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Infectious pleural effusions can be identified by sTREM-1 levels

R.M. Determann [a,b,*], A.A. Achouiti [a], A.A. El Solh [c,d], P. Bresser [e], J. Vijfhuizen [f], P.E. Spronk [a,g], M.J. Schultz [a,b]

[a] *Academic Medical Center, Department of Intensive Care, G3-228, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands*
[b] *Laboratory of Experimental Intensive Care and Anesthesiology (L.E.I.C.A.), Amsterdam, The Netherlands*
[c] *Department of Medicine, Western New York Respiratory Research Center, State University of New York at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY, USA*
[d] *Division of Pulmonary, Critical Care, and Sleep Medicine, Western New York Respiratory Research Center, State University of New York at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY, USA*
[e] *Department of Pulmonology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands*
[f] *Department of Microbiology, Gelre Ziekenhuizen, Gelre Hospitals (Lukas site), PO Box 9014, 7300 DS Apeldoorn, The Netherlands*
[g] *Department of Intensive Care Medicine, Gelre Ziekenhuizen, Gelre Hospitals (Lukas site), PO Box 9014, 7300 DS Apeldoorn, The Netherlands*

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Summary
Background and objective: Conventional methods to establish pleural infection are time-consuming and sometimes inadequate. Biomarkers may aid in making rapid diagnosis of infection. In an observational study we evaluated and compared the diagnostic value of pleural fluid levels of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), C-reactive protein and procalcitonin in intensive care patients with pleural effusions.
Methods: Thirty-six patients with de novo pleural effusions were included and 20 patients with pleural effusions after cardiothoracic surgery and 20 patients with pleural effusions after esophagus surgery acted as controls. Levels of sTREM-1, C-reactive protein and procalcitonin were measured in pleural effusions.

Abbreviations: sTREM-1, soluble triggering receptor expressed on myeloid cells-1; CRP, C-reactive protein; PCT, procalcitonin.

* Corresponding author. Tel.: +31 20 5666345; fax: +31 20 5669568.
E-mail addresses: r.m.determann@amc.uva.nl (R.M. Determann), achmeda75@gmail.com (A.A. Achouiti), solh@buffalo.edu (A.A. El Solh), p.bresser@amc.uva.nl (P. Bresser), j.vijfhuizen@gelre.nl (J. Vijfhuizen), p.spronk@gelre.nl (P.E. Spronk), m.j.schultz@amc.uva.nl (M.J. Schultz).

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Results: Levels of sTREM-1 were highest in empyemas, followed by infectious exudates. Levels of sTREM-1 were low in transudates and non-infectious exudates. C-reactive protein levels were highest in exudates and empyemas, while procalcitonin levels were highest in exudates. Pleural fluid with positive culture results contained higher sTREM-1 and C-reactive protein levels as compared to samples with negative culture results. A cut-off level of 50 pg/ml sTREM-1 yielded a sensitivity of 93% and a specificity of 86%, while these were 87% and 67% respectively for a cut-off value of 7.5 μg/ml C-reactive protein, and 60% and 64% respectively for a cut-off value of 0.15 ng/ml procalcitonin.
Conclusion: sTREM-1 is superior to C-reactive protein and procalcitonin in detecting infection.

Introduction

Pleural effusions are an important complication of several diseases and may cause additional morbidity, also in patients admitted to the intensive care.[1] The clinical presentation of a patient is important in establishing the cause of an effusion.[1] However, if the cause of pleural effusion is uncertain or unknown, further diagnostical procedures are warranted. Diagnosing infection is important as delayed antibiotic therapy may result in additional morbidity.[2] Current guidelines recommend routine microbiological studies to aid in the decision of antimicrobial treatment and the need for drainage.[1] However, Gram stains are not always conclusive and awaiting culture results may result in delayed diagnosis and therapy resulting in complicated parapneumonic effusions or pleural empyema.[2] By contrast, treating all patients with antimicrobial agents before culture results are available will lead to overuse of antimicrobial agents with the associated risk of antibiotic resistance and higher costs.[3]

