

# Biomarkers of infection for the differential diagnosis of pleural effusions

**J. M. Porcel\***, **M. Vives<sup>+</sup>**, **G. Cao<sup>#</sup>**, **S. Bielsa\***, **A. Ruiz-González\***, **A. Martínez-Iribarren<sup>#</sup>** and **A. Esquerda<sup>#</sup>**

## AFFILIATIONS

\*Pleural Disease Unit, Department of Internal Medicine,

<sup>#</sup>Department of Laboratory Medicine, Arnau de Vilanova University Hospital, IRBLLEIDA, Lleida, Spain.

<sup>+</sup>Service of Internal Medicine, Clinica Recoletas, Albacete, Spain.

## CORRESPONDENCE

José M. Porcel, Dept of Internal Medicine, Arnau de Vilanova University Hospital, Avda Alcalde Rovira Roure 80, 25198 Lleida, Spain.

Fax: 34 973 248754

E-mail: [jporcelp@yahoo.es](mailto:jporcelp@yahoo.es)

## SUPPORT STATEMENT

This study was supported by a grant from the Instituto de Salud Carlos III (FIS 06/0725). Dr. Bielsa is a Research Fellow supported by a grant from the Fondo de Investigación Sanitaria (FIS CM07/00020).

## STATEMENT OF INTEREST

The authors have no conflicts of interest to disclose.

**Short title:** Infectious biomarkers in pleural effusions

**ABSTRACT: We aimed to investigate whether pleural fluid concentrations of biomarkers for bacterial infection, namely triggering receptor expressed on myeloid cells (sTREM-1), procalcitonin (PCT), lipopolysaccharide-binding protein (LBP) and C-reactive protein (CRP) might identify infectious effusions and discriminate between complicated (CPPE) and uncomplicated parapneumonic effusions (UPPE).**

**Stored pleural fluid samples from 308 patients with different causes of pleural effusion were used to measure the four biomarkers. ROC analysis determined the accuracy of the new tests.**

**Median pleural fluid levels of CRP, sTREM-1 and LBP were significantly higher in CPPE compared with those in other etiologies. The AUC for distinguishing infectious (parapneumonics and tuberculosis) from non-infectious effusions was 0.87 for CRP, 0.86 for sTREM-1, 0.57 for PCT and 0.87 for LBP. Regarding the discrimination of non-purulent CPPE vs. UPPE, a multivariate analysis found that pleural fluid glucose  $\leq 60$  mg/dL, LBP  $\geq 17$   $\mu$ g/mL and CRP  $\geq 80$  mg/L were the best parameters. Individually, none of the new biomarkers achieved better performance characteristics than pH, glucose or lactate dehydrogenase in labeling CPPE.**

**In conclusion, elevated pleural fluid levels of CRP, sTREM and LBP identify patients with infectious effusions, particularly those with CPPE. PCT has no value for the differential diagnosis of pleural effusions.**

**KEY WORDS: C-reactive protein, empyema, lipopolysaccharide-binding protein, pleural effusion, procalcitonin, triggering receptor expressed on myeloid cells**

Rapid and accurate diagnosis remains the major clinical challenge in patients with suspected pleural infection. Since clinical data may be elusive, measurement of biomarkers in pleural fluids might provide a reliable tool for estimating the probability of an infection [1]. In addition, the search for biomarkers of poor prognosis in parapneumonic effusions (PPE) is critical for better identifying patients in which drainage of the pleural space is mandatory [2].

In general, C-reactive protein (CRP) is considered the biomarker of choice to detect an inflammatory state, whether or not it is triggered by an infection. Newer circulating markers such as soluble triggering receptor expressed on myeloid cells (sTREM-1), procalcitonin (PCT), and lipopolysaccharide binding protein (LBP) seem to have a pivotal role in the inflammatory host response to microbial infections [3,4]. Therefore, we hypothesized that when measured in pleural fluid, they might be useful for diagnosing or ruling out infectious effusions and for identifying complicated parapneumonic effusions (CPPE).

The aims of the present study were to measure the pleural fluid concentrations of CRP, sTREM, PCT and LBP in patients with different causes of pleural effusions and to explore their usefulness in both predicting an infectious etiology and discriminating between subgroups of PPE.

## **METHODS**

### ***Study population***

Since 1994, we have been prospectively maintaining a database on all patients who undergo diagnostic thoracentesis at the Arnau de Vilanova University Hospital (Lleida, Spain), as well as a pleural bank of fluid specimens that are processed and stored at -80°C. The indication for diagnostic thoracentesis was the presence of a clinically significant pleural effusion of uncertain origin. In patients submitted to more than one thoracentesis, during the hospitalization period, only the results of the first tap were considered. Samples of pleural fluid were immediately analyzed for routine biochemistries (e.g., pH, glucose, lactate dehydrogenase –LDH-) while supernatants were frozen within 4 hours of collection until the measurement of the biomarkers at the end of the study.

For the present study, 308 pleural fluid samples collected from 2004 to 2008 were randomly selected for biomarkers determination. Patients were classified into 7 groups according to the cause of the pleural effusion: transudates (40 patients), malignant effusions (40 patients), tuberculous pleurisy (50 patients), uncomplicated parapneumonics -UPPE- (60 patients), non-purulent CPPE (68 patients), empyemas (30 patients) and miscellaneous exudates (20 patients). Primary tumors in malignant effusions were lung (14), breast (8), unknown primary (6), lymphoma (4), ovary (3), gastrointestinal (3) and mesothelioma (2). Among the miscellaneous exudates, there were 5 post-traumatic, 3 idiopathic, 3 post-coronary artery bypass surgery, 2 pericarditis, 2 pulmonary embolism, 2 abdominal abscess, 1 pancreatitis, 1 post-abdominal surgery and 1 systemic lupus erythematosus.

The local Ethics Committee approved this study, and all patients gave informed consent for the analysis of stored specimens for future research.

