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ORIGINAL

# Circulating biomarkers may be unable to detect infection at the early phase of sepsis in ICU patients: the CAPTAIN prospective multicenter cohort study

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## Abstract

**Purpose:** Sepsis and non-septic systemic inflammatory response syndrome (SIRS) are the same syndromes, differing by their cause, sepsis being secondary to microbial infection. Microbiological tests are not enough to detect infection early. While more than 50 biomarkers have been proposed to detect infection, none have been repeatedly validated.

**Aim:** To assess the accuracy of circulating biomarkers to discriminate between sepsis and non-septic SIRS.

**Methods:** The CAPTAIN study was a prospective observational multicenter cohort of 279 ICU patients with hypo- or hyperthermia and criteria of SIRS, included at the time the attending physician considered antimicrobial therapy. Investigators collected blood at inclusion to measure 29 plasma compounds and ten whole blood RNAs, and—for those patients included within working hours—14 leukocyte surface markers. Patients were classified as having sepsis or non-septic SIRS blindly to the biomarkers results. We used the LASSO method as the technique of multivariate analysis, because of the large number of biomarkers.

**Results:** During the study period, 363 patients with SIRS were screened, 84 having exclusion criteria. Ninety-one patients were classified as having non-septic SIRS and 188 as having sepsis. Eight biomarkers had an area under the receiver operating curve (ROC-AUC) over 0.6 with a 95% confidence interval over 0.5. LASSO regression identified CRP and HLA-DRA mRNA as being repeatedly associated with sepsis, and no model performed better than CRP alone (ROC-AUC 0.76 [0.68–0.84]).

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Minou Adib-Conquy: Deceased.
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**Conclusions:** The circulating biomarkers tested were found to discriminate poorly between sepsis and non-septic SIRS, and no combination performed better than CRP alone.

**Keywords:** Sepsis, Cohort, CRP, HLA-DRA mRNA

## Introduction

The systemic inflammatory response syndrome (SIRS) is a generalized inflammatory response in organs remote to an initial insult. Infection is caused by the invasion of normally sterile tissue, fluid, or body cavity by a potentially pathogenic microorganism [1]. When the insult causing SIRS is an infection, it is called sepsis [2]. Sepsis affects about 18 million people a year and its mortality is estimated between 35% and 55% [3].

When infection is due to bacteria or fungi, urgent antimicrobial treatment is required to reduce mortality [4, 5]. Optimization of early antimicrobial agent requires one to confirm infection as soon as patients present clinical severity, such as a high quick SOFA score or serum lactate levels [6]. As clinical information alone is rarely sufficient to detect sepsis, many biomarkers have been studied, but few were consistently associated with infection in this context [7]. The most studied biomarkers were procalcitonin (PCT) [8], C-reactive protein (CRP), soluble triggering receptor expressed on myeloid cell-1 (sTREM1), neutrophil expression of the high-affinity immunoglobulin-Fc fragment receptor I (cluster of differentiation 64, CD64) [9–11], interleukin-6 (IL-6), IL-1 receptor antagonist (IL-1ra), pro-vasopressin (or copeptin), and pro-adrenomedullin (pro-ADM). CRP and PCT have been shown to help to decrease antibiotic use [8, 12]. To improve the diagnostic accuracy of these biomarkers, several [10, 13, 14] attempted to develop biomarker combinations but none have been repeatedly validated.

Discrepancies in the results of the published studies may be due to heterogeneity in infection locations, initial severity, pathogens, or populations studied [15]. A biomarker able to distinguish between sepsis and non-septic SIRS would help in reducing use of antibiotics and searching for other causes of the patients' vital dysfunctions [15].

The aim of our study was to assess, in intensive care unit (ICU) patients with SIRS, the accuracy of individual or combined circulating biomarkers to discriminate between sepsis and non-septic SIRS. Our hypothesis was that, by studying a large panel of markers, it would be possible to obtain a predictive model which performs better than those previously published, and specifically procalcitonin [16].

### Take-home message

The circulating biomarkers tested were found to discriminate poorly between sepsis and non-septic SIRS. No combination performed better than CRP alone.

## Methods

### Study design

We set up the CAPTAIN study (Combined Approach for The eArly diagnosis of INfection in sepsis) as an observational multicenter prospective cohort of ICU patients with SIRS criteria [1], as soon as they were considered for antibiotic therapy. We designed and conducted this study according to STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) guidelines [17] (electronic supplementary material (ESM) Table 1). The protocol was approved by the Comité de Protection des Personnes Ile de France XI (#2010-A00908-31-10056) on September 13, 2010 and registered as NCT01378169 on clinicaltrials.gov.

