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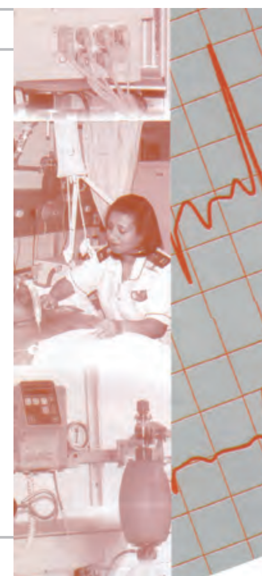
# Soluble triggering receptor expressed on myeloid cells (s-TREM-1) from endotracheal aspirates in critically ill patients: A potential marker of the dynamic inflammatory burden of the lower respiratory tract



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**Objectives.** The study was designed to evaluate the role of soluble triggering receptor expressed on myeloid cells (s-TREM-1) measured in samples of endotracheal aspirates from critically ill, intubated patients as a marker of inflammation or pneumonia.

**Methods.** The Clinical Pulmonary Infection Score (CPIS), a commonly utilised clinical predictor of ventilator-associated pneumonia (VAP), was calculated for each patient at the same time as endotracheal aspirates were obtained using sterile techniques, in order to correlate the CPIS with s-TREM-1 concentrations determined in the laboratory using a validated enzyme-linked immunosorbent assay (ELISA) procedure.

**Results.** Thirty patients with intensive care unit stays ranging from 2 to 39 days were included in the study. s-TREM-1 was detectable in endotracheal aspirates from all patients, and a wide range of concentrations from 13 to >4 000 pg/ml was observed. The mean s-TREM-1 concentrations for patients with a CPIS <6 ($N$=15) and for those with a CPIS ≥6 were 592 (standard error of the mean (SEM) 288) and 382 (SEM 119) pg/ml, respectively ($p$>0.05).

**Conclusions.** s-TREM-1 is readily detectable and quantifiable in endotracheal aspirates from critically ill patients, but does not correlate with the CPIS. The wide range of measured s-TREM-1 concentrations suggests that this pro-inflammatory marker may reflect a progressive increase in the dynamic inflammatory burden of the lower respiratory tract as colonisation by microbial pathogens leads to ventilator-associated tracheobronchitis (VAT) and ultimately VAP. Serial determinations of s-TREM-1 in this setting may therefore be of greater value than the CPIS in differentiating VAT from VAP and provide an alternative threshold for the initiation of empiric antimicrobial therapy.

Ventilator-associated pneumonia (VAP) remains a significant cause of mortality in critically ill patients, claiming the lives of up to 50% of those who develop this complication.[1]

VAP occurs commonly in the intensive care unit (ICU) setting, with an estimated incidence of 1% per day in the ICU.[2] The high incidence and considerable mortality attributable to VAP mean that clinicians

need to maintain a high index of suspicion in any critically ill ventilated patient manifesting a new fever. The survival of patients with VAP is critically dependent on the early administration of appropriate antimicrobial agents.[3] However, the diagnosis of VAP is fraught with difficulty owing to the lack of objective diagnostic criteria and wide-ranging expert opinions on the merits of invasive (bronchoscopically guided) versus non-invasive (endotracheal aspirates) diagnostic procedures.[4] The Clinical Pulmonary Infection Score (CPIS) is commonly utilised as a clinical predictor of VAP, and although lacking in specificity a CPIS >6 can be used as a threshold for the institution of empiric antibiotic therapy.[5]

Recently, soluble triggering receptor expressed on myeloid cells (s-TREM-1), which is up-regulated on the surfaces of inflammatory cells in the presence of bacterial infection, has been used as a diagnostic marker of infection in patients with pleuritis, peritonitis[6] and VAP.[2] Measurement of s-TREM-1 in broncho-alveolar lavage fluid (BALF) predicted VAP with a sensitivity and specificity of 98% and 90%, respectively.[2] As many ICUs are not able to perform bronchoscopy, interest has emerged in the role of non-invasive diagnostic techniques incorporating objective measurements of s-TREM-1 in these samples for detecting VAP.[7] Furthermore, it is likely that ventilator-associated tracheobronchitis (VAT) is an intermediate stage between colonisation of the respiratory tract of intubated patients and subsequent VAP.[8]

The current study was designed to evaluate the role of s-TREM-1 concentrations measured in samples of endotracheal aspirates from critically ill intubated patients as a marker of inflammation or pneumonia, and to correlate these with the CPIS determined at the same time as the endotracheal aspirates were obtained.

