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Acute phase markers for the differentiation of infectious and malignant pleural effusions

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Summary

Acute-phase markers, such as C-reactive protein (CRP), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α), have been studied in inflammatory and malignant disorders. We examined the diagnostic value of these markers for the differentiation among parapneumonic, tuberculous and malignant effusions.

We studied 124 patients with pleural effusions, classified as exudates [total ($n = 97$), parapneumonic ($n = 15$), tuberculous ($n = 25$), malignant ($n = 57$)] and transudates due to congestive heart failure ($n = 27$). CRP, IL-6 and TNF- α were measured in pleural fluid and serum.

Pleural fluid CRP was higher in parapneumonic compared to tuberculous and malignant effusions, providing 100% sensitivity for a cut-off point of 5.3 mg/dL. IL-6 was higher in both parapneumonic and tuberculous compared to malignant effusions. TNF- α was higher in tuberculous compared to malignant effusions, providing 96.0% sensitivity, and 93.0% specificity for a cut-off point of 88.1 pg/mL. Pleural fluid CRP levels were lower than serum in all groups, probably reflecting systemic inflammation, whereas IL-6 and TNF- α were higher in pleural fluid indicating local production.

Our data suggest that these markers may provide useful information for the differentiation of infectious and malignant effusions in clinical practice. However, further studies are needed for the validation of these findings in usual clinical circumstances.

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Introduction

The diagnosis and management of pleural effusions remains a clinical challenge bearing a significant cost both to the patients and the health care system.¹ In everyday clinical practice a variety of laboratory tests are in use for the differential diagnosis of pleural effusions; nevertheless, a significant proportion remain undiagnosed.¹ A major problem remains the differentiation between malignant and infectious effusions, due to their different outcome and management; thus, the need for markers that may help in this differentiation is imperative. Acute-phase proteins, such as C-reactive protein (CRP), have been implicated in various infectious inflammatory and advanced malignant states, and these proteins are regulated by proinflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α).²

C-reactive protein is currently widely used as a marker of inflammation and tissue injury.² CRP is synthesized in the liver in response to various stimuli³ and its production is enhanced by IL-6 and TNF- α .⁴ Increased serum CRP levels have been found in a number of pulmonary disorders, including bacterial infections, malignancies, and pulmonary thromboembolism.^{5,6} However, only a few studies have focused on the role of CRP in pleural effusions.⁷⁻⁹ IL-6 is the chief stimulator of the production of most acute-phase proteins and other cytokines, and regulates the synthesis of TNF- α and CRP.¹⁰ TNF- α is another proinflammatory cytokine with a cardinal role in various inflammatory responses.^{11,12} TNF- α is released by mesothelial cells in pleura and interacts with them leading to cytokine production,¹³ especially in infectious pleural effusions.¹⁴ Pleural fluid IL-6 and TNF- α levels have not been thoroughly studied and the results up to date are contradictory.¹⁵⁻¹⁸

The primary aim of this study was to assess the levels of three major acute phase response markers, i.e. CRP, IL-6 and TNF- α , in the pleural fluid and serum of patients with strictly characterized pleural effusions, in order to evaluate their usefulness in the differentiation among parapneumonic, tuberculous and malignant effusions. Additionally, we investigated the performance of these biomarkers for the differentiation between exudates and transudates.

Materials and methods

Study subjects

We studied prospectively 170 consecutive patients admitted with pleural effusions between September 2003 and February 2005. Forty six of the 170 patients that were initially evaluated were excluded from this study for the following reasons: diagnosis other than congestive heart failure, parapneumonic, tuberculous or malignant etiology was established; inability to establish a definite diagnosis; or presence of more than one plausible causes of pleural effusions. Empyemas and complicated parapneumonic effusions were additionally excluded from our study as in those cases the final diagnosis is easy to achieve and the natural history of these effusions is completely different from that of uncomplicated parapneumonic, tuberculous or malignant effusions. The study protocol was approved by the local

ethics committee and all subjects provided written informed consent.

Study design

Pleural fluid samples were obtained with the first successful thoracentesis before treatment, immediately after admission. Simultaneously, 10 mL of venous blood were obtained. Samples were analyzed for total and differential cell count, glucose, total protein and LDH. Additionally, cytologic examinations and cultures for common pathogens and *Mycobacterium tuberculosis* were routinely performed in all pleural fluid samples. Aliquots of pleural fluid and blood samples were immediately centrifuged at 1500g for 15 min at 4 °C and the supernatants were stored at -80 °C for CRP, IL-6 and TNF- α measurements.