### Setting

Patients were recruited in seven ICUs located in five hospitals in the Paris area. They were included from December 2011 to April 2013. Blood samples for biomarkers were collected at inclusion. Blood samples for cell surface biomarkers were collected from those patients included when the research laboratory was open, owing to the necessity to perform extemporaneous analysis.

### Participants

ICU patients with hypothermia (below 36.0 °C) or hyperthermia (over 38.0 °C) and at least another criterion of SIRS were eligible as soon as the physician considered antibiotic therapy. Other inclusion criteria were age over 18 years, affiliation to the national health insurance system, no treatment limitation, and no underlying immunosuppression (AIDS, immunosuppressive therapy, ongoing malignancy, organ or bone marrow transplant, or drug-induced leukopenia). In a preliminary protocol, admission to the ICU was required to be less than 12 h (clinicaltrials.gov), but this criterion was abandoned before starting the study. Patient consent was collected if he/she was conscious and able to understand. The consent was waived in other situations.

#### Outcome definition: sepsis and non-septic SIRS diagnosis

Patients were classified as having sepsis in the presence of bacterial or fungal infection, or non-septic SIRS in other cases [1]. We called infections the diseases for which antibiotics were genuinely found to be useful. These diseases are a subgroup of the diseases for which antibiotics are recommended (i.e., suspicions of infections) and a larger group than the group of patients with bacteremia. For this reason, we had to refer to infections which are confirmed a posteriori, based on criteria which confirm infection as much as possible, either with or without positive cultures. A so-called gold standard for infection does not exist. In fact, as previously stated, the definition of infection and its causal link with SIRS required medical interpretation [18] and were based on Infectious Diseases Society of America (IDSA) guidelines. They were adjudicated blindly to the studied biomarkers, by two investigators (FP and BM). They reviewed the patients' records, including clinical history, results of routine morphologic, biological, or microbiological tests, and response to therapies during the days following inclusion. Among the biomarkers studied, CRP and PCT were not collected for routine purpose in two units (17.6% of the patients), were collected occasionally but were not considered as part of the diagnostic of sepsis in four units (71.7%), and were collected in routine and usual part of the sepsis diagnosis in one unit (10.8%). CRP and PCT were not provided to the two adjudicators. Strains were considered as infecting, colonizing, or contaminants. Infection could be considered as present despite the absence of a positive microbiological sample, e.g., in cases of abscess or pneumonia. When bacteremia was present, it was linked to the most probable anatomical focus of infection. Viruses were only searched for in case of influenza suspicion and were classified as non-septic SIRS. Disagreements on classification were resolved after discussion between the two adjudicators.

#### Study biomarkers

Fifty-three biomarkers were selected because they had been proposed in prior publications. Of these, 14 cell surface biomarkers were assessed only for samples collected and delivered to the laboratory during open hours. Blood samples were collected in PAXgene®, EDTA and heparinized tubes and transferred to Institut Pasteur's laboratory within 2 h after collection, in a cool bag. The methods of quantification were ELISA and multiplex analysis for 7 and 22 plasma biomarkers, respectively, real-time polymerase chain reaction (RT-PCR) for 10 whole blood RNA biomarkers, and flow cytometry for 14 cell surface biomarkers (ESM Table 2). The analytic methods are reported in ESM Section 2.5. The kits used, the limits of quantification, and the primer and probe designs for mRNAs are reported on ESM Tables 3, 4, and 5.

#### Clinical and routine biological data

Demographics, reasons for ICU admission, underlying diseases, simplified acute severity score (SAPS 2) [19], physiological data, Sequential Organ Failure Assessment (SOFA) score [20], and length of organ failure supply were collected at admission to the ICU, at inclusion in the study, and over the ICU stay.

#### Data management and statistics

##### *Sample size*

The area under the receiver operating characteristic curve (ROC-AUC) of PCT for sepsis diagnosis was reported to be 0.78 [95% CI 0.73–0.83] in 2007 [16]. We hypothesized that the ROC-AUC of the best combination tested in our population would reach 0.85. Three hundred patients needed to be included (150 in each group, type I error risk = 0.05, power = 0.80, correlation between the two ROC-AUCs = 0.4) to find a significant difference between the biomarker combination ROC-AUC and the ROC-AUC of PCT alone [21] under this hypothesis. As we could not be sure that the ratio of sepsis to non-septic SIRS patients would be 1:1 and as we expected missing data, we decided to screen 360 patients.

