Journal of Infection (2012) **65**, 490—495

BIA
British Infection Association

www.elsevierhealth.com/journals/jinf

Combination of biomarkers for the discrimination between bacterial and viral lower respiratory tract infections

Jaap ten Oever [a,b,*], Mirjam Tromp [a,b], Chantal P. Bleeker-Rovers [a,b], Leo A.B. Joosten [a,b], Mihai G. Netea [a,b], Peter Pickkers [b,c], Frank L. van de Veerdonk [a,b]

[a] *Department of Internal Medicine, Radboud University Nijmegen Medical Centre, The Netherlands*
[b] *Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Radboud University Nijmegen Medical Centre, The Netherlands*
[c] *Department of Intensive Care Medicine, Radboud University Nijmegen Medical Centre, The Netherlands*

Accepted 7 August 2012
Available online 13 August 2012

KEYWORDS
Biomarkers;
Lower respiratory tract infection;
Emergency department;
Sepsis;
C-reactive protein

Summary *Objectives:* To investigate whether additional determinations of plasma lipopolysaccharide binding protein (LBP), procalcitonin (PCT), interleukin-6 (IL-6), IL-18, or soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) to C-reactive protein (CRP) improve the discrimination between bacterial and viral lower respiratory tract infections (LRTI).
Methods: Out of 342 patients visiting the emergency department because of a suspected infection and ≥2 clinical signs of sepsis, 56 patients with proven bacterial ($n = 39$) or viral ($n = 17$) LRTI were included. The area under the ROC curves (AUC) of the five possible combinations of CRP with one other biomarker were compared with the AUC of CRP alone. Next, the same analysis was performed in the group of patients with a CRP concentration with <95% specificity for bacterial LRTI.
Results: While CRP, PCT, IL-6, sTREM-1, and LBP concentrations were significantly different between patients with bacterial or viral LRTI, the AUC of CRP (0.82, 95%CI 0.70—0.93) did not increase after combination analyses. After exclusion of patients with a CRP >150 mg/l, biomarker panel analyses did not improve diagnostic accuracy of CRP either.
Conclusions: Combining CRP with LBP, PCT, IL-6, IL-18, or sTREM-1 does not improve differentiation between patients with a bacterial or viral LRTI compared with CRP alone.

* Corresponding author. Department of Internal Medicine, Radboud University Nijmegen Medical Centre, Internal Postal Code 463, PO box 9101, 6500 HB Nijmegen, The Netherlands. Tel.: +31 24 3614198; fax: +31 24 3541734.
E-mail address: j.tenoever@aig.umcn.nl (J. ten Oever).

0163-4453/$36
http://dx.doi.org/10.1016/j.jinf.2012.08.004

Introduction

Morbidity and mortality associated with lower respiratory tract infections (LRTI) remains significant, despite improved diagnostic and therapeutic treatment strategies in recent years.[1] The early initiation of antibiotic therapy has a major impact on the clinical outcome of critically ill patients.[2,3] Several laboratory diagnostic tests are currently used for establishing an aetiologic diagnosis. However, difficulty in obtaining relevant specimens, the low sensitivity or specificity of the used tests, high costs, and the absence of test results within the critical window for initiating adequate treatment, often result in prescription of antibiotic therapy in the absence of a bacterial infection. This may have potentially deleterious consequences such as anaphylactic reactions, antibiotic resistance, and high costs.[4] Rapid tests that provide additional insight in the bacterial/viral aetiology of infection may guide appropriate use of antibiotics and are urgently needed.

