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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*

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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).

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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),

according to the instructions of the manufacturer with a lower detection limit of 62.5 pg/ml.

Statistical analysis

Data are expressed as medians with interquartile range. The Mann–Whitney *U*-test was used to determine the difference of each marker between the two groups of patients. ROC curve statistics were applied for each single marker. Logistic regression analysis was used to estimate the predicted probabilities for CRP alone and in a model with CRP in combination with one of the other five biomarkers. These data were used for the generation of ROC curves. The area under the ROC curves (AUC) of the five possible combinations of CRP with one other biomarker was compared with the AUC of CRP alone. The method described by Hanley and McNeil was used for comparing the AUC.[22] As very high CRP values are highly specific for bacterial infections[23] and no additional biomarkers are needed, we additionally analysed the combination of markers in the subgroup of patients with a moderately increased CRP that may benefit the most from panel analysis. Therefore, the cut-off value of CRP leading to a specificity >95% for a bacterial LRTI (at the top end of CRP values) was determined. Next, a ROC curve of the CRP with one other biomarker was constructed in a similar way as described above for the subgroup of patients with a CRP below this cut-off value. All tests were two-sided, and $p < 0.05$ was considered statistically significant.

Data were analysed using SPSS version 18.0 (IBM, New York, USA) and MedCalc version 11.3.1.0 (MedCalc Software, Mariakerke, Belgium).

Results

A total of 342 patients with a suspected infection and ≥ two clinical signs of sepsis were admitted to the ED. Of these patients, 123 had a pulmonary focus for infection of whom 58 had a microbiologically confirmed LRTI. Two patients were excluded because of a fungal infection (*Pneumocystis jirovecii*). We included 39 patients with a bacterial LRTI and 17 patients with a viral LRTI for further analysis. No patients with both a bacterial and a viral infection were diagnosed. With the number of patients included, this study has 80% power to pick up an increase of the AUC of 10% in the whole group and an increase of the AUC of 20% in the subgroup of patients with a CRP concentration <150 mg/l.

The demographic and clinical parameters of the two groups are shown in Table 1. Table 2 shows the microbiological data. CRP, IL-6, LBP, PCT, and sTREM-1 were significantly higher in the bacterial group compared with the viral group. IL-18 did not differ between the bacterial and the viral group (Fig. 1).

The ROC of CRP had an AUC of 0.82 (95%CI 0.70–0.93). The other biomarkers did not have a larger AUC. The combination of CRP with any of the other makers had no significant effect on the AUC compared to CRP alone (Table 3).

For CRP, a level of > 150 mg/l was highly specific for a bacterial infection (95%CI 0.80–1.0). However, only 49% ($n = 19$) (95%CI 0.32–0.65) of the patients with a bacterial LRTI had concentrations >150 mg/l. In the lower range, considerable overlap of CRP concentrations existed between patients with viral ($n = 17$) and bacterial infections ($n = 20$), which had a negative impact on the specificity of the test and impeded the generation of a cut-off value with an acceptable specificity for viral LRTI. Apart from the patients with bacterial infection and a CRP >150 mg/l (highly specific for bacterial LRTI), the combination of the CRP-value with any one of the other biomarkers in the remaining patients with a CRP <150 mg/l ($n = 37$) did not increase the AUC to discriminate between a viral and bacterial LRTI in this subgroup of patients (Table 4).

Discussion

The main finding of the present study is that while several inflammatory markers are significantly different between a group of bacterial and viral LRTI patients, addition of these markers to CRP was not superior to CRP alone in discriminating between bacterial and viral LRTI in septic patients. CRP as a single biomarker is a useful parameter to suggest the bacterial aetiology of an infection, since a concentration >150 mg/l is highly specific for a bacterial infection. However, lower concentrations of CRP are often observed during both viral and bacterial infections. Unfortunately, biomarker panel analyses in this subgroup of patients did not improve diagnostic accuracy of CRP either and are therefore not suitable to guide therapy for the individual patient.

Antibiotics are the cornerstones in the treatment of bacterial infections and early antibiotic administration has been a crucial part of the surviving sepsis campaign.[24] On the other hand, a dramatic increase in antibiotic resistance

Table 1 Patient characteristics.