##### *Data analysis*

A statistical analysis plan (SAP) was prepared prior to data collection in the protocol. It included the selection of biomarkers associated with sepsis in bivariate analyses with a $p$ value < 0.2, followed by a multivariate logistic regression to identify the biomarkers independently linked with the outcome. This first analytical strategy identified CRP as the only biomarker being significantly linked with sepsis (data not shown). However, missing data were numerous and we decided to use a more powerful analytical strategy to handle the large number of biomarkers (logistic LASSO regression) alongside with missing data (multiple imputations) (ESM 2.7.2.1) [22]. Data were described using median [IQR] and frequency (%). We used three imputation methods for values below the lower limit of quantification (LLoQ), over the upper limit of quantification (ULoQ), and for missing data. Collinearity among continuous markers was assessed using Spearman correlation coefficient. The ROC-AUC was used to evaluate diagnostic accuracy of each continuous marker in univariate analyses. Rubin's rules [23] were applied to combine correlations, ROC-AUCs, and their 95% associated confidence intervals over the imputed data sets. A $p$ value of 0.05 was considered significant in all comparisons.

#### *Modeling strategy*

LASSO is a modelling method [22, 24] (ESM 2.7.3) used to select a subset of the strongest predictors associated with a given outcome, according to a shrinkage parameter $\lambda$. It includes the construction of multiple predictive train and test models by bootstrap, the determination and application of a $\lambda$ value giving the best compromise between high ROC-AUC values and low optimism, and the selection of the biomarkers that are the most frequently selected in the predictive models. Independent variables are entered altogether in the model, and a subset of them is selected as output. The number of selected variables depends on $\lambda$: the higher $\lambda$, the fewer variables selected. Optimism is observed when performances of a predictive model are estimated for samples that were used to build this model. Model coefficients are optimized to fit as much of the data used to train it as possible. It is expected that the performance of a model applied to new data that were not used to train the model will be lower, but more representative of the performance one can expect in real life. Additional methodological and statistical details can be found in the ESM.

## Results

### Participants

A total of 363 patients were screened and 279 were included. Non-inclusions were due mostly to refusal of consent or to absence of an inclusion criterion (Fig. 1). A total of 188 were classified as sepsis and 91 as non-septic SIRS. The adjudicators similarly classified 239 patients (85.6%), and 40 (14.3%) were classified by consensus after initial disagreement. All patients had exploration of plasma circulating biomarkers and 110 (77 sepsis and 33 non-septic SIRS patients) had exploration of cell surface biomarkers. These patients had a slightly higher severity at inclusion (SOFA score = 11 [9–14] versus 9 [9, 10]). While the number of inclusions was lower than expected in the non-septic SIRS group, we decided to assess the ROC-AUC of PCT, because it was the basis of our sample size calculation. The ROC-AUC of PCT was 0.55 [0.47–0.62], which was lower than expected [16]. This made our sample size estimate excessive and we decided to stop including patients.

Inclusion occurred 0 [0–1] days after ICU admission. Forty-two patients (15%, 34 sepsis and 8 non-septic SIRS) were included after day 2 (one patient was included on day 248). Patients' characteristics at ICU admission and at study inclusion are shown in Tables 1 and 2. The SAPS 2 score at ICU admission was 55 [50–61] in both groups. Temperature was higher and PaO2, serum lactate, and hematocrit were lower in sepsis than in non-septic SIRS patients. The causes of non-septic SIRS were mainly circulatory insufficiency or inflammatory states (ESM Table 7), and the anatomic locations of infection in sepsis patients were mainly lung and abdomen (ESM Table 8). The causal pathogens of these infections are provided in ESM Table 9. A combination of pathogens was found in 46 (24%) and neither bacterium nor fungus was found in 27 (14%) patients with sepsis, among whom 24 had lung and three had abdominal infections. The mortality of the two groups at the end of the hospital stay was similar (63/188 [33%] versus 28/91 [31%], $p = 0.54$). The ICU stay after inclusion and the hospital stay after ICU discharge were longer in the sepsis than in the non-septic SIRS patients (9 [4–22] versus 4 [2–11] days, $p < 0.001$, and 24 [9–48] versus 13 [7–24] days, $p < 0.001$).

