Diagnostic accuracy of presepsin (sCD14-ST) and procalcitonin for prediction of bacteraemia and bacterial DNAaemia in patients with suspected sepsis

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Early diagnosis and prompt targeted therapy are essential for septic patients’ outcome. Procalcitonin (PCT) has been shown to predict bacteraemia and bacterial DNAaemia. Presepsin, the circulating soluble form of CD14 subtype, increases in response to bacterial infections, and is considered a new, emerging, early marker for sepsis. We evaluated the diagnostic accuracy of presepsin in predicting bacteraemia and bacterial DNAaemia in 92 patients with suspected sepsis, and we compared it with that of PCT and C-reactive protein (CRP). Presepsin median values were significantly higher in bacteraemic vs non-bacteraemic patients [1290 pg ml⁻¹, interquartile range (IQR) 1005–2041 vs 659 pg ml⁻¹, IQR 381–979; P<0.001] and in patients with vs patients without bacterial DNAaemia (1297 pg ml⁻¹, IQR 1001–2046 vs 665 pg ml⁻¹, IQR 381–940; P<0.001). Receiver operating characteristics analysis showed an area under the curve (AUC) for presepsin of 0.788 [95% confidence interval (CI): 0.687–0.889; P<0.001] in predicting bacteraemia and of 0.777 (95% CI: 0.676–0.878; P<0.001) in predicting bacterial DNAaemia, lower, but not significantly different, than those of PCT (0.876, P=0.12 and 0.880, P=0.07, respectively). Both biomarkers performed significantly better than CRP, which had an AUC for bacteraemia of 0.602 and for DNAaemia of 0.632 (all P values <0.05). In conclusion, in patients with suspected sepsis, presepsin and PCT showed a good diagnostic accuracy in predicting both bacteraemia and bacterial DNAaemia, superior to CRP.

INTRODUCTION

Sepsis, a systemic deleterious host response to infection that leads to organ dysfunction (Dellinger et al., 2013), is a major cause of morbidity and mortality (Mayr et al., 2014). Early diagnosis and prompt targeted therapy are essential for patients’ outcome (Kumar et al., 2006).

Blood culture (BC) is the gold standard for pathogen detection, although false-negative results, especially in antibiotic pre-treated patients, can occur (Opota et al., 2015). New approaches to speed sepsis diagnosis include molecular assays and the use of specific biomarkers (Reinhart et al., 2012). Molecular technologies, allowing a rapid detection of microbial DNA, could represent an important additional tool for the aetiological diagnosis of bloodstream infections, particularly in antibiotic pre-treated patients (Dark et al., 2015; Mongelli et al., 2015; Pasqualini et al., 2012). Indeed, real-time PCR (RT-PCR) can detect not only viable microorganisms in patient’s blood (bacteraemia) but also circulating bacterial DNA (DNAaemia) that persists for several days in blood during antimicrobial treatment or may be released into the bloodstream from the primary infectious foci (Øvstebø et al., 2004; Navarro et al., 2006; Peters et al., 2007).

During recent years, many biomarkers have been evaluated and compared to find the best predictor of sepsis (Cho & Choi, 2014). Procalcitonin (PCT) has been identified as having a good diagnostic accuracy for predicting bacteraemia and pathogen DNAaemia in different clinical settings (Koivula et al., 2011; Mencacci et al., 2012), representing an advance over C-reactive protein (CRP) (Barati et al., 2008; Leli et al., 2014; Yu et al., 2010). CD14, a glycoprotein expressed on the membrane surface of monocytes and macrophages, has a role as a recognition molecule in the innate immune response against micro-organisms (Henriquez-Camacho & Losa, 2014). During inflammation, plasma

Abbreviations: AUC, area under the curve; BC, blood culture; CRP, C-reactive protein; ED, emergency department; IQR, interquartile range; PCT, procalcitonin; ROC, receiver operating characteristics; RT-PCR, real-time PCR; SIRS, systemic inflammatory response syndrome.

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protease activity generates soluble CD14 fragments (Henriquez-Camacho & Losa, 2014). One of them, the circulating soluble form of CD14 subtype (sCD14-ST) or presepsin, increases in response to bacterial infections and is considered a new, emerging, early marker for sepsis (Chenevier-Gobeaux et al., 2015), capable of predicting bacteraemia (Kweon et al., 2014; Rabensteiner et al., 2014; Romualdo et al., 2014; Shozushima et al., 2011).

To the best of our knowledge, no study described presepsin diagnostic accuracy in predicting both bacteraemia and bacterial DNAaemia, which was the first aim of this study. The second aim was to compare the diagnostic accuracy of presepsin with that of PCT and CRP.

