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A Novel Combination of Calprotectin and CXCL12 for Predicting Malignancy in Patients with Exudative Pleural Effusion

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Abstract: Pleural effusion (PE) remains a significant challenge and public health problem, which needs novel noninvasive biomarkers for the precise diagnosis. The aim of this study was to further determine the clinical efficacy and diagnostic accuracy of a novel combination of calprotectin and CXCL12 for predicting malignancy in patients with exudative PE.

Calprotectin and CXCL12 concentrations were measured in 95 individuals of exudative PE, with 39 malignant PE (MPE) and 56 benign PE (BPE). The accuracy of calprotectin and CXCL12 levels for discriminating MPE from BPE or tuberculous PE were evaluated using receiver-operating characteristic (ROC) curves. Univariate and multivariate logistic regression analyses were performed to test the association between calprotectin and CXCL12 levels and MPE.

Calprotectin and CXCL12 levels of patients with MPE were significantly lower than that of BPE and tuberculous PE ($P < 0.05$). The area under the curve (AUC) of calprotectin and CXCL12 was 0.683 and 0.641 in MPE and BPE, and a combination of calprotectin ≤500.19 ng/mL and CXCL12 ≤6.11 ng/mL rendered a sensitivity and specificity of 48.72% and 78.57%, respectively. While in MPE and tuberculous PE, the AUC of calprotectin and CXCL12 was 0.696 and 0.690, and a combination of calprotectin ≤421.73 ng/mL and CXCL12 ≤3.71 ng/mL presented a sensitivity and specificity of 25.64% and 95.45%, respectively. Multivariate logistic regression demonstrated that both calprotectin and CXCL12 were independent predictors of MPE.

Calprotectin and CXCL12 in pleural fluid are informative diagnostic biomarkers for predicting patients with MPE.

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Abbreviations: ADA = adenosine deaminase, anti-TB = anti-tuberculosis, AUC = area under the curve, BPE = benign pleural effusion, CEA = carcinoembryonic antigen, CI = confidence interval, CTPA = computed tomographic pulmonary angiography, ELISA = enzyme-linked immunosorbent assay, GLU = glucose, HIV = human immunodeficiency virus, LDH = lactate dehydrogenase, LR− = negative likelihood ratio, LR+ = positive likelihood ratio, MAPK = mitogen-activated protein kinase, MPE = malignant pleural effusion, NPVs = negative predictive values, NSCLC = nonsmall cell lung cancer, OR = odds ratio, PE = pleural effusion, PPVs = positive predictive values, ROC = receiver-operating characteristic, SCLC = small cell lung cancer, SD = standard derivation, SDF-1α = stromal cell-derived factor-1α, TST = tuberculin skin test.

INTRODUCTION

Pleural effusion (PE) remains a significant challenge and public health problem, which can occur as a consequence of more than 50 recognized etiologies. It can be divided into benign pleural effusion (BPE) and malignant pleural effusion (MPE). MPE is a cause of significant symptoms and distress in patients with end-stage malignancies,[1] which can be observed as a complication of nearly all malignancies, with more than 75% are lung, breast, ovary cancer, or lymphomas,[2,3] and is associated with a poor prognosis, with a median survival of 4 to 6 months, regardless of the underlying cancer cell type.[4]

Thoracentesis, closed pleural cytology, and thoracoscopy are commonly used techniques in establishing MPE diagnosis.[5] Thoracentesis is rendered as the initial diagnostic approach when MPE is suspected. It facilitates the distinction of exudative from transudative effusion by the fluid analysis, and further helps to undergo cytological, microbiological, and biochemical examinations.[6] Although cytological analysis of the pleural fluid exhibit a diagnostic yield, ranging from 60% to 90%, more invasive techniques are often warranted due to its inconsistent outcome reports in different diseases.[7,8] Closed pleural biopsy is usually conducted when a suspected MPE cannot be diagnosed by pleural cytology, but the reported sensitivity range widely varying between 7% and 72% due to the influence from the variation in procedures performed.[9,10] Preceding the thoracic surgery, thoracoscopy may be required when the etiology still remains unknown, because of the high sensitivity and specificity (90% and 100%, respectively).[11,12] However, this procedure requires general anesthesia and can induce pneumothorax, which may be difficult to tolerate for patients with impaired lung function and may not be available at all facilities.

