Toll-like receptor and associated regulators in pneumonia and sepsis

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Chapter 8

Plasma soluble ST2 as a novel biomarker in community acquired pneumonia

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Abstract

Biomarkers may assist in stratifying patients with community-acquired pneumonia (CAP) according to etiology and severity. We evaluated plasma soluble (s)ST2 as a potential biomarker in CAP. sST2 was measured in 75 patients with CAP of microbiologically defined etiology: influenza A(H1N1) (n=9), Coxiella burnetii (n=29) or Streptococcus pneumoniae (n=38). sST2 was elevated in all subgroups; the highest levels were measured in bacteremic S. pneumoniae infections and severe pneumonia. sST2 levels positively correlated with the established CAP biomarkers C-reactive protein, and interleukins 6, 8 and 10. These data suggest that plasma sST2 may be a biomarker of disease severity in CAP.
Introduction

Pneumonia remains one of the most prevalent and potentially lethal manifestations of infectious disease [1-3]. Adequate diagnostics and risk assessment are important for reducing the morbidity and mortality associated with community-acquired pneumonia (CAP) [4, 5]. Though etiologies of community acquired pneumonia (CAP) may vary by geographic region, Streptococcus pneumoniae is the predominant global causative agent [1, 2, 6]. A prospective study in Spain identified Coxiella burnetii as the second most common cause of CAP, followed by Mycoplasma pneumoniae, respiratory viruses and other agents [4]. Biomarkers may help to establish a more detailed and accurate estimation of individual patient pathology, risk and prognosis [7].

Toll-like receptors (TLRs) play an essential role in protective immunity during infection [8]. Membrane bound ST2 (ST2L) functions as a negative regulator of TLR signaling and the receptor for interleukin (IL)-33 [9]. A soluble form of ST2 has been found in the circulation of healthy humans. Elevated plasma soluble (s) ST2 levels have been reported in patients with leptospirosis [10] and sepsis [11], suggesting that circulating sST2 could serve as a biomarker for disease activity in infection. These previous studies prompted us to measure plasma sST2 and IL-33 in patients with microbiologically defined CAP and its possible value as a biomarker of disease severity.

Materials and methods

Study design and population

CAP patients were recruited from the emergency department (ED) of the Jeroen Bosch Hospital in 's-Hertogenbosch in the Netherlands between November 2007 and January 2010 [12]. CAP was defined as an acute lower respiratory tract infection with at least two of the following clinical symptoms: new onset of cough, sputum production or change in color of respiratory secretions in patients with chronic cough, fever or hypothermia, or a physical examination consistent with pneumonia and a chest X-ray that demonstrated new lung infiltrates. Exclusion from the study was based on: age <18yrs, residency in a nursing home or transfer from another hospital. Study design and subjects have been described in detail before [12]. The Pneumonia Severity Index (PSI) [13] was determined on ED admission. EDTA-anticoagulated blood was obtained on admission and in 82% of patients on day 28 for further analysis, as were nose and throat swabs, sera, sputum and urine specimens for pathogen diagnosing (National Institute for Public Health and the Environment: RIVM, Bilthoven, the Netherlands) as described [12]. For the current investigation, CAP patients (n=76) with microbiologically confirmed infections caused by Streptococcus pneumoniae, Coxiella burnetii or influenza A(H1N1) were selected. Informed consent was obtained from all patients. Control plasma was
drawn from 12 healthy volunteers. The study was approved by the Medical Ethical Review Committee in Tilburg, the Netherlands.

**Assays**

Measurements were conducted using a cytometric bead array multiplex assay (BD Biosciences, San Jose, CA; IL-6, IL-8, IL-10 and tumor necrosis factor (TNF)-α) or enzyme-linked immunosorbent assays (R&D systems, Abingdon, UK; sST2 and IL-33). C-reactive protein (CRP) levels were measured by enzyme-linked immunoassay using an Aeroset 2.0 analyzer (Abbott Diagnostics, Santa Clara, CA).

