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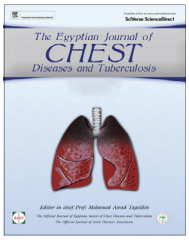

ORIGINAL ARTICLE

Diagnostic utility of soluble triggering receptor expression on myeloid cells-1 in complicated parapneumonic pleural effusion

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KEYWORDS

Diagnosis;
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Abstract *Background:* The differentiation between complicated parapneumonic effusions (CPPE) or empyema, which require chest tube drainage, and uncomplicated parapneumonic effusions (UCPPE), which respond to antibiotic therapy alone, is sometimes unclear. Delay in diagnosis results in substantial delay in the commencement of treatment and may contribute to the high mortality of this infection.

The aim of the study: Evaluation of the utility of soluble triggering receptor expression on myeloid cells-1 (sTREM-1) as an early marker in the diagnosis and management of complicated parapneumonic effusions and empyema.

Patients and methods: This study included 58 patients who were diagnosed as having PPE and admitted to the Chest Department, Zagazig University Hospitals during the period from March 2012 to March 2013. Patients were diagnosed PPE if they had a pleural effusion and showed one or more clinical manifestations typical of pneumonia, including acute febrile illness, sputum production, chest pain, leukocytosis and infiltrate(s) on chest X-ray. They were divided into two groups.

Group (1): Complicated parapneumonic effusion (22 patients), according to at least one of the following criteria on pleural fluid examination: macroscopic pus, presence of organisms on Gram-

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stain or culture, fluid pH < 7.2 with normal peripheral blood pH, or fluid glucose concentrations < 40 mg/dL.

Group (2): Uncomplicated parapneumonic effusion (36 patients), according to the following criteria: pleural effusion associated with a non purulent pleural fluid, negative fluid microbiological studies; fluid pH > 7.2 with normal peripheral blood pH and fluid glucose > 40 mg/dL.

Exclusion criteria: A history of pleural disease or any underlying disease that could potentially cause pleural effusions, such as tuberculosis, malignancy, heart failure, systemic lupus erythematosus and chronic renal failure, were excluded. Pleural fluid samples were examined for level of sTREM-1, pH, LDH and glucose. The sTREM-1 levels were expressed as pg/mL. Microbiological studies included: Gram and Ziehl–Neelsen stains and cultures on conventional media for aerobic and anaerobic micro-organisms in the pleural fluid samples.

Results: The median sTREM-1 level in pleural fluid was significantly higher in the bacterial PPE (688 ± 398 pg/mL) than in the non-bacterial PPE (45 ± 79 pg/mL). The cut-off value of pleural fluid sTREM-1 for diagnosis of bacterial PPE was 130 pg/mL with 93% sensitivity and 92% specificity, while it was 7.237 for pleural fluid pH with 91% sensitivity and 96% specificity and 640 mg/L for pleural fluid glucose with 92% sensitivity and 86% specificity and 800 IU/L for pleural fluid LDH with 81% sensitivity and 90% specificity.

In conclusion: Combination of classical criteria with pleural fluid sTREM-1 could be useful in discrimination between nonpurulent complicated and non complicated parapneumonic pleural effusions and hence early pleural drainage in patients with complicated parapneumonic effusions which may affect disease outcome.

Introduction

Pleural effusion is a common clinical entity that occurs in a great variety of diseases [1]. Various studies listing the etiologies of pleural effusions have reported that parapneumonic effusions account for 11–40% of all pleural effusions [2]. Unfortunately, the differentiation between complicated parapneumonic effusions (CPPE) or empyema, which require chest tube drainage, and uncomplicated parapneumonic effusions (UCPPE), which respond to antibiotic therapy alone, is sometimes unclear [3].

Delay in diagnosis results in substantial delay in the commencement of treatment and may contribute to the high mortality of this infection. Treatment of all patients with suspected pleural effusion with antibiotics while waiting for microbiological results is not a good option since this practice increases antibiotic resistance. Diagnosis and differential diagnosis of parapneumonic effusions pose a great problem. Biochemical parameters are often non-specific and Gram stain has a low sensitivity. Pleural fluid cultures, even though being specific, may take days to reveal a positive culture and in 30–35% of cases, the organism fails to be cultured [1].

