Research Article

Pleural Fluid Soluble Triggering Receptor Expressed on Myeloid Cells-1 in Complicated and Uncomplicated Parapneumonic Pleural Effusions

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Received 12 July 2011; Accepted 4 August 2011

Background. Soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) has recently been found to be high in infected pleural fluid (PF). Objectives. Diagnostic accuracy of PF sTREM-1 for differentiating uncomplicated parapneumonic effusions (UPPEs) from complicated parapneumonic effusions (CPPEs) was evaluated prospectively. Methods. Serum and PF sTREM-1 were measured for 68 patients with parapneumonic and transudative pleural effusion. Results. PF (but not serum) sTREM-1 concentrations were significantly higher in CPPE than in UPPE. Serum and PF sTREM-1 levels were higher in parapneumonic than in transudative groups. PF sTREM-1 had a sensitivity of 85.19% and a specificity of 83.33% at cutoff value of 250.5 pg/mL for differentiating CPPE and UPPE with area under the curve (AUC) of 0.9336. After excluding purulent CPPE cases, sensitivity and specificity became 90.48% and 83.33%, respectively (at the same cutoff value) with AUC of 0.9444. Conclusion. High concentrations of PF sTREM-1 (above 250.5 pg/mL) help to early diagnose and differentiate CPPE from UPPE.

1. Introduction

There are millions of patients hospitalized worldwide each year with pneumonia (about one million patients yearly in USA). Of those hospitalized, about 20 to 40% have a parapneumonic pleural effusion. The mortality is higher in patients with pneumonia who have a pleural effusion [1].

Parapneumonic effusion (PPE) is any pleural effusion secondary to pneumonia or lung abscess. A complicated parapneumonic effusion (CPPE) is a PPE for which an invasive procedure, such as tube thoracostomy, is necessary for its resolution, or a PPE on which the bacterial cultures are positive [2]. Uncomplicated parapneumonic effusions (UPPEs) are not infected and do not usually need tube thoracostomy. An effusion is called an “empyema” when the concentration of leucocytes becomes macroscopically evident as a thick and turbid fluid (pus) [3].

The pleural fluid with a PPE is an exudate with a predominance of neutrophils. If the pleural fluid has mononuclear cells predominantly, an alternative diagnosis should be sought. In cases of PPE, initially, the fluid is smear and culture negative then, the bacteriology becomes positive. As a PPE progresses, the pH and glucose levels become progressively lower, whereas the pleural fluid lactic dehydrogenase (LDH) becomes progressively higher [1].

The recently identified triggering receptor expressed on myeloid cells-1 (TREM-1) is a pattern recognition receptor of the immunoglobulin superfamily that is upregulated on the surfaces of neutrophils, monocytes, and macrophages after exposure to extracellular bacteria and fungi [4, 5]. Signal transduction pathways activated by ligand binding to TREM-1 are associated with enhanced production of proinflammatory cytokines and amplification of the immune response. The expression of TREM-1 reportedly allows...
2. Subjects and Methods

This study was done in Chest Department, Tanta University Hospital, Egypt, in the period from October 2007 to January 2009.

2.1. Study Design. Cases of parapneumonic pleural effusions (PPEs) who were admitted during the study period were enrolled and were divided into uncomplicated parapneumonic effusion (UPPE) and complicated parapneumonic effusion (CPPE). The cases of transudative pleural effusion (TE) who were admitted during the same period were included in the study as a control group.

2.2. Inclusion Criteria of the Patients with TE.

(1) Clinical and radiological (ultrasound, X-ray, or CT) evidence of unilateral or bilateral pleural effusions, 
(2) the effusions being proved to be transudates according to Light’s criteria [2]: (a) a pleural-fluid-to-serum protein ratio less than 0.5, (b) a pleural fluid lactic dehydrogenase (LDH) less than 200 IU, and (c) a pleural-fluid-to-serum LDH ratio and pleural fluid-to-high normal serum LDH ratio less than 0.6.

2.3. Exclusion Criteria of the Patients with TE.

(1) History or evidence of respiratory tract infection during the month before their enrollment in the study, 
(2) exudative pleural effusions.