### ***Diagnostic criteria***

A pleural effusion was categorized as malignant if malignant cells were detected on cytologic examination of the pleural fluid or biopsy specimen. Tuberculous pleuritis was diagnosed if Löwenstein cultures of pleural fluid, sputum or pleural biopsy tissue samples were positive (26 patients), a pleural biopsy specimen showed granulomas in the parietal pleura (5 patients) or an exudative lymphocytic effusion with high adenosine deaminase levels ( $> 40$  U/L) cleared in response to antituberculous therapy (19 patients). Transudates were effusions secondary to heart failure (30 patients), cirrhosis (6 patients), nephrotic syndrome (2 patients) or atelectasis (2 patients). PPEs referred to those associated with bacterial pneumonia and were classified into three groups: UPPE (resolution with antibiotic treatment alone), non-purulent CPPE (requirement of an invasive procedure such as tube thoracostomy for cure) and empyema (pus into the pleural space). Other causes of pleural effusions were determined by well-established clinical criteria.

### ***Measurement of biomarkers***

Assays for the four inflammatory markers, namely CRP, sTREM, PCT and LBP, were performed on the stored cell-free supernatants of pleural fluid samples. All samples were tested in random order and by technicians who were blinded to the clinical diagnosis of patients at the end of the study.

Pleural fluid CRP was measured using a particle-enhanced immunoturbidimetric assay (CRPLX, Tina-quant, Roche Diagnostics GmbH, Mannheim, Germany) on Roche automated clinical chemistry analyzers. The sTREM-1 concentrations in pleural fluid were measured by a sandwich ELISA (DuoSet ELISA, R&D Systems Europe, Abingdon, UK), PCT by a two-site immunoluminometric assay (Liaison Brahms PCT, DiaSorin, Saluggia, Italy) and LBP with a solid-phase, enzyme-labeled chemiluminescent immunometric assay (Immulite 2000 LBP, Siemens, Los Angeles, USA), according to the manufacturer's instructions. The samples for sTREM-1 were assayed in duplicate.

### ***Data analysis***

Continuous data are presented as medians (quartiles). Between-group comparisons were performed with Kruskal-Wallis tests and Dunn's multiple comparison post-hoc tests for continuous variables, and Chi square tests with post-hoc analysis of adjusted residuals for categorical variables. Receiver-operating characteristic (ROC) curves were constructed to illustrate the predictive value of various cutoff points of CRP, sTREM-1, PCT and LBP. The point with the largest sum of sensitivity and specificity was chosen as a threshold. We compared the performances of the four pleural fluid inflammatory biomarkers for discriminating infectious (bacterial and mycobacterial) vs. non-infectious effusions, tuberculosis vs. malignancy, PPE vs. non-PPE, and CPPE vs. UPPE. Measures of test efficacy included sensitivity, specificity and likelihood ratios. To adjust for confounders, a backward conditional step-wise logistic regression model estimated the simultaneous impact of each biomarker, along with other biochemical fluid findings, in the prediction of non-purulent CPPE. The level of significance was set at  $P < 0.05$  using two-tailed tests. Data



were analyzed using a statistical software package (SPSS version 13.0; SPSS Inc, Chicago, IL).

## RESULTS

### ***Pleural fluid levels of the biomarkers***

The demographic data and the median pleural fluid levels of CRP, sTREM-1, PCT and LBP in each of the 7 groups are shown in table 1. The levels of CRP, sTREM-1 and LBP were significantly higher in the pleural fluid from patients with CPPE and lower in the transudate and malignant groups (fig. 1). The levels of PCT showed the same trend but with a large overlap of values.

### ***Discriminating infectious from non-infectious effusions***

The thresholds of the four biomarkers that best discriminate between infectious (PPE and tuberculosis) and non-infectious effusions were as follows: CRP  $\geq 20$  mg/L, sTREM-1  $\geq 80$  pg/mL, PCT  $\geq 0.25$  ng/mL and LPB  $\geq 7$   $\mu$ g/mL (table 2). While PCT had no discriminative properties (AUC = 0.57), sTREM-1 (AUC = 0.86), CRP and LBP (both AUCs = 0.87) showed good abilities to separate infectious from non-infectious states. However, the overlapping 95% confidence interval values for these three biomarkers indicated that no single one could be identified as being superior to the others.

### ***Discriminating tuberculous from malignant effusions***

The AUCs for each pleural fluid biomarker (calculated with the cutoffs described above) in differentiating between tuberculous and malignant effusions are displayed in table 3. In contrast to the PCT (AUC = 0.52), which was not useful, CRP, sTREM-1 and LPB had better AUCs that were similar in value (all AUCs > 0.80).

### ***Discriminating PPE from other etiologies***

In discriminating PPE from the other etiologies, the respective AUCs generated from the use of CRP, sTREM-1, PCT and LBP were 0.83, 0.79, 0.63 and 0.80 (table 4). Therefore, PCT lacked efficacy as a marker of PPE.

### ***Discriminating UPPE from non-purulent CPPE***

Table 5 shows the diagnostic accuracies of the four biomarkers in the differentiation of UPPE and non-purulent CPPE at the optimum cutoff points. They were compared with the performances of pH, glucose and LDH at the cutoff values recommended in the literature. All parameters, with the exception of PCT (AUC = 0.59), performed well in discriminating between groups (AUCs about 0.80). Nevertheless, the overlap of the 95% CI values for the AUC and likelihood ratios did not allow for a selection of a superior test that clearly had the highest discriminative properties for non-purulent CPPE.

When all the variables listed in table 5 were entered into a multivariate stepwise logistic regression model, glucose (adjusted likelihood ratio (LR) + 5.2, 95% CI 2.1-8; adjusted LR - 0.10, 95% CI 0.02-0.44), LBP (adjusted LR + 4.6, 95% CI 2.1-7.1; adjusted LR - 0.14, 95% CI 0.04-0.44) and CRP (adjusted LR + 3.4, 95% CI 1.4-6.1; adjusted LR - 0.22, 95% CI 0.07-0.69) were the parameters that best discriminated non-purulent CPPE from UPPE. This model had an AUC of 0.88 (95% CI 0.82-0.94).

