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Chapter IV

Membrane-Bound and Soluble Triggered Receptor Expressed on Myeloid Cells 1 (TREM-1) Discriminates Sepsis from Other Causes of Systemic Inflammation

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Abstract

Background and Objective: Accurate differential diagnosis between sepsis and other, non-infectious, causes of systemic inflammatory response syndrome (SIRS) is crucial for timely intervention and survival; however, no single biomarker can confirm pathogen presence in such patients. In this context, we assessed Triggered Receptor Expressed on Myelocytes-1 (TREM-1, soluble and membrane-bound forms), a molecule known to propagate systemic inflammation, in regard to its diagnostic value.

Patients and Methods: Seventy patients were enrolled in total. Group A consisted of 37 adult patients with sepsis, mean age 69.7±15.2 years, diagnosed according to the 2011 Sepsis International Conference criteria (10 with severe sepsis/septic shock and 27 without any organ damage). Group B included 16 patients with systemic autoimmune inflammation (non-infectious SIRS), 8 of them with active rheumatoid arthritis (RA, mean age 65±21 years, Disease Activity Score=4.8±0.8) and 8 with active systemic lupus erythematosus (SLE, mean age 45±5 years, SLE Disease Activity Index=8±2). Group C consisted of 17 healthy individuals for establishing a reference point. Traditional biomarkers, such as C-reactive protein (CRP) and procalcitonin (PCT) were available in the majority of patients. TREM-1 expression on CD14+ monocytes was assessed with flow-cytometry whereas soluble TREM-1 was measured with enzyme-linked immunosorbent assay (ELISA). Statistical analysis was performed with Mann-Whitney U test; $p<0.05$ was considered significant.

Results: TREM-1 expression on CD14+ macrophages was significantly enhanced in septic patients (50.19±15.79%) in comparison to autoimmune patients (28.2±14%, $p<0.05$) and healthy controls (16.2±10%, $p<0.05$). Likewise, soluble TREM-1 levels were significantly higher in group A (124.6±36.1pg/ml) than group B (43.6±17.4pg/ml, $p<0.05$) and healthy individuals (5±3.3pg/ml, $p<0.05$). Of note, both membrane-bound and soluble TREM-1 did not differ significantly between patients with severe sepsis/septic shock and those without any overt organ damage.

Conclusion: Patients with sepsis and severe sepsis/septic shock have significantly higher levels of the membrane-bound and soluble forms of TREM-1 compared to active autoimmune patients and healthy individuals. TREM-1 may accurately discriminate infectious from non-infectious SIRS causes.

Keywords: Sepsis, biomarker, triggered receptor expressed on myeloid cells-1

Introduction

Sepsis is among the most common causes of death in hospitalized patients; its incidence is increasing approximately 8.7%/year, resulting in 240.4 cases per 100000 inhabitants in 2000, probably due to the aging of population and the increased use of immunosuppressive medications or chemotherapy. [1] Although considerable progress in the management of critically ill patients has been achieved, sepsis remains an ominous diagnosis. In-hospital mortality ranges from 28.3 to 41.1% in North America and Europe. [1, 2] One third of the septic patients will die from direct complications, such as remote organ dysfunction (severe sepsis) and/or arterial hypotension (septic shock). Many patients may deteriorate unrecognized, as occurrence of infection and related organ dysfunction eludes from physician' s attention. The high rates of delayed diagnosis are frustrating, since it is well documented that every hour of delay in management of sepsis may increase mortality by 10%. [3]

Up-to-date sepsis diagnosis relies on the identification of Systemic Inflammatory Response Syndrome (SIRS) and a severe, uncontrolled infection. SIRS criteria are a) tachycardia >90/min, b) tachypnea >20 breaths/min or PaCO2 <32 mmHg c) fever (>38°C) or hypothermia (<36°C) and d) altered white blood cell count (WBC>12,000 cells/mm^3 or <4000 cells/mm^3) or presence of >10% immature neutrophils on peripheral blood smear. Sepsis is defined as SIRS resulting from infection, whether of bacterial, viral, fungal or parasitic origin. Diagnosis is reached when 2 or more of the above conditions are met. [4]