2.4. Inclusion Criteria of the Patients with PPE.

(1) All patients having criteria of pneumonia [10]: the presence of a new chest radiograph infiltrate with at least one of the following: cough, sputum, dyspnoea, fever >38°C, abnormal breath sounds, leukocytosis >10,000 cells/mm³, and leukopenia <4,000 cells/mm³, 
(2) clinical and radiological (ultrasound, X-ray, or CT) evidence of pleural effusion on the same side of pneumonia, pleural effusions being exudates according to Light’s criteria [2]: (a) a pleural fluid-to-serum protein ratio greater than 0.5, (b) a pleural fluid LDH greater than 200 IU, or (c) a pleural fluid-to-serum LDH ratio or pleural-fluid-to-high normal serum LDH ratio greater than 0.6, 
(3) microbiological evidence of bacterial origin on either gram stain or bacterial culture from sputum, blood, bronchial lavage, or pleural fluid, 
(4) CPPE being identified if one or more of the following criteria were present [11]: (1) presence of organism in pleura on Gram stain or culture, (2) macroscopic pus, (3) fluid pH < 7.2 with normal peripheral blood pH, or (4) pleural fluid glucose concentration <40 mg/dL.

2.5. Exclusion Criteria of the Patients with PPE.

(1) Pleural effusions due to other causes (transudative, tuberculous, malignant, connective tissue diseases, etc.), 
(2) nonbacterial pneumonia.

None of the patients (in both groups) had received antibiotics 3 days before the study. All of them signed an informed written consent to share in the study. The local institutional research ethics committee approved the study protocol.

Detailed history taking and thorough clinical examination were done for all subjects. Chest X-ray posteroanterior and lateral views were done to them (in some cases chest CT and/or ultrasound were required). Complete blood count was done also for all the patients.

Aspirations of pleural fluid were done under aseptic technique and local anesthesia with lidocaine 2%. Pleural-fluid-samples were analyzed for pleural fluid protein, pleural fluid to serum protein ratio, pleural fluid lactate dehydrogenase (LDH), pleural-fluid-to serum LDH ratio, pleural fluid Gram stain, pleural fluid bacterial cultures, pleural fluid acid fast bacilli stain and culture, pleural fluid pH, and pleural fluid glucose level. Pleural fluid cytology was done to exclude malignant effusions.

Pleural fluid samples were centrifuged at 1100 rpm for 5 minutes before 0.5 to 1 mL of supernatant was withdrawn, labeled, and frozen, at −70°C, before measurement of sTREM-1. sTREM-1 concentrations were measured in serum and pleural fluid using a capture enzyme-linked immunosorbent assay (ELISA) procedure using a commercial kit (DuoSet ELISA kit; human TREM-1 catalog no. DY1278; Quantikine R&D Systems, Minneapolis, Minn, USA) according to the manufacturer’s instructions. The sTREM-1 levels were expressed as pg/mL. Briefly, 100 μL of sample was transferred to the well of a microplate precoated with monoclonal murine immunoglobulin G directed against human sTREM-1. After 2 hours of incubation at room temperature, the plates were washed, and bound sTREM was detected and quantified by the serial addition to the wells of a biotin-labeled second antibody, streptavidin-labeled horseradish peroxidase, and enzyme substrate.

2.6. Statistical Analysis. The means and the standard deviations were used to describe the sample. Group differences
were assessed with unpaired students \( t \)-tests, whereas comparison for categorical variables was done using the chi-square test. Both \( t \)-test and chi-square test were done using Minitab for Windows Version 12.1; 1998 Minitab Inc.

Receiver-operating characteristic (ROC) curve was constructed to illustrate the predictive value of cutoff point of sTREM-1. The point with the largest sum of sensitivity and specificity was chosen as a threshold. It was done by using SigmaPlot for Windows Version 11.0; 2008 Systat Software, Inc.

3. Results

The total number of patients with transudative pleural effusion (TE) and parapneumonic pleural effusion (PPE) during the study period who fulfilled the inclusion and exclusion criteria mentioned in the methodology and agreed to share in the study was 68 patients. 17 of them had TE and 51 had PPE. PPE patients were divided into uncomplicated parapneumonic effusion (UPPE) and complicated parapneumonic effusion (CPPE). The number of patients with UPPE was 24 and the number of patients with CPPE was 27.

Demographic data of the patients are shown in Tables 1 and 2. Age, sex, smoking index, and associated comorbidities of patients did not show any statistically significant difference between TE group and PPE group and also between UPPE and CPPE.

