Levels of Soluble Triggering Receptor Expressed on Myeloid Cells 1 in Infectious Exacerbations of Chronic Obstructive Pulmonary Disease

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Key Words
Biomarker · Chronic obstructive pulmonary disease · Exacerbation · Virus · Pathogen · Inflammation

Abstract
Background: Soluble triggering receptor expressed on myeloid cells 1 (sTREM-1) is an activating receptor on inflammatory cells upregulated by microbial products. Elevated levels of sTREM-1 have been associated with the diagnosis and prognosis of patients with sepsis, severe pneumonia and chronic obstructive pulmonary disease (COPD). Objectives: The aim of this study was to define the role of sTREM-1 in acute exacerbations of COPD (AE-COPD) and to investigate the ability of sTREM-1 to differentiate between infectious triggers of AE-COPD. Methods: Smokers without COPD (SM), patients with stable COPD (sCOPD) and patients with AE-COPD were prospectively recruited. sTREM-1 levels were determined by ELISA in serum. Potentially pathogenic bacteria were analyzed by sputum culture, and polymerase chain reaction was used to determine the presence of respiratory viruses. Results: One hundred and ninety-five subjects were included: 64 sCOPD patients, 118 AE-COPD patients and 13 SM. In 62 (52.6%) AE-COPD patients, a respiratory pathogen was detected. Serum levels of sTREM-1 were barely detectable in SM but were significantly increased in patients with sCOPD [97.5 (interquartile value 76.6) pg/ml] and AE-COPD [110.9 (98.5) pg/ml; p < 0.001]. There was no significant difference in sTREM-1 between sCOPD and AE-COPD (p = 0.277). However, in AE-COPD, sTREM-1 was significantly lower in patients with virus detection [87.5 (97.3) pg/ml] compared to those without [120.3 (99.7) pg/ml; p = 0.015]. No difference was found in AE-COPD patients with or without bacterial detection. Conclusions: The present study shows an increase in sTREM-1 in patients with COPD compared to SM but not in AE-COPD compared to sCOPD. Viral exacerbations showed significantly lower sTREM-1 levels than non-viral exacerbations.

Introduction
Chronic obstructive pulmonary disease (COPD) is characterized by progressive and for the most part irreversible airflow obstruction that involves an abnormal airway inflammatory response [1]. COPD also has a systemic inflammatory component, as several inflammatory mediators such as C-reactive protein and interleukin-6 have been found to be increased in blood of COPD pa-
tients [2, 3]. One interesting new inflammatory marker is the triggering receptor expressed on myeloid cells 1 (TREM-1), an activating receptor on neutrophil granulocytes, monocytes and macrophage subsets [4, 5]. The expression of TREM-1 is upregulated by microbial products, i.e. by Toll-like receptor ligands such as lipoteichoic acid of Gram-positive or lipo polysaccharide of Gram-negative bacteria. Activation of TREM-1 occurs in synergy with Toll-like receptor agonists upon activation of receptor-bearing cells resulting in the release of inflammatory mediators such as tumor necrosis factor-α and interleukin-8 and the initiation of neutrophil respiratory burst [4, 6–8]. TREM-1 is also produced in a soluble form [9] and released in patients with various inflammatory conditions. Elevated levels of soluble TREM-1 (sTREM-1) have been associated with the diagnosis and prognosis of patients with sepsis [10], severe pneumonia [11] and community-acquired pneumonia (CAP) [12]. Furthermore, sTREM-1 is detectable in patients with non-microbial inflammatory settings such as acute pancreatitis where it is associated with inflammation, but not with infection [13, 14], and also in autoimmune disorders like rheumatoid arthritis [15] and inflammatory bowel disease [16, 17]. Increased serum levels of sTREM-1 have also been observed in patients with stable COPD (sCOPD) compared with healthy controls, suggesting that sTREM-1 might be a useful biomarker for COPD [18].

The clinical course of COPD is typically interrupted by acute exacerbations, a major cause for the majority of disease-related morbidity and mortality. During these exacerbations, airway inflammation is increased and, in addition, in some patients, an increased systemic inflammatory response is detectable [19, 20]. Exacerbations may have many different triggers, the most common being microbial infection, bacterial [21] or viral [22, 23]. The use of antibiotics during acute exacerbation is often debated due to the fact that bacterial infections cannot always be proven. So far, the choice of antibiotic therapy is dependent on clinical symptoms including dyspnea and the amount and color of sputum [24] indicating the need for better markers of infection in acute exacerbations of COPD (AE-COPD).