SIRS criteria are characterized by a high sensitivity but low specificity, thus resulting in relatively poor diagnostic accuracy. [5] Besides overwhelming infections, other causes of SIRS include trauma, acute pancreatitis, burns and, rarely, autoimmune diseases such as systemic lupus erythematosus (SLE) and highly active rheumatoid arthritis (RA). [6] In typical cases, severe infections are well differentiated from aseptic patients with signs of SIRS; however, sometimes, differential diagnosis is challenging. Traditional markers of systemic inflammation, such as WBC, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) have been proven of limited value in such patients due to their poor sensitivity and specificity for infection. [7] Moreover, microbiological (bodily fluid and tissue) cultures, the conventional gold standard for sepsis diagnosis, are time consuming and, thus, unsuitable for early diagnosis. Additionally, any widely available laboratory

investigation cannot precisely determine the host response in cases of SIRS/sepsis or the exact onset of organ dysfunction whereas, sometimes, is misleading with false-positive or false-negative results. [8] Given this evidence, differential diagnosis of sepsis from other causes of SIRS has become a hot topic in late years. [9] These shortcomings in both clinical and laboratory tests have driven researchers to find other, more sensitive and specific markers.

Though CRP and procalcitonin (PCT) are currently used as biochemical indicators of inflammation and infection, several other biomarkers have been investigated for their ability to detect sepsis in an early, reversible stage. [10] Diagnostic accuracy of PCT has been investigated in meta-analyses, showing a modest capability to discriminate sepsis, since the estimated likelihood ratio of PCT was approximately 7.5, value of modest performance. [11]

Nonetheless, identification of an ideal biomarker (or panel of biomarkers), capable of making a clear distinction between sepsis and SIRS, is imperative. [10] Several biomarkers such as interleukins, pro-vasopressin, CD64 etc. have been tested for the diagnosis, assessment and prognosis of sepsis. Except for PCT, however, their clinical value is still uncertain or controversial. [9, 10. 12, 13, 14]

In 2004, triggering receptor expressing on myeloid cells-1 (TREM-1) was evaluated for its accuracy in the diagnosis and evaluation of sepsis by Gibot et al. [15] TREM-1 is involved in the activation of innate immunity cells and the inflammatory response. It belongs to the immunoglobulin superfamily, engaged as a cell membrane receptor; mainly in the family of myeloid cells. [16] Its expression on monocytes and neutrophils is altered when a pattern recognition receptor (PRR), such as Toll-like receptor (TLR)-4, is activated by a pathogen-associated molecular pattern (PAMP). In vitro activation of TREM-1, in the presence of TLR-2 or TLR-4 ligands, amplifies the production of proinflammatory cytokines, such as tumour necrosis factor (TNF-α), IL-1β and granulocyte–macrophage colony-stimulating factor (GM-CSF). Simultaneously, it inhibits the release of the regulatory cytokine IL-10, further promoting the inflammatory response. [16, 17] The ligand of TREM-1 has not been identified yet. Its action is mediated through the DAP12 protein, whereas other studies have shown that TREM-1 is activated through a phosphatidylinositol-3-kinase-dependent (PI3K) pathway. In parallel, when TLRs are stimulated, the production of the soluble form of TREM-1 (sTREM-1) is augmented. [18] sTREM-1 could originate from the shedding of the membrane bound receptor (mTREM-1) by a metalloprotease or an alternative splicing of the mRNA.[16] Soluble TREM-1 acts as a decoy receptor,

sequestering TREM-1 ligand and dampening its activation. Moreover, up-regulation of mTREM-1 may result in marked elevation of the soluble form in plasma. [12]

In mice, engagement of TREM-1 with monoclonal agonist antibodies was shown to stimulate the production of pro-inflammatory cytokines and chemokines, such as IL-8, monocyte chemoattractant protein-1 (MCP)-1 and -3, and macrophage inflammatory protein-1 (MIP-1), leading to rapid neutrophil degranulation and oxidative burst. [17] Pro-inflammatory cytokine secretion (IL-8, IL-6 and TNF-α) was significantly compromised when leukocytes were treated with the synthetic peptide LP17, which may act as a decoy receptor, by blocking interaction of TREM-1 with its ligand. The inflammatory response mediated by TLR-2 and TLR-4 stimulation is amplified by the engagement of mTREM-1. [18] Modulation of TREM-1 signaling pathway, with the use of small synthetic peptides, confers considerable survival advantages during experimental septic shock in mice, even if they are administered late after the onset of sepsis. [19, 20]

mTREM-1 expression on circulating CD14+ monocytes and tissue macrophages is up-regulated, when infection by Gram-positive and Gram-negative bacteria occurs. During sepsis, in particular, the expression of mTREM-1 is greatly increased on monocytes, albeit reduced on neutrophils. [21]