Among the 27 cases of CPPE, 18 patients (66.67%) had pleural fluid glucose less than 40 mg%, 6 patients (22.22%) had pleural fluid with macroscopic pus (empyema), 17 patients (62.96%) had pleural fluid pH less than 7.2, and 15 patients (55.56%) had positive pleural fluid culture or gram stain.

Both pleural fluid pH and glucose were significantly lower in PPE group in comparison to TE group (Table 1), and they were significantly lower in CPPE in comparison to UPPE (Table 2).

The serum and pleural fluid levels of sTREM-1 were significantly higher in PPE group than in TE group (Table 1). There was no significant difference in serum levels of sTREM-1 between UPPE and CPPE, while the pleural fluid levels of sTREM-1 were significantly higher in CPPE than in UPPE (Table 2).

There was no significant difference between serum and pleural fluid sTREM-1 in TE group \((t = 0.501 \text{ and } P = 0.62)\), whereas pleural fluid levels of sTREM-1 were significantly higher than serum levels of sTREM-1 in PPE group \((t = 9.191 \text{ and } P < 0.001)\). (Figure 1).

Receiver-operating characteristic (ROC) curve analysis showed that pleural fluid sTREM-1 had a sensitivity of 85.19% (95% confidence interval \(\text{CI}: 69.62\%–95.81\%) \text{ and a specificity of } 83.33\% (95\% \text{ CI}: 62.62\%–95.26\%) \text{ for differentiating between UPPE and CPPE at an optimal cutoff value of } 250.5 \text{ pg/mL. The likelihood ratio positive } \text{(LR+) was 5.11, and the likelihood ratio negative } \text{(LR–) was 0.1777. The area under the ROC curve } (\text{AUC}) \text{ was 0.9336 (Figure 2).}

After excluding purulent CPPE (6 cases of empyema) from the analysis, the sensitivity and specificity of pleural fluid sTREM-1 for differentiating between UPPE and CPPE at the same cutoff value \((250.5 \text{ pg/mL}) \text{ became } 90.48\% \text{ (95\% CI: 69.62\% to 98.83\%) \text{ and 83.33\% (95\% CI: 62.62\% to 95.26\%) \text{, respectively. The LR+ was 5.4277, and the LR– was 0.1142. AUC was 0.9444 (Figure 3).}

4. Discussion

A lot of pleural fluid measurements have been used to assess the severity and predict the course of a parapneumonic effusion (PPE) as low pH, low glucose, high lactic dehydrogenase (LDH) values [11, 12], high procalcitonin, and high C-reactive protein [13].

Triggering receptor expressed on myeloid cells-1 (TREM-1), a receptor of the immunoglobulin superfamily, amplifies the inflammatory response through its overexpression and subsequent activation of neutrophils and monocytes/macrophages in response to microbial products. It has been found to increase significantly in some conditions associated with inflammation as acute pancreatitis [14], pneumonia [6], and COPD [15] as a marker of systemic inflammation in these conditions.

In the present study, serum levels of soluble TREM-1 (sTREM-1) were significantly higher in patients with parapneumonic pleural effusion than in those with transudative effusion, while their levels showed no significant difference between uncomplicated parapneumonic effusion (UPPE) and complicated parapneumonic effusion (CPPE).

In accordance with our results, Phua et al. in 2006 [16] found that serum levels of sTREM-1 were elevated in pneumonia. They stated that there elevated levels had moderate but insufficient accuracy as a surrogate marker for the need for antibiotics in lower respiratory tract infections.

On the other hand, Richeldi et al. in 2004 found that the expression of TREM-1 on peripheral blood neutrophils may not distinguish pneumonia from noninfectious causes of interstitial lung disease, suggesting that biological fluids from the site of infection may provide more useful diagnostic information [17].

Significantly higher concentrations of sTREM-1 were associated with the presence of diverse microbial pathogens and may be useful in predicting the presence of microbial pathogens in pleural fluid samples [18].

In this context, our results showed that pleural fluid levels of sTREM-1 were significantly higher in parapneumonic effusion patients than in transudative effusion patients and were significantly higher in CPPE than in UPPE.

Similar results were found by Liu et al. in 2007 [8]. They reported that concentrations of sTREM-1 were significantly higher in infectious pleural effusions than in transudates. Among infectious effusions, the sTREM-1 levels were significantly higher in parapneumonic than in tuberculous effusions.