Recent studies report that at least a quarter of exacerbations are of bacterial, another quarter of viral and a third quarter of mixed (bacterial + viral) origin [25, 26]. Many COPD patients and especially those with more severe disease show colonization of lower airways by bacteria [27]. Persistence of respiratory viruses, particularly adenoviruses [28] and respiratory syncytial virus (RSV) [29], in lower airways and lung parenchyma has also been recently described. The impact of bacterial colonization and/or viral persistence on local and systemic inflammation in COPD has not yet been fully clarified.

It has been proposed that plasma levels of sTREM-1 in patients with pneumonia might have a high accuracy and sensitivity in detecting microbial infections as underlying disease [11, 30, 31].

The hypothesis of this study is that sTREM-1 is increased in AE-COPD and that sTREM-1 may serve as a marker of infection in AE-COPD.

Therefore, we used an sTREM-1-specific assay to assess the amount of sTREM-1 in patients with sCOPD, patients with AE-COPD and smokers without COPD (SM). Furthermore, we investigated the ability of sTREM-1 measured in serum to differentiate between infectious triggers of AE-COPD.

Methods

Subjects

Three different groups were studied: (1) hospitalized subjects with sCOPD, (2) hospitalized patients with AE-COPD and (3) SM. The groups were defined as previously published [32]. Briefly, sCOPD patients did not have an exacerbation within the last 30 days prior to hospital admission, had no changes in therapy within the last 14 days (including inhaled and oral medication) and had been admitted for medical reasons other than pulmonary diseases to departments of internal medicine. None of these patients suffered from inflammatory conditions. The most frequent reasons for admission were cardiovascular disease in 39 patients and gastroenterologic disorders in 4 patients (reflux disease, n = 2; ulcus ventriculi, n = 1; poliposis coli, n = 1).

AE-COPD patients suffered from COPD as defined by GOLD [33]. Acute exacerbation was characterized by worsening in dyspnea, cough and expectoration. A routine posterior-anterior chest radiograph was evaluated on admission by expert radiologists to exclude other reasons for increased symptoms as pneumonia, tuberculosis, pulmonary fibrosis, bronchiectasis, bronchial carcinoma or congestive heart failure. AE-COPD patients were enrolled into the study immediately at presentation to the hospital (to the emergency department in all cases) in order to assure that all AE-COPD patients were enrolled at the maximum of their symptoms. Smokers had been smoking more than 10 pack-years, could have chronic symptoms such as cough and phlegm but did not report dyspnea and did not have bronchial obstruction [forced expiratory volume in 1 s (FEV1)/forced vital capacity (FVC) >70%, FEV1 >80% predicted]. None of the smokers had a history of COPD or asthma, or was using systemic or topic pulmonary medication. The smokers were recruited either from our smoking cessation initiative or by newspaper advertisement.

Both sCOPD patients and smokers had to be free from lower respiratory tract infection (LRTI). In case of sCOPD patients, this was assured by the clinical criteria defining stability of disease pointed out above in addition to the absence of radiographic signs...
of LRTI. In smokers, this could be only assured clinically by absence of symptoms suggesting LRTI and a normal lung auscultation.

The study was approved by the ethical committee of the Ruhr University of Bochum, Germany. Written informed consent was obtained from all patients and control subjects before inclusion in the study.

**Diagnostic Methods**
Clinical evaluation, spirometric tests, collection of peripheral blood, detection of respiratory viruses in nasal lavage and induced sputum were carried out as described before [22, 29, 34, 35].

**Quantification of sTREM-1 Levels in Serum**
Blood for sTREM-1 analysis was drawn from SM and sCOPD patients once. In all 118 AE-COPD patients, blood was drawn at admission, and in 78 patients, blood was additionally drawn before discharge.

For the detection of sTREM-1, anti-TREM-1 monoclonal antibody (Hycult Biotechnology, Uden, The Netherlands) was coated at 0.5 µg/ml in PBS, then blocked by addition of 100 µl of 15% BSA for 2 h at 37°C and washed as previously described [18]. Afterwards, the standard (recombinant TREM-1:immunoglobulin G1 in 7.5% BSA-PBS) and the samples were added and the plates were incubated for 2 h at 37°C. For analysis of patient samples, sera were diluted 1:10 in 5% BSA prior to addition to the plates. After incubation, plates were washed and the biotinylated detection polyclonal antibody anti-TREM-1 (5 µg/ml in 7.5% BSA-PBS; R&D Systems) was added for 2 h at 37°C. Plates were then washed again and developed using the Tetramethylbenzidine Peroxidase Substrate System (KPL, Gaithersburg, Md., USA). The absorbance was measured at 450 nm. Results are shown as means with SD of triplicates. The lower detection limit was defined by 2 SD of the blank values (5 pg/ml). The recovery rate was 90.6 ± 1%. Intra-assay variation was 7 ± 1%, interassay variation was 6 ± 5%.