METHODS

Patients and samples. This prospective observational study was conducted using clinical and laboratory data routinely collected from the Clinical Microbiology Unit of the General Hospital of Perugia, Italy, from January to April 2015. Patients with suspected sepsis (Bone et al., 1992) were included in the study. Inclusion criteria were as follows: (1) fulfilment of at least two of the systemic inflammatory response syndrome (SIRS) criteria [temperature ≥38 °C or ≤36 °C; heart rate >90 beats/min; respiratory rate >20 breaths/min or pCO2 <32 mm Hg; white blood cell count ≥12 000 mm−3 or <4000 mm−3 or >10% immature neutrophils]; (2) infection documented or suspected on clinical presentation; (3) samples for presepsin drawn simultaneously with those for BC, RT-PCR, PCT, and CRP; and (4) age above 18 years. For each patient, only data from the first samples, collected during the same infectious episode, were considered. Exclusion criteria were lack of at least one of the above samples or samples not drawn simultaneously from the same patient.

Blood culture. For each sample, an aliquot of 5 to 10 ml whole blood was inoculated into BACTEC aerobic and anaerobic bottles (Becton Dickinson). BACTEC Plus bottles were used for patients under antibiotic therapy and standard bottles were used for untreated patients. Two sets from two different sites were collected at the same time. The bottles were incubated in BACTEC FX automated BC system (Becton Dickinson). All bottles flagged positive were removed from the instrument and an aliquot was taken for Gram stain and culture on solid media for subsequent analysis. Identification of micro-organisms was performed with conventional methods and with the matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (Bruker Daltonics).

Blood RT-PCR. For blood RT-PCR, a 3-ml K3 EDTA blood sample was collected, and 1.5 ml was processed for DNA extraction and PCR testing by means of the SeptiFast (SF) molecular assay (Roche Diagnostics), as described previously (Pasqualini et al., 2012). Briefly, samples were mechanically lysed, and internal extraction and amplification controls were included for each sample. A negative control supplied by the manufacturer was included in each extraction series. Using the LightCycler SF kit MGRADE, RT-PCR was performed in a LightCycler 2.0 instrument (Roche Diagnostics). Three different primer mixes were used to amplify Gram-positive and Gram-negative bacteria, and fungi. The internal transcribed spacer region was the specific target for the detection of bacterial and fungal pathogens. Species identification (melting temperature analysis of specimens and controls in each channel) and report generation was obtained using the SF identification software (Roche Diagnostics). The micro-organisms identified by SF have been listed elsewhere (Lehmann et al., 2008). The analytical sensitivity of the assay ranged between 3 and 100 cells ml−1, depending on the individual micro-organisms (Lehmann et al., 2008).

Presepsin determination. Presepsin was measured by non-competitive chemiluminescence enzyme immunoassay on the fully automated PATHFAST immunoanalyser (Mitsubishi Chemical Medience Corporation) in K3 EDTA whole blood samples. The assay had an analytical sensitivity <20.0 pg ml−1 and a limit of quantitation ≤51.0 pg ml−1.

PCT and CRP determination. PCT levels were measured in sera via the automatic analyser VIDAS PCT assay (bioMérieux), according to the manufacturer’s instructions. The lower limit of detection of the assay was 0.05 ng ml−1 and the functional assay sensitivity was 0.09 ng ml−1 (VIDAS B.R.A.H.M.S. PCT package insert; bioMérieux). Plasma CRP levels were measured using the latex-enhanced CRP assay (Dade Behring High Sensitivity CRP Assay).

Definitions of pathogen. Micro-organisms detected by BC and RT-PCR were considered responsible for the sepsis episode (true pathogens) whenever the results of BC analysis and PCR assay coincided. If BC and RT-PCR were positive for different micro-organisms, or if a micro-organism was detected by only one of the two tests, then culture results from additional microbiological samples, collected from patient’s suspected infectious foci during the same infectious episode, were evaluated. If the same micro-organism was isolated also from these additional samples, it was considered a pathogen. If a micro-organism was detected without any other culture support, it was considered a contaminant or a contaminant according to the attending physician’s final decision, based on the occurrence of clinical and laboratory findings of sepsis (Bone et al., 1992). Coagulase-negative staphylococci, Corynebacterium species and other skin commensals were considered contaminants when isolated from only one set of BC, in the absence of clinical and/or laboratory data suggesting their pathogenic role (Weinstein et al., 2003).

Statistical analysis. Values were expressed as count and percentages or median and interquartile range (IQR). Statistical significance was assumed if a null hypothesis could be rejected at a P value of ≤0.05. The chi-square test was used to analyse associations between categorical variables. Median values were compared across groups using the Mann–Whitney U test. Receiver operating characteristics (ROC) curve analysis was used to define the diagnostic ability of the various cut-offs, and Youden index was calculated to find the best discriminatory cut-off (Youden index = sensitivity + specificity − 1). SPSS statistical package, release 13.0 (SPSS), was used for all statistical analyses.

