

# HUMAN SOLUBLE TREM-1: LUNG AND SERUM LEVELS IN PATIENTS WITH BACTERIAL VENTILATOR ASSOCIATED PNEUMONIA

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**SUMMARY** – Human soluble triggering receptor expressed on myeloid cells (sTREM-1) is a glycoprotein of the immunoglobulin superfamily. In normal lung tissue, sTREM-1 is selectively expressed in lung alveolar macrophages specialized for pathogen clearance and is up-regulated in the presence of bacteria and fungi. The aim of this study was to assess sTREM-1 levels in serum and lungs of patients with ventilator associated pneumonia (VAP) and to evaluate its potential diagnostic role. The study cohort included 31 patients meeting the criteria for VAP, including clinical, microbiological, radiological and laboratory findings in patients on mechanical ventilation for more than 48 hours and with Clinical Pulmonary Infection Score (CPIS) >6. Serum and lung levels of sTREM-1 were obtained and tested for differences. The samples were analyzed using ELISA technique and the values were expressed in pg/mL. The samples for lung sTREM-1 were obtained from direct bronchial lavage fluid and serum samples from peripheral blood. Differences were tested by Mann Whitney U test with  $P < 0.05$  considered significant. In patients with bacterial VAP, a statistically significant difference was found between serum and lung sTREM-1 levels ( $P < 0.05$ ), with very high levels of sTREM-1 recorded in lung samples (mean value 1565 pg/mL). There was no statistically significant difference in pulmonary sTREM-1 level between the polymicrobial and monomicrobial VAP groups. In conclusion, sTREM-1 is present in a high concentration in the lungs of patients with bacterial VAP. sTREM-1 levels can help in making the diagnosis of bacterial pneumonia as a standalone marker.

**Key words:** *TREM1 protein, human; Pneumonia, ventilator associated; Receptors, immunologic*

## Introduction

Human soluble Triggering Receptor Expressed on Myeloid Cells (sTREM-1), a hydrophobic signal peptide, is a 26.4-kDa glycoprotein of immunoglobulin superfamily. This relatively recently discovered molecule activates neutrophils and macrophages by signaling transmembrane receptor complex with the adapter

protein DAP 12 chain subunit. The sTREM-1 enhances Toll-like receptor (TLR) response against bacterial, fungal and viral microbes and potentiates the secretion of proinflammatory chemokines and cytokines. The main sTREM-1 ligands are still not known but intracellularly it induces phosphorylation of the ERK1 and ERK2 kinases and C-gamma phospholipase, induces up-regulation of adhesion molecules, and has a strong effect on apoptotic mechanism and secretion of inflammatory cytokines, in particular TNF- $\alpha$  and IL-8<sup>1,2</sup>. In normal lung tissue, sTREM-1 is selectively expressed in lung alveolar macrophages specialized for pathogen clearance. Up-regulation in

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the presence of bacteria and fungi has been demonstrated in some studies, suggesting that sTREM-1 may turn out to be a good predictive marker for respiratory infections<sup>3-7</sup>. The aim of this study was to assess the levels sTREM-1 in serum and lungs of patients with proven ventilator associated pneumonia (VAP) of bacterial source.

## Patients and Methods

The study included 36 surgical Intensive Care Unit (ICU) patients meeting the criteria for bacterial VAP. Five patients were excluded for the presence of both fungal and bacterial source of pneumonia. The analysis included clinical, microbiological, radiological and laboratory findings of mechanically ventilated patients for more than 48 hours, with the Clinical Pulmonary Infection Score (CPIS) >6. Inclusion criteria are shown in Table 1. CPIS has been reported to have relevant sensitivity and specificity in the detection of VAP<sup>8-13</sup>.

There were 13 (42%) male and 18 (58%) female patients, age range 34–66 years. X-ray revealed unilateral and bilateral lung infiltrate in 65% and 35% of patients, respectively. All patients were treated with antibiotics prescribed according to the culture antibiotic sensitivity report. Figure 1 shows ventilator dependent days until meeting the criteria for VAP.