Gibot et al. investigated whether sepsis alters mTREM-1 expression, using experimental models of polymicrobial infection induced by caecal ligation and puncture (CLP) in mice. In sham-operated animals, TREM-1 was present at low levels on the surface of peripheral monocytes and neutrophils, peritoneal macrophages and neutrophils, as well as splenic macrophages. Sepsis induced a marked (three-fold to five-fold) increase in TREM-1 expression on the surface of CD14+ cells, with the most pronounced increase observed on peritoneal macrophages. Furthermore, the release of sTREM-1 was substantially increased in peritoneal lavage fluid from septic animals but it was barely detectable in sham-operated animals. [22] Other studies have shown that its expression is dramatically increased in skin, biological fluids and tissues infected by Gram-positive and Gram-negative bacteria and fungi. [23, 24] Additionally, elevated broncho-alveolar lavage fluid sTREM-1 concentrations were found in patients with bacterial or fungal pneumonia and increased pleural fluid sTREM-1 levels were detected in patients with infectious pleural effusions. [25]

Gibot and Cravoisy found that sTREM-1 displayed greater diagnostic value when compared to CRP and PCT and could represent a useful biomarker

for early diagnosis of sepsis. [26] Other investigators found that TREM-1 increase may positively correlate to the critical condition and prognosis of the patients. [27]

Accordingly, other studies have shown that TREM-1 is not elevated in the serum of patients with non-infectious inflammatory disorders such as ulcerative colitis or immune-complex vasculitis, proposing TREM-1 as the ideal biomarker for the discrimination between septic and aseptic causes of SIRS. [12, 16, 28] On the contrary, reports that increased plasma sTREM-1 concentrations were also observed in patients with acute pancreatitis and non-infectious inflammations after traumatic lung contusion or pulmonary aspiration syndromes have been published. [29] Prucha et al. compared levels of mTREM-1 (assessed by flow cytometry) in septic patients and patients after CNS elective surgery and came to the conclusion that TREM-1 was increased in both entities. [30] The diagnostic and prognostic performance of sTREM-1 in sepsis and septic shock was reported to be variable and controversial, according to other clinical studies that reaffirmed the role of traditional laboratory parameters, such as CRP, IL-6 and PCT, in distinguishing sepsis from non-infectious SIRS. [31, 32] Latour-Pérez et al. assessed the accuracy of sTREM-1 and concluded that the area under the curve (AUC) of sTREM-1 for the diagnosis of sepsis was 0.62 [95% confidence interval (CI) 0.51-0.72]. Furthermore, the alterations of sTREM-1 had a modest diagnostic accuracy in the first three days after admission (P=0.047, sensitivity=47%, specificity=78%). [33]

In 2012, Gibot et al. introduced the concept of Bioscore (BS), which is a composite index, based on the measurement of three different biomarkers: sTREM-1, PCT and CD64. The authors assessed the ability of BS to achieve greater diagnostic accuracy in early sepsis. Three hundred intensive care unit (ICU) patients were studied and levels of these biomarkers were determined in 2 different blood samples that were obtained at 12 and 24 hours after admission. They defined a lower threshold (cut-off) for each biomarker (PCT: 1.55 ng/ml, sTREM-1: 755 pg/ml, PMNCD64 index: 1.62). Scoring of all parameters above the respective threshold in a combined BS confers a considerable diagnostic accuracy (AUC 0.97). [34] Moreover, a recent systematic review and meta-analysis evaluated the accuracy of plasma sTREM-1 for sepsis diagnosis in systemic inflammatory patients. The positive likelihood ratio, negative likelihood ratio and diagnostic odds ratio were 4.0 (95% CI, 2.4 to 6.9), 0.26 (95% CI, 0.14 to 0.48) and 16 (95% CI, 5 to 46) respectively. [35]

Given these discrepancies, aim of the present study was to compare the levels of TREM-1 expression on CD14+ cells and in serum in three groups of subjects: in patients with sepsis, in patients with autoimmune systemic inflammation and in healthy controls, in order to evaluate the diagnostic accuracy of TREM-1 in the discrimination between infectious and non-infectious causes of SIRS.