Bishara et al. in 2009 [19] found that the mean levels of sTREM-1 were significantly higher in empyema than in postthoracotomy pleural effusion and in effusions of other etiologies.

Huang et al. in 2008 [9] reported that the concentrations of sTREM-1 in bacterial pleural effusion were significantly
Table 1: Patients’ profile and some laboratory findings in patients of the two studied groups.

| Age (years) | Transudative pleural effusion | Parapneumonic pleural effusion | t or χ² | P |
|-------------|-------------------------------|---------------------------------|---------|---|
| Mean ± standard deviation | No. = 17 | No. = 51 | t or χ² | P |
| Sex: | | | | |
| (i) Male | 52.0 ± 6.14 | 49.7 ± 11.855 | t = 0.757 | 0.452 |
| (ii) Female | 49.7 ± 6.14 | 49.7 ± 11.855 | t = 0.757 | 0.452 |
| Comorbidities: | | | | |
| (i) Liver diseases | 6 | 6 | 5 | 5 | 0.219 | 0.640 |
| (ii) Heart diseases | 5 | 5 | 5 | 5 | 0.219 | 0.640 |
| (iii) Renal diseases | 4 | 4 | 4 | 4 | 0.219 | 0.640 |
| (iv) COPD | 0 | 0 | 0 | 0 | 0.219 | 0.640 |
| (v) Bronchial asthma | 0 | 0 | 0 | 0 | 0.219 | 0.640 |
| (vi) Malignant diseases | 3 | 3 | 3 | 3 | 0.219 | 0.640 |
| Smoking index | 172.353 ± 248.99 | 182.745 ± 273.847 | t = 0.138 | 0.89 |
| Pleural fluid pH | 7.462 ± 0.0788 | 7.056 ± 0.514 | t = 3.224 | <0.001* |
| Pleural fluid glucose (mg/dL) | 152.765 ± 39.113 | 86.608 ± 72.349 | t = 3.567 | <0.001* |
| Serum sTREM-1 (pg/mL) | 22.529 ± 6.644 | 48.373 ± 19.03 | t = 5.466 | <0.001* |
| Pleural fluid sTREM-1 (pg/mL) | 23.941 ± 9.542 | 290.686 ± 187.323 | t = 5.839 | <0.001* |

*: Significant.

Table 2: Patients’ profile and some laboratory findings in patients of uncomplicated parapneumonic effusion (UPPE) and complicated parapneumonic effusion (CPPE).

| Age (years) | UPPE No. = 24 | CPPE No. = 27 | t or χ² | P |
|-------------|---------------|--------------|---------|---|
| Mean ± standard deviation | | | | |
| Sex: | | | | |
| (i) Male | 48.292 ± 12.21 | 50.963 ± 11.614 | t = 0.8 | 0.427 |
| (ii) Female | 48.292 ± 12.21 | 50.963 ± 11.614 | t = 0.8 | 0.427 |
| Comorbidities: | | | | |
| (i) Liver diseases | 6 | 6 | 5 | 5 | 0.336 | 0.562 |
| (ii) Heart diseases | 2 | 2 | 2 | 2 | 0.336 | 0.562 |
| (iii) Renal diseases | 3 | 3 | 3 | 3 | 0.336 | 0.562 |
| (iv) COPD | 1 | 1 | 1 | 1 | 0.336 | 0.562 |
| (v) Bronchial asthma | 2 | 2 | 2 | 2 | 0.336 | 0.562 |
| (vi) Malignant diseases | 1 | 1 | 1 | 1 | 0.336 | 0.562 |
| Smoking index | 198.333 ± 289.28 | 168.899 ± 264.13 | t = 0.38 | 0.706 |
| Pleural fluid pH | 7.408 ± 0.117 | 6.743 ± 0.53 | t = 6.009 | <0.001* |
| Pleural fluid glucose (mg/dL) | 141.167 ± 70.166 | 38.111 ± 22.821 | t = 7.22 | <0.001* |
| Serum sTREM-1 (pg/mL) | 45.125 ± 17.86 | 51.259 ± 19.897 | t = 1.153 | 0.255 |
| Pleural fluid sTREM-1 (pg/mL) | 145.5 ± 111.766 | 419.741 ± 140.275 | t = 7.656 | <0.001* |

*: Significant.

higher than those in malignant, tuberculous, and transudative groups. Porcel et al. in 2009 [13] found that sTREM-1 was significantly higher in the pleural fluid from patients with CPPE and lower in the transudate and malignant groups.