Accurate markers to establish the presence of infection are needed. Several proteins that are elevated in sera of patients with systemic infection have been suggested as markers of infection in pleural effusions.[4–10] Recently, soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) has been proposed by us and others as a specific marker of infection in patients with sepsis, pneumonia or bacterial meningitis.[11–14] Experimental studies have shown that TREM-1 is up-regulated on myeloid cells if these cells make contact with bacterial components.[15] Simultaneously, a soluble form of TREM-1 is released.[15] Studies in patients with pneumonia and bacterial meningitis have shown that if this protein is measured locally, i.e. on the site of infection, its occurrence is strongly correlated with active infection.[12–14] Indeed, two recent studies have shown that levels of sTREM-1 are elevated in infectious pleural effusions.[4,5] In parallel, other locally detectable markers of inflammation were suggested to be useful in the diagnosis of pleural infection. C-reactive protein (CRP) levels were shown to be higher in patients with infectious pleural effusions as compared to effusions in the context of tuberculosis or malignancy.[7] The differentiating capacity of CRP for infection was demonstrated to be superior to that of the inflammatory cytokines interleukin-6 and tumor necrosis factor-α.[7] Furthermore, pleural effusion levels of procalcitonin (PCT) were shown to be higher in patients with tuberculosis as compared to patients with effusions associated to malignancy.[6]

As previous studies have shown that locally measured sTREM-1 levels are highly correlated with the presence of infection, we evaluated the diagnostic value of pleural fluid levels of sTREM-1 as a biological marker of infection in intensive care patients with pleural effusions. Moreover, the diagnostic value of pleural fluid levels of sTREM-1, CRP and PCT has been studied separately only. Therefore, in the present study we evaluated and compared the diagnostic capacity of each of these markers.

Methods

Patients

Critically ill patients with a de novo presentation of a pleural effusion during the course of their stay on the intensive care were eligible for the study. Only patients in whom pleural effusions were drained were included in the study. Indications for drainage were diagnostical purposes in case an exudate was suspected (e.g., in case of suspected infection) or for respiratory failure considered to be the consequence of the pleural effusion. Patients with a pleural effusion within 24 h after cardiothoracic or esophagus surgery served as control patients. In view of the observational nature of the study the institutional ethical committee of the Academic Medical Center mandated neither retrospective patient consent nor formal assent from relatives to be required.

Study protocol

Drainage was performed either by insertion of a sterile Pneumo-cath (Intra special catheters GmbH, Rehlingen-Siersburg, Germany) or a sterile thorax drain (Tyco Healthcare, Tullamore, Ireland). In control patients who had a thorax drain after cardiothoracic or esophagus surgery, pleural fluid was collected from the indwelling thorax drain by aspiration with a sterile needle. Immediately after fluid had been obtained, samples were sent to the bacteriological department and the clinical hospital laboratory for routine analyses. These consisted of culture and measurement of levels of lactate dehydrogenase, total protein and cholesterol, and leukocyte count with differentiation. For study purposes fluid was centrifuged at

1500 × *g* for 10 min at 4 °C, and supernatant was collected and stored at −80 °C until measurement of levels of sTREM-1, CRP and PCT.

Classification of pleural effusions

All pleural effusions were classified into transudates or exudates according to the criteria of Light or as empyema based on the macroscopic presence of pus.[1] In case an exudates was positive on bacteriological culture than this effusion was classified as infectious. Classification of effusions was performed before analysis of biological markers.

Measurements

Levels of sTREM-1 were measured with an enzyme-linked immunosorbent assay as described previously.[14] The sensitivity of this assay is 10 pg/ml and results can be obtained within 4 h. Levels of CRP were measured with an automated immunoturbid assay. The sensitivity of this assay is 1 μg/ml and results are obtained within 1 h. PCT was measured with a sensitive luminescence immunoassay (Brahms, Berlin, Germany). This assay takes 2 h and the sensitivity is 0.05 ng/ml. All measurements were made in duplicate.