### Biomarkers

#### *Univariate analyses*

Missing data ranged from 11% to 16% for the 39 plasma and RNA biomarkers in 279 patients, and from 3% to 54% for the 14 cell surface biomarkers available in 110 patients (ESM Table 10). Most missing data were due to insufficient volume of the blood sample. Thirteen plasma and three RNA biomarkers were significantly different between sepsis and non-septic SIRS patients: CRP, suPAR, PSP, G-CSF, IL-6, IL-8, IP-10, MIP-1α, MIP-1β, MMP8, PCT, and S100A9 were higher, while MIF, RANTES, HLA DR mRNA, and CD74 mRNA were lower. Four cell surface biomarkers were significantly different between sepsis and non-septic SIRS patients: CD64-Neutrophil-MFI, and MFI intraTLR4 in CD56Dim were higher, while HLA-DR on CD14 high and low were lower (ESM Table 2).

Figure 2 describes the ROC-AUC of the 28 biomarkers treated as quantitative data and computed using each of the three imputation methods. The different imputation methods did not impact the results and only eight markers had a ROC-AUC above 0.6 and a 95% confidence lower limit above 0.5. These were CRP (ROC-AUC = 0.73 [0.65–0.81]), HLA-DRA mRNA (0.65 [0.58–0.77]), pancreatic stone protein (PSP) (0.63 [0.54–0.71]), CD74 mRNA (0.62 [0.53–0.72]), metalloproteinase 8 (MMP8) (0.62 [0.54–0.70]), suPAR (= 0.62 [0.53–0.71]), IL-6 (0.60 [0.51–0.68]), and S100A9 (0.62 [0.50–0.67]).

#### *LASSO statistics*

Among the 12,500 predictive models computed, optimism between train and test samples was close to 0 for a $\lambda$ between 0.1 and 0.16 (ESM Fig. 4). ROC-AUCs decreased slightly when $\lambda$ increased from 0.1 to 0.16 (ESM Fig. 5). We chose a $\lambda = 0.1$ because it allowed the best compromise between ROC-AUC and optimism. For $\lambda = 0.1$, CRP was selected in the LASSO logistic regression model in 95–99% of the 500 samples and HLA-DRA mRNA was selected in 33–58% of the 500 samples,