Variable	Bacterial infection ($n = 39$)	Viral infection ($n = 17$)
Patient characteristics		
Gender, % male	69	47
Age, yrs [median (IQR)]	60 (50–70)	54 (32–63)
Number of SIRS criteria [median (IQR)]	2 (2–3)	2 (2–3)
Patients admitted to nursing ward or ICU [*n* (%)]	35 (90)	16 (94)
Length of hospital stay, days [median (IQR)]	8 (5–20)	7 (3–18)
In-hospital mortality rate [*n* (%)]	2 (5%)	0 (0%)
Bacteraemia [*n* (%)]	11 (28%)	0 (0%)

Table 2 Isolated microorganisms.

Bacterial infection ($n = 39$)		Viral infection ($n = 17$)	
Streptococcus pneumoniae	10	Influenza A	5
Coxiella burnetii	9	Influenza B	4
Haemophilus influenzae	6	Respiratory syncytial virus	1
Mycoplasma pneumoniae	3	Metapneumovirus	1
Pseudomonas aeruginosa	3	Parainfluenza virus	3
Staphylococcus aureus	1	Influenza A + respiratory syncytial virus	2
Legionella pneumophila	1	Picornavirus + rhinovirus	1
Citrobacter freundii	1		
Corynebacterium propinquum	1		
Escherichia coli	1		
Streptococcus pneumoniae + Moraxella catarrhalis	1		
Haemophilus influenzae + Pseudomonas aeruginosa	1		
Staphylococcus aureus + Streptococcus pneumoniae	1		

has emerged without the prospect of development of novel classes of antimicrobial agents.[4,25] Therefore, reduction of the unnecessary use of antibiotics is mandatory.

Unfortunately, symptoms and signs routinely used in the diagnosis of LRTI have limited value in predicting the requirement of antibiotic therapy.[23] As a consequence, a multitude of biomarkers gained a lot of attention in differentiating bacterial from viral infections and in prognostication. Among these, CRP and PCT have found their way into daily practice.[5,6] LRTI caused by various classes of microorganisms are characterized by different concentrations of PCT and CRP.[16,23,26,27] However, the high a-priori chance of having a bacterial infection,[28,29] together with the considerable overlap between the biomarkers in the lower range, complicates the exclusion of a bacterial cause of an infection by the use of individual biomarkers.[16,23,29]

To overcome the problem with single marker analysis, some studies advocated panel analysis.[16–19,29,30] While some promising results have been reported,[17,18] studies including only patients with LRTI found no relevant effects of combining markers on diagnostic accuracy.[16,19,29,30] We used the addition of a second biomarker to CRP to examine the discriminatory power of laboratory testing, both in the group as a whole and in a subgroup of patients with relatively moderately increased CRP levels that potentially would benefit most from this approach. In contrast with

