SOLUBLE TRIGGERING RECEPTOR EXPRESSED ON MYELOID CELLS (s-TREM-1) IN SPUTUM OF PATIENTS WITH COMMUNITY-ACQUIRED PNEUMONIA OR PULMONARY TUBERCULOSIS: A PILOT STUDY

GREGORY R TINTINGER*, VAN DER MERWE JJ*, FICKL H°, RHEEDER P*, FELDMAN C, ANDERSON R°

*Department of Internal Medicine, Faculty of Health Sciences, University of Pretoria, South Africa; °Medical Research Council Unit for Inflammation and Immunity, Department of Immunology, University of Pretoria, and Tshwane Academic Division of the National Health Laboratory Service, Pretoria, South Africa; and Division of Pulmonology, Department of Internal Medicine, Charlotte Maxeke Johannesburg Academic Hospital and University of the Witwatersrand, Johannesburg South Africa.

Correspondence	Dr GR Tintinger
	Departments of Internal Medicine/Immunology
	P O Box 2034
	Pretoria, 0001
	South Africa
Telephone	012 319 2425
Telefax	012 323 0732
e-mail	grtintinger@gmail.com

Abstract

Purpose. Soluble Triggering Receptor Expressed on Myeloid Cells is upregulated on the surface of inflammatory cells in the presence of bacterial infections apparently excluding those due to *Mycobacterium tuberculosis*. Therefore, sputum concentrations of Soluble Triggering Receptor Expressed on Myeloid Cells (s-TREM-1) may be of value in distinguishing bacterial pneumonia from pulmonary tuberculosis (PTB) in patients with respiratory infections. The current pilot study was designed to evaluate whether s-TREM-1 concentrations measured in the sputum of patients with suspected community-acquired pneumonia (CAP) allowed differentiation of those patients with PTB from other causes of pneumonia and to correlate s-TREM-1 with the CURB-65, a marker of disease severity.
Methods. Soluble s-TREM-1 concentrations were measured in sputum samples from patients admitted to a tertiary hospital with CAP or PTB by means of an ELISA procedure.
Results. Soluble-TREM-1 was readily detectable and quantifiable in sputum samples from patients with both CAP and PTB, with concentrations of 234 $\pm$ 47 and 178 $\pm$ 36 pg/ml respectively, but did not differ significantly between the 2 groups. However, patients with PTB had significantly lower leukocyte counts, 9 $\pm$ 1.3 *versus* 15 $\pm$ 1.4 x 10^9/l compared to those without PTB. Interestingly, sputum s-TREM-1 concentrations correlated significantly with the CURB-65 pneumonia severity score calculated at the time of admission.
Conclusions. Soluble-TREM-1 expression is upregulated in patients with both CAP and PTB, but does not differentiate these two conditions. Sputum concentrations of s-TREM-1 may predict the severity of disease in patients with CAP.

Keywords: Sputum s-TREM-1, Community-acquired pneumonia, Pulmonary Tuberculosis and CURB-65

Introduction

Infection of the pulmonary parenchyma results in the activation of macrophages and neutrophils which participate in the rapid, innate immune response aimed at eliminating microbial pathogens. Triggering receptor expressed on myeloid cells (s-TREM-1) is upregulated on the surface of these cells with consequent amplification of their pro-inflammatory activity [1]. The soluble form of the receptor, s-TREM-1, can be measured in plasma and other body fluids such as pleural or peritoneal fluid [2] and may differentiate infectious from non-infectious causes of the systemic inflammatory response syndrome (SIRS). Soluble-TREM-1may be useful in distinguishing pneumonia from other causes of pulmonary infiltrates such as interstitial lung disease [3]. Although s-TREM-1 is markedly upregulated in the presence of bacterial or fungal infections, its role during mycobacterial infections remains controversial. Initial *in vitro* studies suggested that mycobacteria do not upregulate s-TREM-1[4], while subsequent clinical observations have not supported this finding [2, 5].