To strengthen the evidence of MPE, pleural fluid biomarkers for carcinoma are sometimes identified. Carcinoembryonic antigen (CEA) is a typical tumor marker, which was reported to have a diagnostic accuracy of 83% in the pleural fluid.[13] Recently, many studies have investigated some novel

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biomarkers of cancers in pleural fluid for discriminating between MPE and BPE, but there is still a lack of candidate diagnostic markers of MPE with sufficient sensitivity and specificity,[6] which drove us to find and test novel biomarkers. Calprotectin and CXCL12 are both inflammatory markers, which is a noncovalent heterodimer formed by the calcium-related proteins S100-A8 and S100-A9 and a CXC chemokine which has chemotactic activity for broad range of hematopoietic cell linage, respectively,[14–16] but recent studies demonstrated their novel potential as tumor markers and potential treatment targets,[17,18] which appeal us to evaluate the efficacy of these 2 novel markers in the distinction of MPE from BPE.

Calprotectin performs significant roles in the cancer evolutions. Hermani et al and Cross et al showed that S100A8 and S100A9 could regulate cell proliferation, which was further demonstrated by Mulligan et al that up-regulation of S100A9 in breast cancer cells could lead to reduced tumor cell proliferation.[19–21] Rafii and Lyden[22] illuminated that S100A8 and S100A9 could act as chemoattractants to facilitate the homing of tumor cells to premetastatic sites as was expressed by the primary tumors in the myeloid and endothelial cells within the lung preceding the tumor metastasis. S100A8 and S100A9 were also found to accelerate the motility of circulating cancer cells by p38 mitogen-activated protein kinase (MAPK)-mediated activation of tumor cell pseudopodia.[23] So as CXCL12, also known as stromal cell-derived factor-1α (SDF-1α), which was found to increase proliferation, cell adhesion, motility, and change morphology in small cell lung cancers.[18] In summary, it is paramount to detect calprotectin and CXCL12 because they could be somehow targeted to prevent and inhibit the metastasis of tumors.

However, to our knowledge, studies that evaluate the roles of calprotectin and CXCL12 in differentiating MPE are still scarce, and the clinical significance of these 2 biomarkers in MPE has never been elucidated. Therefore, on the foundation of studies by Sánchez-Otero et al[24] and Kohmo et al,[25] we further identified the clinical efficacy and diagnostic accuracy of the combination of calprotectin and CXCL12 for predicting malignancy in Chinese patients with exudative PE.

MATERIALS AND METHODS

Study Population

From April to October 2013, pleural fluid of 124 consecutive patients with PE, who were admitted to the Department of Respiratory and Critical Care Medicine, West China Hospital of Medicine, Sichuan University, were collected. Finally, a total of 95 consecutive nonselected patients with a specific diagnosis for exudative PE, as well as an established diagnosis for the etiologies of the PE, were enrolled in this prospective study. The study protocol had been approved by the Institutional Ethical Committee for Clinical and Biomedical Research of West China Hospital (Sichuan, China), and all participants provided written informed consent.

Inclusion and Exclusion Criteria

Patients with PE observed via X-ray or chest computerized tomography were eligible for inclusion. The predefined exclusion criteria were as follows: use of antituberculous treatment for more than 3 days; use of immunosuppression drugs or chemotherapy for carcinoma; and patients with human immunodeficiency virus (HIV), diabetes mellitus, respiratory failure, autoimmune disease, or severe cardiac, hepatic, or renal dysfunction.