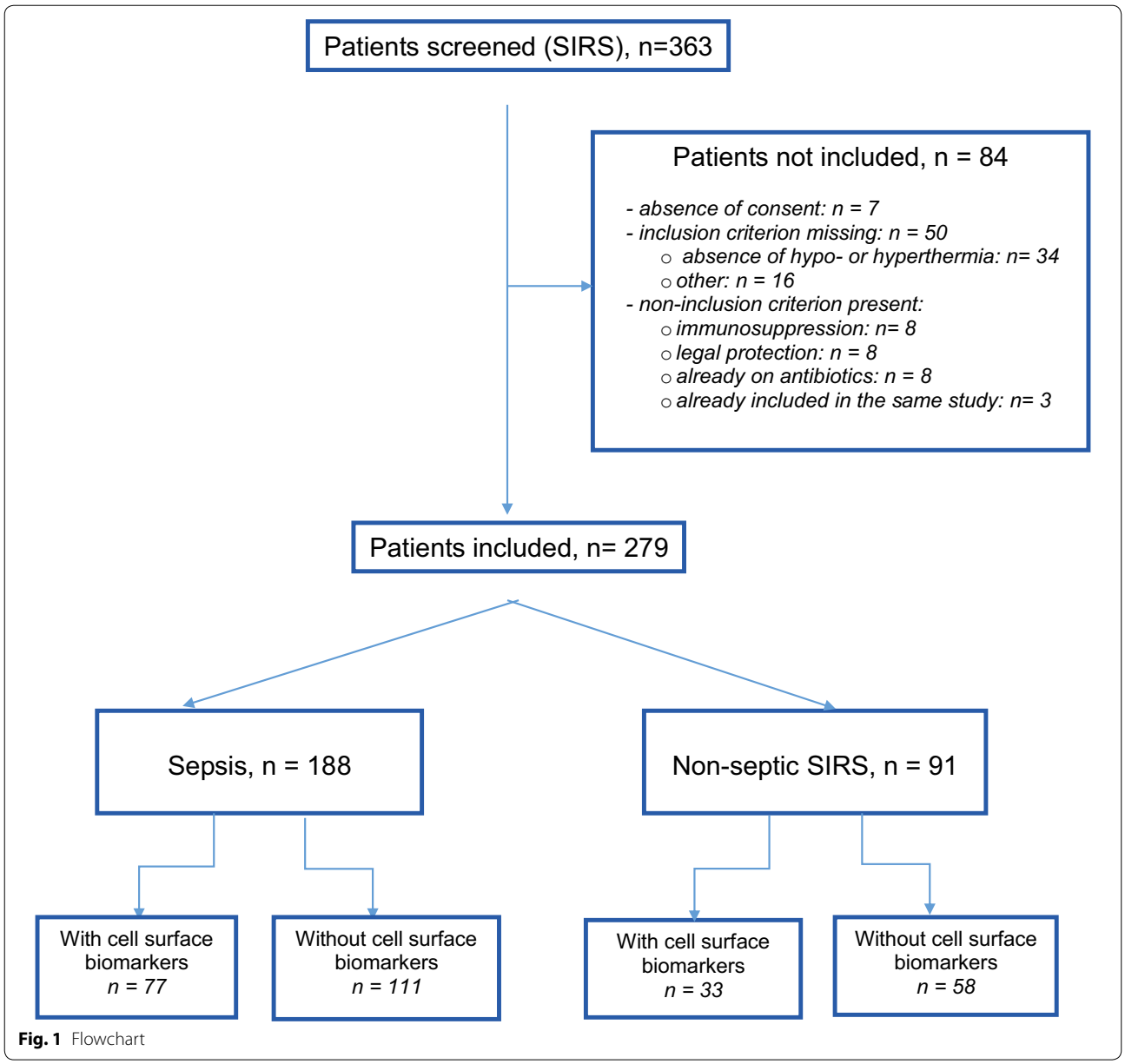


**Fig. 1** Flowchart

depending on the imputation method. None of the other markers were selected in more than 30% of the samples (ESM Fig. 6). The mean train ROC-AUC of the model was 0.76 [0.68–0.84] while mean test ROC-AUC was 0.72 [0.57–0.83], whatever the imputation method. The ROC-AUCs of two previously published combinations [25, 26] are displayed in Table E6, and another one [10] could not be assessed because of an insufficient number of values for one of the parameters (PMN CD64 index).

## Discussion

We assessed plasmatic and whole blood RNAs in a prospective multicenter cohort of 279 ICU patients with criteria of SIRS, and assessed cell surface biomarkers in 110 of these patients. Two-thirds of the patients were diagnosed as having sepsis and one-third as having non-septic SIRS, blindly to the results of the biomarkers. The ROC-AUC of PCT, which we had used as the reference value to calculate the sample size, was much lower in our series (median 0.55) than in Tang et al's meta-analysis (mean 0.78) [16]. This discrepancy may be due to differences in gold- standard in the individual studies [27]

**Table 1 Characteristics of the patients at ICU admission**

| | Sepsis $N=188$ | Non-septic SIRS $N=91$ | $p$ value | Missing values $n$ (%) |
|---|---|---|---|---|
| Age (years) | 64.7 [52.5–77.7] | 65.5 [51.0–78.8] | 0.98 | 0 |
| Male sex, $n$ (%) | 124 (66.0) | 55 (60.4) | 0.38 | 0 |
| SAPS 2 score | 55 [50–61] | 55 [50–61] | 0.81 | 0 |
| Reason for admission to ICU, $n$ (%) | | | 0.31 | 0 |
| Acute respiratory failure | 67 (35.6) | 30 (33.0) | | |
| Septic shock | 25 (13.3) | 0 (0.0) | | |
| Hypovolemic shock | 7 (3.7) | 3 (3.3) | | |
| Other shock | 8 (4.3) | 10 (10.1) | | |
| Convulsive state | 9 (4.8) | 8 (8.8) | | |
| Coma | 23 (12.2) | 16 (17.6) | | |
| Surgical abdomen | 7 (3.7) | 2 (2.2) | | |
| Other | 42 (22.3) | 22 (24.2) | | |
| Location of origin, $n$ (%) | | | 0.43 | 0 |
| Emergency department | 67 (35.6) | 29 (31.9) | | |
| Direct admission | 53 (28.2) | 37 (70.7) | | |
| Ward | 47 (25.0) | 18 (19.8) | | |
| Operating room | 12 (6.4) | 4 (4.4) | | |
| Other ICU | 9 (4.8) | 3 (3.3) | | |
| Underlying disease, $n$ (%) | | | | 2 (0.7) |
| Chronic obstructive pulmonary disease | 38 (20.2) | 12 (13.2) | 0.25 | |
| Cardiac insufficiency | 21 (11.2) | 11 (12.1) | 0.85 | |
| Diabetes | 40 (21.3) | 17 (18.7) | 0.88 | |
| Chronic renal insufficiency | 15 (8.0) | 9 (9.9) | 0.38 | |
| Cancer | 29 (15.4) | 8 (8.8) | 0.15 | |
| Hematological malignancy | 2 (1.0) | 0 (0.0) | 1.00 | |
| Chronic liver disease | 14 (7.4) | 6 (6.6) | 0.38 | |
| McCabe score, $n$ (%) | | | 0.18 | 3 (1.1) |
| 1—No fatal disease | 143 (76.1) | 75 (82.4) | | |
| 2—Estimated vital prognosis less than 5 years | 42 (22.3) | 15 (16.5) | | |
| 3—Estimated vital prognosis less than 1 year | 1 (0.5) | 0 (0.0) | | |