Serum and lung levels of sTREM-1 were determined and the difference was tested. Both samples were analyzed using ELISA sandwich technique (Human TREM-1 RWD Systems Inc., Minneapolis, USA) and values were expressed in pg/mL. Specimens for lung sTREM-1 and microbe detection were obtained using bronchoscopic directed bronchoalveolar lavage (BAL) fluid from x-ray evident side of the lung involved. Serum specimens were obtained from peripheral blood with sample venepuncture or central venous catheter. Monomicrobial VAP was found in 41% and polymicrobial VAP in 59% of cases. Methicillin resistant *Staphylococcus aureus* (MRSA) and *Streptococcus pneumoniae* predominated in the former, while *Staphylococcus aureus*, *Escherichia coli* and *Haemophilus influenzae* were most frequent in the latter. Differences were tested by Mann Whitney U test using the Statistica 7 and MedCalc statistical software, with  $P < 0.05$  considered statistically significant.

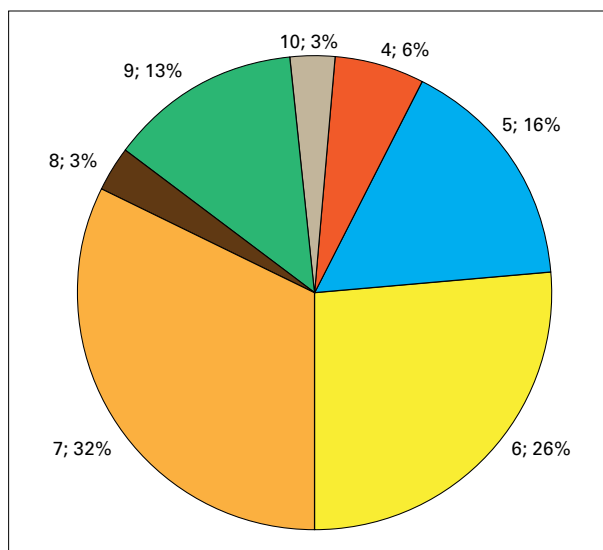


Fig. 1. Days on ventilation with all criteria for bacterial VAP completed.

## Results

There was a statistically significant difference between serum and lung sTREM-1 levels in patients with bacterial VAP ( $P < 0.05$ ). Very high levels of sTREM-1 were recorded in lung specimens, maximum 6542 pg/mL and minimum 155 pg/mL (mean 1565 pg/mL). Serum levels of sTREM-1 were considerably lower, ranging from minimum 27 pg/mL to maximum 529

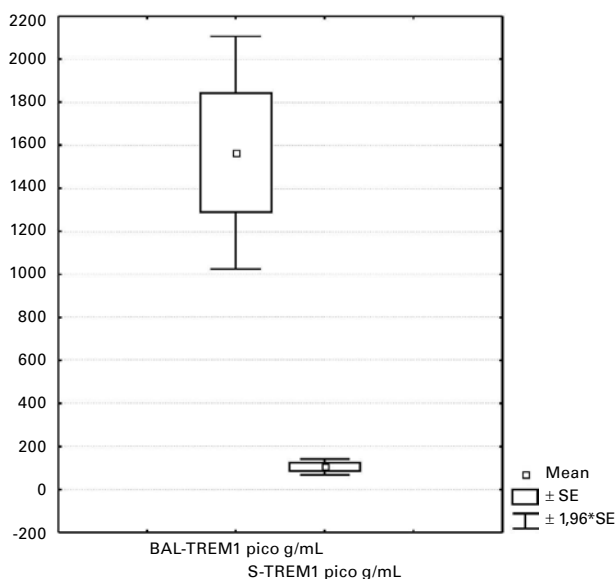


Fig. 2. Bronchoalveolar lavage (BAL) TREM-1 (pg/mL) vs. serum TREM-1 (pg/mL).

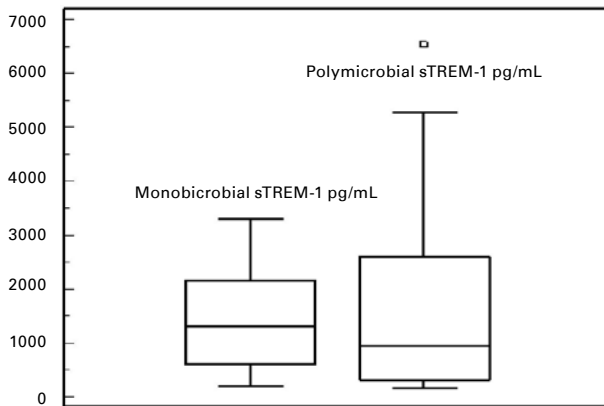


Fig. 3. Monomicrobial VAP vs. polymicrobial VAP sTREM-1 level (pg/mL).

pg/mL (mean 70 pg/mL). In all pair samples, lung sTREM-1 levels were several times greater than serum sTREM-1 levels, indicating local lung infection. Relations between serum and local lung sample levels are shown in Figure 2.