Diagnosis Criteria for Pleural Fluid

In accordance with the PE diagnosis protocol released by British Thoracic Society, we initiated the first thoracentesis for common biochemical, microbiological, and cytological analysis in patients with PE identified by X-ray or chest computed tomography.[6] Exudative and transudative effusions were established based on the ''light'' criteria, that is the ratio of pleural fluid to serum lactate dehydrogenase (LDH) >0.6; pleural fluid LDH >1/3 of the upper limit of the normal serum LDH value; or ratio of pleural fluid to serum protein >0.5.[26] If the initial pleural fluid cytological study did not differentiate the etiology, a second thoracentesis and/or a percutaneous pleural (lung) biopsy or thoracoscopy was further conducted. Depending on the diagnostic suspicion and clinical condition, complementary tests were also recommended, such as fibrobronchoscopy, tuberculin skin test (TST), computed tomographic pulmonary angiography (CTPA), or immunologic tests. The causes of PE in all enrolled patients were identified by the aforementioned methods.

We divided the enrolled patients into 2 principal groups: MPE and BPE. MPE was defined as the presence of malignant cells in the pleural fluid or pleural tissues upon cytological or histological examination. According to the sites of origin, patients with MPE were further divided into 2 subgroups: lung carcinoma and metastatic lung carcinoma. Lung carcinoma was then divided into nonsmall cell lung cancer (NSCLC) and small cell lung cancer (SCLC), according to the histopathological types. Patients with BPE consisted of tuberculous PE, parapneumonic PE, and PE secondary to pulmonary thromboembolism or pulmonary paragonimiasis. The diagnosis of tuberculous PE was established when one of the following 3 criteria was achieved: *Mycobacterium tuberculosis* was identified based on the stain or culture of pleural fluid, sputum, or pleural biopsy; typical caseating granuloma was found in the pleural tissue; or a remarkable response to antituberculosis (anti-TB) treatment was presented. PE associated with pneumonia and response to antibiotics was classified as parapneumonic effusion.

Pleural Effusion Sampling and Biochemical Procedures

Pleural fluid samples (10 mL) were obtained individually, centrifuged at 3000 revolutions per minute (rpm) for 10 minutes, and immediately frozen in 2-mL aliquots at −80°C.

Calprotectin and CXCL12 were independently measured by Detection Center of USCN Life Science Inc. Wu Han, via enzyme-linked immunosorbent assay (ELISA) with SEK504Hu kit and SEA122Hu kit, respectively. The laboratory test of these 2 biomarkers was conducted completely separately from clinical diagnosis and treatment. The assay was conducted according to the manufacturer's guidelines.

Statistical Analysis

Normality and homogeneity of variances were tested by the Shapiro–Wilk and Levene tests, respectively. Receiver-operating characteristic (ROC) curves and area under the curve (AUC) were calculated to evaluate the accuracy of calprotectin and CXCL12 levels for discriminating MPE from BPE or tuberculous pleural effusions. Cut-off points were calculated as the point when Youden index (sensitivity + specificity-1) reached the maximum. On the basis of the cut-off points, we calculated the sensitivity, specificity, positive predictive values