Patients and Methods

Seventy individuals were enrolled and subdivided as follows. Group A consisted of 37 adult patients (mean age 69.7±15.2 years) with sepsis, diagnosed according to the 2012 Sepsis International Conference criteria. [4] Septic patients were recruited between February 2010 and August 2012 from the Emergency Department (11 patients), the 2nd Department of Internal Medicine and the ICU of Hippokration General Hospital (26 patients). Exclusion criteria were age <14 years, pregnancy and use of immunosuppressive agents. The most frequent diagnosis, at the time of admission, was community acquired pneumonia (CAP) in 32% (n=12) of patients, followed by urinary tract infection in 25% (n=9), skin and soft tissue infection in 16% (n=6), catheter-related infection in 16% (n=6) and infection of unknown origin in 11% (n=4). Ten of these patients were suffering from severe sepsis/septic shock (subgroup A1) and 27 did not have any sign of end organ damage at the time of diagnosis (subgroup A2). Subdivision was made in accordance with the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference for sepsis and severe sepsis/septic shock. [4]

Severe sepsis group was defined by the sepsis patients with more than one of organ dysfunction signs including (1) cardiovascular: arterial systolic blood pressure less than 90mmHg or mean arterial pressure less than 70mmHg that responds to administration of intravenous fluid, (2) renal: urine output less than 0.5mL/kg/hour for 1 hour despite adequate fluid resuscitation, (3) respiratory: PaO_2/FiO_2 less than 250, (4) hematologic: platelet count less than 80000/μL or 50% decrease in platelet count from highest value recorded over the previous 3 days, (5) unexplained metabolic acidosis: pH<7.30 or a base deficit>5.0 mEq/L and a plasma lactate level more than 1.5 times the upper limit of normal for reporting laboratory, (6) pulmonary artery wedge pressure less than 12 mmHg or central venous pressure less than 8 mmHg with

adequate fluid resuscitation and (7) acute alteration of mental status. Septic shock was defined as sepsis with hypotension (systolic blood pressure below 90 mmHg or its reduction by 40 mmHg or more from baseline in the absence of other causes) despite adequate fluid resuscitation along with organ dysfunction signs or need of inotropes or vasopressors for maintaining a systolic blood pressure of more than 90 mmHg or mean arterial pressure of more than 70 mmHg. Laboratory tests such as CRP, PCT and blood, sputum and urine cultures were performed as a routine. The percentage of patients with positive blood cultures was 70%.

Table 1. Demographic characteristics, SIRS criteria and types of infections in our patients

Parameter	Group A (n=37)	Group B (n=16)
Age (years, mean±SD)	69.7±15.2	55±11
Female/male ratio	20/17	14/2
SIRS criteria		
Fever	35/37 (94%)	8/16 (50%)
Tachycardia	30/37 (81%)	12/16 (75%)
Tachypnea	10/37 (27%)	1/16 (6%)
White blood cells disturbances	30/37 (81%)	12/16 (75%)
Infection characteristics		
Septic shock	10/37 (27%)	
Community acquired pneumonia (CAP)	12/37 (32%)	
Skin and soft tissue infection	6/37 (16%)	
Urinary tract infection	9/37 (25%)	
Catheter-related infection	6/37 (16%)	
Infection of unknown origin	4/37 (11%)	

Group B included 16 patients with systemic autoimmune inflammation (non-infectious SIRS), 8 of them with active RA (mean age 65±21 years, Disease Activity Score=4.8±0.8) and 8 with active SLE (mean age 45±5 years, SLE Disease Activity Index=8±2). These patients were recruited from the Clinical Immunology Outpatient Clinic of the 2nd Department Internal Medicine and were newly diagnosed, without taking immunosuppressive medications. All RA patients had destructive arthritis and extra-articular manifestations as well (3 had rheumatoid nodules, 3 had rheumatoid lung and 2 had serositis). SLE patients had fever, hematologic abnormalities and

visceral disease (2 with neurological manifestations, 3 with nephritis, and 3 with serositis). Diagnosis was made according to EULAR and ACR revised criteria respectively. [36, 37] SIRS criteria were satisfied in all patients.

Group C consisted of 17 healthy individuals (mean age 55±30 years) for establishing a reference point. Health status was confirmed by clinical examination and routine laboratory tests. Patients' characteristics are shown in Table 1.

Laboratory Methods

Flow Cytometry

Immunophenotypic analysis was performed on peripheral blood using three-colour flow cytometry (EPICS COULTER XL®). Monoclonal antibodies used were: FITC-labeled anti-TLR4 (Clone 610015, R&D Systems®, Minneapolis, USA), PE-labeled anti-TREM-1 (Clone 193015, R&D Systems®, Minneapolis, USA) and PC5-labeled anti-CD14 (Clone RMO52, Beckman-Coulter®, Switzerland). Whole blood was incubated with the antibodies at 18°C for 15 minutes in the dark. Lysing and WBC membrane stabilization was performed by treatment of the specimen with prepared solutions (Immunoprep Reagent System, Beckman-Coulter®). A minimum of 2,000 cells was analyzed in each sample. Stained IgG_1 FITC and PE monoclonal antibodies mixed with CD14 PC5 antibody were used to determine negative control fluorescence for TLR4 and TREM-1 on macrophages.