Methods

The determination of the etiology of pleural effusions was based on widely accepted criteria, as previously described.¹⁹ Briefly, *parapneumonic effusions* were characterized by coexistence of pneumonia, response to antibiotics and/or pleural fluid neutrophilia (empyemas and complicated parapneumonic effusions that needed chest drainage were excluded); *malignant effusions* were diagnosed by cytologic or histologic examination; *tuberculous (TB) effusions* were diagnosed with the presence of positive stain or culture for *Mycobacterium tuberculosis* in the pleural fluid, sputum or pleural biopsy; or in the presence of typical caseating granulomas in pleural biopsy. In two of the patients without positive cultures or histopathological evidence, the diagnosis of tuberculous pleurisy was based on the following criteria: (i) adenosine deaminase levels in pleural fluid greater than 40 U/L;²⁰ (ii) exclusion of any other cause of pleural effusion; and (iii) response to antituberculous therapy. All *transudative effusions* were transudates in patients diagnosed with congestive heart failure (CHF). The differentiation between exudates and transudates was made by two experienced physicians (K.K. and K.I.G.) blinded to the results of the CRP, IL-6 and TNF- α measurements at the time of the diagnosis. The classification was based on Light's criteria, using serum and pleural fluid total protein and LDH measurements,²¹ and was further confirmed by the final diagnosis. Specifically, parapneumonic, tuberculous and malignant effusions were exudates, whereas effusions due to CHF were transudates.

CRP, IL-6 and TNF- α measurements

Pleural fluid and serum CRP measurements were performed by immunonephelometry with the Behring Nephelometer Analyzer II (BNII), using the N High Sensitivity kit (Dade Behring GmbH, Germany). The appropriate control and standard sera were provided by the same company, according to the manufacturer's instructions. IL-6 and TNF- α levels were measured with a commercially available enzyme-immunosorbent assay kit (Biosource Europe S.A.) according to the manufacturer's protocol. The lower limits of detection for IL-6 and TNF- α were 2 and 3 pg/mL, respectively.

Reproducibility of CRP, IL-6 and TNF- α measurements

The reproducibility of CRP, IL-6 and TNF- α measurements was assessed in two consecutive measurements on the same day in pleural fluid samples from a subgroup of 15 patients, including 4 patients with transudative, 3 patients with parapneumonic, 4 patients with tuberculous, and 4 patients with malignant effusions.

Statistical analysis

Data are presented as mean \pm standard deviation (SD) for data with normal distribution, and as median with interquartile ranges in parenthesis for skewed data. Normality of distribution was checked with Shapiro–Wilk's test. Comparisons between two different groups were performed using Mann–Whitney *U*-tests. Comparisons among more than two groups were performed using the non-parametric Kruskal–Wallis test. For the evaluation of the reproducibility of CRP, IL-6 and TNF- α in pleural effusions the method described by Bland and Altman²² was used. *P*-values <0.05 were considered statistically significant.

For the evaluation of the diagnostic performance of CRP, IL-6 and TNF- α levels as markers for the differential diagnosis between pleural effusions of different origin, receiver operator characteristics (ROC) analysis was performed for all significant differences between groups. ROC curves were generated by plotting the sensitivity against 1-specificity, and the area under the curve (AUC) with 95% confidence intervals (95% CI) was calculated. The optimum cut-off point from the ROC analysis was established by selecting the value that provides the greatest sum of sensitivity and specificity, i.e. the point closest to the upper left point of the ROC plot. For the optimum cut-off point provided by each ROC analysis, sensitivity, specificity, positive likelihood ratio (+LR), negative likelihood ratio

(–LR), positive predictive value (PPV), and negative predictive value (NPV) were calculated using standard formulas. Once these terms would be complementary for each pair of disorders, we refer to them regarding the first of the two disorders mentioned. For the calculation of the ROC curves and AUCs we have used the MedCalc version 6.15 software (MedCalc, Mariakerke, Belgium) and for the rest of the analyses the GraphPad Prism version 4.00 software (GraphPad Software, CA, USA).

Results

General characteristics of pleural effusions

The demographic data and the pleural fluid characteristics of the 124 patients that were included in the study are presented in Table 1. The levels of CRP, IL-6 and TNF- α in pleural fluid and serum of the study subjects are presented in Table 2 and in Fig. 1(A–C).

Pleural fluid levels of CRP, IL-6 and TNF- α in exudates

CRP measurements

Pleural fluid CRP levels were significantly higher in patients with parapneumonic compared to tuberculous ($P < 0.0001$) or malignant ($P = 0.001$) effusions. Additionally, pleural fluid CRP was higher in tuberculous compared to malignant effusions ($P = 0.02$) (Table 2, Fig. 1A). No significant differences were observed in CRP measurements between malignant effusions due to lung cancer and other malignant effusions.

The diagnostic performance from the ROC analysis of pleural fluid CRP values is presented in Table 3. Pleural fluid

Table 1 Demographic data and pleural fluid characteristics of the study population ($n = 124$).