Ethical considerations. All samples were collected as part of standard care. In our hospital, patients are routinely requested to consent to the possible anonymous use of clinical, radiological and laboratory data for research. Data included in the study database were de-identified before access and no personal information was stored in the database. No intervention on patients’ management was made based on the results of the study. For these reasons, the study was exempt from the institutional review board.

RESULTS

During the 4-month study period, 3851 BC sets from 1102 patients were processed. In 109/1102 patients, blood samples for RT-PCR were also collected. Pressepsin, PCT and CRP samples were also collected simultaneously in 92/109 patients. Therefore, according to the inclusion criteria, data from 92 patients were included in the study.

Median age was 73 years (IQR 59–81): 62/92 patients (67.5%) were hospitalized in internal medicine wards, 12/92...
(13 %) in oncohaematology, 12/92 (13 %) in intensive care units and 6/92 (6.5 %) in surgery wards. A total of 54/92 patients (58.7 %) fulfilled two out of the four SIRS criteria considered for the inclusion in the study, 23/92 (25 %) fulfilled three criteria and 15/92 (16.3 %) fulfilled four criteria. The infectious focus was identified in 61/92 patients (66.3 %) of which: 25/61 (41.0 %) were urinary tract infections, 13/61 (21.3 %) were pneumonia, 8/61 (13.1 %) were endocarditis, 7/61 (11.5 %) were skin/soft tissues infections, 3/61 (4.9 %) were abdominal abscesses, 3/61 (4.9 %) were osteomyelitis, and 2/61 (3.3 %) were central line-associated bloodstream infections. A total of 68/92 (73.9 %) patients were receiving empiric antibiotic therapy at the time of blood sampling.

BC and RT-PCR gave concordant results in 82/92 (89.1 %) patients: 60/92 (65.2 %) were concordant negative and 22/92 (23.9 %) were concordant positive. Discordant results were obtained in 10/92 (10.9 %) patients, in which pathogens were detected by only BC or only RT-PCR. Pathogens responsible of sepsis were identified in 32 (34.8 %) patients, 22 were identified by both BC and RT-PCR and 10 were identified by only one method (Table 1). Among the latter 10 pathogens, one Streptococcus pneumoniae micro-organism detected only by RT-PCR was considered a pathogen because of positive Streptococcus urine antigen; one Corynebacterium striatum isolate from BC was also isolated from patient’s groin surgical wound infection; three Escherichia coli organisms detected only by RT-PCR were also isolated from patient’s urine samples; one E. coli isolate identified only by BC was also cultured from patient’s diabetic foot and one Bacteroides fragilis isolate in a patient with diverticulitis was considered a true pathogen according to physician’s final decision. Finally, micro-organisms detected by only RT-PCR in polymicrobial bloodstream infections were also cultured from patients’ abdominal aspirates: Klebsiella pneumoniae and Enterobacter cloacae, K. pneumoniae and E. coli and K. pneumoniae and Pseudomonas aeruginosa.

No contamination was reported for the molecular test, while two BCs positive for Staphylococcus epidermidis isolates and one BC positive for Staphylococcus hominis and Rhodotorula mucilaginosa isolates were considered contaminated according to the criteria described in ‘Methods’ and were excluded from subsequent analyses that were finally performed on 89 BC and 92 RT-PCR results.

Table 2 describes the demographic, clinical and laboratory characteristics of the population evaluated, according to BC and RT-PCR results. Median values of presepsin and PCT were significantly higher in both BC and RT-PCR positive groups, compared to negative ones.

ROC analysis was performed to evaluate the diagnostic accuracy of the studied biomarkers in predicting bacteraemia and bacterial DNAaemia (Fig. 1). No significant difference was found between the areas under the curve (AUCs) of presepsin and PCT for both bacteraemia (AUC 0.788 vs 0.876, respectively, P = 0.12) and DNAaemia (AUC 0.777 vs 0.880, respectively, P = 0.07). Both

<table>
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<th>Only BC</th>
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<td>Monomicrobial infections</td>
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bacterial vs non-bacterial infections in a population of 859 patients with suspected sepsis, found an AUC of 0.605 for presepsin in predicting bloodstream infections, not significantly different from that of PCT (Rabensteiner et al., 2014). Likewise, in another study in 226 ED patients, AUCs of 0.750 for presepsin and 0.783 for PCT were obtained for discrimination of bacteremic from non-bacteremic SIRS patients (Romualdo et al., 2014), with presepsin median value comparable to that of the present study.

Presepsin showed a good diagnostic accuracy in discriminating bacterial vs non-bacterial infections in a population of 185 patients with at least one of the diagnostic criteria of SIRS (Bone et al., 1992), while, differently from our results, no significant differences in presepsin levels were found between BC-positive and BC-negative patients with bacterial infections (Endo et al., 2012).

