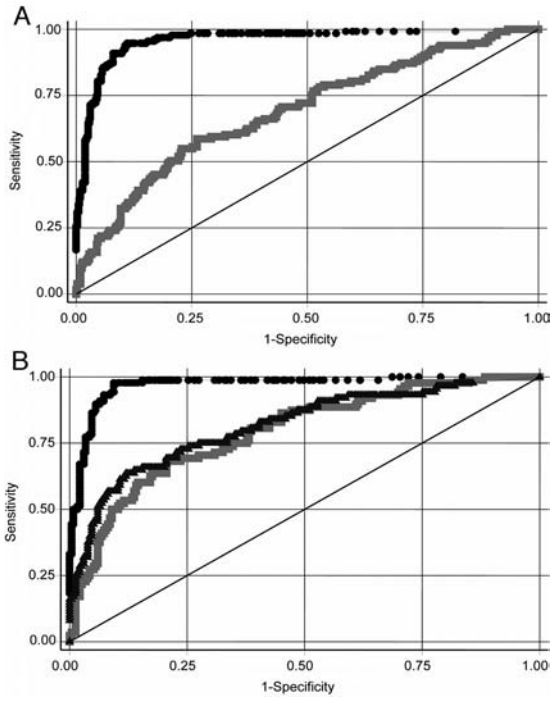


Figure 1.—Prediction of severe sepsis-septic shock by *a posteriori* analysis. Areas under the ROC curves. A) C-reactive protein (squares) 0.682 (0.629-0.735) and procalcitonin (circles) 0.952 (0.931-0.973) for 26 patients and 592 patient days, P<0.0001; B) C-reactive protein (squares) 0.792 (0.736-0.847), procalcitonin (circles) 0.970 (0.951-0.989) and IL-6 (triangles) 0.814 (0.759-0.870) in 332 patient days and the last 16 patients, P<0.0001. With day-by-day classification (data not shown in figure), ROC areas were 0.590 (0.536-0.639) for CRP, 0.824 (0.796-0.869) for PCT (592 days) and 0.753 (0.707-0.819) for IL-6 (332 days), P<0.0001.

In patients with infection but who never experienced severe sepsis or septic shock, CRP, PCT and IL-6 levels dropped with time (GLM within group: CRP -2.362 mg/dL/day, P<0.0001; PCT -0.027 ng/mL/day, P=0.001; IL-6 -2.212 pg/mL/day, P=0.015). Those who recovered from severe sepsis or septic shock showed a reduction in the markers; however, this reduction was only significant for CRP (mean reduction -4.320 mg/dL/day, P<0.0001) and PCT (-0.391 ng/mL/day, P=0.001). None of the markers fell in patients who died in sepsis.

Values of inflammatory markers at the start and at the last day of antibiotic treatment courses, the average daily values during treatment and during the period without antibiotics, and the values on the day before additional antibiotic treatment courses are shown in Table V.

Inflammatory markers significantly rose from No-SIRS to septic shock days.

Overlapping among levels, due to high variability, was consistent, apart from PCT values of days classified as sepsis when compared to those of the more severe septic strata (Table II). The predictive diagnostic discriminative ability of each marker for days with severe sepsis-septic shock *vs*. days without severe sepsis–septic shock is shown in Figure 1. Including more than one inflammatory marker did not improve the discriminative model.

The ability of the markers to discriminate between sepsis and SIRS not-related to infection, colonization and No-SIRS patient's days (*a posteriori* analysis) was 0.631 (0.572-0.689) for CRP, 0.737 (0.687-0.787) for PCT (assessed in 448 days) and 0.720 (0.645-0.795) for IL-6 (240 days). Adopting the day-by-day classification, areas under the ROC curves were as follows: 0.608 (0.551-0.665) for CRP, 0.575 (0.516-0.634) for PCT