	Transudates ($n = 27$)	Parapneumonic ($n = 15$)	Tuberculous ($n = 25$)	Malignant [§] ($n = 57$)
Age (years)	70 \pm 9	50 \pm 22	55 \pm 16.5	70 \pm 11
Gender (male/female)	21/6	11/4	17/8	39/18
Pleural fluid cells	858 \pm 405	10010 \pm 6006*	2767 \pm 1301*	1997 \pm 1419*
Pleural fluid lymphocytes (%)	50.4 \pm 21.4	12.8 \pm 6.7 [#]	77.1 \pm 13.0 [‡]	60.8 \pm 18.0 [‡]
Pleural fluid neutrophils (%)	13.2 \pm 9.0	73.8 \pm 18.6 [#]	11.5 \pm 6.7	17.3 \pm 11.9
Pleural fluid glucose (mg/dL)	126.3 \pm 32.5	49.2 \pm 48.1 [#]	90.7 \pm 22.1	111.8 \pm 41.8
Total protein pleural fluid (g/L)	2.1 \pm 0.7	5.0 \pm 0.6*	5.2 \pm 0.7*	4.5 \pm 0.9*
Total protein serum (g/L)	6.0 \pm 1.1	7.1 \pm 0.7	7.0 \pm 0.8	6.4 \pm 1.0
Total protein pleural fluid/serum ratio	0.34 \pm 0.1	0.71 \pm 0.1*	0.74 \pm 0.1*	0.70 \pm 0.1*
Pleural fluid LDH (U/L)	112 \pm 34	2563 \pm 2400*	555 \pm 274*	316 (224, 469)*
Serum LDH (U/L)	218 \pm 70	203 \pm 55	226 \pm 69	309 \pm 173
LDH pleural fluid/serum ratio	0.50 \pm 0.10	14.60 \pm 18.80*	2.60 \pm 1.50*	1.39 (0.87, 1.80)*

Data are presented as mean \pm SD for normally distributed data or median (interquartile ranges) for skewed data. PF: pleural fluid; PF/S: pleural fluid to serum ratio.

[§]Malignant effusions include effusions due to lung cancer ($n = 28$) and other malignant effusions ($n = 29$). Other malignancies include breast ($n = 7$), ovary ($n = 6$), renal cell carcinoma ($n = 1$), urinary ($n = 4$) and unknown origin ($n = 11$). All transudates are due to congestive heart failure.

*Statistically significant difference ($P < 0.05$) between exudates (parapneumonic, tuberculous or malignant effusions) vs. transudates.

[#]Statistically significant difference ($P < 0.05$) between parapneumonic vs. all other types of effusions.

[‡]Statistically significant difference ($P < 0.05$) between tuberculous or malignant effusions vs. parapneumonic or transudates.

Table 2 Levels of CRP, IL-6 and TNF- α in the pleural fluid and serum of patients with transudative or exudative pleural effusions.

	Transudates (n = 27)	Exudates (n = 97)	Parapneumonic (n = 15)	Tuberculous (n = 25)	Malignant (n = 57)
CRP (mg/dL)					
PF	0.84±0.76	2.9 (1.40, 7.35) *	12.25±5.32 ^{*,#,\$}	4.24±2.40 * [†]	1.9 (0.85, 3.60) *
Serum	2.22±1.61	7.2 (3.0, 15.8) *	24.17±9.30 ^{*,#,\$}	9.04±5.58 * [†]	3.7 (2.05, 11.70) *
PF/S	0.50±0.63	0.44 (0.28, 0.60)	0.53±0.23	0.53±0.24	0.41 (0.22, 0.60)
IL-6 (pg/mL)					
PF	814±594	14526±10881 *	22762±8124 ^{*,#,\$}	24268±8640 ^{*,†}	8085±7191 *
Serum	43.8±42.6	90.2 (49.6, 173.3) *	150.3±125.6 ^{*,#,\$}	133.8±99.3 ^{*,†}	72.1 (32.5, 181.5) *
PF/S	19.0 (12.1, 29.0)	114.3 (62.5, 203.6) *	225.9±167.8 ^{*,#,\$}	240.8±129.9 ^{*,†}	76.5 (40.2, 141.5) *
TNF-α (pg/mL)					
PF	18.8±11.8	53.9 (33.3, 129.0) *	90.2±52.8 ^{*,#,\$}	215.3±124.2 ^{*,†}	45.4±32.7 *
Serum	20.7±14.2	24.9 (18.1, 36.4) *	28.3±15.2 ^{*,#}	37.3±19.2 ^{*,†}	25.1±12.1 *
PF/S	1.04±0.6	2.2 (1.46, 4.56) *	3.6±3.1 ^{*,#,\$}	6.6±3.8 ^{*,†}	1.5 (1.2, 2.4) *

Data are presented as mean \pm SD for normally distributed data or median (interquartile ranges) for skewed data. PF: pleural fluid; PF/S: pleural fluid to serum ratio.

*: Statistically significant difference ($P < 0.05$) between exudates (parapneumonic, tuberculous or malignant effusions) and transudates.

#: Statistically significant difference ($P < 0.05$) between parapneumonic and tuberculous effusions.

§: Statistically significant difference between parapneumonic and malignant effusions.

†: Statistically significant difference ($P < 0.05$) between tuberculous and malignant effusions.