Enzyme Linked Immunosorbent Assay (ELISA)

Levels of sTREM-1 in peripheral whole blood were measured by commercially available specific ELISA kits (USCN Life Sciences®) following the manufacturer's protocol. Each 100µl serum sample was transferred to the microstrip wells of the ELISA plate and subsequently incubated for 2 hour at 37°C; then the first reagent was added. After this, the plate was again incubated for 1 hour at 37°C. After 3 washing steps, the detection antibody was added and the reaction system was incubated for 30 min at 37°C. Antibody binding was detected using streptavidin-conjugated horseradish

peroxidase and developed using a substrate solution. The reaction was then stopped by the addition of sulphoric acid solution and the optical density was determined spectrometrically using a microplate reader set at 450±10nm with a wavelength correction set at 570 nm to subtract background. Soluble TREM-1 concentration was measured according to a calibration curve using a human sTREM-1 standard. According to manufacturer's instructions, a standard curve was generated using a four-parameter logistic curve fit for each set of samples assayed. The lower limit detection of the test was 3.8 pg/ml. All the values were within the linear portion of the standard curve. The inter- and intra-assay coefficient of variation for the sTREM-1 tests were <10%.

Statistical Analysis

Results are expressed as mean±SD. Kruskal-Wallis test for independent variables was applied for differences' identification between the three groups.

The Mann-Whitney U-test was performed to assess the significant differences; $p<0.05$ was considered statistically significant. Spearman test was used for correlation assessment. All analyses were performed using the SigmaStat 4.0 software.

Results

TREM-1 expression on CD14+ macrophages (mTREM-1) was significantly enhanced in septic patients (mean value 50.19±15.79%) in comparison to autoimmune patients (mean value 28.2±14%, $p<0.05$) and healthy controls (mean value 16.2±10%, $p<0.05$), Table 2 and Figure 1. Likewise, soluble TREM-1 (sTREM-1) levels were significantly higher in group A (124.6±36.1 pg/ml) than group B (43.6±17.4 pg/ml, $p<0.05$) and healthy individuals (5±3.3 pg/ml, $p<0.05$), Table 2 and Figure 2).

Table 2. Expression of membrane TREM-1 on CD14+ monocytes of the peripheral blood and soluble receptor concentration in serum in the three groups

	Group A	Group B	Group C
Membrane TREM-1 on CD14+ cells (%)	50.19±15.79	28.2±14	16.2±10
sTREM-1 (pg/ml)	124.6±36.1	43.6±17.4	5±3.3

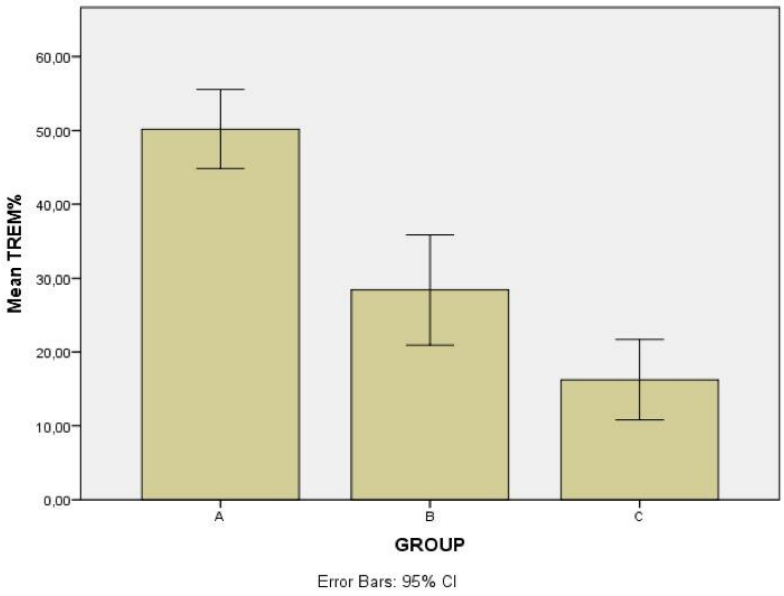

Figure 1. Cellular expression of membrane TREM-1 in the three groups.