TABLE VI.—*Discriminative ability (a posteriori classification) for severe sepsis-septic shock (upper panel: all days), and for sepsis (lower panel) severe sepsis-shock days excluded) of C-reactive protein (CRP), procalcitonin (PCT), and interleukin-6 (IL-6).*

| | Study days | Best threshold | Sensitivity (95% CI) % | Specificity (95% CI) % | Correct classification (95% CI) % | Likelihood ratio + | Likelihood ratio – |
|---|---|---|---|---|---|---|---|
| *Severe sepsis-septic shock* | | | | | | | |
| CRP | 592 | 99.0 | 59.4 (55.5-63.4) | 65.5 (61.7-69.3) | 64.0 (60.2-67.9) | 1.7 (1.4-2.1) | 0.6 (0.5-0.8) |
| PCT | 592 | 1.58 | 83.7 (80.7-86.7) | 94.6 (92.7-96.4) | 91.9 (89.7-94.1) | 15.4 (10.3-23.1) | 0.2 (0.1-0.2) |
| IL-6 | 332 | 192.4 | 63.0 (57.8-68.2) | 88.7 (85.3-92.1) | 81.6 (77.5-85.8) | 5.6 (3.8-8.3) | 0.4 (0.3-0.5) |
| *Sepsis* | | | | | | | |
| CRP | 448 | 95.6 | 53.5 (48.9-58.1) | 73.4 (69.4-77.5) | 66.2 (61.8-70.6) | 2.0 (1.6-2.6) | 0.6 (0.5-0.8) |
| PCT | 448 | 0.43 | 61.8 (57.3-66.3) | 81.6 (78.0-85.2) | 74.7 (70.7-78.7) | 3.4 (2.6-4.4) | 0.5 (0.4-0.6) |
| IL-6 | 240 | 105.1 | 65.5 (59.4-71.6) | 72.5 (66.8-78.3) | 70.8 (65.0-76.7) | 2.4 (1.8-3.2) | 0.5 (0.5-0.7) |

(assessed for 394 days) and 0.654 (0.577-0.730) for IL-6 (assessed for 213 days).

The best thresholds (*a posteriori* analysis) dividing severe sepsis-septic shock from other diagnoses (sepsis, SIRS not-related to infection, colonization and No-SIRS), and sepsis from SIRS not-related to infection, colonization and No-SIRS are shown in Table VI.

## Discussion

Diagnosis of infection and sepsis is a subjective clinical judgment based on the presence of the criteria for systemic inflammatory reaction, which, albeit highly sensitive, is not sufficiently specific and is often misleading in intensively treated patients. For example, fever could be the result of a variety of factors, such as skin hypoperfusion, drug use or an excessive amount of calorie substrates, and heart and respiratory rate can vary based on non-sepsis related factors, such as respiratory support and sedation level. Moreover, microbiological results, which are determinant for the diagnosis of infection on ICU admission, but are much less specific in patients with long-term ICU stays, are often not available when infection is suspected. Therefore, diagnosis of full-blown sepsis at ICU admission is relatively easy, while assessment for the presence and level of an inflammatory/infectious state is much more difficult when a patient, treated with multiple organ system support, shows new symptoms that may possibly be due to superinfection. Moreover, it is also difficult to appreciate the backward shift to sepsis or to SIRS when the septic state improves, as the patient would still present with residual organ failure, a need for inotropic or vasoactive drugs or show a rising bilirubin. In this scenario, the usual diagnostic criteria frequently fail.

"Objective" biomarkers, determined daily in a prospective manner throughout the patient's entire ICU stay,[6, 19] would be particularly helpful in this scenario as a part of an overall clinical assessment.[7] Measurable criteria could allow for better insight into the changes in infection states during the evolution of a patient's disease and would aid in the appropriate and timely clinical diagnosis of presence/levels of sepsis.[4, 9-13, 15, 20-23] This would improve specific therapeutic decision-making in clinical practice.[24] In the retrospective analysis assessing the diagnostic value of CRP, PCT and IL-6, rather than the prospective clinical diagnosis made day-by-day at the bedside, we chose as the reference standard an *a posteriori* classification of each study day. The knowledge of the overall evolution of organ failure, infectious complications and other confounding factors, even if not feasible in clinical practice, allows for the most appropriate infectious classification of the patient day and is the best reference for investigating the various markers' contribution to the grading of an infectious inflammatory reaction.