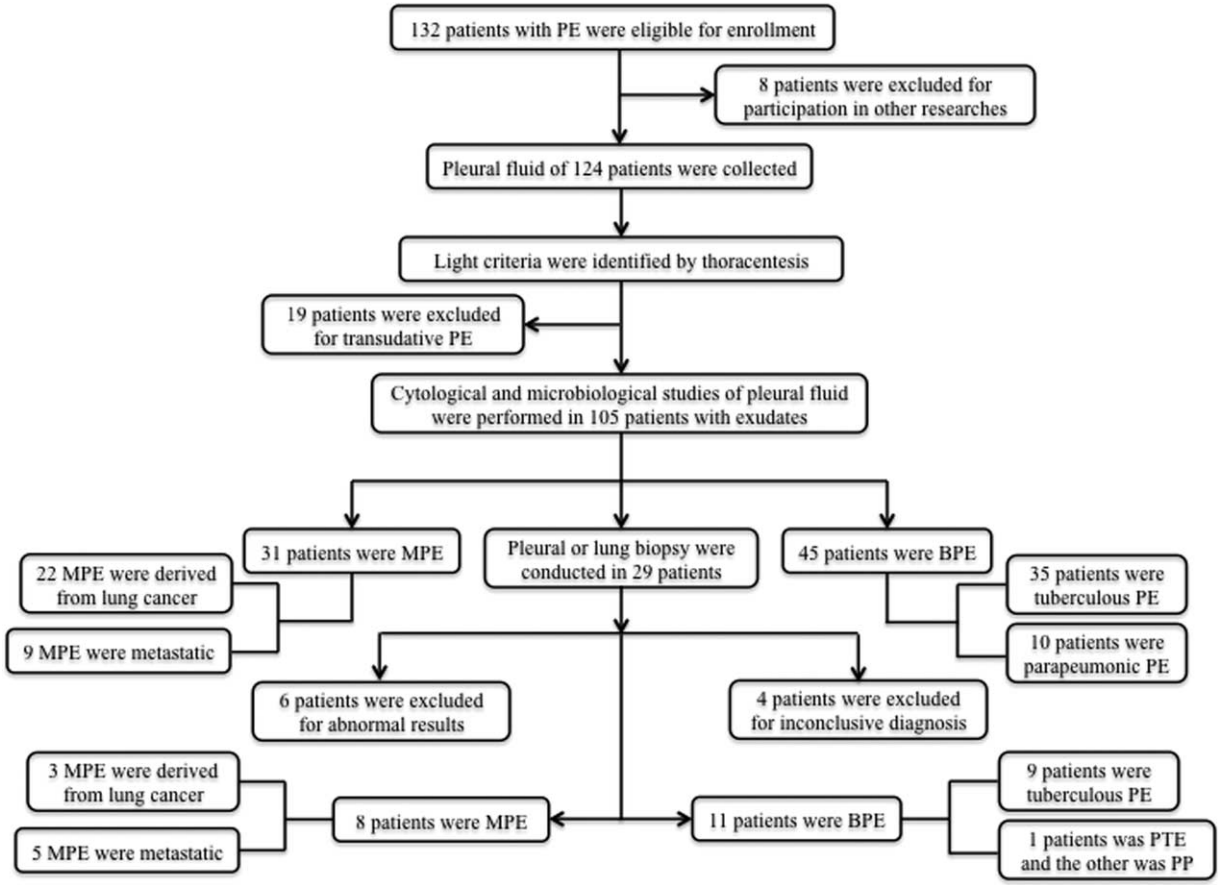

FIGURE 1. Study flow chart of enrolling patients. BPE = benign pleural effusion, MPE = malignant pleural effusion, PE = pleural effusion, PP = pulmonary paragonimiasis, PTE = pulmonary thromboembolism.

(PPVs), negative predictive values (NPVs), as well as likelihood ratios of calprotectin and CXCL12 in different subgroups. Univariate logistic regression with unadjusted odds ratio (OR) and 95% confidence intervals (CI) was performed to test the prediction of calprotectin and CXCL12 for MPE, and significant predictors with $P < 0.2$ in the univariate analysis were then analyzed by multivariate logistic regression to assess the independence of calprotectin and CXCL12 levels in predicting MPE.

Data analysis was performed using the SPSS Statistics version 16.0 (Copyright (c) SPSS Inc., 1989–2007). For normal distribution data, results of continuous variables were compared by independent-samples *t* test, whereas categorical variables were compared by chi-square test. For non-normal distribution data, results were compared by Mann–Whitney *U* test. A *P* value <0.05 was considered statistically significant.

RESULTS

Patient Characteristics

A total of 95 patients, with 39 patients in the MPE group (41.05%) and 56 patients in the BPE group (58.95%), were eventually enrolled in our study (Fig. 1). The MPE group consisted of 25 patients (64.10%) with lung cancer (21 NSCLC and 4 SCLC) and 14 patients (35.90%) with metastatic lung cancer. The patients with BPE were secondary to TB (n = 44, 78.57%), pneumonia (n = 10, 17.85%), pulmonary thromboembolism (n = 1, 1.79%), and pulmonary paragonimiasis (n = 1, 1.79%).