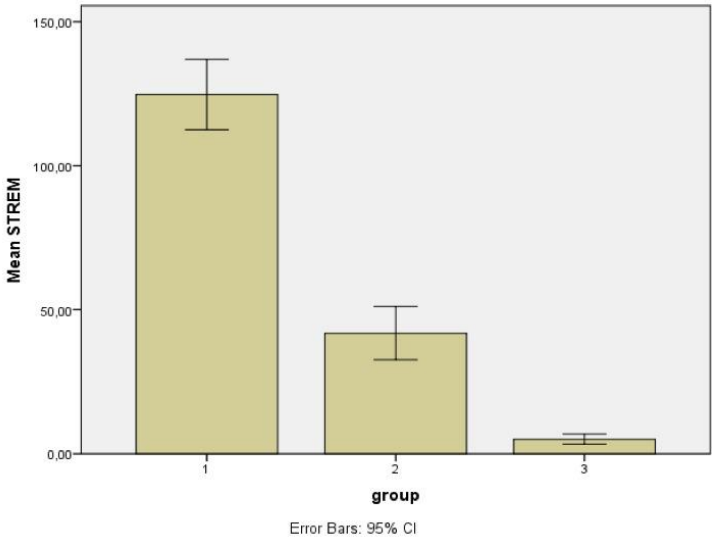

Figure 2. Serum concentrations of TREM-1 in the three groups.

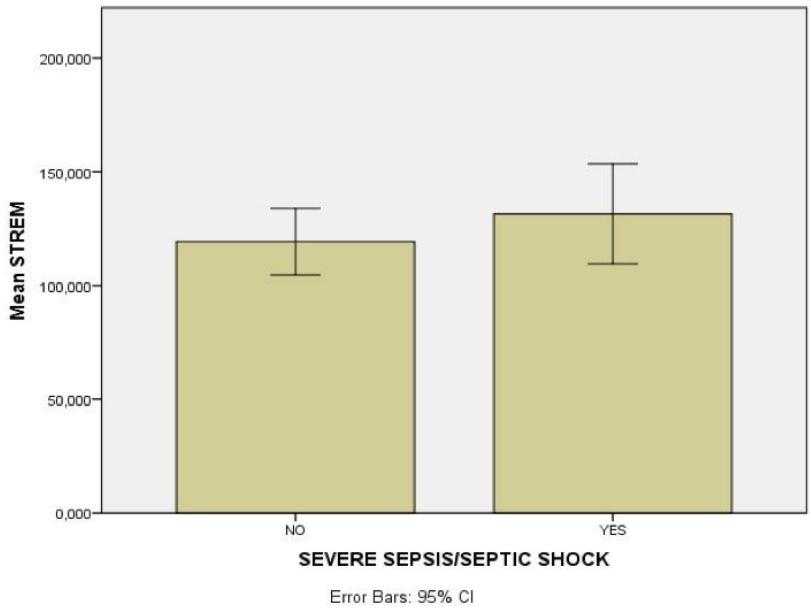

Figure 3. No statistical difference in sTREM-1 concentrations between subgroups A1 and A2.

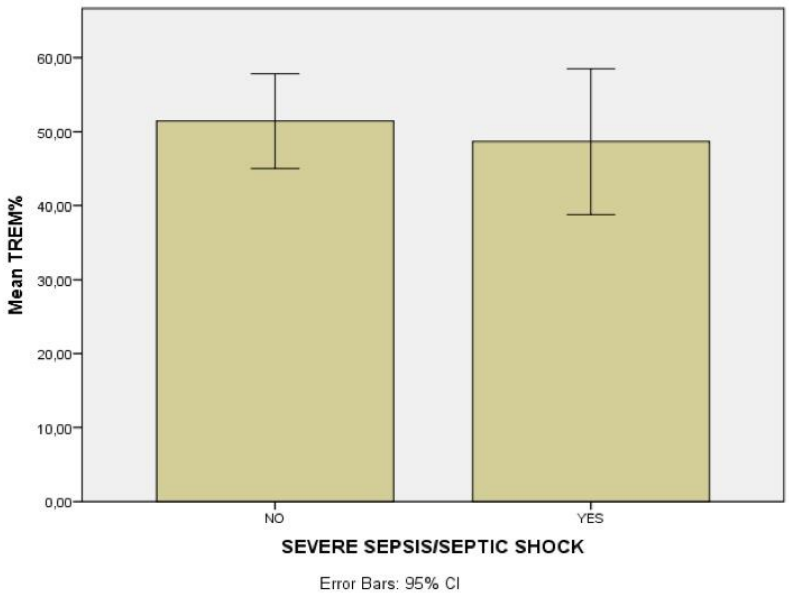

Figure 4. No statistical difference in mTREM% between subgroups A1 and A2.