Statistical analysis

Data are presented as medians [range] or means (± standard deviation) as appropriate. Baseline characteristics were evaluated with analysis of variance or the Kruskal Wallis test as appropriate. Differences in levels of sTREM-1, CRP or PCT between groups were analyzed with post-hoc analysis (Dunn's multiple comparison test). Categorical data were analyzed with the χ^2- or Fisher's exact test. Relations between sTREM-1, CRP and PCT levels and clinical or biological features were assessed with use of the Spearman's correlation test. To estimate the ability to discriminate between infectious and non-infectious pleural effusions, receiver operating characteristic (ROC) analysis was performed and the area under the curve (AUC) was calculated. In this analysis, a model with an AUC between 0.7 and 0.8 is considered clinically useful. A model with an AUC between 0.8 and 0.9 has excellent diagnostic accuracy. If the AUC approaches 1.0, the model approaches 100% sensitivity and specificity. All analyses were done with SPSS, version 14.0 and GraphPad Prism, version 4.03. A P-value < 0.05 was considered as statistically significant.

Results

Patients

Thirty-six consecutive patients with a de novo presentation of pleural effusion were included from January until December 2006; 20 patients with pleural effusions after cardiothoracic surgery and 11 patients with pleural effusions after esophagus surgery were included as control patients. Patients with a de novo presentation were 55 ± 13 years of age and 16 patients (44%) were male. The control patients were of comparable age (57 ± 13 years, $P = 0.52$) but more patients were male (79%, $P = 0.01$). Of the 36

Table 1 Baseline characteristics of patients with de novo pleural effusion.

	Transudate ($n = 13$)	Exudate ($n = 38$)	Infection ($n = 5$)	Complicated infection ($n = 11$)	P-value
Age (years)	54 ± 10	66 ± 16	63 ± 6*	47 ± 10	<0.01
Male sex (n, %)	5 (38%)	25 (66%)	4 (80%)	4 (36%)	0.11
Admission diagnosis					
Pancreatitis		1	1		
Bowel perforation		1	2		
Trauma		2			
Aspiration pneumonia		3			
Community-acquired pneumonia			2	11	
Resuscitation after ventricular fibrillation	4				
Heart failure	9				
Cardiac surgery		20			
Esophagus cardia resection		11			
Blood leukocyte count (x 10^9/l)	11.6 ± 4.0	12.5 ± 4.2	12.2 ± 6.4	16.0 ± 8.9	0.60
PE leukocyte count (x 10^9/l)	0.5 [0–1.1]	3.6 [0–10.9]	2.0 [0.1–10.1]*	–	<0.01
PE LDH (IU/l)	204 [103–891]	995 [243–3933]	2197 [570–3787]*	–	<0.01
PE cholesterol (mmol/l)	Not detectable	1.6 ± 0.7	0.7 ± 0.9	–	
PE total protein (g/l)	Not detectable	28.5 ± 18.9	16.0 ± 22.0	–	<0.01
PE glucose (mmol/l)	7.5 ± 1.3	6.4 ± 2.3	2.8 ± 2.3*	–	<0.01
PE pH	7.4 ± 0.04	7.4 ± 0.08	7.3 ± 0.09*	–	0.01

PE pleural effusion; LDH lactate dehydrogenase; *$P < 0.05$ vs. all other groups on post-hoc analysis (Bonferroni).

Table 2 Isolated pathogens from pleural effusions and associated illness.

	Infection ($n = 5$)	Complicated infection ($n = 11$)
Coagulase negative Staphylococci	1	2
Enterococcus faecium		2
Escherichia coli	2	
Porphyromonas species		1
Serratia marescens	1	
Staphylococcus aureus		2
Streptococcus pneumoniae		3
Streptococcus viridans	1	
No micro-organism cultured		1

patients with a de novo presentation, 11 patients presented with macroscopic pus in their effusion. The baseline characteristics of the 25 patients presenting with a transudate or exudate are presented in Table 1.