## **DISCUSSION**

A number of biochemical markers of bacterial infection have been identified, though the validity of their measurement in pleural fluid remains unclear. Our findings in a large series of patients provide data on the value and accuracy of some of these biomarkers (i.e., CRP, sTREM and LBP) measured in the pleural fluid to diagnose infectious effusions, particularly from CPPE.

### ***C-reactive protein***

CRP, an acute-phase reactant released from the liver, is a common diagnostic test within hospital laboratories for the screening or monitoring of infections and non-infectious inflammatory diseases. CRP levels have been studied in pleural fluid and have been found to be higher in PPE than in other types of exudative or transudative effusions [5-7]. We showed that a pleural fluid CRP level above 80 mg/L argues for the presence of a PPE (LR + = 7.4), whereas CRP levels below 20 mg/L are a strong indicator against an infectious pleural effusion, whether of bacterial or mycobacterial nature (LR - = 0.22). Indeed, a number of studies provide support for the use of CRP as a diagnostic aid in tuberculous pleuritis; low pleural CRP levels (< 30 mg/L) make this diagnosis unlikely while being more indicative of a malignancy in patients with exudates [8-10]. In addition, our findings complement the scarce previous literature on the application of CRP for identifying CPPE [11-12]. This biomarker emerged as an independent predictor of non-purulent CPPE in the multivariate analysis, although its adjusted LR + was lower than those for glucose and LBP.

### ***Soluble triggering receptor expressed on myeloid cells-1***

TREM-1, a receptor of the immunoglobulin superfamily, amplifies the inflammatory response through its over-expression and subsequent activation of neutrophils and monocytes/macrophages in response to microbial products. TREM-1 is also shed by the membrane of activated phagocytes and can be found in a soluble form in body fluids.

Few studies have investigated the clinical significance of sTREM-1 in pleural effusions [13-18]. All included small sample sizes (45 to 109 patients), measured sTREM-1 by ELISA or other techniques 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 cutoff point. For example, in one study, a sTREM-1 cutoff 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 empyemas) from 88 effusions with other etiologies [17]. In contrast, our findings yielded an AUC of 0.79 for identifying PPE at the best cutoff of 80 pg/mL. The over-representation of empyemas in the first series (74% vs. 19% of PPE) may help explain these discrepancies, while different sTREM-1 concentrations and cutoffs probably reflect the lack of standardization of the ELISA technique. In a second study, a cutoff 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 empyemas from 72 pleural effusions of other etiologies [18]. We think there is no point in trying to identify empyema by measuring sTREM-1, because this diagnosis is easily achieved by simple inspection. Finally, in another study, 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 (n = 22) from tuberculous pleuritis (n = 16) [14]. Based on our results (sensitivity 80%, specificity 56% at a cutoff of 180 pg/mL, AUC 0.64; data not shown), we do not support the use of sTREM-1 measurements for this particular indication. In addition, the ability to differentiate between PPE and tuberculous effusions does not normally pose a problem in clinical practice.

Taken together, our data indicate that pleural fluid levels of sTREM-1 may be accurate enough to help differentiate between infectious and non-infectious effusions (AUC = 0.86). Notably, two studies demonstrated that the levels of sTREM-1 in pleural fluid greatly exceeded those in serum [16-17], suggesting that recruited inflammatory cells in the pleural space produce the sTREM-1 locally.

### ***Procalcitonin***

PCT, an acute-phase hormone, has been reported to be more accurate for differentiating between bacterial infections and non-infectious causes of inflammation than other biomarkers [19]. Serum levels of PCT increase in severe bacterial infections. A PCT serum concentration of 0.5 ng/mL has been recommended in clinical practice guidelines to rule out bacterial infection [1]. There is little useful information on PCT levels in pleural fluid. One study found that the median pleural fluid PCT levels were significantly higher in 26 patients with bacterial infection (0.67 ng/mL) than in 80 malignant effusions (0.14 ng/mL), 33 cardiac effusions (0.06 ng/mL) and 17 viral effusions (0.007 ng/mL) [20]. However, the authors did not fully describe the diagnostic criteria of the study population or calculate any discriminatory cutoff values for PCT. A recent investigation showed that pleural fluid PCT levels greater than 0.18 ng/mL

discriminated 45 PPE from 37 non-PPE with a sensitivity of 67%, a specificity of 77% and an AUC of 0.752 [21]. Interestingly, serum PCT had better diagnostic accuracy than pleural PCT and both correlated with the severity of pneumonia.

Our study failed to demonstrate any firm relationship between pleural fluid levels of PCT and the specific cause of the pleural effusion. These disappointing results could be explained by considering PCT as a potential biomarker of a state or a syndrome (e.g., severe sepsis) rather than an indicator of a disease. In fact, some studies have reported that, as a marker of bacterial infection, PCT was no better than CRP [22]. However, in itself, it was considered quite useful as a marker regarding the severity of infection.

### ***Lipopolysaccharide-binding protein***

LBP, a glycosylated 58-kDa protein produced predominantly in hepatocytes, has recently been described as a promising novel diagnostic marker of bacterial infection [22]. To the best of our knowledge, pleural fluid LBP concentrations have not been previously evaluated. We observed that pleural fluid LBP levels of less than 7  $\mu\text{g/mL}$  substantially reduced the probability of an infectious etiology (LR – 0.22). This may have implications, for instance, in the differential diagnosis of tuberculous and malignant effusions. Moreover, a LBP greater than 17  $\mu\text{g/mL}$  conferred an adjusted LR + of 4.6 in identifying non-purulent CPPE, a performance which was similar to the currently used tests, such as glucose or pH.