Baseline characteristics of the patients in different groups were summarized in Table 1. The mean ± standard derivation (SD) age was 61.00 ± 13.19 years in patients with MPE and 47.09 ± 20.67 years in patients with BPE. The difference was significant ($p = 0.012$), indicating that carcinoma tends to occur in older people. In pleural fluid, the protein and adenosine deaminase (ADA) levels were significantly lower in the MPE than in the BPE group ($P = 0.002$ and $P = 0.012$, respectively), whereas the CEA levels were significantly higher in the MPE group ($P = 0.002$). When comparing MPEs and tuberculous PEs, similar results were found.

Calprotectin and CXCL12 Levels in PE

Both calprotectin and CXCL12 levels did not distribute normally, but the variances were homogeneous. Table 2 showed the calprotectin and CXCL12 concentrations, expressed as ng/mL, in different diagnostic groups. The median (95% CI) calprotectin and CXCL12 levels of the MPE were 447.15 (254.36, 714.12) ng/mL and 4.12 (1.83, 6.08) ng/mL, and both were significantly lower than that of the BPE ($P = 0.003$ and $P = 0.020$, respectively) and that of tuberculous PE ($P = 0.002$ and $P = 0.003$, respectively). No significant difference was

TABLE 1. Patient Baseline Characteristics

Characteristics	MPE	BPE	P^*	Tuberculous PE	$P^\dagger$
Sex (male) (%)	25 (64.1)	34 (60.7)	0.738	27 (61.4)	0.797
Age (y)	61.00 ± 13.19	47.09 ± 20.67	0.000	45.16 ± 20.66	0.000
Height (cm)	163.29 ± 7.15	164.98 ± 7.41	0.275	164.74 ± 7.37	0.371
Weight (kg)	58.97 ± 10.01	58.36 ± 10.43	0.776	57.51 ± 9.36	0.496
Smoking (%)	20 (58.8)	19 (38.8)	0.072	12 (31.6)	0.020
Symptoms					
Fever (%)	5 (13.9)	28 (51.9)	0.000	22 (52.4)	0.000
Cough (%)	30 (81.8)	42 (75.0)	0.492	30 (68.2)	0.187
Chest pain (%)	18 (48.6)	27 (49.1)	0.967	17 (39.5)	0.413
Dyspnea (%)	26 (70.3)	30 (53.6)	0.107	25 (56.8)	0.212
Serology					
ESR (mm/h)	63.66 ± 33.82	66.33 ± 38.08	0.754	67.34 ± 37.38	0.674
CRP (mg/L)	62.98 ± 69.14	64.17 ± 68.99	0.944	61.55 ± 46.25	0.924
Neutrophils (106/mL)	6.17 ± 2.81	7.03 ± 13.30	0.697	7.45 ± 14.92	0.606
Lymphocytes (106/mL)	1.35 ± 0.74	1.38 ± 1.80	0.920	1.45 ± 2.02	0.780
Pleural fluid					
Protein (g/L)	38.22 ± 6.33	44.35 ± 10.50	0.002	45.28 ± 10.24	0.001
GLU (mmol/L)	5.73 ± 2.48	5.07 ± 2.30	0.198	5.07 ± 1.85	0.179
ADA (IU/L)	9.22 ± 4.29	22.92 ± 32.10	0.012	16.85 ± 17.96	0.014
LDH (IU/L)	612.30 ± 876.30	1377.02 ± 3896.33	0.245	382.47 ± 241.23	0.107
CEA (ng/mL)	1.97 ± 296.23	1.02 ± 0.53	0.002	0.91 ± 0.36	0.005

ADA = adenosine deaminase, BPE = benign pleural effusion, CEA = carcinoembryonic antigen, CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, GLU = glucose, LDH = lactate dehydrogenase, MPE = malignant pleural effusion, PE = pleural effusion.
* Comparison between MPE and BPE.
† Comparison between MPE and tuberculous PE.

found between MPE and parapneumonic PE, or among different MPE subgroups ($P > 0.05$).