Of the patients presenting with new effusions, 11 effusions were classified as empyema, 5 as infectious, 7 as exudate and 13 as transudate. All of the effusions of control patients were classified as exudates. In 15 patients (10 with an empyema and 5 with an exudate) bacterial culture of the pleural fluid was positive and in one patient with an exudate no culture was performed. The isolated pathogens are listed in Table 2. One patient with a macroscopic empyema had a negative culture result. In this patient empyema was drained after initiation of antibiotic treatment.

Levels of sTREM-1, CRP, and PCT in pleural fluid (Figure 1)

In empyemas, levels of sTREM-1 were significantly higher (median, 922 [range, 0—2972] pg/ml) as compared to exudates (0 [0—189] pg/ml) and transudates (0 [0—110] pg/ml). Levels of sTREM-1 in infectious effusions (287 [131—395] pg/ml) were comparable to empyemas and significantly higher compared to transudates and non-infectious exudates.

Levels of CRP were higher in empyemas (86 [8—232] μg/ml) and infectious effusions (42 [11—164] compared to transudates (7 [1—83] and non-infectious exudates (2 [1—118]. In post-hoc analysis all comparisons (empyema vs. transudates and exudates, and infectious vs. transudates and exudates) were statistically significant.

Levels of PCT were highest in infectious exudates (0.58 [0.05—30.00] ng/ml; $P < 0.01$). In contrast, PCT levels in empyemas were low and not different from the levels in transudates and non-infectious exudates.

Levels of sTREM-1, CRP and PCT in relation to the presence of infection (Figure 2)

In patients with positive culture results, levels of sTREM-1 were significantly higher (302 [0—2972] pg/ml compared to the levels in non-infected effusions 0 [0—1671] pg/ml; $P < 0.01$). In one patient with pleural empyema but a negative culture result, an sTREM-1 level of 1671 pg/ml was found and the sTREM-1 level was 75 pg/ml in the patients with an exudate of which no culture was performed. This patient survived without antibiotic treatment. In patients with positive culture results, CRP levels tended to be higher as compared to patients with negative results (78 [1—232] pg/ml versus 29 [1—160] pg/ml; $P = 0.11$). In contrast, no differences were found in PCT levels between patients with positive or negative culture results.

Receiver operating characteristic curves (Figure 3)

As a next step, we constructed receiver operating characteristic (ROC) curves to study the capacities of sTREM-1, CRP and PCT to differentiate between infectious and non-infectious pleural effusions. For the first analysis, all patients and controls were included. The AUCs for sTREM-1 and CRP were 0.93 (95% CI 0.84—1.00; $P < 0.01$) and 0.81 (95% CI 0.69—0.93; $P < 0.01$) respectively. The AUC for PCT was 0.62 (95% CI 0.45—0.80; $P = 0.17$). For the identification of infection, a cut-off value of 50 pg/ml for sTREM-1 resulted in a sensitivity of 93% (95% CI 75—99) and a specificity of 86% (95% CI 76—92). Sensitivity was 87% (95% CI 67—96) and specificity 71% (95% CI 59—80) for a cut-off value of 10 μg/ml