Other authors found presepsin to be superior to PCT in predicting the diagnosis of sepsis. Liu et al. (2013), in a population of 859 patients with suspected sepsis, found an AUC for presepsin superior to that of PCT. Nevertheless, in that study, BCs were not collected, and the final diagnosis of sepsis was made on clinical features, laboratory findings and imaging tests. Shozushima et al. (2011) found presepsin superior to PCT in discriminating between SIRS and sepsis in a population of 41 septic patients, with ~30% of cases of severe burns and multiple trauma, conditions in which, in the early stage of traumatic insult, PCT does not increase in an infection-specific manner (O’Neill et al., 1992; Wanner et al., 2000).

However, in a multicentre prospective study in ED patients, PCT diagnostic accuracy in the recognition of sepsis and severe sepsis/septic shock, diagnosed according to the Surviving Sepsis Campaign Criteria (Dellinger et al., 2013), was superior to that of presepsin, although presepsin retained a significant prognostic role and closely correlated with in-hospital mortality of septic patients (Ulla et al., 2013). Differently from our study, the role of presepsin in predicting positive BC was not evaluated.

We found that presepsin and PCT were not significantly different in predicting bacteremia and bacterial DNAemia. To what extent the non-significant difference could be due to the small sample size of this study needs to be verified in a larger population. Nevertheless, the results of this study are in line with those of a large observational study on 35 343 consecutive patients, in which PCT was highly effective excluding bloodstream infections regardless of pathogen categories, with the highest PCT concentration observed in patients with BCs growing Gram-negative bacteria (Oussalah et al., 2015). Likewise, in a recent systematic review and meta-analysis on 16 514 patients, an AUC of 0.79 was found for PCT in predicting bacteremia (Hoeboer et al., 2015). Moreover, PCT AUCs found in this study match our previous results (Mencacci et al., 2012; Leli et al., 2014), highlighting its accuracy in identifying not only bacteremia but also bacterial DNAemia.

**DISCUSSION**

The main result of this study is that presepsin and PCT showed a good diagnostic accuracy in predicting bacteremia and bacterial DNAemia in patients with suspected sepsis superior to CRP.

The ability of presepsin to predict bacteremia has already been demonstrated by recent studies. Rabensteiner, in a group of 300 emergency department (ED) patients, found an AUC of 0.605 for presepsin in predicting bloodstream infections, not significantly different from that of PCT (Rabensteiner et al., 2014). Likewise, in another study in 226 ED patients, AUCs of 0.750 for presepsin and 0.783 for PCT were obtained for discrimination of bacteremic from non-bacteremic SIRS patients (Romualdo et al., 2014), with presepsin median value comparable to that of the present study.

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However, PCT failed to optimally predict bloodstream infection in a population of 898 patients, although its values were significantly higher in patients with bloodstream infection than in those without (Hoenigl et al., 2014). To the best of our knowledge, this is the first study showing a good diagnostic accuracy of presepsin in predicting bacterial DNAaemia in patients with suspected sepsis. This is an important issue, given the diagnostic role of DNAaemia, which can be detected in septic patients even in the case of negative BC (Pasqualini et al., 2012; Mongelli et al., 2015). Moreover, it has been demonstrated that bacterial DNA load (viable and non-viable bacteria) correlates with disease severity and with the risk of developing septic shock (Darton et al., 2009; Ho et al., 2009; Kirkbright et al., 2011; Rello et al., 2009; Waterer & Rello, 2011). As conceivable, we found presepsin superior to CRP in predicting not only bacteraemia, as already demonstrated (Kweon et al., 2014; Rabensteiner et al., 2014; Romualdo et al., 2014), but also DNAaemia. The same result was also found for PCT, in line with previous reports (Barati et al., 2008; Leli et al., 2014; Yu et al., 2010).

The small sample size, the lack of data on patients’ medical history and the single-centre design are the main limitations of this study. Our results need to be confirmed in other well-characterized clinical settings in larger populations.

In conclusion, in patients with suspected sepsis, presepsin and PCT showed a good diagnostic accuracy in predicting both bacteraemia and bacterial DNAaemia, superior to CRP.

ACKNOWLEDGEMENTS

The authors declare that there is no conflict of interests regarding the publication of this paper.

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<td>&lt;0.001</td>
<td>843.5</td>
<td>0.88</td>
<td>0.71</td>
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<td>0.602</td>
<td>0.480–0.725</td>
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<td>9.3</td>
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<tr>
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<td>Presepsin (pg ml⁻¹)</td>
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Fig. 1. ROC curves for BC and blood RT-PCR results along with sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR) and negative likelihood ratio (–LR), at the best cut-offs of the following parameters: presepsin, PCT and CRP.

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