**Statistical analysis**

Comparisons between groups were performed using the Kruskall-Wallis test, followed by Mann Whitney U test where appropriate. Comparisons between day 0 and 28 levels were done by Wilcoxon matched pairs tests. Analysis were done using Graphpad Prism version 5.01 (San Diego, CA). Correlations were calculated using the Spearman rho test via SPSS version 16.0 (Armonk, NY). P-values < 0.05 were considered statistically significant.

**Results**

**Patient characteristics**

This study comprised 9 influenza A(H1N1) patients (nose/throat swab PCR positive for influenza A(H1N1)), 29 patients with acute Q-fever (serum PCR positive for C. burnetii, serology negative), 16 patients with sputum culture confirmed S. pneumoniae infection, and 22 patients with blood culture confirmed S. pneumoniae infection (Table 1).

**Enhanced inflammatory markers in CAP patients**

EDTA plasma samples were taken directly at presentation and 28 days later. As expected CRP, IL-6, IL-8 and IL-10 were raised at time of presentation in all pneumonia patients when compared to healthy blood donors (Table 1). TNF-α was detectable in only a minority of patients (not significantly different from controls) (data not shown). All cytokine levels had relatively normalized 28 days later in all pneumonia patients when compared to healthy donors, with the exception of IL-10 in S. pneumoniae bacteremic patients (data not shown). When comparing IL-6, IL-8 and IL-10 levels between the different pneumonias S. pneumoniae infection displayed the highest circulating cytokine levels, especially in bacteremic patients. This difference was significant when comparing S. pneumoniae bacteremia with Q-fever (IL-6 P <0.05; IL-8 and IL-10 P <0.001) or influenza (IL-6 P <0.01). CRP levels were also higher in S. pneumoniae bacteremic patients when compared with influenza patients (P <0.01) and S. pneumoniae sputum positive pneumonia patients (P <0.01).
Table 1: Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Acute Q-fever n=29</th>
<th>Influenza A n=9</th>
<th>Streptococcus pneumoniae n=16</th>
<th>S. pneumoniae with bacteremia n=22</th>
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<tbody>
<tr>
<td><strong>Demographics</strong></td>
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<tr>
<td>Age (years)</td>
<td>54 (44-60)</td>
<td>51 (47-56)</td>
<td>69 (60-74)</td>
<td>61 (53-70)</td>
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<tr>
<td>Male sex (n, %)</td>
<td>20 (69%)</td>
<td>1 (11%)</td>
<td>8 (50%)</td>
<td>13 (59%)</td>
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<td><strong>Signs</strong></td>
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<tr>
<td>Temperature (°C)</td>
<td>38.7 (37.2-39.6)</td>
<td>37.7 (37.6-38.3)</td>
<td>38.2 (37.6-38.7)</td>
<td>38.2 (37.8-38.4)</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>90 (81-100)</td>
<td>95 (82-96)</td>
<td>82 (75-99)</td>
<td>84 (77-95)</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>95 (91-110)</td>
<td>85 (78-89)</td>
<td>102 (92-120)</td>
<td>93 (89-103)</td>
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<tr>
<td>Respiratory rate (brpm)</td>
<td>20 (18-30)</td>
<td>27 (24-29)</td>
<td>20 (20-24)</td>
<td>24 (20-30)</td>
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<td><strong>Severity and outcome</strong></td>
<td></td>
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<tr>
<td>PSI class</td>
<td></td>
<td></td>
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<tr>
<td>Low risk &lt; IV</td>
<td>20 (69%)</td>
<td>6 (67%)</td>
<td>8 (50%)</td>
<td>9 (41%)</td>
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<tr>
<td>Medium/High risk ≥ IV</td>
<td>4 (14%)</td>
<td>1 (11%)</td>
<td>5 (31%)</td>
<td>8 (36%)</td>
</tr>
<tr>
<td>Missing</td>
<td>5 (17%)</td>
<td>2 (22%)</td>
<td>3 (19%)</td>
<td>5 (23%)</td>
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<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1 (3%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Inflammatory markers</strong></td>
<td></td>
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<tr>
<td>CRP (µg/ml)</td>
<td>230 (177-326)</td>
<td>130 (16-170)</td>
<td>106 (51-192)</td>
<td>317 (156-445)</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>30.4 (13.5-50.0)</td>
<td>7.7 (3.5-14.2)</td>
<td>76.4 (6.4-2144)</td>
<td>95.3 (185.0-1651.0)</td>
</tr>
<tr>
<td>IL-8 (pg/ml)</td>
<td>3.7 (1.4-5.6)</td>
<td>5.2 (3.2-8.0)</td>
<td>9.9 (4.1-33.8)</td>
<td>24.6 (11.0-53.0)</td>
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<tr>
<td>IL-10 (pg/ml)</td>
<td>0.9 (0.5-2.0)</td>
<td>5.1 (1.6-6.1)</td>
<td>2.6 (1.4-7.5)</td>
<td>12.7 (4.7-21.0)</td>
</tr>
</tbody>
</table>