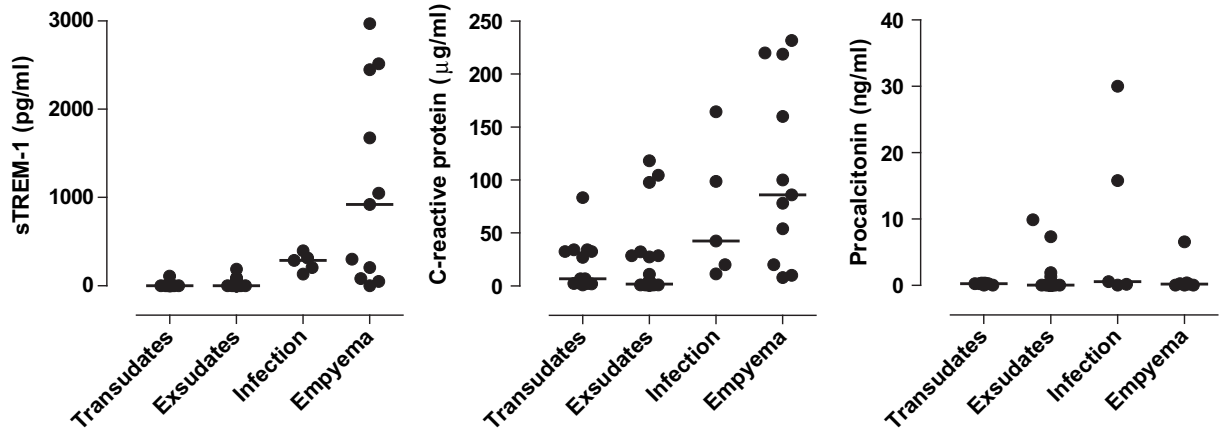

Figure 1 Levels of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), C-reactive protein and procalcitonin in patients with transudative pleural effusions, exudative pleural effusions, infectious pleural effusions and empyema.

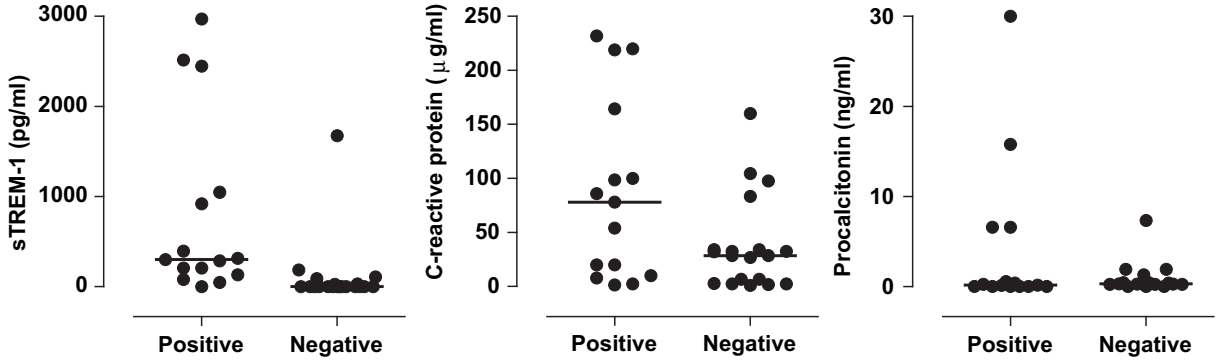

Figure 2 Levels of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), C-reactive protein and procalcitonin in patients with positive or negative culture results of pleural effusions.

for CRP and 60% (95% CI 39—78) and 64% (95% CI 52—74) respectively for a cut-off value of 0.15 ng/ml for PCT.

Discussion

Early diagnosis of infection in patients with pleural effusions is essential for optimal treatment. While a definite diagnosis of infection requires results of cultures of obtained pleural fluid, use of biomarkers of infection may result in earlier diagnosis and thus more appropriate treatment. While recent studies have suggested sTREM-1, CRP and PCT as biological markers for infection in pleural effusions,[4—8] we compared these markers in one cohort and explored the diagnostic capacities. Local levels of sTREM-1 and CRP were elevated both in patients with empyemas or exudates. Local PCT levels were only found to be elevated in patients with infectious exudates. Moreover, sTREM-1 was demonstrated to be superior to CRP and PCT to detect local infection.

Several limitations of our study have to be acknowledged. First, we included a limited number of patients. While the present study was designed to explore the differences between sTREM-1, CRP and PCT we may not have included a sufficient number of patients to calculate sensitivities and specificities that can be generalized to all intensive care patients. Second, in fact we only included intensive care patients with an acute presentation of pleural effusion, but our main goal was to explore whether the studied markers can be used to diagnose acute infection in this setting. Therefore, our results cannot be extended to other patient groups. Third, we did not measure the parameters in plasma samples. This may be considered an important drawback because plasma levels might reveal infection without the need to aspire fluid. However, increased plasma sTREM-1 levels are not specific for any kind of infection and aspiration of fluid may still be needed to rule out infection in the pleural cavity.