Triggering receptor expressed on myeloid cell (TREM) proteins are a family of cell surface receptors expressed broadly on myeloid cells. The first TREM identified (TREM-1) is a recently-discovered cell surface molecule expressed by neutrophils and monocytes. TREM-1 is a 30-kDa glycoprotein belonging to the immunoglobulin super family, and its expression is upregulated by various ligands for Toll-like receptors (TLRs). The initial characterization of TREM-1 demonstrated that TREM-1 expression is upregulated in response to lipopolysaccharide and other microbial products. TREM-1 acts synergistically with receptors for pathogen-associated molecular patterns, including both TLRs and Nod-like receptors. Activation of TREM-1 expressed on neutrophils and monocytes by an agonistic monoclonal antibody has been shown to stimulate the expression of various proinflammatory cytokines, chemokines, and cell surface molecules. TREM-1 exists in both a membranous and a soluble form (soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) [4].

TREM-1 is shed by the membrane of activated phagocytes after exposure to bacteria and fungi and, its soluble form, sTREM-1 can be detected in the body fluids [5]. sTREM-1 is a diagnostic marker for sepsis and inflammation. It has been described as a diagnostic marker with a high accuracy and sensitivity in detecting microbial infections as underlying disease in critically ill patients [6]. The levels of sTREM-1 have previously been investigated in plasma, bronchoalveolar lavage fluid and exhaled breath [7].

Few studies have investigated the clinical significance of sTREM-1 in pleural effusions and found that patients with PPE or empyema exhibited the highest pleural fluid concentrations of this biomarker. However, there were discrepancies regarding its discriminative properties as well as its optimal cut-off point [8]. Taking into account that the management of complicated pyogenic bacterial effusions may be delayed using classic diagnostic procedures the aim of this work was to evaluate the utility of sTREM 1 as an early marker in the diagnosis and management of complicated parapneumonic effusions and empyema.

Patients and methods

Patients

The study included 58 patients diagnosed PPE admitted to the Chest Department, Zagazig University Hospital during the period from March 2012 to March 2013. A written informed consent was obtained from all patients. Patients were diagnosed as having PPE if they had a pleural effusion and showed one or more clinical manifestations typical of pneumonia, including acute febrile illness, sputum production, chest pain,

leukocytosis and infiltrate(s) on chest X-ray. Patients were divided into two groups. *Group (1):* Complicated parapneumonic effusion (22 patients), according to at least one of the following criteria on pleural fluid examination: macroscopic pus, presence of organisms on Gram-stain or culture, fluid pH < 7.2 with normal peripheral blood pH, or fluid glucose concentrations < 40 mg/dL. *Group (2):* Noncomplicated parapneumonic effusion (36 patients), according to the following criteria: pleural effusion associated with a non purulent pleural fluid, negative fluid microbiological studies; fluid pH > 7.2 with normal peripheral blood pH and fluid glucose > 40 mg/dL.

Exclusion criteria

A history of pleural disease or any underlying disease that could potentially cause pleural effusions, such as tuberculosis, malignancy, heart failure, systemic lupus erythematosus and chronic renal failure, were excluded.

Methods

Diagnostic plural fluid samples using standard thoracocentesis technique were collected in heparinized tubes from each patient and subjected to:

Biochemical analysis

Biochemical measurements were carried out using a Hitachi 919 automatic analyser (Boehriger Mannheim, GMbH, Mannheim, Germany), using the method of Biuret for proteins, hexokinase for glucose, and pyruvate-to-lactate reduction at 37° for LDH. For determination of pH, pleural fluid was collected directly into a heparinized blood-gas syringe and was maintained anaerobically. The syringe containing the pleural fluid was immediately placed on ice and transferred to the laboratory. Pleural-fluid pH was measured within 20 min after thoracocentesis using a selective pH electrode (Chiron Diagnostics 860; Ciba Corning Diagnostics Corp., Medfield, MA, USA). For sTREM-1 determinations the concentrations of sTREM-1 in pleural fluid were measured using ELISA kits according to the manufacturer's protocol (R&D Systems Inc., Minneapolis, MN, USA). The sTREM-1 levels were expressed as pg/mL.