Comparison of lung sTREM-1 levels between polymicrobial and monomicrobial VAP groups showed no statistically significant difference. Median value was 955 pg/mL in the former and 1290 pg/mL in the latter, with greater dispersal in the polymicrobial group (minimal value 155 pg/mL and maximal value 6500 pg/mL). Relations of lung sTREM-1 values between monomicrobial and polymicrobial VAP are shown in Figure 3.

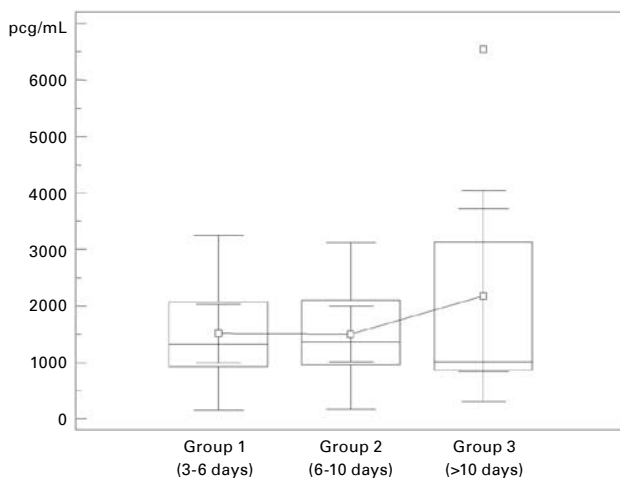


Fig. 4. Lung sTREM-1 level according to days on ventilation.

Comparison of pulmonary sTREM-1 levels in three patient groups according to the length of dependence on ventilation before developing VAP (group 1, day 3 to day 6; group 2, day 6 to day 10; and group 3, >day 10) showed slightly higher sTREM-1 levels in group 3, however, without statistical significance. Relations of sTREM-1 levels in these three patient groups are shown in Figure 4.

## Discussion

The aim of this study was to determine whether our patients with bacterial VAP showed increased levels of sTREM-1 in local lung samples in case of severe bacterial VAP and whether these levels showed correlation with sTREM-1 blood levels.

Our study results pointed to significant difference between sTREM-1 levels in local (BAL) and systemic (blood) samples in patients with strong evidence of bacterial VAP, suggesting that sTREM-1 might be used as a potential biomarker in these patients. The statistically significant difference between sTREM-1 levels in serum and lungs of patients with bacterial VAP indicated a strong local immune process. Some recent studies in the field report controversial results. Since the time of Gibot *et al.*<sup>3</sup>, who were the first to point to the rapid detection of sTREM-1 in BAL fluid in pneumonia patients, the early and exact diagnosis remains a problem and many clinicians point to it<sup>14,15</sup>. Four recent works in the field report doubtful results. Nitin *et al.* and Horonenko *et al.* evaluated sTREM-1 concentrations in BAL fluid and found no statistically significant difference between VAP patients and non-pneumonia subjects, suggesting that sTREM-1 may not be able to discriminate with strong potency between patients with infection and those without infection<sup>16,17</sup>. Another two studies by Huh *et al.*<sup>18</sup> and Determan *et al.*<sup>4</sup> report opposite results. Our findings are consistent with these two reports, implying good correlation between local sTREM-1 levels and presence of pneumonia. Such results may have been consequential to the fully developed and clinically overt bacterial VAP in our patients, admitting that in normal subjects sTREM-1 levels may not have been as high; a limitation of our study was that we did not include a clinical borderline group. In their studies, Nitin *et al.* and Horonenko *et al.* included mixed cases with clinically relevant and clinically vague presenta-

Table 1. Modified Clinical Pulmonary Infection Score (CPIS)<sup>10</sup>

CPIS points	0	1	2
Tracheal secretions	Rare	Abundant	Abundant + purulent
Infiltrate on chest x-ray	None	Diffuse	Localized
Temperature (°C)	36.5-38.4	38.5-38.9	<36 or >39
WBC count (1000/mm <sup>3</sup> )	4-11	<4 or >11	<4 or >11 + >500 bands
PaO <sub>2</sub> /FiO <sub>2</sub>	Without clinical ARDS or with P/F >240		Without clinical ARDS and with P/F <240