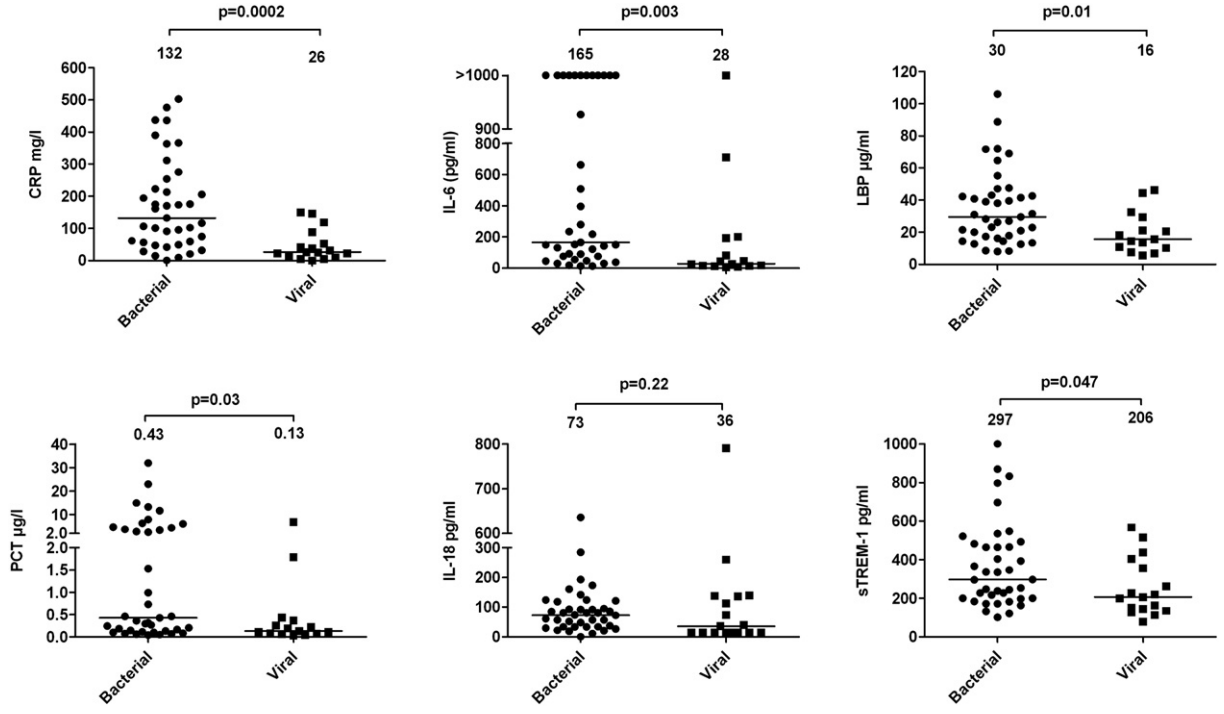

Figure 1 Plasma concentrations of CRP, IL-6, LBP, PCT, sTREM-1, and IL-18 in patients with viral lower respiratory tract infection (LRTI) compared with LRTI of bacterial origin ($n = 56$). Horizontal bars represent medians of the concentrations; the median is reported above the scatter plots in the different figures.

Table 3 Area under the curve (AUC) of the ROC for diagnosing bacterial lower respiratory tract infection of CRP and CRP in combination with other markers ($n = 56$). *P*-value for the comparison with the AUC of CRP.

Marker	AUC	(95%CI)	*P*-value
CRP	0.82	(0.70–0.93)	
CRP + LBP	0.85	(0.74–0.95)	0.7
CRP + IL-6	0.84	(0.74–0.95)	0.8
CRP + PCT	0.84	(0.73–0.95)	0.7
CRP + sTREM-1	0.83	(0.72–0.94)	0.9
CRP + IL-18	0.82	(0.70–0.93)	0.6

most previous studies, we included both proven bacterial and viral markers aimed at increasing the discriminative power. For example, IL-18 is a cytokine playing an important role in antiviral immunity,[9–12] and was expected to be a sensitive marker for diagnosing viral infections, while other markers are mechanistically related to the immune response against bacterial infections.

Most biomarkers examined in previous studies are stimulated both by bacterial and viral pathogens, but reach higher values during bacterial invasion. Therefore, moderately increased concentrations of these biomarkers can be found in both categories. Combination of these bacterial markers may only be beneficial if they do not correlate well with each other. Furthermore, a potentially suitable biomarker for combination analysis in future studies should demonstrate increased levels during viral infections. Bystanders of viral replication or proteins released upon virus recognition may be reasonable candidates.

There are several limitations of this study. First, the sample size is relatively small, especially for the viral infection group, although our study had enough power to detect a clinically relevant difference between the single and panel analyses. Second, this was a subset analysis of a prospective study in patients with LRTI that had a microbiological diagnosis. For an explorative study, we deliberately included only patients with a confirmed diagnosis. Because of the outbreak of acute Q-fever in the Netherlands between 2007 and 2010 *Coxiella burnetii* infections represented a large part of the bacterial pathogens. Patients with acute Q-fever are indistinguishable from other bacterial infection on clinical grounds.[31] Furthermore, in the present study the distribution of the inflammatory markers was not importantly influenced by the inclusion of acute Q-fever patients. Third, viral infections predispose to bacterial super infections and these cannot be ruled out in all patients diagnosed as having a viral LRTI. In most of the patients admitted to the hospital, because of the severity of illness, antibiotic treatment was initiated, making it difficult to determine in hindsight the presence or absence of a bacterial co-infection. Fourth, although the results of our study may be different in LRTI patients with <2 clinical signs of sepsis, in general practice the vast majority of patients with a LRTI fulfil ≥ 2 of the sepsis criteria and consequently satisfy the sepsis definition. Therefore, the results of our study apply to most patients presenting with signs of LRTI to the emergency department. Finally, a group of control patients with other types of non-infectious inflammatory reactions was not included in our cohort, as our primary aim was to investigate whether additional biomarkers could improve the differentiation between viral and bacterial LRTI's and not to determine their value to establish the presence of an infection.