and to the populations studied. In fact, as procalcitonin is a marker of severity [28], high ROC-AUC may be due to lower severity [27] or mortality [27, 29] in non-septic SIRS patients from previous series, secondary to differences in inclusion criteria. We did not expect this result, which made our sample size estimate inadequate for this population. Therefore, we decided to stop inclusions as initially planned. The biomarker with the highest observed ROC-AUC to discriminate between sepsis and non-septic SIRS was CRP, and no combination of biomarkers was found to improve its diagnostic accuracy.

Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection [2]. In case of infection, early antibiotic therapy is associated with a reduction of mortality [4], while in the absence of infection the search for an alternate diagnosis must be aggressive to initiate appropriate therapy [30, 31]. This justifies searching discriminatory biomarkers available in routine use. Such biomarkers have been studied in prior studies. As a gold standard definition of infection does not exist, the groups may be heterogeneous across studies. We chose to include patients with SIRS, for whom the attending clinician was suspecting infection and considering antibiotic therapy. To initiate early therapy, such an a priori suspicion of sepsis was in accordance with the recommendations of the Surviving Sepsis Campaign [5]. While our study was not designed to evaluate compliance with these guidelines, differences in individual practices may have influenced the time of inclusion. We classified patients a posteriori using two blind adjudicators, and used conventional definitions [10, 30, 32]. Our series comprises 67% infected patients. Pneumonia was the predominant location of infection and the strains responsible for infections were predominantly Gram-negative bacteria, consistent with epidemiology in ICUs [33, 34]. Unlike prior series [10, 14], as measured with

**Table 2 Clinical and biological characteristics of the patients at study inclusion**

| Characteristics | Sepsis $N=188$ $n=188$ | Non-septic SIRS $N=91$ $n=91$ | *p* value | Missing values *n* (%) |
|---|---|---|---|---|
| Time from ICU admission to inclusion (days) | 0 [0–1] | 0 [0–1] | 0.05 | 0 |
| Clinical data | | | | |
| Temperature (°C) | 38.7 [38.2–39.3] | 38.6 [36.0–39.0] | 0.03 | 0 |
| Respiratory rate (breaths/min) | 27 [23–34] | 26 [21–32] | 0.19 | 15 (3.6) |
| Heart rate (beats/min) | 117 [100–132] | 112 [97–132] | 0.42 | 10 (3.7) |
| Mean arterial pressure (mmHg) | 67.0 [55.5–84.0] | 70.0 [58.5–83.5] | 0.46 | 3 (1.1) |
| Urine output (mL/24 h) | 1250 [700–1970] | 1110 [600–2000] | 0.64 | 14 (5.2) |
| Biological data | | | | |
| PaO2 (mmHg) | 87 [70–114] | 98 [77–134] | 0.02 | 14 (5.2) |
| FiO2 (%) | 40 [30–60] | 45 [30–60] | 0.35 | 14 (5.2) |
| PaCO2 (mmHg) | 40 [34–47] | 40 [34–45] | 0.44 | 14 (5.2) |
| Serum lactate (meq/L) | 1.8 [1.2–2.5] | 2.1 [1.4–5.3] | 0.03 | 66 (24.5) |
| Serum creatinine (mg/L) | 11 [7–22] | 13 [8–22] | 0.13 | 10 (3.7) |
| Blood leukocytes ($\times 10^9$/mL) | 12.7 [9.2–18.6] | 13.6 [9.1–17.0] | 0.92 | 10 (3.7) |
| Blood platelets ($\times 10^9$/L) | 198 [122–287] | 196 [126–269] | 0.86 | 12 (4.5) |
| Hematocrit (%) | 31.0 [27.8–37.4] | 36.0 [29.6–41.6] | 0.001 | 15 (5.6) |
| SOFA score | 9 [8–10] | 9 [9, 10] | 0.06 | 14 (5.2) |
| Organ supply and specific drugs | | | | |
| Mechanical ventilation, *n* (%) | 142 (77.6) | 66 (72.5) | 0.12 | 11 (4.1) |
| Non-invasive mechanical ventilation, *n* (%) | 19 (10.4) | 11 (12.1) | 0.56 | 11 (4.1) |
| Vasopressor use (epinephrin or norepinephrin), *n* (%) | 74 (40.9) | 27 (30.7) | 0.10 | 10 (3.6) |
| Hemodialysis, *n* (%) | 13 (7.2) | 8 (9.1) | 0.40 | 10 (3.6) |
| Antibiotic use, *n* (%) | 158 (87.3) | 59 (67.0) | 0.0001 | 10 (3.6) |
| Steroids for suspected septic shock, *n* (%) | 18 (9.9) | 5 (5.7) | 0.30 | 10 (3.6) |