We tested this approach in the most severe medical and surgical critically ill patients. These patients were characterized by a wide spectrum of diseases, organ failure, levels of infection that resulted in a long ICU stay that was spent, primarily, providing support for vital functions,[16] persistent or recurrent infections and substantial mortality. At ICU admission, seventeen patients had signs and symptoms of severe sepsis or septic shock, and only five had a non-infective illness, while, during the ICU stay,

all developed sepsis and only seven never met the diagnostic criteria for severe sepsis-septic shock (Table I).

Among the 592 collected patient days, half received an *a posteriori* classification of sepsis and severe sepsis or septic shock, which was equal to the day-by-day bedside classification that they received on the ward. Almost all of the remaining non-septic days were classified as colonization, with only 12% being classified as No-SIRS and very few as SIRS days (Table II). The comparison of the daily bedside classification with the *a posteriori* best classification of the overall clinical evolution, quantified an obvious misclassification in the day-by-day approach. Misclassification in the diagnosis of the most and less severe levels of infection was low. Both levels were associated with low sensitivity and good specificity. In such cases, it may be easier to exclude than to make a precise diagnosis, which is likely due to the presence of confounding factors. However, we found a much higher degree of misclassification in distinguishing between sepsis (a diagnosis with low sensitivity and low specificity) and the less severe scenarios, mainly composed of colonization days (Table III). More specifically, several severe septic or septic shock days turned into sepsis, perhaps because residual organ failures or supportive care were ascribed to sepsis in progress. Several sepsis (a misleading interpretation of variables from the SIRS definition in the presence of positive cultures) and No-SIRS-No-infection days (based on the microbiological results available after the physician's diagnosis) turned into colonization. In particular (Table IV), in patients re-defined as being without clinical signs of SIRS or positive cultures, over-triage could explain very few treatment decisions out of the first antibiotic treatments (only four courses started without clinical signs of sepsis). Indeed, 56% of the additional antibiotic treatment courses started without signs of sepsis! On the other hand, a quarter of the first antibiotic treatment courses were stopped when there was a possibility that the patient might still have been in sepsis. Should the antibiotic treatment have been prolonged until bacterial eradication? These findings highlight that the day-by-day clinical differentiation of levels of sepsis, particularly in long-stay ICU patients with persistent or recurrent infections and with concomitant, secondary or independent, organ failure, is difficult.

As expected, the time-course for the level of all three biomarkers mirrored the clinical trend; they declined with time during the ICU stay in patients who were never severely septic and in those who recovered from severe sepsis (PCT and CRP only), while the values remained high in patients who subsequently died. Accordingly, antibiotic treatment reduced the very high levels of the biomarkers by 60-70% (but only PCT was normalized). After antibiotic withdrawal, the levels continued to decrease (particularly for PCT). Of note, the average values on the first day of the additional antibiotic treatment course were significantly higher than the lowest values reached in the preceding period without antimicrobial treatment, confirming the likely shift from no-infection or colonization to re-infection in at least 44% of the patients who had a clinical diagnosis of sepsis at the start of the new treatment (Table V).

As previously reported in children [14] and adults,[10-12] the median values of the three markers differed significantly among the levels of infection severity, but with wide and overlapping interquartile ranges, indicating the scant clinical utility of these differences across groups. By contrast, PCT showed no overlap between interquartile ranges for sepsis and severe sepsis values. A PCT value of 0.91 ng/mL was actually able to separate the two groups of days (Table II).