Table 4 Area under the curve (AUC) of the ROC for diagnosing bacterial lower respiratory tract infection of CRP and CRP in combination with other markers of patients with a CRP $<$ 150 mg/l ($n = 37$). *P*-value for the comparison with the AUC of CRP.

Marker	AUC	(95%CI)	*P*-value
CRP	0.64	(0.45–0.84)	
CRP + LBP	0.68	(0.50–0.86)	0.9
CRP + IL-6	0.70	(0.53–0.88)	0.8
CRP + PCT	0.69	(0.50–0.88)	0.7
CRP + sTREM-1	0.64	(0.46–0.83)	1.0
CRP + IL-18	0.62	(0.43–0.81)	0.7

In conclusion, while different markers of inflammation show statistically significant higher levels in the group of patients with a bacterial infection compared to the patients with a viral LRTI, the combination of CRP with LBP, PCT, IL-6, IL-18, or sTREM-1 does not improve the prediction of microbiological aetiology in patients with LRTI, when compared with CRP as a single marker. Ruling out a bacterial infection remains troublesome and future studies should aim to identify better diagnostic markers.

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References

1. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med* 2003;**348**(16):1546–54.
2. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Crit Care Med* 2006;**34**(6):1589–96.
3. Houck PM, Bratzler DW, Nsa W, Ma A, Bartlett JG. Timing of antibiotic administration and outcomes for Medicare patients hospitalized with community-acquired pneumonia. *Arch Intern Med* 2004;**164**(6):637–44.
4. World Health OrganizationAnti-Infective Drug Resistance Surveillance and Containment Team. *WHO global strategy for containment of antimicrobial resistance*. Geneva: World Health Organization; 2001.
5. Cals JW, Butler CC, Hopstaken RM, Hood K, Dinant GJ. Effect of point of care testing for C reactive protein and training in communication skills on antibiotic use in lower respiratory tract infections: cluster randomised trial. *BMJ* 2009;**338**. b1374.