## Materials and methods

Endobronchial aspirates were obtained by nursing staff using a sterile technique from critically ill patients (surgical or medical) admitted to the medical ICU at Pretoria Academic Hospital following informed consent from patients' relatives. Endobronchial aspirates are sent routinely twice weekly for all patients, as well as for any patient who develops a new fever or signs of sepsis (systemic inflammatory response syndrome, SIRS) and those with suspected VAP. The physician's decision to obtain a bronchial aspirate for culture was based on standard practice criteria in our ICU. A CPIS was calculated for each patient immediately before or after collection of the aspirate. The CPIS score was determined for each patient by assigning a score as shown in Table I.

The sample obtained was sent for microscopy and culture to the microbiology laboratory. Microscopy allows an estimate of the quality of the sample by determining the relative numbers of polymorphonuclear leucocytes and squamous epithelial cells. A score of 0 - 3 is assigned to each sample by the microbiologist, with 3 representing the highest quality while 0 indicates significant contamination with saliva9 (Table II). The remaining sample was submitted to the immunology laboratory for further processing. Samples sent to immunology were frozen at -20°C until the specified number (30) had been received. Sputum samples were processed according to a modification of the method described by Pizzichini *et al.*,[10] which has been shown to be reliable with good repeatability and validity. The principle underlying this method is based on the use of dithiothreitol (DTT, 0.1%), the mucolytic properties of which convert the viscid, gel form of sputum to a liquid phase. After centrifugation, the supernatant liquid phase has been used to measure

**Table I. Modified Clinical Pulmonary Infection Score (CPIS)**

| | Points | | |
|---|---|---|---|
| | 0 | 1 | 2 |
| Tracheal secretions | Rare | Abundant | Abundant + purulent |
| Chest X-ray infiltrates | None | Diffuse | New localised |
| Temperature (°C) | ≥36.1 and ≤38.4 | ≥38.5 and ≤38.9 | ≥39.0 or ≤36.0 |
| Leucocyte count ($\times10^9$/µl) | ≥4 and ≤11 | ≤4 and ≥11 | ≤4 or ≥11 plus band forms ≥0.5 |
| $PaO_2/FiO_2$ | >240 or ARDS | | ≤240 and no evidence of ARDS |
| Microbiology | Negative | Pathogen cultured | Gram stain and culture positive for same organism |

ARDS = acute respiratory distress syndrome.

**Table II. Bartlett's grading system for assessing the quality of sputum samples**

| | Grade |
|---|---|
| No. of neutrophils per 10 × low-power field | |
| <10 | 0 |
| 10 - 25 | +1 |
| >25 | +2 |
| Presence of mucus | +1 |
| No. of epithelial cells per 10 × low-power field | |
| 10 - 25 | -1 |
| >25 | -2 |
| Final score | Total |

eosinophil cationic protein, tryptase, albumin and various cytokines.

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 4 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 equal to the volume of DTT was added to and mixed with the liquefied sputum by rocking for 5 minutes. The sample was centrifuged at 790 *g* (2 250 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. A capture enzyme-linked immunosorbent assay (ELISA) procedure (Quantitative RMD Systems) was used to quantify s-TREM-1.

In addition, the white blood cell count (WCC), serum C-reactive protein (CRP), procalcitonin (PCT) and lactate concentrations were recorded for each patient.

## Statistical methods

Results are expressed as means (standard error of the mean (SEM)), together with the median and range (10th - 90th percentile). Levels of statistical significance were calculated using the Mann-Whitney U-test for comparison of non-parametric data, and the Pearson correlation was used to measure the degree of dependency between variables. $p<0.05$ was considered significant.

## Results

A total of 30 patients were included in the study. Their demographic data and clinical diagnoses are shown in Table III.

The bronchial aspirates from 2 patients were spoiled and could not be used. Endobronchial aspirates were representative of the lower respiratory tract with an overall quality score (Bartlett) of 2.3 (SEM 0.15). Most of the patients included in the study (22) had been in the ICU for >48 hours and were therefore at risk for developing VAP. Three patients with community-acquired pneumonia (CAP) were included in the study and samples were submitted within 24 hours of admission to the ICU.