### ***Selecting tests***

The choice of one among different diagnostic tests depends not only on its accuracy, but also on the financial costs and technical considerations. The cost of one measurement for CRP, sTREM-1, PCT and LBP is about 1, 4, 14 and 6 euros, respectively. On the other hand, CRP and LBP are less labor-intensive than PCT and sTREM-1 measurements. Therefore, given the lack of utility of measuring PCT and the similarity in the accuracy of the other three tests, cost-effectiveness and technical reasons would argue for the diagnostic use of CRP, if a selection needs to be done.

### ***Study limitations***

This study should be interpreted in the context of certain limitations. First, the use of physician judgment as the gold standard for indicating the need for pleural drainage in CPPE may have resulted in patients' misclassifications, thus compromising the diagnostic accuracy of the pleural fluid tests. However, satisfactory correction for the bias introduced by an imperfect gold standard does not exist. Second, except for the identification of non-purulent CPPE, we have not compared the advantages of the new tests over alternative tests; that is, we have not answered the question of whether the new tests are better than the current diagnostic standard. Nevertheless, our primary goal was just to determine whether the new biomarkers accurately and reliably identified cases of obvious infectious pathology in a well-defined population. Whether these biomarkers offer additional information beyond that which is normally available is a matter of speculation. Finally, our findings, which are based on a retrospective analysis, deserve further prospective evaluation in an independent validation cohort.



## **CONCLUSIONS**

In summary, CRP, sTREM-1 and LBP measurements may be useful as an additional tool in the assessment of pleural effusions to support the differential diagnosis between infectious and non-infectious etiologies. However, in the most challenging clinical setting, such as the identification of a non-purulent PPE needing a tube thoracostomy, the accuracy of the three biomarkers is comparable with that of the classical pleural fluid biochemistries, such as pH, glucose or LDH. For this reason, we predict that even if these new tests are adopted, they will not have a major impact on clinical practice.

## REFERENCES

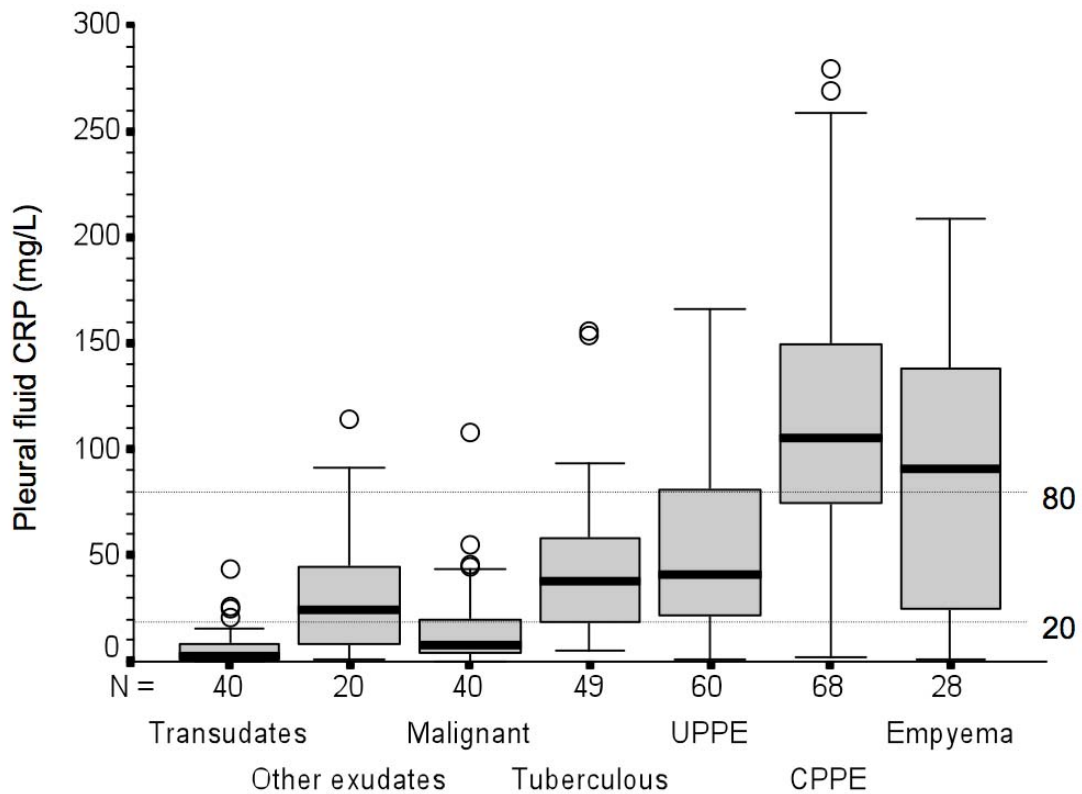
1. Christ-Crain M, Müller B. Biomarkers in respiratory tract infections: diagnostic guides to antibiotic prescription, prognostic markers and mediators. *Eur Respir J* 2007; 30: 556-573.
2. Rahman NM, Davies RJO. Effusions from infections: parapneumonic effusion and empyema. *In: Light RW, Gary Lee YC, eds. Textbook of Pleural Diseases. Hodder Arnold, London, 2008; pp. 341-366.*
3. Herzum I, Renz H. Inflammatory markers in SIRS, sepsis and septic shock. *Curr Med Chem* 2008; 15: 581-587.
4. Sharif O, Knapp S. From expression to signaling: roles of TREM-1 and TREM-2 in innate immunity and bacterial infection. *Immunobiology* 2008; 213: 701-713.
5. Yilmaz Turay U, Yildirim Z, Türköz Y, et al. Use of pleural fluid C-reactive protein in diagnosis of pleural effusions. *Respir Med* 2000; 94: 432-435.
6. García-Pachon E, Llorca I. Diagnostic value of C-reactive protein in exudative pleural effusions. *Eur J Intern Med* 2002; 13: 246-249.
7. Kiropoulos TS, Kostikas K, Oikonomidi S, et al. Acute phase markers for the differentiation of infectious and malignant pleural effusions. *Respir Med* 2007; 101: 910-918.
8. Chierakul N, Kanitsap A, Chaiprasert A, Viriyataveekul R. A simple C-reactive protein measurement for the differentiation between tuberculous and malignant pleural effusion. *Respirology* 2004; 9: 66-69.
9. Garcia-Pachon E, Soler MJ, Padilla-Navas I, Romero V, Shum C. C-reactive protein in lymphocytic pleural effusions: a diagnostic aid in tuberculous pleuritis. *Respiration* 2005; 72: 486-489.