6. Schuetz P, Christ-Crain M, Thomann R, Falconnier C, Wolbers M, Widmer I, et al. Effect of procalcitonin-based guidelines vs standard guidelines on antibiotic use in lower respiratory tract infections: the ProHOSP randomized controlled trial. *JAMA* 2009;**302**(10):1059—66.
7. Oshita H, Sakurai J, Kamitsuna M. Semi-quantitative procalcitonin test for the diagnosis of bacterial infection: clinical use and experience in Japan. *J Microbiol Immunol Infect* 2010;**43**(3): 222—7. http://dx.doi.org/10.1016/S1684-1182(10)60035-7.
8. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. *Clin Infect Dis* 2004;**39**(2):206—17.
9. van de Berg PJ, Heutinck KM, Raabe R, Minnee RC, Young SL, van Donselaar-van der Pant KA, et al. Human cytomegalovirus induces systemic immune activation characterized by a type 1 cytokine signature. *J Infect Dis* 2010;**202**(5):690—9.
10. Ahmad R, Sindhu ST, Toma E, Morisset R, Ahmad A. Elevated levels of circulating interleukin-18 in human immunodeficiency virus-infected individuals: role of peripheral blood mononuclear cells and implications for AIDS pathogenesis. *J Virol* 2002;**76**(24):12448—56.
11. Mustafa AS, Elbishbishi EA, Agarwal R, Chaturvedi UC. Elevated levels of interleukin-13 and IL-18 in patients with dengue hemorrhagic fever. *FEMS Immunol Med Microbiol* 2001;**30**(3): 229—33.
12. van de Veerdonk FL, Wever PC, Hermans MH, Fijnheer R, Joosten LA, van der Meer JW, et al. IL-18 serum concentration is markedly elevated in acute EBV infection and can serve as a marker for disease severity. *J Infect Dis* 2012;**206**(2):197—201.
13. Jiyong J, Tiancha H, Wei C, Huahao S. Diagnostic value of the soluble triggering receptor expressed on myeloid cells-1 in bacterial infection: a meta-analysis. *Intensive Care Med* 2009; **35**(4):587—95.
14. Schumann RR, Leong SR, Flaggs GW, Gray PW, Wright SD, Mathison JC, et al. Structure and function of lipopolysaccharide binding protein. *Science* 1990;**249**(4975):1429—31.
15. Ubenauf KM, Krueger M, Henneke P, Berner R. Lipopolysaccharide binding protein is a potential marker for invasive bacterial infections in children. *Pediatr Infect Dis J* 2007;**26**(2):159—62. http://dx.doi.org/10.1097/01.inf.0000253064.88722.6d.
16. Ip M, Rainer TH, Lee N, Chan C, Chau SS, Leung W, et al. Value of serum procalcitonin, neopterin, and C-reactive protein in differentiating bacterial from viral etiologies in patients presenting with lower respiratory tract infections. *Diagn Microbiol Infect Dis* 2007;**59**(2):131—6. http://dx.doi.org/10.1016/j.diagmicrobio.2007.04.019.
17. Nuutila J, Hohenthal U, Laitinen I, Kotilainen P, Rajamaki A, Nikoskelainen J, et al. Quantitative analysis of complement receptors, CR1 (CD35) and CR3 (CD11b), on neutrophils improves distinction between bacterial and viral infections in febrile patients: comparison with standard clinical laboratory data. *J Immunol Methods* 2006;**315**(1—2):191—201. http://dx.doi.org/10.1016/j.jim.2006.07.021.
18. Kofoed K, Andersen O, Kronborg G, Tvede M, Petersen J, Eugen-Olsen J, et al. Use of plasma C-reactive protein, procalcitonin, neutrophils, macrophage migration inhibitory factor, soluble urokinase-type plasminogen activator receptor, and soluble triggering receptor expressed on myeloid cells-1 in combination to diagnose infections: a prospective study. *Crit Care* 2007;**11**(2):R38.
19. Don M, Valent F, Korppi M, Canciani M. Differentiation of bacterial and viral community-acquired pneumonia in children. *Pediatr Int* 2009;**51**(1):91—6.
20. Tromp M, Lansdorp B, Bleeker-Rovers CP, Gunnewiek JM, Kullberg BJ, Pickkers P. Serial and panel analyses of biomarkers do not improve the prediction of bacteremia compared to one procalcitonin measurement. *J Infect* 2012. http://dx.doi.org/10.1016/j.jinf.2012.06.004.
21. Levy MM, Fink MP, Marshall JC, Abraham E, Angus D, Cook D, et al. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. *Crit Care Med* 2003;**31**(4):1250—6.
22. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristic curves derived from the same cases. *Radiology* 1983;**148**(3):839—43.
23. Stolz D, Christ-Crain M, Gencay MM, Bingisser R, Huber PR, Muller B, et al. Diagnostic value of signs, symptoms and laboratory values in lower respiratory tract infection. *Swiss Med Wkly* 2006;**136**(27—28):434—40.
24. Dellinger RP, Levy MM, Carlet JM, Bion J, Parker MM, Jaeschke R, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock. *Crit Care Med* 2008;**36**(1):296—327.
25. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. *Clin Infect Dis* 2009;**48**(1):1—12.
26. Kruger S, Ewig S, Papassotiriou J, Kunde J, Marre R, von Baum H, et al. Inflammatory parameters predict etiologic patterns but do not allow for individual prediction of etiology in patients with CAP: results from the German competence network CAPNETZ. *Respir Res* 2009;**10**:65.
27. Masia M, Gutierrez F, Llorca B, Navarro JC, Mirete C, Padilla S, et al. Serum concentrations of lipopolysaccharide-binding protein as a biochemical marker to differentiate microbial etiology in patients with community-acquired pneumonia. *Clin Chem* 2004;**50**(9):1661—4.
28. Jennings LC, Anderson TP, Beynon KA, Chua A, Laing RT, Werno AM, et al. Incidence and characteristics of viral community-acquired pneumonia in adults. *Thorax* 2008; **63**(1):42—8.
29. Toikka P, Irjala K, Juven T, Virkki R, Mertsola J, Leinonen M, et al. Serum procalcitonin, C-reactive protein and interleukin-6 for distinguishing bacterial and viral pneumonia in children. *Pediatr Infect Dis J* 2000;**19**(7):598—602.
30. Korppi M. Non-specific host response markers in the differentiation between pneumococcal and viral pneumonia: what is the most accurate combination? *Pediatr Int* 2004;**46**(5):545—50.
31. Keijmel SP, Krijger E, Delsing CE, Sprong T, Nabuurs-Franssen MH, Bleeker-Rovers CP. Clinical presentation of patients with acute Q fever to the hospital. *Clin Microbiol Infect* 2012;**18**(s3):525.