**Statistical Analysis**
The primary objective of this study was to compare the levels of sTREM-1 in serum between COPD patients with or without an acute exacerbation and SM.

Data are presented as the median and the interquartile value, unless otherwise stated. Differences between groups were assessed by the Kruskal-Wallis test. To further analyze significant differences between two individual groups, a pair-wise comparison by two-sided Mann-Whitney U test was performed. Differences of paired variables within a group were analyzed by the Wilcoxon test. For discrete variables, frequencies were reported and compared by χ² test or Fisher’s exact test, as appropriate. The Yates correction procedure was applied to all comparisons.

Bivariate correlations for normally distributed variables were analyzed by Pearson’s test; for not normally distributed variables, Spearman’s test was used and the respective two-tailed significance was reported.

All significance levels were set to 5%. Data were analyzed and processed using SPSS version 12.0 on a Windows XP operating system.

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**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th></th>
<th>sCOPD</th>
<th>AE-COPD</th>
<th>SM</th>
<th>Kruskal-Wallis test p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects</td>
<td>64</td>
<td>118</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>67 (19)</td>
<td>66 (13)</td>
<td>47.5 (4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sex, M/F</td>
<td>56/8</td>
<td>95/25</td>
<td>7/6</td>
<td>0.032 (χ)</td>
</tr>
<tr>
<td>BMI</td>
<td>27.5 (7.7)</td>
<td>26.9 (6.3)</td>
<td>26.5 (6.6)</td>
<td>0.757</td>
</tr>
<tr>
<td>Smoking history pack-years</td>
<td>30 (47)</td>
<td>30 (32)</td>
<td>40.5 (26.8)</td>
<td>0.375</td>
</tr>
<tr>
<td>FEV₁</td>
<td>1.32 (0.8)</td>
<td>1.0 (0.5)</td>
<td>3.3 (1.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV₁%</td>
<td>45.4 (29.6)</td>
<td>35.1 (20.9)</td>
<td>98.4 (13.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FVC</td>
<td>2.5 (1.2)</td>
<td>1.9 (1.1)</td>
<td>4.2 (1.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FVC%</td>
<td>67.7 (20.4)</td>
<td>50.9 (26.1)</td>
<td>108 (16.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>54.2 (21.6)</td>
<td>46 (18.8)</td>
<td>80.6 (8.1)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as the median and the interquartile value (in parentheses), unless stated differently. BMI = Body mass index.

**Table 2. Detection of bacteria in induced sputum of AE-COPD patients**

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>5</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>4</td>
</tr>
<tr>
<td><em>Serratia</em> spp.</td>
<td>4</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>3</td>
</tr>
<tr>
<td><em>Proteus</em> spp.</td>
<td>3</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Morganella morganii</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Enterobacter cloacae</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Citrobacter freundii</em></td>
<td>1</td>
</tr>
<tr>
<td><em>Ochrobactrum anthropi</em></td>
<td>1</td>
</tr>
</tbody>
</table>

**Results**

**Subjects**
A total of 195 subjects were included into the study: 64 patients with sCOPD, 118 patients with AE-COPD and 13 SM. SM were significantly younger than COPD patients. They were comparable to COPD patients in terms of body mass index and smoking history. None of the SM showed airway obstruction (table 1), whereas patients with sCOPD and AE-COPD showed a significantly decreased FEV₁ and FVC in terms of absolute and % pre-
dicted values (table 1). In patients with AE-COPD, FEV$_1$ as well as FVC were also significantly lower compared to sCOPD patients.

Additionally, sputum cultures and nasal lavage were also analyzed in 118 patients with AE-COPD. In 62 (52.6%) of these patients, a bacterial pathogen was detected in sputum cultures. Gram-negative bacteria were the most frequent bacteria (table 2). Respiratory viruses were detected in 53 patients (44.9%). The most frequently discovered viral pathogens were RSV and influenza A virus (table 3). Twenty-eight patients were positive for both bacteria and respiratory viruses.