CRP proved a good marker for the differentiation of parapneumonic effusions from tuberculous and/or malignant effusions. Using a cut-off point of 5.3 mg/dL, CRP presented 100% sensitivity and 79.0% specificity for the diagnosis of parapneumonic vs. combined tuberculous and malignant effusions (Fig. 2A). Comparable findings were observed for the differentiation between parapneumonic and malignant effusions using a cut-off point of 5.0 mg/dL, whereas CRP values > 8.7 mg/dL presented 80.0% sensitivity but 100% specificity for the differentiation between parapneumonic and tuberculous effusions.

IL-6 measurements

Pleural fluid IL-6 levels were similar in tuberculous and parapneumonic effusions ($P = ns$) and significantly higher in those two groups compared to malignant effusions ($P < 0.0001$ for both comparisons). (Table 2, Fig. 1B) No significant differences were observed in IL-6 measurements between malignant effusions due to lung cancer and other malignant effusions.

The diagnostic performance from the ROC analysis of pleural fluid IL-6 values is presented in Table 4. Pleural fluid IL-6 proved a good marker for the differentiation of parapneumonic and/or tuberculous effusions from malignant effusions. Using a cut-off point of 13750 pg/mL, IL-6 presented 96.0% sensitivity and 80.7% specificity for the differentiation between tuberculous and malignant effusions. Additionally, using a cut-off point of 12680 pg/mL, IL-6 presented 94.9% sensitivity and 75.9% specificity for the differentiation of infectious (parapneumonic and tuberculous) vs. malignant effusions (Fig. 2B); similar findings were observed with the same cut-off point regarding the differentiation of parapneumonic and malignant effusions.

TNF- α measurements

Pleural fluid TNF- α levels were significantly higher in patients with tuberculous compared to malignant ($P < 0.0001$) and parapneumonic ($P = 0.0007$) effusions. Additionally, TNF- α levels were higher in parapneumonic compared to malignant effusions ($P = 0.0001$) (Table 2, Fig. 1C). No significant differences were observed in TNF- α measurements between malignant effusions due to lung cancer and other malignant effusions.

The diagnostic performance from the ROC analysis of pleural fluid TNF- α values is presented in Table 5. Pleural fluid TNF- α proved a good marker for the differentiation of tuberculous from malignant and parapneumonic effusions. Using a cut-off point of 88.1 pg/mL, TNF- α presented 96.0% sensitivity and 93.0% specificity for the differentiation between tuberculous and malignant effusions (Fig. 2C). Similar performance was observed for the differentiation of tuberculous vs. combined parapneumonic and malignant effusions.

Pleural fluid and serum levels of CRP, IL-6, and TNF- α

Pleural fluid CRP levels were lower than serum levels in all exudates ($P = 0.0002$ for parapneumonic, $P = 0.0003$ for tuberculous, and $P < 0.0001$ for malignant effusions, Table 2). Interestingly, the ratio of pleural fluid to serum CRP did not differ among the different groups ($P = ns$), presenting mean values that approximate 0.5 in all types of effusions. In contrast, pleural fluid levels of IL-6 and TNF- α were higher than serum levels (IL-6: $P < 0.0001$ for all types of effusions; TNF- α : $P = 0.0001$ for parapneumonic, and $P < 0.0001$ for tuberculous and malignant effusions; Table 2).