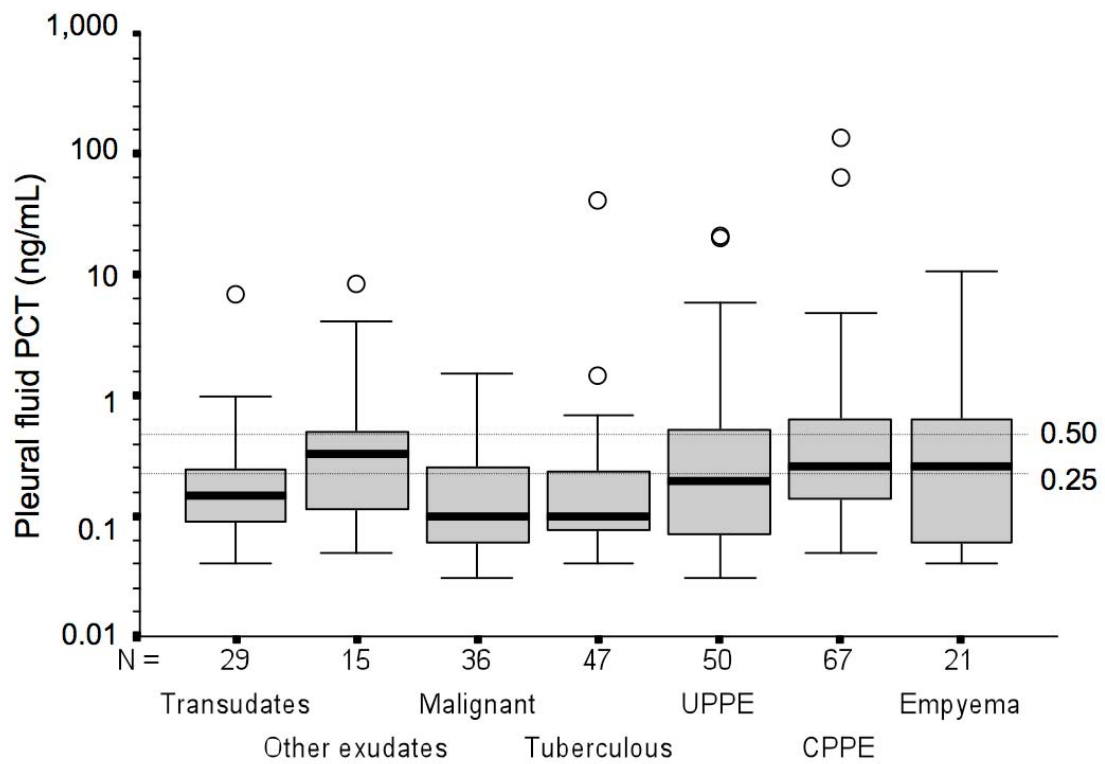
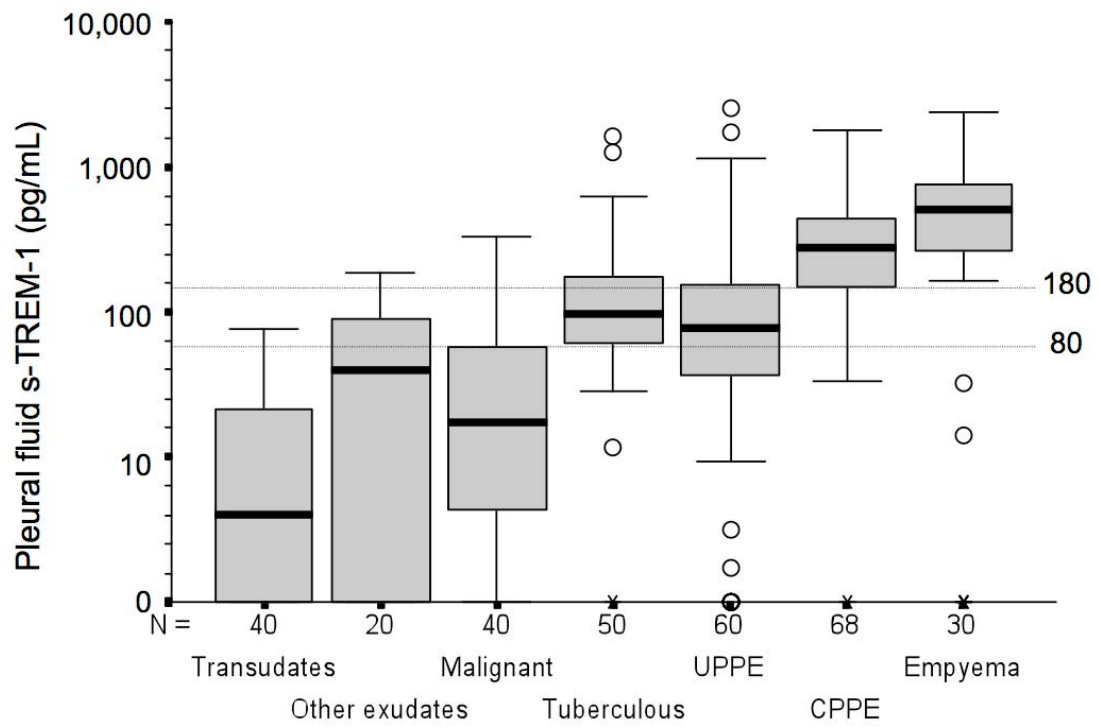
10. Daniil ZD, Zintzaras E, Kiropoulos T, et al. Discrimination of exudative pleural effusions based on multiple biological parameters. *Eur Respir J* 2007; 30: 957-964.
11. Chen SC, Chen W, Hsu WH, Yu YH, Shih CM. Role of pleural fluid C-reactive protein concentration in discriminating uncomplicated parapneumonic pleural effusions from complicated parapneumonic effusion and empyema. *Lung* 2006; 184: 141-145.
12. Porcel JM, Galindo C, Esquerda A, et al. Pleural fluid interleukin-8 and C-reactive protein for discriminating complicated non-purulent from uncomplicated parapneumonic effusions. *Respirology* 2008; 13: 58-62.
13. Liu CL, Hsieh WY, Wu CL, Kuo HT, Lu YT. Triggering receptor expressed on myeloid cells-1 in pleural effusions: a marker of inflammatory disease. *Respir Med* 2007; 101: 903-909.
14. Chan MC, Chang KM, Chao WC, et al. Evaluation of a new inflammatory molecule (triggering receptor expressed on myeloid cells-1) in the diagnosis of pleural effusion. *Respirology* 2007; 12: 333-338.
15. Kim JH, Park EY, Kim WH, et al. Soluble triggering receptor expressed on myeloid cells-1: role in the diagnosis of pleural effusions. *Tuberc Respir Dis* 2007; 62: 290-298.
16. Sim YS, Lee JH, Chun EM, Chang JH. Diagnostic utility of pleural fluid soluble triggering receptor expressed on myeloid cells 1 in patients with exudative pleural effusion. *Tuberc Respir Dis* 2007; 62: 499-505.
17. Huang LY, Shi HZ, Liang QL, Wu YB, Qin XJ, Chen YQ. Expression of soluble triggering receptor expression on myeloid cells-1 in pleural effusion. *Chin Med J (Engl)* 2008; 121: 1656-1661.