Microbiological studies

Gram and Ziehl–Neelsen stains were carried out and cultures were performed on conventional media for aerobic and anaerobic micro-organisms (Phedebact Pneumococcus test; Boule Diagnostics, Huddinge, Sweden). Total white blood cell counts were carried out with a Coulter®-s-Plus IV Counter Izasa, Spain. To differentiate between leukocytes, the sample was centrifuged (Cytospin® 2, Shandon Southern Products Ltd, UK) at 2000 rpm for 8 min, and the preparation obtained was stained with May-Grunwald-Giemsa.

Statistical analysis

Statistical analysis was performed with SPSS version19 software package (SPSS, Inc. Chicago). Categorical variables were expressed as proportions, and continuous variables that were or were not normally distributed were expressed as means ± SD or medians (quartiles), respectively. The *t*-test or Mann–Whitney test was used to compare means or medians between different groups, for variables that were or were not normally distributed, respectively. Receiver Operator Curves (ROC) were designed to assess sensitivity, specificity, positive and negative predictive values for the estimated parameters to predict CPPE. For all analyses, a two-tailed *P* value < 0.05 was considered significant.

Results

The study included 58 patients (41 males and 17 females), of these, 22 had CPPE (16 males and 6 females, with a mean age of 63 ± 12 years) and 36 had UCPPE (25 males and 11 females, with a mean age of 64 ± 13 years) (Table 1).

The median sTREM-1 levels in pleural fluid were significantly higher in the bacterial PPE (688 ± 398 pg/mL) than in the non-bacterial PPE (45 ± 79 pg/mL). Others, LDH, glucose levels and TLC of pleural fluid also demonstrated significant differences among the two groups, but pleural protein level did not (Table 2).

The cut-off value of pleural fluid sTREM-1 for the diagnosis of bacterial PPE (from the ROC curve; AUC = 0.92) was 130 pg/mL. This corresponded to 93% sensitivity and 92% specificity, while it was 7.237 for pleural fluid pH with 91% sensitivity and 96% specificity and 640 mg/L. For pleural fluid glucose with 92% sensitivity and 86% specificity and 800 IU/L for pleural fluid LDH with 81% sensitivity and 90% specificity (Table 3).

Discussion

The differentiation between non purulent complicated parapneumonic effusions, which require chest tube drainage, and uncomplicated parapneumonic effusions, which respond to antibiotic therapy alone, is sometimes difficult because biochemical parameters used are often nonspecific. Pleural fluid cultures are specific, but results may take days. A rapid microbiologic tool is the Gram stain, but its sensitivity is low, approximately 50% [1].

Table 1 Characteristics of the patients with complicated or uncomplicated parapneumonic effusions.

	CPPE (n = 22)	UCPPE (n = 36)	P value
Age, years	63 ± 12	64 ± 13	$P > .05$
Males, n (%)	16 (72.7%)	25 (69.4%)	$P > .05$
Female, n (%)	6 (27.3%)	11 (30.6%)	$P > .05$
Comorbidities, n (%)	11 (50%)	9 (25%)	$P > .05$
Diabetes mellitus	5	1	$P < .05$
COPD	5	6	$P > .05$
Neuropsychiatric disease	1	2	$P > .05$

Table 2 Laboratory characteristics of pleural fluid of the patients with complicated and uncomplicated parapneumonic effusions.

	CPPE (n = 22)	UCPPE (n = 36)	P value
sTREM-1 (pg/mL)	688 ± 398	45 ± 79	$P < .05$
Protein (mg/dL)	2.8 (2.1–3.8)	2.6 (1.8–3.2)	$P > .05$
Glucose (mg/dL)	89.6 (20.1–168.7)	170.3 (97.4–212.61)	$P < .05$
LDH (IU/L)	353.2 (222–1023.3)	232 (140–501.3)	$P < .05$
pH	6.79 (6.36–7.00)	7.40 (7.35–7.42)	$P < .05$
TLC	13.24 (4.2–34.5)	7.6 (3.1–11.1)	$P < .05$

Table 3 Accuracy of biomarkers for identification of complicated parapneumonic effusions.

	Cut-off value	Sensitivity, % (95% CI)	Specificity, % (95% CI	AUC (95% CI)
LDH	800 IU/L	81 (64–94)	90 (67–99)	0.90 (0.81–0.99)
pH	7.237	91 (72–97)	96 (76–100)	0.92 (0.85–1.00)
Glucose	640 mg/L	92 (74–99) s	86 (62–97)	0.91 (0.76–1.00)
sTREM-1	130 pg/mL	93 (75–96)	92 (68–98)	0.92 (0.77–1.00)

AUC, area under the curve; CI, confidence interval.