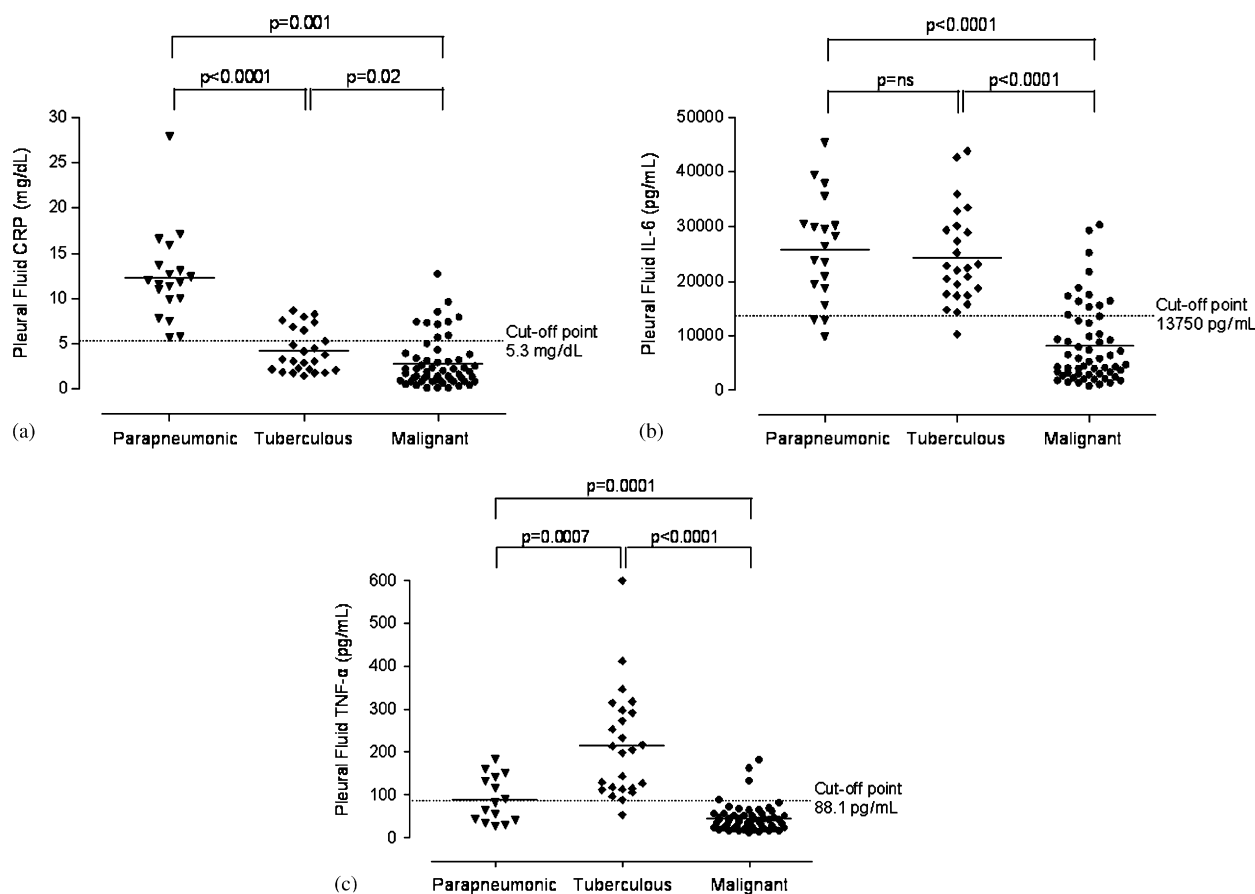


Figure 1 Acute phase markers in the pleural fluid of patients with parapneumonic ($n = 15$ ▲), tuberculous ($n = 25$ ◆), and malignant ($n = 57$ ●) effusions. Each symbol represents one individual and horizontal bars represent mean values. (A) Pleural fluid CRP levels. The dotted line represents the optimal cut-off point for the differentiation between parapneumonic vs. combined tuberculous and malignant effusions. (B) Pleural fluid IL-6 levels. The dotted line represents the optimal cut-off point for the differentiation between infectious (parapneumonic and tuberculous) vs. malignant effusions. (C) Pleural fluid TNF- α levels. The dotted line represents the optimal cut-off point for the differentiation between tuberculous vs. malignant effusions.

Table 3 Diagnostic performance of pleural fluid CRP for the differential diagnosis of infectious and malignant effusions at the optimal cut-off points of the ROC analysis curves.

	Optimal cut-off point (mg/dL)	Sensitivity (%)	Specificity (%)	+LR	-LR	PPV (%)	NPV (%)	AUC (95% CI)
PE vs. TB	> 8.7	80.0	100.0	> 20.00	0.20	100.0	89.3	0.955 (0.837–0.994)
PE vs. MAL	> 5.0	100.0	82.5	5.70	0.00	60.0	100.0	0.965 (0.892–0.993)
TB vs. MAL	> 1.4	100.0	45.6	1.84	0.00	44.6	100.0	0.719 (0.609–0.813)
PE vs. TB and MAL	> 5.3	100.0	79.0	4.76	0.00	46.9	100.0	0.961 (0.901–0.990)
TB vs. PE and MAL	> 1.4	100.0	36.1	1.57	0.00	35.2	100.0	0.579 (0.474–0.678)
PE and TB vs. MAL	> 1.7	97.5	41.9	1.92	0.05	57.4	96.6	0.811 (0.719–0.884)

PE, parapneumonic effusions; TB, tuberculosis; MAL, malignancy; +LR, positive likelihood Ratio; -LR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; CI: confidence intervals.

Differentiation between exudates and transudates

Pleural fluid CRP, IL-6 and TNF- α levels were significantly higher in patients with exudates compared to transudates (Table 2). However, in the ROC analyses, only IL-6 presented acceptable performance as a marker for the differentiation

between exudates and transudates, presenting 95.8% sensitivity, 92.6% specificity and AUC 0.983 (95% CI 0.942–0.997) in the ROC analysis for a cut-off point of 1375 pg/mL. Pleural fluid CRP and TNF- α were poor predictors for the differentiation between exudates and transudates (data not presented).