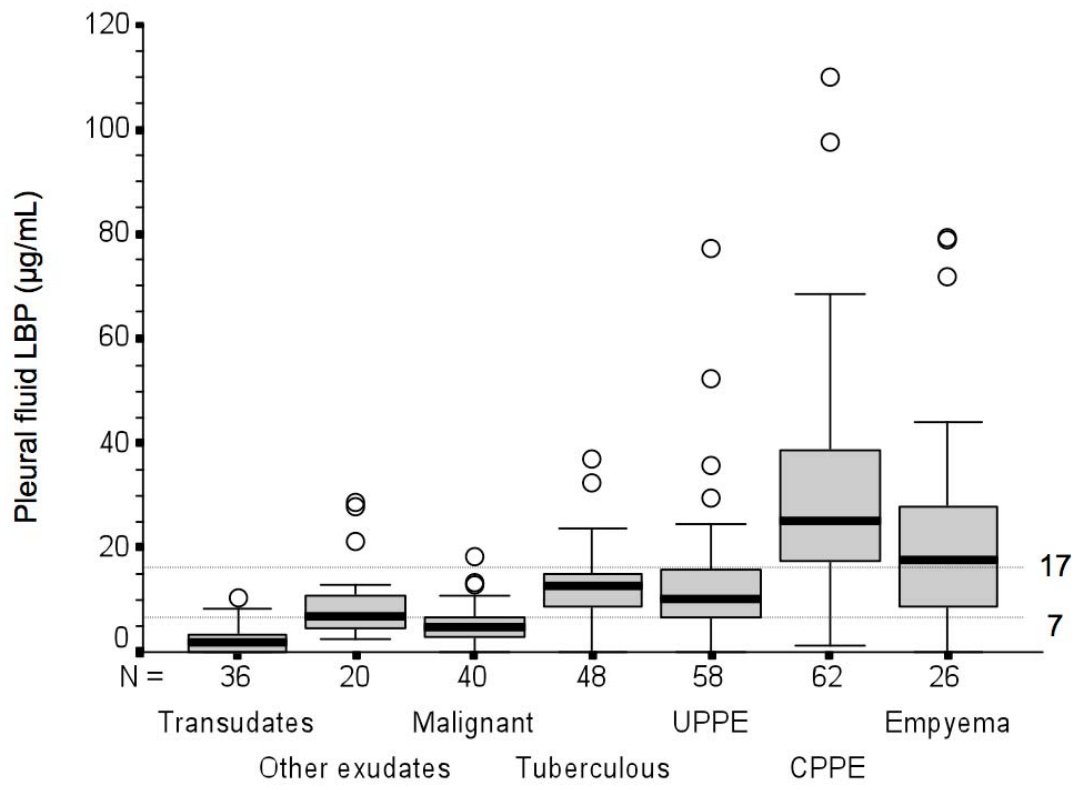
18. Bishara J, Goldberg E, Ashkenazi S, et al. Soluble triggering receptor expressed on myeloid cells-1 for diagnosing empyema. *Ann Thorac Surg* 2009; 87: 251-254.
19. Becker KL, Snider R, Nylén ES. Procalcitonin assay in systemic inflammation, infection, and sepsis: clinical utility and limitations. *Crit Care Med* 2008; 36: 941-952.
20. Topolcan O, Holubec L, Polivkova V, et al. Tumor markers in pleural effusions. *Anticancer Res* 2007; 27: 1921-1924.
21. Lin MC, Chen YC, Wu JT, Ko YC, Wang CC. Diagnostic and prognostic values of pleural fluid procalcitonin in parapneumonic pleural effusions. *Chest* 2009 Mar 2. [Epub ahead of print]
22. Gaïni S, Koldkjaer OG, Pedersen C, Pedersen SS. Procalcitonin, lipopolysaccharide-binding protein, interleukin-6 and C-reactive protein in community-acquired infections and sepsis: a prospective study. *Crit Care* 2006; 10: R53.