At variance with authors who pooled days with sepsis, severe sepsis and septic shock [9-15], we considered it more useful to draw a line between severe sepsis and the less severe scenario and a line between sepsis and SIRS, colonization or No-SIRS. The predictive diagnostic ability for severe sepsis-septic shock (*a posteriori* assessment: Figure 1) was poor for CRP, fine for IL-6 (ROC area 0.81, comparable to 0.82 for pooled postoperative sepsis [9]) and excellent for PCT (ROC area 0.95). Of note, the markers' diagnostic ability was more accurate with the *a posteriori* classification than with the day-by-day one. Our best threshold value (*a posteriori* analysis) for PCT (≥1.59 ng/mL, satisfactory sensitivity, high specificity, correct classification and positive likelihood ratio with low negative likelihood ratio) eventually coupled with IL-6 (≥192.4 pg/mL) strongly supports a clinical

diagnosis of severe sepsis-septic shock (Table VI). The PCT cut-off was in the range of those reported (from 1.1 to 34 ng/mL [9, 10, 12-15, 23]) for the so-called "sepsis group", a mix of sepsis, severe sepsis and septic shock days, whose wide range depends on the composition of the three different clinical conditions that cover a broad range of severity.

On the contrary, in our case-mix, CRP was completely unreliable in distinguishing sepsis from colonization (82% of days) and non-septic days; PCT and IL-6 had low diagnostic accuracy with a best cut-off of ≥0.43 ng/mL and ≤105 ng/mL, respectively. Low diagnostic accuracy in separating the sepsis from no-sepsis scenario was recently described for PCT in a general critical care case-mix,[7] probably because PCT (and perhaps IL-6 levels) in surgery, trauma, pancreatitis and acute left heart failure are increased by factors other than bacterial infection,[4, 6, 7] as well as in patients still in ICU after the resolution of a primary septic episode.[25] Both of those scenarios were evident in our case mix.

Hence, in critically ill adult patients, PCT, more than CRP or IL-6 offers an effective contribution to the day-by-day clinical classification of a sepsis state, as a part of an overall clinical assessment, and may aid in therapeutic decision-making. PCT values <0.43 ng/mL moderately support SIRS not-related to infection or colonization rather than sepsis, while values ≥0.43 moderately confirm the likelihood of a clinical diagnosis of sepsis. Values ≥1.58 ng/mL offer strong support for the bedside clinical diagnosis of severe sepsis. A gray area concerning decision-making exists in between the last two values. A PCT value of approximately 1 ng/mL, at least in this specific context, was suggested as the threshold to safely discontinue antibiotic therapy when associated with improved clinical signs and symptoms of infection.[26] However, 0.91 ng/mL, our upper interquartile value for sepsis, was very different from the lower interquartile for severe sepsis (1.79 ng/mL, Table II). The value of 1.0 ng/mL, even if not the best cut-off for severe sepsis-septic shock, is associated with a 90.2% (87.8%-92.6%) correct classification rate, good sensitivity (92.9% [90.8%-95.0%] ) and specificity (89.4% [86.9%-91.8%]), and a moderate likelihood of correct severe sepsis-septic shock classification (Likelihood ratio +: 8.74 [5.0-15.3], Likelihood ratio -: 0.08 [0.06-0.1]). Therefore, values of 1 ng/mL could add to the clinical propensity for severe sepsis. However, in cases of clinical uncertainty associated with PCT values below 1 ng/mL, the tight daily monitoring of clinical conditions and PCT may be useful in making correct treatment decisions at the appropriate time.

## Conclusions

Choosing an *a posteriori* re-evaluation of overall clinical evolution (based on ACCP-SCCM classification) as a standard reference for sepsis in a complicated ICU case mix allowed us to quantify the inevitable misclassification made at the bedside in day-by-day evaluations. Furthermore, we could compare the diagnostic performance of biomarkers, with the aim of incorporating biomarkers in the overall clinical assessment of patients admitted to the ICU. Diagnostic performance was weak for CRP, intermediate for IL-6 and acceptable for PCT.

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