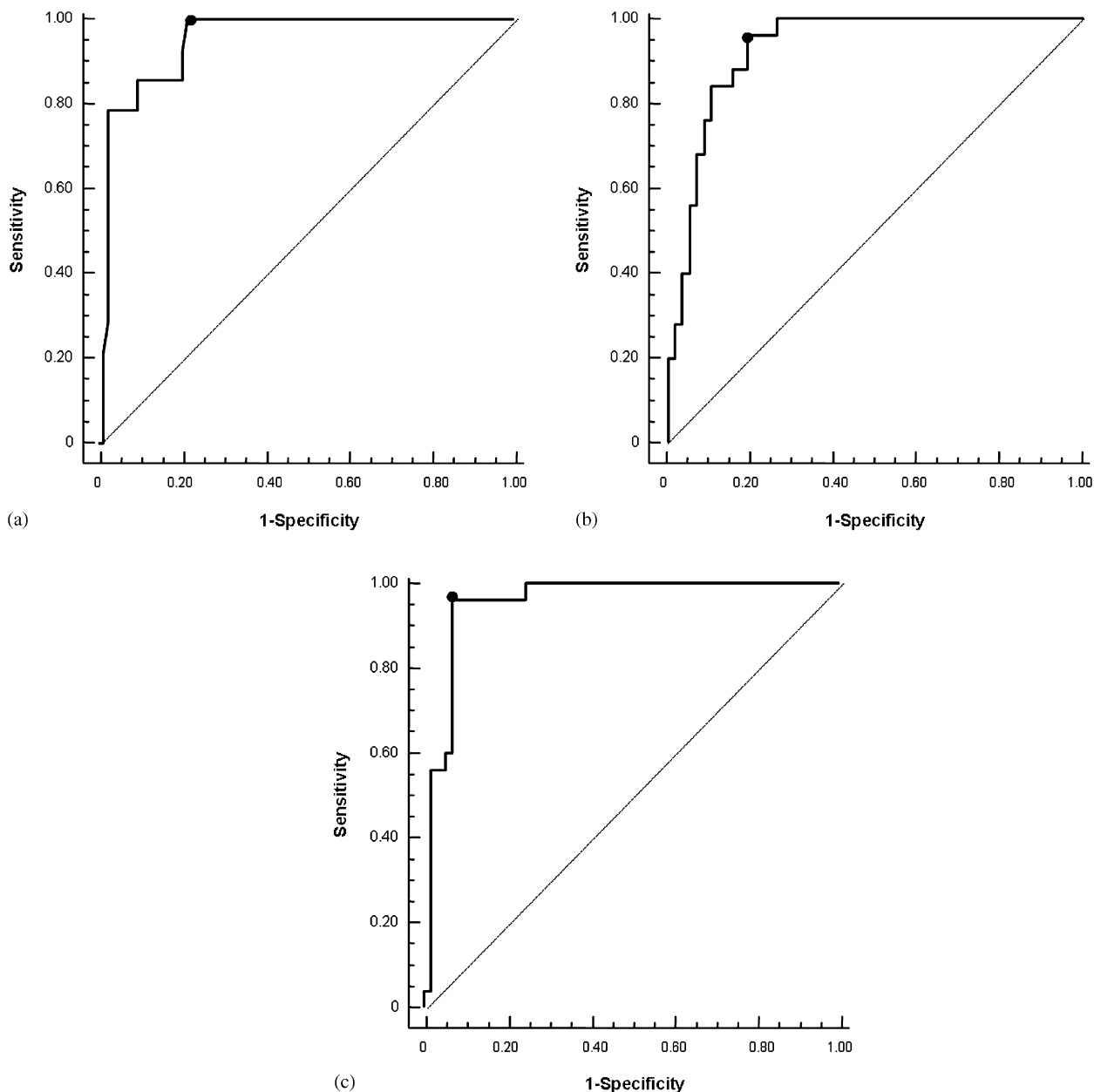


Figure 2 Receiver operator characteristic (ROC) analysis curves of acute phase markers in pleural fluid for the differentiation of infectious and malignant effusions. (A) ROC curve of CRP for the differentiation of parapneumonic vs. tuberculous and malignant effusions (optimal cut-off point ●, 5.3 mg/dL). (B) ROC curve of IL-6 for the differentiation of parapneumonic and tuberculous vs. malignant effusions (optimal cut-off point ●, 12,680 pg/mL). (C) ROC curve of TNF- α for the differentiation of tuberculous vs. malignant effusions (optimal cut-off point ●, 88.1 pg/mL).

Reproducibility of acute phase markers in pleural fluid

The measurements of acute phase markers in pleural fluid samples were highly reproducible. CRP levels on two consecutive measurements were 5.92 ± 5.17 mg/dL and 5.83 ± 5.01 mg/dL, respectively. The mean difference with limits of agreement was 0.09 ± 0.90 (mean ± 2 sd) and all values were within the limits of agreement in the Bland and Altman plot.

IL-6 levels on two consecutive measurements were 12.502 ± 10.873 and 12.339 ± 10.831 pg/mL, respectively. The mean difference with limits of agreement was 163 ± 642 (mean ± 2 sd) and all values were within the limits of agreement in the Bland and Altman plot.

TNF- α levels on two consecutive measurements were 84.9 ± 99.8 and 84.7 ± 99.4 pg/mL, respectively. The mean difference with limits of agreement was 0.25 ± 3.87 (mean ± 2 sd) and all values were within the limits of agreement in the Bland and Altman plot.