## FIGURE LEGENDS

**Figure 1.** Box plots of pleural fluid CRP (A), sTREM-1 (B), PCT (C) and LBP (D) concentrations in different etiologies of pleural effusion. Pleural sTREM-1 and PCT levels are plotted on a log scale. Horizontal dotted lines represent cutoff values with discriminatory properties.







**TABLE 1.** Demographics and pleural fluid biomarkers in patients (n=308)

|                                 | Transudates               | Malignant                   | Tuberculosis             | UPPE             | CPPE                        | Empyema                    | Miscellaneous exudates  | P#     |
|---------------------------------|---------------------------|-----------------------------|--------------------------|------------------|-----------------------------|----------------------------|-------------------------|--------|
| <b>Demographic data</b>         |                           |                             |                          |                  |                             |                            |                         |        |
| Subjects, n                     | 40                        | 40                          | 50                       | 60               | 68                          | 30                         | 20                      |        |
| Age, yrs                        | 78 (67-83)                | 72 (62-79)                  | 38 (27-52) <sup>  </sup> | 66 (43-78)       | 50 (40-64) <sup>  </sup>    | 53 (41-72) <sup>  </sup>   | 70 (43-79)              | <0.001 |
| Male gender (%)                 | 23 (57)                   | 17(42) <sup>  </sup>        | 38 (76) <sup>+</sup>     | 35 (58)          | 49 (72)                     | 22 (73)                    | 10 (50)                 | 0.009  |
| <b>Pleural fluid biomarkers</b> |                           |                             |                          |                  |                             |                            |                         |        |
| CRP, mg/L                       | 3 (1-9) <sup>  </sup>     | 8 (4-20) <sup>  </sup>      | 37 (19-59)               | 41 (21-82)       | 105 (74-150) <sup>+</sup>   | 91 (24-141) <sup>+</sup>   | 25 (9-47)               | <0.001 |
| sTREM-1, pg/mL                  | 4 (0-23) <sup>  </sup>    | 18 (4-58) <sup>  </sup>     | 96 (61-181)              | 77 (36-158)      | 279 (145-444) <sup>+</sup>  | 515 (251-785) <sup>+</sup> | 39 (0-95) <sup>  </sup> | <0.001 |
| PCT, ng/mL                      | 0.15 (0.08-0.24)          | 0.10 (0.05-0.25)            | 0.10 (0.07-0.26)         | 0.19 (0.07-0.51) | 0.26 (0.14-0.63)            | 0.19 (0.06-0.63)           | 0.33 (0.11-0.50)        | <0.001 |
| LBP, µg/mL                      | 1.8 (0-3.4) <sup>  </sup> | 4.8 (2.8-6.8) <sup>  </sup> | 12.5 (8.5-15.2)          | 10.3 (6.6-15.6)  | 25 (17.3-38.9) <sup>+</sup> | 17.7 (7.5-29)              | 7 (4.4-11.4)            | <0.001 |

Values are presented as medians (quartiles) unless otherwise stated

#Chi-square with *post-hoc* analysis of adjusted residuals (qualitative variables) or Kruskal-Wallis test with *post-hoc* Dunn test (quantitative variables)

<sup>||</sup>Significantly lower than the respective values in other groups by *post-hoc* test (p<0.05)

<sup>+</sup>Significantly higher than the respective values in other groups by *post-hoc* test (p<0.05)



CPPE: complicated parapneumonic effusion; CRP: C-reactive protein; LPB: lipopolysaccharide binding protein; PCT: procalcitonin; sTREM-1: soluble triggering receptor expressed on myeloid cells; UPPE: uncomplicated parapneumonic effusion

**TABLE 2.** Measures of diagnostic accuracy for pleural fluid tests that identify an infectious effusion\*

|                           | <b>Sensitivity, %<br/>(95% CI)</b> | <b>Specificity, %<br/>(95% CI)</b> | <b>LR +<br/>(95% CI)</b> | <b>LR –<br/>(95% CI)</b> | <b>AUC, %<br/>(95% CI)</b> |
|---------------------------|------------------------------------|------------------------------------|--------------------------|--------------------------|----------------------------|
| <b>CRP ≥ 20 mg/L</b>      | 83 (78-88)                         | 76 (67-85)                         | 3.5 (2.4-4.9)            | 0.22 (0.16-0.31)         | 0.87 (0.84-0.91)           |
| <b>sTREM-1 ≥ 80 pg/mL</b> | 71 (64-77)                         | 89 (82-96)                         | 6.4 (3.7-11.3)           | 0.33 (0.26-0.41)         | 0.86 (0.82-0.90)           |
| <b>PCT ≥ 0.25 ng/mL</b>   | 45 (38-53)                         | 72 (61-82)                         | 1.6 (1.1-2.3)            | 0.77 (0.63-0.92)         | 0.57 (0.50-0.64)           |
| <b>LBP ≥ 7 µg/mL</b>      | 83 (77-88)                         | 78 (69-87)                         | 3.8 (2.6-5.6)            | 0.22 (0.16-0.30)         | 0.87 (0.82-0.91)           |

\*Infectious pleural effusion refers to parapneumonic and tuberculous etiologies

AUC: area under curve; CI: confidence interval; CRP: C-reactive protein; LPB: lipopolysaccharide binding protein; LR +: likelihood ratio positive; LR -: likelihood ratio negative; PCT: procalcitonin; sTREM-1: soluble triggering receptor expressed on myeloid cells