SAPS 2 score, our two groups had similar severity. This may result from our using the time when the physician was about to initiate antibiotic therapy among the inclusion criteria. The ICU mortality of our patients was high (31% and 33%) and consistent with their SAPS 2 scores at ICU admission [19]. Similarly, the SOFA score, which has become part of the sepsis description [2], was high (9 [8–10)] and similar in our two groups, allowing us to discard severity as a confounding factor. We observed differences between the two groups: the high prevalence of hypoxemia in our sepsis group may be due to the frequency of pneumonia [33]; higher serum lactate—a marker of cellular hypoxia—and lower hematocrit in non-septic SIRS patients may result from the frequency of circulatory dysfunction and is consistent with the role of tissue hypoxia, ischemia, and reperfusion in the pathophysiology of SIRS [35]. Lastly, sepsis patients had longer ICU and hospital stays than non-septic SIRS patients. This may reflect that source control [5] and resolution of infection are slower to achieve than resolution of events such as ischemia–reperfusion syndrome.

An important result of our study is that the circulating biomarkers did not differ substantially whether the stimulus for clinical inflammation was microbial or not. This may relate to the similarity of the mediator cascade and cell interaction profiles, whether the involved stimuli are pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) [36]. Considering the large amount of literature promoting the use of PCT to guide antibiotic prescription [15], our results were unexpected. This may have been due to the similar level of severity of our two groups and the strong link between PCT and mortality, as reported in a recent large international cohort [28]. The good performance of CRP relative to other markers is consistent with previous studies [9, 11, 13] and with the fact that CRP use was found to successfully decrease the duration of antibiotic therapy [12]. In two important studies, it was not assessed [10] or was used as part of the criteria of infection [14] precluding its assessment as a diagnostic marker. Also, the fact that we could not improve the diagnostic accuracy of CRP with a combination of biomarkers is consistent with one prior study [11], while two studies concluding differently either addressed distinction between bacterial and viral infections [13] or were not reproduced [25, 26] (ESM Table 11).