Table 4 Diagnostic performance of pleural fluid IL-6 for the differential diagnosis of infectious and malignant effusions at the optimal cut-off points of the ROC analysis curves.

	Optimal cut-off point (pg/mL)	Sensitivity (%)	Specificity (%)	+LR	−LR	PPV (%)	NPV (%)	AUC (95% CI)
PE vs. TB	> 12949	20.0	96.0	5.00	0.83	75.0	66.7	0.531 (0.367–0.690)
PE vs. MAL	> 12680	93.3	77.2	4.09	0.09	51.9	97.8	0.910 (0.819–0.964)
TB vs. MAL	> 13750	96.0	80.7	4.97	0.05	68.6	97.9	0.930 (0.851–0.974)
PE vs. TB and MAL	> 9313	100.0	48.8	1.95	0.00	26.3	100.0	0.776 (0.680–0.854)
TB vs. PE and MAL	> 17215	87.5	76.7	3.76	0.16	55.3	94.9	0.852 (0.766–0.916)
PE and TB vs. MAL	> 12680	94.9	75.9	3.93	0.07	72.5	95.7	0.919 (0.845–0.964)

PE, parapneumonic effusions; TB, tuberculosis; MAL, malignancy; +LR, positive likelihood ratio; −LR, negative likelihood ratio; PPV, positive predictive value; NPV, negative predictive value; AUC, area under the curve; CI: confidence intervals.

Table 5 Diagnostic performance of pleural fluid TNF- α for the differential diagnosis of infectious and malignant effusions at the optimal cut-off points of the ROC analysis curves.

	Optimal cut-off point (pg/mL)	Sensitivity (%)	Specificity (%)	+LR	−LR	PPV (%)	NPV (%)	AUC (95% CI)
PE vs. TB	> 89.8	66.7	92.0	8.33	0.36	83.3	82.1	0.856 (0.709–0.946)
PE vs. MAL	> 43.9	68.7	62.5	1.83	0.50	34.4	87.5	0.701 (0.581–0.803)
TB vs. MAL	> 88.1	96.0	93.0	13.68	0.04	85.7	98.1	0.954 (0.883–0.987)
PE vs. TB and MAL	> 27.1	100.0	22.0	1.28	0.00	19.0	100.0	0.556 (0.452–0.657)
TB vs. PE and MAL	> 88.1	92.3	85.9	6.55	0.09	70.6	96.8	0.920 (0.847–0.965)
PE and TB vs. MAL	> 88.1	75.0	93.0	10.69	0.27	88.2	84.1	0.872 (0.789–0.931)

PE, Parapneumonic effusions; TB, tuberculosis; MAL, malignancy; +LR, positive likelihood ratio; −LR, negative likelihood ratio; PPV, positive predictive value; NPV, Negative Predictive value; AUC, area under the curve; CI: confidence intervals.

Discussion

In this prospective study we have validated the diagnostic performance of three acute phase response markers, i.e. CRP, IL-6 and TNF- α , in the serum and pleural fluid of a well-characterized population of patients with infectious and malignant pleural effusions. The main observation of this study is that these acute phase response markers may be helpful for the differentiation between infectious and malignant pleural effusions. Specifically, pleural fluid CRP is increased in parapneumonic effusions compared to tuberculous and malignant effusions, pleural fluid IL-6 is increased in both tuberculous and parapneumonic effusions compared to malignancy, and pleural fluid TNF- α may represent a good marker for the differentiation between tuberculous and malignant effusions. Additionally, pleural fluid IL-6 presented acceptable performance for the differentiation between exudates and transudates, comparable with that of Light's criteria.²¹ Finally, the acute phase markers measurements were highly reproducible in the pleural fluid.

The findings of the present study may further support a pathophysiological mechanism of the acute phase response during the formation of infectious and malignant effusions. Pleural fluid CRP levels are likely to reflect serum levels, as they were significantly lower compared to serum levels in all study groups; in contrast, IL-6 and TNF- α were higher in pleural fluid than serum in all groups of exudates. These findings may suggest that a local inflammatory process leads

to production of IL-6 and TNF- α in the pleural cavity, and the consequent systemic inflammatory response is expressed by the serum and pleural fluid levels of CRP. Based on these findings we may hypothesize that the major proportion of CRP is not produced in the pleural cavity and its presence in pleural fluid may be the result of diffusion from the blood. This is supported by previous reports suggesting that CRP is produced mainly in the liver and may arrive in the pleural space from plasma.^{2,7} This theory is further supported by the fact that the ratio of pleural fluid to serum CRP did not differ among study groups, being around 0.5 in mean values. This observation is in agreement with the findings of a previous paper by Castano-Vidriales and Amores Antequera⁷ with the exception of a lower mean value of pleural fluid to serum CRP ratio in transudates reported by these authors (0.26 vs. 0.50 in our study). The results of both studies, especially in exudative effusions, suggest that pleural fluid CRP is likely to reflect systemic inflammation that may have been induced by the local production of IL-6 and TNF- α in the pleural cavity. An additional implication of our findings is that serum CRP levels may be of comparable value to pleural fluid levels for the differentiation between parapneumonic vs. tuberculous and/or malignant effusions, and this is in accordance with our findings (data not shown in detail).