Parapneumonic effusions are generally considered to be uncomplicated when pleural pH is >7.2 and pleural glucose is >60 mg/dL, since they resolve following antibiotic therapy, whereas empyema and complicated parapneumonic effusions require early drainage with or without instillation of local fibrinolytic agents to prevent the development of pleural complications [9].

The detection of frank pus or Gram-positive fluid or culture dictates the need for placement of a chest tube drainage, but management is not as clear-cut when fluid is non purulent, and Gram stain and culture are negative. A number of classifications have been proposed to differentiate bacterial effusions that require pleural drainage from those that resolve with antibiotic therapy alone [10]. In 1995 Heffner et al. [11] established that a pleural pH of 7.2 was the most useful marker for draining parapneumonic pleural effusions, but later they suggested that pleural pH < 7.1 and pleural glucose <40 mg/dL or LDH >1000 are better criteria to determine drainage of these effusions. Sahn [12] also recommended drainage when pleural fluid pH is <7.1. The classification of Light [9] recommended drainage of effusions with pleural pH < 7.2 or glucose <40 mg/dL; however, since 1995 drainage is recommended when pleural pH or pleural glucose is <7 or <40 mg/dL, respectively. Borderline complicated parapneumonic pleural effusions with pleural pH between 7 and 7.2 should be managed with daily therapeutic thoracentesis, and chest tubes should be used if pleural pH falls <7 or if glucose falls <40 mg/dL [5].

Despite these classification systems, the diagnosis of complicated pyogenic bacterial pleural effusions with classic markers is still delayed in some patients [5,6]. Thus, it would be beneficial to find a marker, which, when associated with classic pleural fluid biochemical analysis, would help in the early identification of pyogenic bacterial effusions so that pleural drainage could be quickly initiated to prevent the development of local complications. sTREM-1 is a diagnostic marker for sepsis and inflammation. It has been described as a diagnostic marker with a high accuracy and sensitivity in detecting microbial infections as underlying disease in critically ill patients [6].

The levels of sTREM-1 have previously been investigated in plasma, bronchoalveolar lavage fluid and exhaled breath [13]. Few studies have investigated the clinical significance of sTREM-1 in pleural effusions and found that patients with complicated PPE or empyema exhibited the highest pleural fluid concentrations of this biomarker. However, there were discrepancies regarding its discriminative properties as well as its optimal cut-off point [14].

So this study was done to assess the usefulness of sTREM-1 for the identification of complicated PPE that requires drainage. When complicated and uncomplicated parapneumonic effusions were compared, sTREM-1 levels were significantly higher in complicated parapneumonic effusions Table 2 this result agrees with the result of previous studies.

This study demonstrated that sTREM-1 cut-off value of 130 pg/mL had a sensitivity of 93% and a specificity of 95 % for identification of complicated PPE that requires drainage (Table 3). Previous studies have evaluated the use of sTREM-1 for the same purpose. Bishara et al. [15] reported that a cut-off value of 114 pg/mL for pleural sTREM-1 achieved a sensitivity of 94% and a specificity of 93% (AUC 0.966) in differentiating 17 patients with empyema from 72 pleural effusions of other aetiologies. Furthermore, Chan et al. reported that a sTREM-1 at a cut-off 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 (n = 22) from tuberculous pleuritis (n = 16) [13]. In another study sTREM-1 cut-off value of 768.1 pg/mL had a sensitivity of 86%, specificity of 93% and AUC of 0.93 in differentiating 23 bacterial effusions (including 17 empyema) from 88 effusions with other aetiologies [16]. The differences in representation of empyemas and the lack of standardization of the ELISA technique in these studies may explain the discrepancies in sTREM-1 concentrations and its cut-off points.

In conclusion

Combination of classical criteria with pleural fluid sTREM-1 could be useful in discrimination between nonpurulent compli-

cated and non complicated parapneumonic pleural effusions and for early recommendation of pleural drainage in patients with complicated parapneumonic effusions which may affect disease outcome.

Conflict of interest

None declared.

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