The current human immunodeficiency virus (HIV) epidemic manifest in sub-Saharan Africa has resulted in a dramatic increase in the incidence of pulmonary tuberculosis (PTB). Consequently, numerous patients admitted to hospitals in this region with fever and pulmonary infiltrates may have pulmonary tuberculosis, rather than community-acquired pneumonia. This distinction is important as the therapy for these two conditions is different and delays in appropriate antimicrobial therapy may increase the mortality of CAP [6]. Acid-fast bacilli may

not be detected in the sputum of HIV positive patients [7], which also delays initiation of anti-tuberculous therapy until culture results become available.

The current study was designed to determine whether s-TREM-1 measured in the sputum of patients admitted with respiratory infections can distinguish CAP from PTB and to correlate s-TREM-1 concentrations with inflammatory markers such as C-reactive protein, leukocyte counts and markers of disease severity such as CURB-65. To our knowledge, no previous studies have evaluated the role of s-TREM in sputum samples of patients with respiratory infections. Our results suggest that s-TREM-1 measured in sputum of patients with respiratory infections does not differentiate CAP from PTB, but does correlate with markers of disease severity.

Methods

Sputum for s-TREM-1 measurements was obtained during the routine diagnostic evaluation of patients admitted to the Department of Internal Medicine at Steve Biko Academic Hospital (Pretoria, South Africa) with suspected community-acquired pneumonia. Patients were recruited within 12 hours of admission to hospital, and following informed consent from the patient, a sample of sputum was collected. For those patients who could not easily expectorate sputum, hypertonic saline induction was used to facilitate production of a sputum sample. All patients received empiric antibiotic therapy according to standard guidelines

with most patients receiving intravenous cefuroxime and a macrolide. The sputum sample was sent to the Microbiology Laboratory for microscopy, culture and antimicrobial susceptibility testing. Microscopy allows an estimate of the quality of the sample by determining the relative numbers of polymorphonuclear leukocytes and squamous epithelial cells. A score of 0 – 3 is assigned to each sample by the microbiologist with 3 representing the highest quality, while a score of 0 indicates significant contamination with saliva [8]. The remainder of the sample was transported to the Immunology Laboratory, labeled and frozen at -70°C within 24 hours of collection.

Once the required number of samples had been received, s-TREM-1 concentrations were measured using a capture ELISA procedure (Quantikine R&D Systems). Sputum samples were processed according to a modification of the method described by Pizzichini *et al* [9]. The principle underlying this method is based on the use of dithiothreitol (DTT), which converts the gel form of sputum to a liquid phase due to its mucolytic properties. After centrifugation, the supernatant liquid phase has been used to measure eosinophil cationic protein, tryptase, albumin and various cytokines. This method of sputum processing has been shown to be reliable with good repeatability and validity [10].

The tube containing the sputum sample was weighed which allowed calculation of the weight of the sputum alone, by subtracting the known tube mass from the total mass of tube plus sputum. A volume of 0.1% DTT, equal to four times the weight of the sputum was added to the tube. The sample was agitated in a vortex mixer with gentle aspiration using a Pasteur pipette, to ensure mixing. This was

followed by rocking of the sample with a bench rocker for 15 minutes. A volume of Dulbecco's phosphate buffered saline (D-PBS) equal to the volume of DTT was added to and mixed with the liquefied sputum by rocking for 5 minutes. The sample was then centrifuged at 790 *g* (2250 rpm) for 10 minutes and the fluid phase contents transferred to a clean tube for determination of the s-TREM-1 concentration with the final value corrected for dilutions carried out during the sputum processing.

The presence of acid-fast bacilli (Ziehl-Neelsen staining) and/or culture of mycobacteria was considered diagnostic of pulmonary tuberculosis. In addition, the white cell count and C-reactive protein (CRP) were recorded for each patient and serological testing to determine the HIV status of the patient was carried out at the discretion of the treating physician.