**Table III. Patient characteristics**

| | Age (mean (SEM)) | Diagnoses on admission |
|---|---|---|
| Males (11) | 46 (6) | Status epilepticus, diabetes and myocardial infarction, pneumonia, renal failure, skull fracture, multiple trauma, organophosphate poisoning, extradural haemorrhage |
| Females (19) | 41 (4) | Myasthenia gravis, diabetic keto-acidosis, pneumonia, renal failure, organophosphate poisoning, alveolar haemorrhage, stroke, intracerebral haemorrhage, septic shock, urosepsis, meningitis, chronic obstructive pulmonary disease, *Pneumocystis jirovecii* pneumonia. |

SEM = standard error of the mean.

Microbial pathogens were cultured from 21 of 30 endobronchial aspirate samples. The organisms identified were *Acinetobacter baumanii* (7), *Klebsiella pneumoniae* (3), *Enterobacter* species (3), *Candida* species (3), *Pseudomonas aeruginosa* (1), *Stenotrophomonas maltophilia* (1), *Staphylococcus aureus* (1), *Streptococcus pneumoniae* (1) and *S. epidermidis* (1).

Blood cultures were positive in 11 patients, and the following pathogens were isolated: *S. pneumoniae* (3), *Enterobacter* species (2), *A. baumanii* (1), *K. pneumoniae* (2), *P. aeruginosa* (1), *S. epidermidis* (1) and *Proteus mirabilis* (1).

s-TREM-1 was detectable in all endobronchial samples tested, and the concentrations ranged from 13 to >4 000 pg/ml. The highest s-TREM-1 concentration (>4 000 pg/ml) was obtained from a patient with acute CAP due to *S. pneumoniae*.

When the patients were divided into two groups, namely those with a CPIS <6 (low likelihood of VAP) or ≥6 (higher likelihood of VAP), there were 13 patients in the former group and 15 in the latter. The quality of the endobronchial aspirates in each group was similar (Table IV).

The s-TREM-1 concentrations measured in endobronchial aspirates for each group together with the corresponding values for CRP, PCT, WCC and serum lactate are shown in Table IV.

The CPIS scores for the two groups of patients were significantly different, being 3 (SEM 0.4) and 8 (SEM 0.5). Interestingly, the CPIS values in each group did not correlate with the s-TREM-1 concentrations from endobronchial aspirates, and no significant difference in s-TREM-1 concentrations was observed between patients with a CPIS <6 and those with a score ≥6. Furthermore, when all patients were analysed together as a single group, only the serum lactate concentration correlated with CPIS values ($r$=0.4) ($p$<0.05).

## Discussion

The study evaluated the role of s-TREM-1 measured in endobronchial aspirates from critically ill patients as a marker of inflammation or pneumonia. The CPIS is considered to be of value in the diagnosis of VAP,[1] and despite a wide range of reported sensitivities and specificities[5] it was found to be the best clinical predictor of VAP in critically ill patients (odds ratio of 3).[2] Current guidelines recommend that patients with a CPIS ≥6 should be treated empirically with antimicrobial therapy that covers those pathogens most likely to cause early (<5 days) or late (>5 days) VAP,[11] and that an endobronchial aspirate or broncho-alveolar lavage should be performed at the same time to obtain samples for quantitative cultures.[1,11] As cultures usually require 3 - 4 days to detect the growth of micro-organisms, a diagnostic test that provides more rapid results would assist clinicians in deciding whether or not to initiate empiric antimicrobial therapy. Delayed therapy of VAP is associated with a high mortality,[12] and it has been suggested that adjusting the antibiotic spectrum when culture results become available does not reduce the mortality associated with VAP.[12] Therefore, s-TREM-1 concentrations in bronchial lavage samples that can be determined rapidly in the laboratory have been reported to