In our study, there was no significant difference between serum and pleural fluid sTREM-1 in transudative effusion patients, whereas pleural fluid levels of sTREM-1 were significantly higher than serum levels of sTREM-1 in UPPE and CPPE groups.

In accordance with that, Huang et al. in 2008 [9] found that the concentration of sTREM-1 in pleural effusion greatly exceeds that in serum, suggesting that sTREM-1 is produced locally by recruited inflammatory cells in the pleural space and that sTREM-1 released into pleural effusion does not exude into serum.
Transudative effusion Parapneumonic effusion

- Serum sTREM-1
- Pleural fluid sTREM-1

**Figure 1:** Comparison between serum and pleural fluid levels of sTREM-1 in transudative pleural effusion (TE) group and parapneumonic pleural effusion (PPE) group. There was no significant difference between serum and pleural fluid sTREM-1 in TE group, whereas pleural fluid levels of sTREM-1 were significantly higher than serum levels of sTREM-1 in PPE group.

0 0.2 0.4 0.6 0.8 1

Sensitivity

1-specificity

ROC curve

- Pleural fluid sTREM-1, AUC = 0.9336

**Figure 2:** ROC curve analysis showed that pleural fluid sTREM-1 had a sensitivity of 85.19% and a specificity of 83.33% for differentiating between complicated and uncomplicated parapneumonic pleural effusions at an optimal cutoff value of 250.5 pg/mL. The area under the ROC curve (AUC) was 0.9336.

This can be explained by recruitment of phagocytes, in particular alveolar macrophages and neutrophils in cases of pneumonia [20] in which surface expression of TREM-1 was significantly increased [17].

In the present study, receiver-operating characteristic (ROC) curve analysis showed that pleural fluid sTREM-1 had a sensitivity of 85.19% and a specificity of 83.33% for diagnosing CPPE versus UPPE at an optimal cutoff value of > 250.5 pg/mL. The area under the ROC curve (AUC) was 0.9444.

We think there is no point in trying to identify empyema by measuring sTREM-1, because this diagnosis is easily achieved by simple inspection, so we repeated the ROC curve analysis after exclusion of cases having frank purulent pleural effusion. After doing this we found that the sensitivity and specificity of pleural fluid sTREM-1 for differentiating between nonpurulent complicated and uncomplicated parapneumonic pleural effusions at the same cutoff value (250.5 pg/mL) became 90.48% and 83.33%, respectively. The area under the ROC curve (AUC) was 0.9444.

There were other studies aiming to detect a cutoff point and to evaluate the diagnostic accuracy of pleural fluid sTREM-1 [7, 9, 13, 19]. Bishara et al. in 2009 [19] reported a cutoff value of 114 pg/mL for pleural sTREM-1 achieved a sensitivity of 94% and specificity of 93% in differentiating empyema from pleural effusions of other etiologies.

Huang et al. in 2008 [9] reported a pleural fluid sTREM-1 cutoff value of 768.1 pg/mL having a sensitivity of 86% and a specificity of 93% for differentiating bacterial pleural effusion from pleural effusions due to other etiologies. The AUC was 0.93, with a likelihood ratio of 2.60.

Also, in another study [7], pleural sTREM-1 at a cutoff value of 374 pg/mL yielded a sensitivity of 93.8%, a specificity of 90.9%, and an AUC of 0.93 in discriminating bacterial pleural infection from tuberculous pleuritis.
Porcel et al. in 2009 [13] found that the threshold of the sTREM-1 that best discriminated between nonpurulent CPPE and UPPE was >180 pg/mL with sensitivity of 72%, specificity of 82%, and AUC of 0.79.

In conclusion, the present results indicate that pleural fluid sTREM-1 concentration is a good tool for early differentiation between complicated and uncomplicated parapneumonic pleural fluid. Pleural fluid sTREM-1 concentrations above 250.5 pg/mL are highly suggestive of complicated parapneumonic pleural fluid. Further studies are required to evaluate the prognostic value of using sTREM-1 as a diagnostic tool in cases of parapneumonic pleural effusion on a long-term followup.

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