Previous studies have shown that CRP can adequately differentiate transudates from exudates in case of pleural effusion.[9,10] Sensitivities of 74%—94% and specificities of 74%—76% have been reported. However, reports on the capacity of CRP to distinguish infectious from non-infectious effusions have not been performed before. However, a recent study suggested that CRP levels in pleural effusions might be used to differentiate between categories of severity of infection.[8] In that study, patients with complicated parapneumonic effusions or empyema had higher local CRP levels as compared to patients with uncomplicated parapneumonic effusions. In our study, patients with empyema indeed had the highest CRP levels, followed by patients with infectious exudates. Our study was not powered to show differences between these subgroups but CRP levels were significantly lower in non-infectious effusions. Some patients with exudative fluid showed high CRP levels. This may have been caused by the fact that we studied intensive care patients, whereas all previous

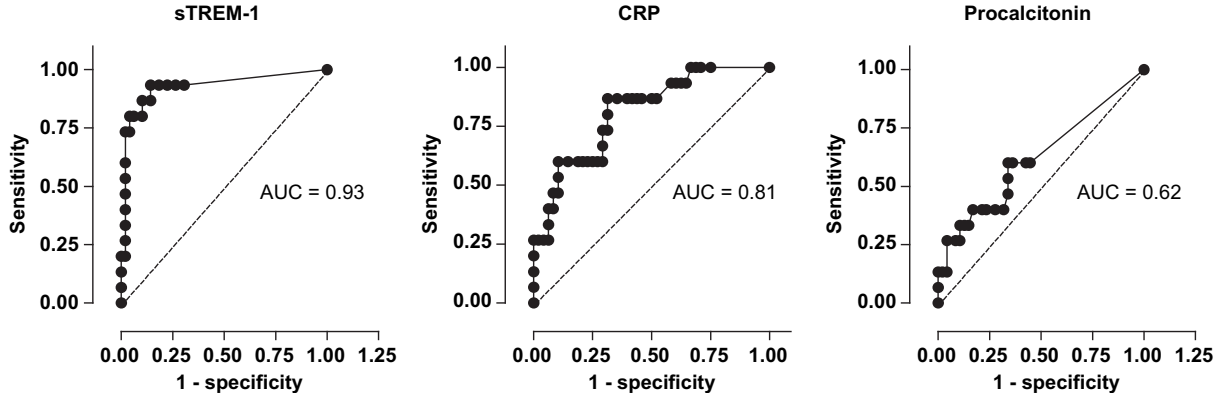

Figure 3 Receiver operating characteristic curves of levels of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1), C-reactive protein and procalcitonin in patients with pleural effusions: differentiation between infectious and non-infectious pleural effusions.

studies on CRP levels in pleural effusions were performed in non-critically ill patients. Intensive care patients may have higher baseline levels of systemic inflammation as compared to the other patients. As CRP is a non-specific marker of inflammation, higher levels in pleural fluid may simply reflect higher systemic levels.

We observed that sTREM-1 was superior to CRP and PCT in differentiating between infected and non-infected pleural effusions. This confirms findings of previous studies on sTREM-1 in pleural effusions, [4,5] and shows that sTREM-1 may be the candidate marker as a diagnostic tool to detect infection. While current chemistry analyses provide information on presence of exudates or transudates, sTREM-1 could be the biological marker that detects infection before bacteriological studies are performed. Future studies may compare Gram-staining with sTREM-1 measurement as a rapid method for detection of infection.

In conclusion, based on the present study, sTREM-1 appears a highly useful marker to differentiate between infectious and non-infectious pleural effusions. Moreover, sTREM-1 appears superior to CRP and PCT for this purpose. Awaiting bacteriological culture results, sTREM-1 may aid in early detection of infection and guide early treatment.

Conflict of interest statement

None of the authors have any conflict of interest to disclose.

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Author contributions:

Conception and design: RMD, AES, MJS.

Acquisition of data, analysis and interpretation: RMD, AAA, PB, PES, JV, MJS.

Drafting the article or revising it critically for intellectual content: RMD, AAA, AES, PB, PES, MJS.

Final approval of the version to be published: RMD, AAA, AES, PB, JV, PES, MJS.

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