**TABLE 3.** Diagnostic accuracy of individual pleural fluid tests for identifying tuberculous as compared with malignant effusions

|                           | <b>Sensitivity, %<br/>(95% CI)</b> | <b>Specificity, %<br/>(95% CI)</b> | <b>LR +<br/>(95% CI)</b> | <b>LR –<br/>(95% CI)</b> | <b>AUC, %<br/>(95% CI)</b> |
|---------------------------|------------------------------------|------------------------------------|--------------------------|--------------------------|----------------------------|
| <b>CRP ≥ 20 mg/L</b>      | 74 (61-87)                         | 77 (63-92)                         | 3.3 (1.8-6)              | 0.34 (0.20-0.55)         | 0.81 (0.72-0.90)           |
| <b>sTREM-1 ≥ 80 pg/mL</b> | 62 (47-76)                         | 85 (73-97)                         | 4.1 (1.9-8.9)            | 0.45 (0.31-0.65)         | 0.83 (0.74-0.92)           |
| <b>PCT ≥ 0.25 ng/mL</b>   | 25 (12-39)                         | 76 (60-91)                         | 1 (0.5-2.2)              | 0.98 (0.77-1.26)         | 0.52 (0.39-0.65)           |
| <b>LBP ≥ 7 µg/mL</b>      | 79 (67-92)                         | 77 (63-92)                         | 3.5 (1.9-6.4)            | 0.27 (0.15-0.48)         | 0.83 (0.74-0.92)           |

AUC: area under curve; CI: confidence interval; CRP: C-reactive protein; LPB: lipopolysaccharide binding protein; LR +: likelihood ratio positive; LR -: likelihood ratio negative; PCT: procalcitonin; sTREM-1: soluble triggering receptor expressed on myeloid cells

**TABLE 4.** Diagnostic accuracy of individual pleural fluid tests for identifying parapneumonic effusions

|                           | <b>Sensitivity, %<br/>(95% CI)</b> | <b>Specificity, %<br/>(95% CI)</b> | <b>LR +<br/>(95% CI)</b> | <b>LR –<br/>(95% CI)</b> | <b>AUC, %<br/>(95% CI)</b> |
|---------------------------|------------------------------------|------------------------------------|--------------------------|--------------------------|----------------------------|
| <b>CRP ≥ 80 mg/L</b>      | 49 (41-57)                         | 93 (89-98)                         | 7.4 (4-13.8)             | 0.54 (0.46-0.64)         | 0.83 (0.78-0.87)           |
| <b>sTREM-1 ≥ 80 pg/mL</b> | 73 (66-81)                         | 72 (64-79)                         | 2.6 (2-3.4)              | 0.37 (0.28-0.49)         | 0.79 (0.74-0.84)           |
| <b>PCT ≥ 0.25 ng/mL</b>   | 52 (43-60)                         | 73 (64-81)                         | 1.9 (1.4-2.6)            | 0.66 (0.54-0.81)         | 0.63 (0.56-0.70)           |
| <b>LBP ≥ 17 µg/mL</b>     | 49 (40-57)                         | 90 (84-95)                         | 4.7 (2.8-7.8)            | 0.57 (0.49-0.68)         | 0.80 (0.75-0.85)           |

AUC: area under curve; CI: confidence interval; CRP: C-reactive protein; LPB: lipopolysaccharide binding protein; LR +: likelihood ratio positive; LR -: likelihood ratio negative; PCT: procalcitonin; sTREM-1: soluble triggering receptor expressed on myeloid cells

**TABLE 5.** Operating characteristics of pleural fluid tests for distinguishing non-purulent CPPE from UPPE

|                            | <b>Sensitivity, %<br/>(95% CI)</b> | <b>Specificity, %<br/>(95% CI)</b> | <b>LR +<br/>(95% CI)</b> | <b>LR –<br/>(95% CI)</b> | <b>AUC, %<br/>(95% CI)</b> |
|----------------------------|------------------------------------|------------------------------------|--------------------------|--------------------------|----------------------------|
| <b>pH ≤ 7.20</b>           | 57 (44-70)                         | 93 (85-100)                        | 8.1 (3.1-21.5)           | 0.46 (0.34-0.62)         | 0.83 (0.75-0.91)           |
| <b>Glucose ≤ 60 mg/dL</b>  | 59 (46-71)                         | 90 (82-98)                         | 5.9 (2.7-12.9)           | 0.46 (0.34-0.62)         | 0.80 (0.72-0.88)           |
| <b>LDH ≥ 1,000 U/L*</b>    | 75 (63-86)                         | 82 (71-92)                         | 4.1 (2.3-7.1)            | 0.31 (0.20-0.48)         | 0.82 (0.75-0.90)           |
| <b>CRP ≥ 80 mg/L</b>       | 68 (56-79)                         | 75 (63-87)                         | 2.7 (1.7-4.3)            | 0.43 (0.30-0.63)         | 0.81 (0.63-0.88)           |
| <b>sTREM-1 ≥ 180 pg/mL</b> | 72 (61-83)                         | 82 (71-92)                         | 3.9 (2.3-6.8)            | 0.34 (0.23-0.51)         | 0.79 (0.70-0.86)           |
| <b>PCT ≥ 0.50 ng/mL</b>    | 33 (21-45)                         | 72 (59-86)                         | 1.2 (0.7-2.1)            | 0.93 (0.73-1.17)         | 0.59 (0.49-0.70)           |
| <b>LBP ≥ 17 µg/mL</b>      | 76 (64-87)                         | 81 (70-92)                         | 4 (2.3-6.9)              | 0.30 (0.19-0.47)         | 0.84 (0.76-0.91)           |

\*In our laboratory, the normal upper limit for serum LDH is 470 U/L

AUC: area under curve; CI: confidence interval; CRP: C-reactive protein; LDH: lactate dehydrogenase; LBP: lipopolysaccharide binding protein; LR +: likelihood ratio positive; LR -: likelihood ratio negative; PCT: procalcitonin; sTREM-1: soluble triggering receptor expressed on myeloid cells