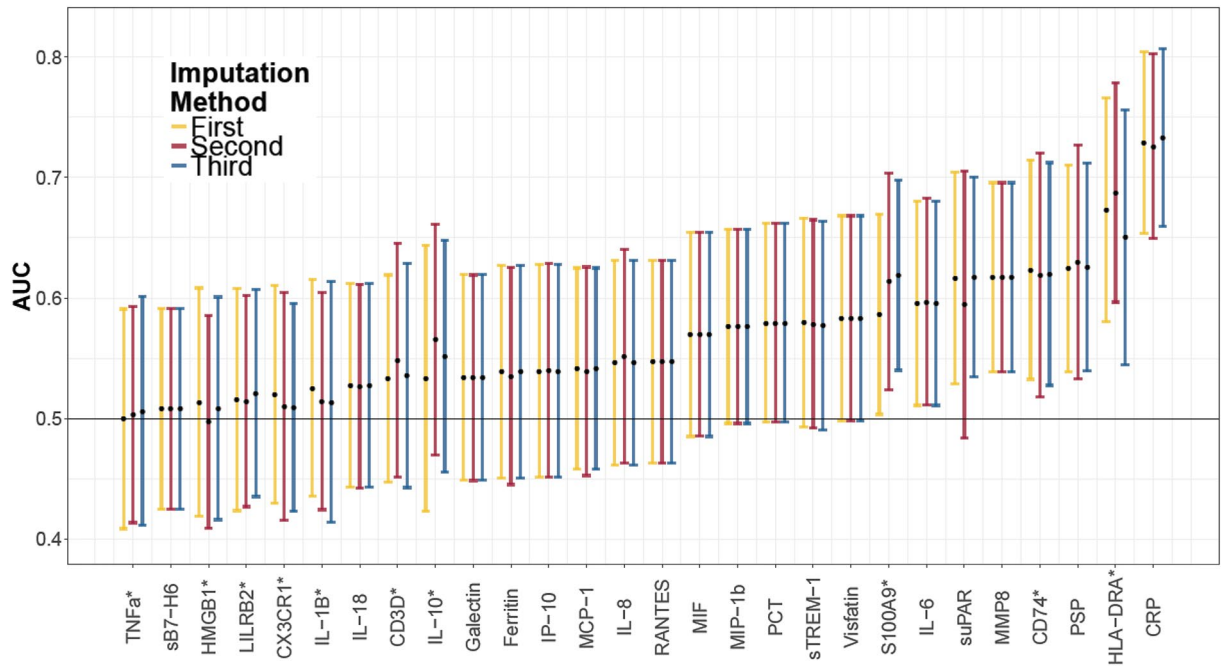


**Fig. 2** Univariate performance of the 28 quantitative markers, according to different imputation methods. X-axis, name of each biomarker; Y-axis, ROC-AUC values of each biomarker ranked between 0 and 1. Each bar corresponds to the 95% confidence interval of the ROC-AUC. For each biomarker, three bars are provided, corresponding to each of the first, second, and third imputation method for values below LLoQ, over ULoQ, and for missing values (ESM Table 6). *RNA

Our study has several limitations. First, many eligible patients may not have been included, particularly because inclusion outside working hours of the research laboratory was discouraged. This may reduce generalization of our results. Second, despite CRP and PCT not being provided to the adjudicators, the interpretations of the physicians in charge of some patients could be based in part on these results and were visible in the patients' records and may have influenced the decisions of the adjudicators. Third, the dispersion of the ROC-AUC of most markers was large, secondary to the relatively small sample size. This sample size is, however, in the higher range of the studies which established the ROC-AUC of PCT [16] and served as a basis for our sample size calculation. Fourth, we could assess cell surface biomarkers in only 40% of our patients, because the cells require immediate treatment in the research laboratory, i.e., during regular working hours. This was due to the fact we had planned to include patients independently of the hours of symptoms occurrence, to favor external validity. Also, we obtained a substantial amount of missing data for these cell surface biomarkers, mainly because of a shortage of blood sample volume. This reduces the validity of our findings regarding these biomarkers. For example, the positive results we observed in our first 28 patients [37] for CD24 on neutrophils were not confirmed in the present whole cohort. Fifth, we had to adjust our initial statistical plan analysis, from a conventional approach with multivariate regression to the LASSO technique, after we observed the large number of missing values. Last, as the study was only conducted in ICUs in the Paris area, the external validity in other countries or smaller hospitals may be decreased.

## Conclusion

In this prospective multicenter cohort, addressing most circulating biomarkers previously tested in the setting of sepsis, and using the LASSO biostatistical method, we found that these biomarkers poorly discriminated sepsis from non-septic SIRS patients and that no one biomarker, alone or in combination, performed better than CRP alone. Because cell surface biomarkers were collected in only 40% of the patients, the validity of our results is weaker for these biomarkers.

**Electronic supplementary material**

The online version of this article (https://doi.org/10.1007/s00134-018-5228-3) contains supplementary material, which is available to authorized users.

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**Author contributions**
AR, LE, SB, VM, JMC, and BM had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: FP, AR, AP, JMC, and BM. Acquisition of data: MP, FP, CF, VP, VM, JPB, DJ, OH, DA, JLD, LL, JMT. Access to data, analysis, and interpretation: MP, FP, AR, LE, SB, VM, MBB, AP, MAC, JMC, and BM. Drafting of the manuscript: FP, MP, AR, SB, VM, JMC, and BM. Critical revision of the manuscript for important intellectual content: MP, FP, AR, SB, VM, DA, JMC, and BM. Statistical analysis: AR, MBB, LE, and SB. Obtained funding: AP, JMC, and BM. Administrative, technical, or material support: CF and JMT.

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**Compliance with ethical standards**

**Conflicts of interest**
Moucadel, Estève, and Pachot are employed by bioMérieux SA, a private company specializing in in vitro diagnostics. The authors declare no other potential conflicts of interest in relation to the subject of the present manuscript.

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