Abbreviations: n total number; N normal reference value; MAP mean arterial blood pressure; bpm beats per minute; brpm breaths per minute; PSI Pneumonia Severity index; score <90= low risk (class I-III), 91-130=medium risk (class IV) 131-395 =high risk (class V); CRP C-reactive protein. Data present either numbers of patients with percentages (percentages given are within study groups) or medians with (interquartile ranges). Reference (normal) values for biomarkers were: CRP < 10 mg/L, IL-6 (0.08 (0.08-0.08) pg/ml), IL-8 (0.49 (0.48-0.49) pg/ml) and IL-10 (0.12 (0.12-0.45) pg/ml)(n = 12). * P < 0.05, ** P < 0.01, *** P < 0.001 when compared to healthy controls.

Plasma sST2 and IL-33 concentrations during CAP

Plasma sST2 levels were elevated on admission in patients with CAP caused by either influenza A (0.14 (0.09-1.01) ng/ml), C. burnetii (0.45 (0.20-0.68) ng/ml) or S. pneumoniae (3.62 (0.51-7.71) ng/ml) when compared to sST2 levels in healthy blood donors (0.01 (0.01-0.01) ng/ml, Figure 1A). sST2 levels were highest in patients with positive blood cultures for S. pneumoniae (4.66 (2.89-7.71) ng/ml versus (0.51 (0.24-9.18) ng/ml in patients with sputum cultures positive for S. pneumoniae). Patients with severe pneumonia (PSI-score≥IV) had higher plasma sST2 concentrations on admission than patients with less severe pneumonia (Figure 1B). At day 28, sST2 concentrations had decreased in all patients from whom a follow up sample was available, except for one patient with S. pneumoniae bacteremia (Figure 1C-F). Co-morbidity (metastasized
malignancy) could explain the 28th day raise in sST2 in this patient [14-16]. Notably, day 28 sST2 levels were still higher than those in healthy controls, significantly so for bacteremic *S. pneumoniae* patients (0.09 (0.04-0.35)ng/ml; P<0.05).

Possible correlations between sST2 and inflammatory markers were calculated. Significant positive correlations existed between admission sST2 and CRP (r=0.46, P<0.001), IL-6 (r=0.81, P<0.001), IL-8 (r=0.78, P<0.001) and IL-10 (r=0.54, P<0.001).

IL-33 could be measured in plasma of approximately one third of healthy controls and patients, and no differences between groups were present (data not shown).

![Figure 1: Plasma sST2 levels in community-acquired pneumonia (CAP). sST2 levels were measured in plasma of healthy controls and patients with CAP caused by influenza A(H1N1), acute Q-fever or *Streptococcus pneumoniae* (sputum or blood culture positive) (A). sST2 levels of patients with CAP (a pneumonia severity index (PSI)-score belonging to class I-III (low risk)) and severe (S)CAP (PSI class IV-V (medium/high risk)) (B). sST2 levels measured in individual influenza A(H1N1) (C), acute Q-fever (D), *S. pneumoniae* sputum culture positive (E) and *S. pneumoniae* blood culture positive (F) patients on emergency department admission and 28 days later. Horizontal lines in panels A and B represent medians. All patients (N=76) were included in all tests with the exception of CAP versus SCAP comparisons, in which the patients lacking PSI scores were excluded. * P < 0.05, ** P < 0.01, *** P < 0.001 when compared to controls (A), PSI <4 (B) and day 0 (C-F). # P < 0.05, ### P < 0.001 when compared to *S. pneumoniae* blood culture positive patients (A).
Discussion