Serum Levels of sTREM-1

Serum levels of sTREM-1 were barely detectable in SM. In contrast, levels of sTREM-1 were significantly increased in patients with sCOPD [97.5 (interquartile value 76.6) pg/ml] and AE-COPD [110.9 (98.5) pg/ml; p < 0.001 compared with smokers] (fig. 1). There was no significant difference in sTREM-1 serum levels between sCOPD and AE-COPD (p = 0.277). However, when analyzing patients with AE-COPD, levels of sTREM-1 were significantly lower in patients with detectable viral infection in nasal lavage fluid [87.5 (97.3) pg/ml] compared with patients without detectable viral infection [120.3 (99.7); p = 0.015] (fig. 2). No such difference was found when AE-COPD patients with or without growth of bacteria in sputum culture were compared [109.5 (102.9) vs. 114.1 (96.4) pg/ml, respectively; p = 0.588]. In a subgroup of 78 AE-COPD patients, serum sTREM-1 levels at hospital admission [101.9 (98.5) pg/ml] were not statistically different from levels assessed before discharge [103.3 (90.9) pg/ml; p = 0.883] (fig. 3). The same was true for AE-COPD due to bacterial infection. Levels of sTREM-1 at admission [104.1 (72.2) pg/ml] were not significantly different to levels before discharge [95.7 (109.8) pg/ml; p = 0.709]. AE-COPD patients stayed in hospital for 13 ± 11 days.

Table 3. Detection of respiratory viruses in nasal lavage of AE-COPD patients

<table>
<thead>
<tr>
<th>Virus</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV</td>
<td>28</td>
</tr>
<tr>
<td>Influenza A</td>
<td>14</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>8</td>
</tr>
</tbody>
</table>

Fig. 1. sTREM-1 levels in sCOPD and AE-COPD patients compared to SM. Box and whisker plot: the central box represents the values from the lower to the upper quartile (25 to 75 percentile), the middle line the median and the whiskers the highest and lowest value, respectively. ** p < 0.001.

Fig. 2. sTREM-1 levels in AE-COPD patients without and with virus detection in nasal lavage. Box and whisker plot. * p = 0.015.

Relationship between sTREM-1 Levels, Inflammation and Airflow Obstruction

Serum levels of sTREM-1 were significantly related to peripheral blood leukocyte numbers ($r = 0.252$, $p = 0.001$), FEV$_1$ % predicted ($r = -0.201$, $p = 0.006$) and FVC % predicted ($r = -0.201$, $p = 0.006$). No other significant relationship of sTREM-1 with other variables was detected.
Levels of sTREM-1 in Infectious Exacerbations of COPD

Stratification according to Anthonisen Criteria

sTREM-1 levels in AE-COPD patients were analyzed after stratification according to Anthonisen criteria [18]. sTREM-1 levels were not significantly different between 43 subjects with Anthonisen type I exacerbation [114.9 (112.9) pg/ml], 42 patients with type II exacerbation [110.0 (79.1) pg/ml] and 30 patients with type III exacerbation [111.4 (103.9) pg/ml; p = 0.994].

Discussion

COPD exacerbations are of critical importance for the natural course of the disease. During AE-COPD, there is increased local and also increased systemic inflammation [36]. In a previous study, Phua et al. [37], using an immunoblot technique, found increased levels of sTREM-1 in patients with COPD exacerbation compared to healthy controls. However, increased serum levels of sTREM-1 have also been described in patients with sCOPD [18]. Therefore, in the present study, we tested sTREM-1 levels using an established ELISA technique in a large and well-defined cohort of patients with AE-COPD and compared them to patients with sCOPD and SM. Similarly to previous studies, we found increased levels of sTREM-1 in serum of patients with sCOPD compared to SM where sTREM-1 was low or not detectable. In patients with hospitalized AE-COPD serum, levels of sTREM-1 were also increased; however, we found no significant difference compared with patients with sCOPD. These findings suggest that sTREM-1 is not a suitable marker for detecting acute exacerbations in patients with AE-COPD. A reason for this might be the fact that sTREM-1 levels are already significantly increased in advanced sCOPD patients, and a further increase during AE-COPD cannot be detected. In this study, we investigated patients with advanced COPD. It is possible that sTREM-1 levels could differentiate between sCOPD and AE-COPD in less advanced COPD. However, sTREM-1 is a marker for COPD, comparable to other inflammatory diseases, such as acute pancreatitis [13, 14], rheumatoid arthritis [15] and inflammatory bowel disease [16, 17]. Our study clearly shows significantly elevated sTREM-1 levels in advanced COPD patients compared to asymptomatic smokers (SM group). Future studies will have to demonstrate whether sTREM-1 could help to differentiate asymptomatic smokers from patients with COPD of all severity grades or whether the differences observed only relate to the extremes of the spectrum. Interestingly, sTREM-1 levels were not different in AE-COPD patients at hospital admission compared to levels before discharge. This indicated that sTREM-1 might well play a role in COPD pathogenesis per se but not in acute exacerbations. Moreover, our findings highlight that specific processes must take place in the transition from asymptomatic smokers to COPD, and sTREM-1 might be involved. Further studies are warranted to better understand the role of sTREM-1 in this regard. Our patients had moderate to severe airflow obstruction and advanced COPD. Future studies should investigate sTREM-1 levels in early, not-advanced COPD (GOLD I–II).