**Table IV. s-TREM-1 concentrations in endobronchial aspirates from patients with a CPIS <6 or ≥6, as well as the blood CRP, PCT, WCC, serum lactate concentrations and sample quality score for each group**

| | | CPIS <6 (*N*=13) | CPIS ≥6 (*N*=15) |
|---|---|---|---|
| s-TREM-1 (pg/ml) | Mean (SEM) | 592 (288) | 382 (119) |
| | Median (10:90) | 295 (94:2667) | 301 (19:1264) |
| CRP (mg/l) | Mean (SEM) | 93 (13) | 148 (28) |
| | Median (10:90) | 93 (56:127) | 179 (13:222) |
| PCT (µl) | Mean (SEM) | 7.7 (6) | 52 (34) |
| | Median (10:90) | 0.8 (0.4:51) | 2.1 (0.4:282) |
| White cell count ($10^9$/µl) | Mean (SEM) | 11 (1.5) | 15 (1.8) |
| | Median (10:90) | 917 (4.9:12.9) | 12.6 (5.2:27.2) |
| Serum lactate (mmol/l) | Mean (SEM) | 1.3 (0.2) | 1.5 (0.24) |
| | Median (10:90) | 1.2 (0.6:1.8) | 1.4 (0.6:3.2) |
| Endobronchial aspirate quality score | Mean (SEM) | 2.54 (0.18) | 2.0 (0.24) |
| | Median (10:90) | (1.4:3) | (0.6:3) |

SEM = standard error of the mean.

predict VAP in critically ill patients.[13] However, this requires more invasive bronchoscopic procedures not available in many ICUs. Furthermore, recent evidence indicates that diagnosing VAP using cultures of blind endobronchial aspirates does not increase the morbidity or mortality of critically ill patients when compared with bronchoscopically obtained samples.[12] Although the simpler procedure of endotracheal aspiration offers some advantages to clinicians, these samples may be contaminated by micro-organisms colonising the upper respiratory tract and therefore may not be truly representative of lower respiratory tract pathogens.

This apparent lack of specificity of cultures of endobronchial aspirates could potentially be overcome by using a quantifiable marker such as s-TREM-1. s-TREM-1 was detectable in endobronchial aspirates from all patients in the current study. The mean s-TREM-1 of 592 (SEM 288) and 382 (SEM 119) pg/ml for patients with a CPIS $<6$ and $\geq 6$, respectively, suggests that inflammatory cells such as monocytes, macrophages or neutrophils are recruited to the lungs of critically ill patients from both groups. Importantly, s-TREM-1 concentrations did not correlate with the calculated CPIS. This may be due to limitations of the CPIS, which can be increased with pulmonary oedema, acute respiratory distress syndrome (ARDS) and respiratory failure not caused by pulmonary infections. The CPIS may therefore overestimate the true incidence of VAP[14] and account for the poor correlation with s-TREM-1. However, as no 'gold-standard' test is currently available to diagnose VAP,[4] the potential predictive value of s-TREM-1 in endobronchial aspirates is difficult to determine.

The CPIS in this pilot study did not correlate with serum PCT concentrations. However, the combination of CPIS and PCT has been reported to predict VAP with a specificity approaching 100%.[15] Future research is required to evaluate the role of s-TREM-1 in endobronchial aspirates combined with the serum PCT and CPIS as predictors of pneumonia and/or mortality in critically ill patients.

Endobronchial aspirates from the majority of patients (73%) in this study yielded positive culture results and the s-TREM-1 concentrations were in the ranges reported for other body fluids such as pleural or peritoneal fluid in the presence of active infection.[6] Interestingly, 3 of these patients had acute CAP with a mean s-TREM-1 concentration of 1 614 pg/ml.

The poor correlation between s-TREM-1 concentrations and the CPIS may also be explained by the presence of inflammation in the lower respiratory tracts of these patients. Recently, the specificity of s-TREM-1 for bacterial and fungal infections has been questioned. The relatively high s-TREM-1 concentrations may therefore be compatible with a state of ongoing bacterial infection and/or inflammation. Soluble-TREM-1 may reflect the burden of inflammation or infection of the lower respiratory tract, increasing progressively as colonisation by microbial pathogens leads to ventilator-associated tracheobronchitis (VAT)[8] and ultimately to VAP. Serial determinations of s-TREM-1 from endobronchial aspirates may detect rising concentrations[7] as colonisation/VAT becomes VAP and thus distinguish the former entities from true infection of the pulmonary parenchyma.

In conclusion, s-TREM-1 is readily quantifiable in endobronchial aspirates and may reflect the dynamic inflammatory burden of the lower respiratory tract in ICU patients. However, confirmation of this hypothesis will depend on serial determinations of this important marker of inflammation which may provide an alternative threshold to the CPIS for the initiation of empiric antimicrobial therapy.


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