ARDS = acute respiratory distress syndrome

tion of VAP<sup>16,17</sup>. They also used a different detection technique and sampling, with a note that their patient populations were highly immunocompromised<sup>16,17</sup>. Also, the study conducted by Horonenko *et al.*<sup>17</sup> was retrospective by design, and both this and the study carried out by Nitin *et al.*<sup>16</sup> implicated better prognostic significance of clinical scores (CPIS) than biomarkers, although some newer studies report different opinion<sup>19,20</sup>. Our results are similar to those reported by Determan *et al.*<sup>4</sup> and Huh *et al.*<sup>18</sup>, however, the present study suffered from a limited number of patients and surgical procedures in their history, which may have contributed to the higher levels of sTREM-1 in all cases. Considering the still controversial and dubious results and opinion about newer biomarkers such as sTREM-1 in detecting pneumonia, large and well-designed multicenter studies may help elucidate the diagnostic role of this molecule in the future.

## Conclusion

sTREM-1 was found to be present in high concentration in lung specimens of patients with bacterial VAP. Blood sTREM-1 values were also greater than normal, indicating enhanced general immune reaction despite much greater local sTREM-1 levels. Almost the same local sTREM-1 levels were found in polymicrobial and monomicrobial VAP, thus denying the ability of stronger influence of some bacterial species on sTREM-1 immune expression in our cases (fungal and viral sources were not included). It seems that lung sTREM-1 levels may be useful in the diagnosis of bacterial pneumonia as a standalone marker. There is no need to obtain serum sTREM-1 for diagnostic verification when other clinical data and local findings (lung sTREM-1) support VAP.

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#### Sažetak

### RAZINE HUMANOG TOPLJIVOG TREM-1 U PLUĆIMA I SERUMU BOLESNIKA S BAKTERIJSKOM UPALOM PLUĆA POVEZANOM S UREĐAJEM ZA UMJETNO DISANJE

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Humani topljivi okidački receptor izražen na mijeloidnim stanicama (sTREM-1) je glikoprotein iz superporodice imunoglobulina. U normalnom plućnom tkivu sTREM-1 je selektivno izražen na makrofazima plućnih alveola, koji su specijalizirani za uklanjanje patogena, te podliježe regulaciji naviše u prisutnosti bakterija i gljiva. Cilj ove studije bio je procijeniti razine sTREM-1 u serumu i plućima bolesnika s upalom pluća povezanom s uređajem za umjetno disanje (VAP), kao i njegovu moguću dijagnostičku vrijednost. Ispitivanje je obuhvatilo 31 bolesnika koji su zadovoljavali kriterije za VAP, uključujući kliničke, mikrobiološke, radiološke i laboratorijske nalaze bolesnika priključenih na mehaničku ventilaciju kroz više od 48 sati i sa zbirom CPIS (*Clinical Pulmonary Infection Score*) većim od 6. Izmjerene su serumske i plućne razine sTREM-1 i izračunate razlike među njima. Uzorci su analizirani pomoću tehnike ELISA, a vrijednosti su izražene u pg/mL. Uzorci za procjenu sTREM-1 u plućima dobiveni su direktnom bronhijalnom lavožom, a uzorci seruma iz periferne krvi. Razlike su testirane Mann Whitneyevim U testom uz razinu značajnosti  $P < 0,05$ . Kod bolesnika s bakterijskom VAP utvrđena je statistički značajna razlika između serumske i plućne razine sTREM-1 ( $P < 0,05$ ), uz vrlo visoke razine sTREM-1 zabilježene u plućnim uzorcima (srednja vrijednost 1565 pg/mL). Nije bilo statistički značajne razlike u plućnoj razini sTREM-1 između skupina s polimikrobnom i monomikrobnom VAP. U zaključku, sTREM-1 je prisutan u visokoj koncentraciji u plućima bolesnika s bakterijskom VAP, pa bi stoga razine sTREM-1 mogle biti korisne u postavljanju dijagnoze bakterijske upale pluća kao jedan jedinstveni biljeg.

**Ključne riječi:** *Protein TREM1, humani; Pneumonija povezana s umjetnom ventilacijom; Receptori, imunološki*