The levels of sTREM-1 in healthy subjects were almost undetectable whereas the estimated expression of the membrane bound receptor was considered to be the basal expression in health.

Of note, both membrane-bound and soluble TREM-1 did not differ significantly between patients with severe sepsis/septic shock and those without any overt organ damage. The mean value of sTREM-1 in group A1 was 110±18 pg/ml whereas for group A2 was 125±28 pg/ml (p>0.05), Figure 3. Membrane bound receptor expression was 52.2±14% for group A1 and 48±10.79% for group A2, Figure 4 and Table 3.

TREM-1 on monocytes did correlate with serum TREM-1 in patients without severe sepsis (r=0.54, p<0.05) but not in uncomplicated sepsis (r=0.33, p>0.05), data not shown.

Table 3. Expression of membrane TREM-1 on peripheral blood CD14+ monocytes and soluble receptor concentration in serum in subgroups A1 and A2

	Group A1	Group A2
Membrane TREM-1 on CD14+ cells (%)	52.2±14	48±10.79
sTREM-1 (pg/ml)	110 ± 18	125±28

Discussion

Despite significant progress in critical care, sepsis continues to show disproportionately high rates of mortality. [1] Delayed diagnosis, even in tertiary hospital centres, consists one of the main reasons for this pitfall. [3] In the absence of sufficient biological markers, the clinician is forced to support the diagnosis of sepsis mainly on clinical grounds (excluding the measurement of white blood cells), which makes it doubtful, since the SIRS criteria have ambiguous diagnostic performance. [4] Furthermore, other non-infectious causes of SIRS, such as SLE or RA mandate different treatment strategies and management could be urgent as well. The long incubation period and modest sensitivity of blood cultures hinders a rapid final diagnosis. [5] Therefore, the need for indicators that can be used in daily clinical practice for the diagnosis or exclusion of sepsis is imperative. Several studies have evaluated the reliability of TREM-1 to diagnose infection in patients with different underlying diseases; most of them reported that it could be used as a marker to

discriminate between sepsis and other causes of SIRS. [22, 23, 32] No previous study, however, has compared serum levels of TREM-1 in sepsis and autoimmune systemic inflammatory diseases, like SLE and RA.

The present study aimed to evaluate mTREM-1 and sTREM-1 in two different conditions —autoimmune SIRS and sepsis— and to determine whether these markers might be used to discriminate between the two entities.

In sepsis, mTREM-1 and sTREM-1 levels were significantly increased compared to active autoimmune patients and healthy controls; in patients with autoimmune SIRS, TREM-1 was, in turn, elevated in relation to healthy controls. To evaluate the weight of the clinical implication of sTREM-1 for differentiating sepsis from autoimmune diseases, we set the cut-off value for sTREM-1 at 100 pg/ml, with sensitivity of 72% and specificity of 99%. [32]

It seems that septic and autoimmune inflammation differs quantitatively regarding TREM-1 induction. This might be due to the different and more potent initiators (PAMPs or DAMPs) of the immune activation when it is induced against infectious agents. It could be considered that the magnitude of inflammatory reaction is augmented and more sustained in septic patients compared to autoimmune diseases. Furthermore, the disproportionate levels of CRP in SLE flares suggest that different immune pathways may be activated or inhibited in autoimmune inflammation. Although it is ambiguous if TREM-1 expression differs significantly between sepsis and other sterile causes of SIRS, there is a significant difference between sepsis and autoimmune SIRS. If there is discrepancy, specifically, between infectious and autoimmune SIRS (due to differences in antigenicity), larger studies need to be done, since few have investigated TREM-1 in autoimmunity. [38, 39, 40, 41]

Regarding RA, to our knowledge, there are no published studies evaluating sTREM-1 in serum; however, high levels of sTREM-1 in synovial fluid were reported by Kuai et al. [42] Furthermore, Collins et al. demonstrated that sTREM-1 concentrations in the synovial fluid were similar in septic arthritis and RA, but measurements were confined to local, articular expression.[41] Although one could expect that these findings could be reflected in peripheral blood, we found that this is not the case. Based on our study, the serum levels of TREM-1 are lower in patients with RA compared to septic patients. Local and peripheral levels of an inflammatory marker could be different, something usual in systemic autoimmune diseases. Probably, differences between serum and body fluids derived from the site of inflammation could be explained by the sequestration of neutrophils and macrophages taken place in situ, accompanied by forced circulation of naive

cells, not yet activated. Soluble immune receptors in RA could concentrate in high amounts in synovium accompanied by a moderate increase in blood.