Both C-reactive protein (CRP) and procalcitonin (PCT) concentrations have been used to initiate and monitor the antibiotic use for LRTI.[5,6] However, the specificity of single biomarkers in terms of aetiologic distinction between bacterial and viral inflammatory insults remains cumbersome,[7,8] and a combination of markers could prove more reliable. The usefulness of IL-18 as a viral marker is supported by the reported high concentrations in HIV, dengue hemorrhagic fever, EBV, and CMV infections.[9–12] Triggering receptor expressed on myeloid cells-1 (TREM-1) is expressed on neutrophils and monocytes upon exposure to bacteria and fungi. Soluble TREM-1 (sTREM-1) has been proposed to be of diagnostic value in bacterial infections.[13] Lipopolysaccharide (LPS)-binding protein (LBP) is an acute phase protein produced by hepatocytes that binds LPS to form a LPS–LBP complex during bacterial infections.[14] In children, LBP has excellent sensitivity for diagnosing invasive bacterial infections.[15] Interleukin-6 (IL-6) is the chief stimulator of the production of most acute phase proteins, such as CRP and LBP. Thus, IL-6 is a potential marker for the early phase of infection.

It is suggested that determination of several biomarkers, or a panel of biomarkers, may improve their predictive value,[16–20] but clinical evidence for this notion is scarce.[16–20] Also, differences in the plasma concentrations between, e.g., viral and bacterial infection groups are frequently reported, while the discriminating power for the individual patient remains unclear. Therefore, in the present study we assessed whether combination of the most commonly used biomarker CRP with LBP, PCT, IL-6, IL-18, or sTREM-1 can improve the discriminating ability in patients with a proven bacterial or viral LRTI.

Patients and methods

Study design

This study was a prospective single centre study, performed at the emergency department (ED) of a 953-bed university hospital in the Netherlands between November 2006 and May 2007. During the study, medical policy at the ED and the nursing wards was based on the standard basic clinical chemistry test results, in combination with a physical and additional examination depending on the clinical suspicion, and not on the results of the novel inflammatory markers described in this manuscript. Prior to the conduct of this study, the local Medical Ethics Committee was informed. Although they waived the need for a written informed consent, patients were informed about the study and the acquisition of supplementary plasma.

Study population

The study inclusion criteria were:

(1) Patients ($\geq$16 years old) visiting the ED because of a suspected infection, who had at least two of the following clinical signs of sepsis: temperature $>$38.3 °C or $<$ 36 °C, heart rate $>$90/min, respiratory rate $>$20/min, chills, altered mental status, systolic blood pressure $<$90 mmHg, mean arterial pressure $<$65 mmHg, hyperglycaemia (plasma glucose $>$ 6.8 mmol/l) in the absence of diabetes mellitus.[21]
(2) Signs of a LRTI: fever, cough with or without sputum, chest pain, dyspnoea, and altered breath sounds on auscultation and/or the presence of an infiltrate on chest X-ray.
(3) Patients with a microbiologically confirmed bacterial or viral infection. Since the primary goal of this study was not to differentiate between patients with or without infection, but to establish the value of biomarkers in discriminating between bacterial and viral LRTI, we deliberately selected only patients with a microbiologically confirmed bacterial or viral infection.

All patients not fulfilling these inclusion criteria were excluded.

Data collection

Cultures from sputum and blood, PCR on nose and throat swabs, antigen tests and serology were used to establish a diagnosis. Blood samples were taken for basic clinical chemistry tests and the measurements of the inflammatory mediators. Two blood cultures for microbiological testing were performed. Only CRP results were known to the attending physician. Blood was collected into two 3 ml lithium-heparin coated tubes for PCT, IL-6, LBP, sTREM-1, IL-18, and for basic clinical chemistry tests including CRP. Plasma was obtained by centrifugation of the blood at 4 °C with 2000 rpm for 15 min after which the plasma was frozen at −80 °C until measurements took place. CRP was measured by use of the Abbott Aeroset® (Abbott Diagnostics, Chicago, USA) with a lower detection limit of 5 mg/l. PCT was measured by use of the Kryptor PCT® (Brahms, Hennigsdorf, Germany) with a detection limit of 0.02 μg/l. IL-6 and LBP were measured by use of the Immulite 2500® (Siemens, Breda, The Netherlands) with a lower detection limit of 2 pg/ml and 1.2 μg/ml, respectively. Circulating IL-18 levels were measured using a commercial Luminex assay (BioRad, Hercules, USA) with a lower detection limit of 15 pg/ml. Circulating sTREM-1 was assessed by a commercial ELISA kit (R&D Systems, Minneapolis, USA),