The clinical suspicion of pneumonia was based on the following criteria: Any patient presenting with recent onset of fever and cough associated with abnormal infiltrates on the chest radiograph [11]. The CURB-65 score was calculated for each patient on admission as a guide to the severity of the presumed pneumonia [12]. This score is derived by allocating one point for the presence of any of the following: age $\geq$ 65 years, confusion, urea > 7 mmol/l, respiratory rate $\geq$ 30/min, and systemic hypotension defined as a systolic blood pressure < 90 mmHg or diastolic blood pressure $\leq$ 60 mmHg. Exclusion criteria included underlying malignancy of the airways, presence of chronic obstructive airways disease or

asthma, critically-ill or ventilated patients, and patients who received antibiotic treatment for more than 24 hours.

The study was approved by the Research Ethics Committee of the Faculty of Health Sciences, University of Pretoria and Steve Biko Academic Hospital.

Statistical Analysis

Results are expressed as the mean ± standard error of the mean (S.E.M.), as well as the median and range (25^{th}:75^{th} percentiles). Levels of statistical significance were calculated using the Mann-Whitney U-test for comparison of non-parametric data. Spearman rank correlation was used to measure the degree of dependency between variables. $P < 0.05$ was considered significant.

Results

Eighty-nine patients were screened and 42 of these met inclusion criteria and were able to provide sputum samples. Two samples were lost during transportation to the laboratory. Three samples could not be analyzed due to poor quality (score = 0). The patient demographic data together with the CURB-65 severity score, duration of hospitalization, temperature, white blood cell counts (WBC) and C-reactive protein (CRP) concentrations, are shown in Table 1.

A diagnosis of pulmonary tuberculosis determined as a positive Ziehl-Neelsen smear for acid-fast bacilli or culture of *M. tuberculosis* from the sputum was confirmed in 16 patients. The mean sputum s-TREM-1 concentrations, sputum quality score (Bartlett) [8], WBC, serum CRP, CURB-65 score and HIV status of this group of patients, as well as the corresponding values for those patients with CAP not due to *M. tuberculosis* (PTB) (n = 19) are shown in Table 1.
The mean sputum s-TREM-1 concentration for those patients with tuberculosis was 178 $\pm$ 36 compared to 234 ± 47 pg/ml for those without tuberculosis ($p > 0.05$). The quality of the sputum samples was acceptable in both groups with mean values for the Bartlett scores of 1.3 $\pm$ 0.2 for those with tuberculosis and 1.3 $\pm$ 0.15 for those without TB.

The CRP did not differ significantly between those patients with pulmonary TB and those without. However, the WBC was significantly lower in those patients with pulmonary tuberculosis ($p < 0.05$).

The sputum s-TREM-1 concentrations for the total group of patients were found to correlate significantly with the calculated CURB-65 scores ($r= 0.78$) ($p < 0.05$) as the latter increased from 0 to 3, indicating more severe disease (Figure 1).

Discussion

The findings of the current study suggest that s-TREM-1 can be easily quantified in sputum samples of patients with CAP or PTB, but does not differentiate these two conditions. Soluble-TREM-1 was significantly upregulated in patients with PTB resulting in similar sputum concentrations of this inflammatory marker in patients with CAP or PTB. This contrasts with initial *in vitro* reports that s-TREM-1 expression is not upregulated by mycobacteria [4], but rather in response to other bacterial as well as fungal infections, and supports our previous findings that s-TREM-1 is markedly upregulated in pleural and peritoneal fluid samples from patients with tuberculous pleuritis and peritonitis [2]. Furthermore, almost all patients with PTB in this study were co-infected with the human immunodeficiency virus (HIV) in keeping with the HIV epidemic in sub-Saharan Africa. Interestingly, sputum s-TREM-1 concentrations were significantly elevated in these HIV-positive patients suggesting that this component of the innate immune system remains fully functional. Not surprisingly, a relative leukopaenia was observed in the HIV-infected patients, but this did not attenuate the magnitude of s-TREM-1 concentrations measured in their sputum.