An additional finding of our study is that pleural fluid CRP levels were higher in parapneumonic compared to tuberculous and malignant effusions. This may be attributed to the fact that CRP plays an important role in inflammation, as it increases profoundly in the region of inflammation.²³

The more intense inflammation of parapneumonic compared to tuberculous or malignant effusions may account for this difference. This finding may provide an additional marker for the diagnosis of parapneumonic effusions, as high levels of pleural fluid CRP present excellent sensitivity and NPV, with acceptable specificity. Additionally, CRP was higher in tuberculous compared to malignant effusions and this finding is consistent with other reports.^{9,24,25} This has been attributed either to local production of CRP in the pleural space that is induced by IL-6,^{24,26} or to leakage of plasma CRP via the inflamed pleura.²³ Our data support the second mechanism, as CRP levels were constantly higher in serum compared to pleural fluid and no correlation was found between pleural fluid IL-6 and CRP (data not shown).

Elevated IL-6 levels have been found in various inflammatory, infectious and malignant disorders.¹⁸ In this study pleural fluid IL-6 levels were significantly higher in infectious (i.e. tuberculous and parapneumonic) compared to malignant effusions. A previous study reported high IL-6 levels in tuberculous and low IL-6 levels in malignant effusions.¹⁵ The same study reported lower levels of IL-6 in parapneumonic compared to tuberculous effusions,¹⁵ and this finding is consistent with the results of a recent study by Lin et al.²⁷ and in disagreement with our findings. However, the study by Lin et al. included complicated parapneumonic effusions and empyemas that presented lower IL-6 levels, probably accounting for the lower IL-6 levels in the whole parapneumonic group.²⁷ Empyemas and complicated parapneumonic effusions were carefully excluded from our study due to their different natural history and their obvious differentiation from tuberculous and malignant effusions, and this may account for the observed higher IL-6 levels in our parapneumonic effusions.

In ROC analysis IL-6 provided good sensitivity and NPV for the differentiation of tuberculous vs. malignant effusions (cut-off level 13,750 pg/mL) and for the differentiation of infectious (i.e. combined parapneumonic and tuberculous) vs. malignant effusions (cut-off level 12,680 pg/mL). Indeed, only in 1 of 25 patients in the tuberculous group IL-6 was lower than 13,750 pg/mL, whereas only in 2 of 40 patients with infectious diseases IL-6 was lower than 12,680 pg/mL. Thus, increased IL-6 is indicative of the presence of infectious effusions; however the low specificity of the method suggests that it is not an optimal marker to be used alone in clinical practice.

Pleural fluid TNF- α levels were higher in tuberculous compared to parapneumonic and malignant effusions. The higher TNF- α levels in tuberculous effusions may be part of a complex immune response in the pleural space, as TNF- α has both protective and deleterious role in tuberculosis.²⁸ Mycobacterial products are potent stimuli for TNF- α production by monocytes,²⁹ providing a plausible explanation for the high levels of TNF- α in tuberculous effusions. Our results are in accordance with other studies,^{30,31} but they are in discrepancy with data reported by Xirouchaki et al.¹⁵ and Gursel et al.³² However, these studies used different methodology and smaller study populations. In the present study we included a strictly characterized population and utilized ROC analysis to demonstrate that pleural fluid TNF- α may be a good marker for the discrimination between tuberculous and malignant effusions. At an optimal cut-off level of >88.1 pg/mL, which has not previously been

evaluated in the diagnostic assessment of pleural effusions, none of the 25 tuberculous effusions was falsely characterized as malignant and only 3 of 57 malignant effusions were misclassified as tuberculous, providing great sensitivity, specificity, NPV and PPV. The gold standard for the diagnosis of malignant effusions still remains the cytologic and/or histologic confirmation; nevertheless, when available, TNF- α may prove useful in clinical practice.

In summary, in this prospective study of acute phase markers in pleural effusions in a strictly characterized population, we report that pleural fluid CRP levels >5.3 mg/dL may have diagnostic utility for the differentiation of parapneumonic vs. tuberculous and malignant effusions, IL-6 is increased in both tuberculous and parapneumonic effusions and may be used to differentiate them from malignancy, and TNF- α levels >88.1 pg/mL may be useful for the differentiation between tuberculous and malignant effusions. From a pathophysiologic point of view, pleural fluid CRP levels seem to reflect the extent of systemic inflammation, whereas IL-6 and TNF- α express the local inflammatory response. These pilot data suggest that these markers combined with clinical and routine laboratory data may provide useful aids for the differentiation of infectious and malignant pleural effusions in everyday clinical practice.

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