We could not replicate the findings of Phua et al. [37] with respect to stratification according to Anthonisen criteria. They measured sTREM-1 in 43 AE-COPD patients, 16 type I and 27 type II/III exacerbations. There was an important overlap in sTREM-1 levels, but the difference was statistically significant. In our cohort of 118 AE-COPD patients, sTREM-1 levels were similar in type I and II/III exacerbations. In their publication, Phua et al. [37] do not give further clinical details of the subgroups, which renders a further analysis of the possible differences in study populations impossible.

Exacerbations of patients with COPD are triggered by different factors. About 75% of exacerbations are believed to be of infectious origin and either viral, bacterial or both pathogens can be detected in these patients. Interestingly, during virus-induced AE-COPD, inflammatory cells detected in induced sputum differ from patients with bacterially induced AE-COPD. Indeed, both bacterial and viral infection lead to increased amounts of neutrophils in induced sputum; however, virally induced ex-
Acute exacerbations are often associated with increased numbers of eosinophils [25]. These differences might also influence sTREM-1 levels.

In the present study, in more than 50% of patients, a pathogen (either viral or bacterial) could be detected. Predominantly isolated pathogens were RSV and influenza, similar to previous reports. Interestingly, patients with AE-COPD and viral detection in nasal lavage showed overall significantly lower levels of serum sTREM-1 compared to AE-COPD patients without viral detection. This suggests that differences in origin and type of inflammation affect the systemic response. Indeed, in patients with sepsis, serum levels of sTREM-1 were associated with patient survival [10]. However, a cross-sectional study is not suited to accurately define the diagnostic relevance of sTREM-1 in this regard. In acute exacerbations of COPD, frequently, several different pathogens are discovered simultaneously [25]. Moreover, COPD patients are frequently colonized with bacteria. All these issues influence the expression of sTREM-1 and cannot be controlled for in a case-control setting. sTREM-1 might become a valuable marker for the differentiation between viral and bacterial infection in the future, but this has to be shown in a large longitudinal cohort with reliable intra-individual pathogen detection before, during and after exacerbation.

In patients with AE-COPD, sTREM-1 levels were evaluated at admission and before discharge. The median hospital stay was 18.2 ± 15.9 days, suggesting that the initial infection should have been cleared. We found no differences in sTREM-1 levels measured at all time points. This is comparable to results in patients with CAP. In this patient group, sTREM-1 serum levels at admission were unchanged during follow-up. Furthermore, sTREM-1 levels at admission in patients with subsequent clinical failure were similar to patients with a subsequent successful outcome [38], suggesting that serum sTREM-1 is not a suitable prognostic parameter for patients with CAP. Similarly, in patients with advanced COPD, it is not possible to differentiate between sCOPD and AE-COPD based on serum sTREM-1 levels. The lack of increase in sTREM1 during AE-COPD may partly be explained by the high baseline levels, probably due to spillover of inflammatory cytokines from the lung into the systemic circulation or due to bacterial colonization present in these patients with moderate to severe COPD. However, bacterial colonization is also found in asymptomatic smokers [39], suggesting that other factors directly related to the pathophysiology of COPD might be responsible for the increase in sTREM-1. Clearly, the mechanism leading to increased levels of sTREM-1 in COPD needs further study, and our findings strongly support future research on this issue.

This study has some limitations. Due to the study design and the retrospective assessment of symptoms in the past of patients in order to define stability of COPD in the sCOPD group, we cannot rule out that sTREM-1 levels in patients with clinically proven stability (a cohort study would be needed to better approach this issue) would have had lower sTREM-1 levels than our sCOPD group. This is clearly an interesting research question and should be addressed in a prospective cohort study design.

In summary, the present study shows an increase in serum sTREM-1 levels in patients with COPD compared to SM. However, sTREM-1 levels were elevated in patients with advanced COPD independently of the presence of acute exacerbation. This might be different in mild to moderate COPD. Viral exacerbations showed significantly lower sTREM-1 levels than non-viral exacerbations. The mechanism behind and the clinical relevance of these findings have to be further characterized.

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Financial Disclosure and Conflicts of Interest

None of the authors declare a possible conflict of interest.

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