Lower respiratory tract infections, in particular pneumonias, are common, potentially life-threatening disorders that still challenge global health [1-3]. Accurate diagnostics, severity assessments and risk stratification of patients are extremely important in the management of CAP [4, 5]. Biomarkers may help in either identifying disease severity, establishing individual disease prognoses or even distinguishing between causative agents [7]. The interleukin 1 receptor-like 1 gene transcripts have divergent functions during infection: as negative regulator of TLR signaling (ST2L), as specific inhibitor of LPS effector functions in certain cells (sST2) and as either the IL-33 receptor (ST2L) or decoy receptor (sST2) [9, 17-21]. Here we sought to investigate a possible additional function for sST2: that of biomarker during CAP.

Elevated circulating sST2 plasma levels were found on ED admission in Influenza A, Q-fever and S. pneumoniae pneumonia patients. The highest levels were measured during S. pneumoniae infection, in particular when bacteremia was present. CAP patients also had elevated plasma levels of CRP, IL-6, IL-8 and IL-10 relative to healthy controls. Admission sST2 concentrations showed significant positive correlations with admission CRP, IL-6, IL-8 and IL-10 levels. CRP, IL-6, IL-8 and IL-10 have been associated with CAP mortality risk and treatment failure [7, 22]. Thus, these data are in accordance with the fact that sST2 levels were higher in patients with higher clinical severity scores. Notably, patients with Q fever had relatively high CRP admission levels although they had a relatively low percentage of severe CAP according to clinical scores. This in particular was evident when comparing patients with Q fever and sputum culture positive S. pneumoniae pneumonia. In contrast, sST2 levels were not different between these two groups; only blood culture positive pneumococcal infections were associated with higher sST2 levels. These data suggest that, unlike sST2, CRP at least partially is dependent on the causative pathogen, with C. burnetii possibly causing a higher CRP response.

Elevated circulating sST2 concentrations have been reported in various disease states. In asthma patients raised serum sST2 levels correlated with the severity of asthma exacerbations [19]. In patients with myocardial infarction high serum sST2 levels were suggested to predict mortality and heart failure [23]. Leptospirosis and sepsis were associated with elevated serum sST2 concentrations, which correlated with disease severity and mortality [10, 11]. Hence, while elevated circulating sST2 levels clearly are not specific for CAP, these previous data taken together with our current results suggest that they do reflect disease severity in different clinical conditions.

A previous study reported detectable serum IL-33 levels in approximately 50% of sepsis patients, with IL-33 concentrations remaining below detection limit in most healthy subjects [24]. Although we used the same ELISA to measure IL-33, we found detectable
IL-33 levels (>4 pg/ml) in only approximately one third of subjects, either CAP patients or healthy individuals, and IL-33 concentrations did not differ between groups. In CAP patients who had detectable IL-33 levels on admission, IL-33 remained measurable on day 28, further suggesting that CAP does not induce IL-33. Additional studies are warranted to obtain further insight in the appearance of IL-33 in the circulation during infections of distinct origins and severities.

Conclusion
We established that CAP caused by either influenza A(H1N1), C. burnetii or S. pneumoniae is associated with elevated sST2 plasma levels on ED admission, with the highest concentrations detected in patients with bacteremic S. pneumoniae infection or the highest clinical severity scores. Several studies have suggested that measurement of a combination of biomarkers (rather than the measurement of a single biomarker) may aid in risk stratification of patients with severe infections [25]. Further investigations are required to establish the value of plasma sST2 levels as a biomarker for disease severity in CAP, including studies examining the value of a set of biomarkers.

Reference list