Sputum s-TREM-1 concentrations correlated significantly with the calculated CURB-65 scores suggesting a relationship between s-TREM-1 and the severity of pneumonia. The concentration of sputum s-TREM-1 increased progressively as the CURB-65 score increased from 0 to 3. Sputum s-TREM-1 concentrations

may therefore be potentially more useful in predicting severity of disease and outcome than commonly measured serum markers such as C-reactive protein or procalcitonin. Indeed, bronchoalveolar lavage fluid (BALF) concentrations of s-TREM-1 have been shown to correlate with mortality in patients with CAP [13]. In addition, sputum s-TREM-1 measured on admission may allow clinicians to institute empiric antimicrobial therapy for those patients considered to be at highest risk.

It is also important to note that mixed infections may occur in HIV-infected patients which may contribute to the increased s-TREM-1 concentrations. However, sputum cultures are not reliable for identifying the pathogen in CAP and therefore it is difficult to determine the number of patients, if any, with combined *M. tuberculosis* and bacterial pneumonia.

Limitations of this pilot study include the relatively small number of patients and the difficulty in obtaining suitable sputum samples from some patients with pneumonia which could have biased the results. Further studies with larger numbers of patients may be useful to confirm these results and correlate sputum s-TREM-1 values with in-hospital mortality.

In conclusion, this study demonstrates that s-TREM-1 is easily quantifiable in sputum samples from patients with pneumonia and that *M. tuberculosis* appears to significantly upregulate s-TREM-1 in both immunocompetent and

immunocompromised (HIV-infected) patients. Sputum s-TREM-1 concentrations correlated significantly with the CURB-65 severity score.

Conflict of interest statement

The authors declare no conflict of interests.

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Table 1: Patient characteristics and serum C-reactive protein (CRP), white blood cell counts (WBC), CURB-65 scores, duration of hospitalization, sputum s-TREM concentrations and HIV status for all patients combined, as well as those with bacterial pneumonia or pulmonary tuberculosis.

		All Patients (n = 35)	Bacterial Pneumonia (n = 19)	Pulmonary Tuberculosis (n = 16)
Age (years)		**40 ± 13**	**45 ± 16**	**36 ± 11**
Gender	**Males**	**13**	**6**	**7**
	Females	**22**	**13**	**9**
CRP (mg/l)	**Mean ± S.E.M**	**198 ±15.7**	**195 ± 16**	**208 ± 26**
	Median (25:75)	**187 (132:243)**	**207 (135:245)**	**179 (138:243)**
WBC (x10^9/l)	**Mean ± S.E.M**	**11.4 ± 1.1**	**15 ± 1.4**	**9 ± 1.3***
	Median (25:75)	**10 (7.5:15.2)**	**15.6 (12:19.5)**	**8.3 (5.8:102)**
CURB-65	**Mean ± S.E.M**	**1.23 ± 0.15**	**1.1 ± 0.19**	**1.4 ± 0.26**
	Median (25:75)	**1 (0:2)**	**1 (0:2)**	**1.5 (0.25:2)**
Hospitalization (Days)	**Mean ± S.E.M**	**7.8 ± 0.7**	**7.8 ± 0.45**	**8 ± 1.2**
	Median (25:75)	**7 (6:9)**	**8 (6:9)**	**7.5 (4.3:8.8)**
Sputum s-TREM-1 (pg/ml)	**Mean ± S.E.M**	**209 ± 30.4**	**234 ± 47**	**178 ± 36**
	Median (25:75)	**159 (67:328)**	**164 (69:359)**	**132 (65:278)**
HIV Co-Infection		**18**	**3**	**15**

Results are expressed as the mean ± SEM and median (25^{th}:75^{th}) percentiles.

*$P < 0.05$ for comparison of patients with community-acquired pneumonia or pulmonary tuberculosis.

Figure 1: Relationship between sputum s-TREM-1 concentrations (mean ± SEM; pg/ml) and CURB-65 scores of patients with pulmonary TB □, or CAP ▨.