Regarding mTREM-1, we found a three-fold increase of its expression on CD14+ monocytes in sepsis compared to healthy controls, as other authors reported. [12, 24, 44] Knapp et al. demonstrated that mTREM-1 on macrophages was increased when healthy volunteers received an injection of LPS, with a dose- and time–dependent manner, both in vivo and in vitro. [45]

Additionally, TREM-1 expression was higher in septic rather than autoimmune patients. Presently, TREM-1 expression was compared between sepsis and sterile systemic inflammation caused by traumatic tissue damage.[43] Ferat-Osorio et al. described an increase of mTREM-1 in sepsis and in non-infectious SIRS, at the same levels. [38] This study included only surgical patients postoperatively and its conclusions could not be transferred in autoimmune patients. This discrepancy gives a clue to hypothesize that the expression of an immune receptor depends upon multiple parameters acting in a dynamic inflammatory milieu. Autoimmune and traumatic systemic inflammation could differ regarding the mechanisms of innate and adaptive immunity involved.

The levels of TREM-1 on peripheral monocytes and serum in autoimmune patients raise questions on the inflammatory pathways involved in RA and SLE. Apparently, the inflammatory reaction is not equally potent to augment TREM-1 expression at the same levels as sepsis provokes. However, its levels are well above the normal values, a finding that comes in agreement with previous studies. Molad et al. reported ten times higher sTREM-1 levels in SLE patients than normal controls.[39] Herein, we confirmed a statistically significant raise in serum sTREM-1 in SLE in comparison to healthy controls; however, the role of TREM-1 as a possible biomarker in autoimmunity warrants further investigation. Indeed, TREM-1 expression depends on the activation of several TLRs or NOD-like receptors, and besides PAMPs, many DAMPs (alarmins, such as high-mobility group box nuclear protein, heat shock proteins and free cyclic AMP) may trigger autoimmune inflammatory reactions through these receptors.

Next, we also divided sepsis patients in two subgroups depending on the presence of organ dysfunction and septic shock. We found no correlation of TREM-1 levels, membrane or soluble, to the severity of sepsis. Latour-Perez et al. conducted a cohort study finding that sTREM-1 was only marginally higher in patients with severe sepsis. [46] On the contrary, several studies have reported a positive predictive value for sTREM-1 regarding sepsis severity and mortality. [44, 45, 46] Poukoulidou et al. suggested that TREM-1 alterations

upon transition from uncomplicated sepsis to septic shock were dependent on the type of infection and the causative pathogen. Additionally, mTREM-1 expression was altered at various stages of sepsis. [44] These data could explain the lack of agreement observed in clinical studies with dissimilarly designed protocols.

Furthermore, we found that there is a strong correlation between mTREM-1 and sTREM-1 levels in severe sepsis group but not in uncomplicated septic patients. Few studies, that measured mTREM-1 and sTERM-1 simultaneously, have reported different patterns of TREM-1 expression, regarding the correlation of mTREM-1 and sTREM-1 levels. [12, 27, 46] We speculate that the kinetics of mTREM-1 expression and its cleavage is altered as sepsis progress and an internal feed-back loop of regulation between mTREM-1 and sTREM-1 could be reinforced as sepsis deteriorates. The mechanisms of this regulation have not been elucidated yet.

The limitations of this study are the small number of patients, especially autoimmune patients due to the rare incidence of SIRS in these diseases. Patients with two distinct diseases were studied although our results do not demonstrate great differences in these parameters between SLE and RA. Secondly, autoimmune patients were not sex-matched to septic patients, although no gender-related difference in TREM-1 expression has been reported.

In conclusion, we can support that membrane bound and soluble TREM-1 is elevated in both septic and aseptic inflammation. In sepsis, its elevation may be more pronounced, like other biomarkers such as CRP, which is usually higher in sepsis rather than in RA or SLE, but a cut-off value has not been yet determined. From the immunological point of view, these patterns of TREM-1 expression could be explained by the different patterns of PRR activation in sepsis and autoimmune inflammation. TREM-1 may be useful for discrimination of infectious from non-infectious SIRS causes. [48, 49, 50] Our results are in accordance with other reports in the literature and the recent meta-analysis. [35] TREM-1 could be comparable to PCT, but the number of available studies for sTREM-1 is still low; its role in the diagnosis of sepsis needs to be clarified. The diagnostic accuracy of TREM-1 in autoimmune diseases, such as RA and SLE, remains an open question. How the alterations in mTREM-1 and/or sTREM-1 could predict the course of an inflammatory systemic disease could be a topic for future research.

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