Prediction of Calprotectin and CXCL12 for MPE

Figure 2 showed the ROC curve of calprotectin and CXCL12 in patients with MPE and BPE. The AUC of calprotectin and CXCL12 was 0.683 (95% CI 0.570–0.795, $P = 0.003$) and 0.641 (95% CI 0.529–0.753, $P = 0.020$), and the cut-off point was calculated to be 500.19 and 6.11 ng/mL. In patients with MPE and tuberculous PE, the ROC curve showed that the AUC of calprotectin and CXCL12 was 0.696 (95% CI 0.581–0.812, $P = 0.002$) and 0.690 (95% CI 0.0.577–0.803, $P = 0.003$), and the cut-off point was 421.73 and 3.71 ng/mL, respectively (Fig. 3).

Table 3 and Table 4 presented the sensitivity, specificity, PPV, NPV, positive likelihood ratio (LR+), and negative likelihood ratio (LR−) of calprotectin and CXCL12 in distinguishing MPE from BPE and tuberculous PE by the corresponding cut-off point. Calprotectin showed a high specificity in discriminating MPE from BPE and tuberculous PE (71.43% and 84.09%), whereas CXCL12 showed a high sensitivity (79.49%) in discriminating MPE from BPE and a high specificity (81.82%) in discriminating MPE from tuberculous PE. The combination of calprotectin and CXCL12 greatly improved the specificity (78.57% and 95.45%).

Influence of Calprotectin and CXCL12 Levels on MPE Prediction

In comparison to patients with MPE and BPE, the univariate logistic regression demonstrated a strong association between the low levels of calprotectin and CXCL12 and MPE (Table 5), with an unadjusted OR of 0.224 ($P = 0.001$) and 0.344 ($P = 0.026$). We also found a significant ($P < 0.2$) association between MPEs and age, smoking, and proteins and glucose (GLU) in pleural fluid. The multivariate logistic regression of the significant predictors, indicating that calprotectin levels ≤500.19 ng/mL and CXCL12 ≤6.11 ng/mL, remained an independent significant predictor, with an unadjusted OR of 0.324 ($P = 0.043$) and 0.204 ($P = 0.026$).

TABLE 2. Calprotectin and CXCL12 Levels (Mean and 95% CI) in PE

PE Origin	MPE	BPE	P^*	Tuberculous PE	$P^\dagger$
Calprotectin (ng/mL)	447.15 (254.36, 714.12)	592.40 (460.17, 768.51)	0.003	600.75 (470.40, 780.76)	0.002
CXCL12 (ng/mL)	4.12 (1.83, 6.08)	5.47 (3.11, 7.66)	0.020	5.89 (4.04, 7.99)	0.003

BPE = benign pleural effusion, CI = confidence interval, MPE = malignant pleural effusion, PE = pleural effusion.
* Comparison between MPE and BPE.
† Comparison between MPE and tuberculous PE.

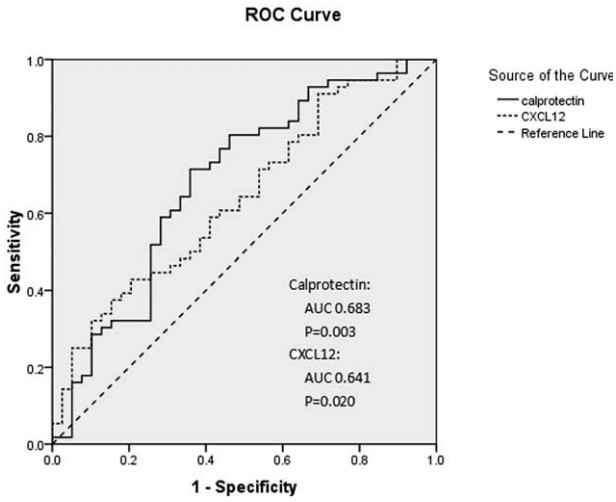

FIGURE 2. ROC curve of calprotectin and CXCL12 in patients with MPE and BPE. ROC curves of calprotectin and CXCL12 in patients with MPE and BPE. Line in black represented calprotectin, whereas dotted line in black represented CXCL12. The AUC of calprotectin and CXCL12 was 0.683 (95% CI 0.570–0.795, $P = 0.003$) and 0.641 (95% CI 0.529–0.753, $P = 0.020$), and the cut-off point was calculated to be 500.18748 and 6.11315 ng/mL, respectively. AUC = area under the curve, BPE = benign pleural effusion, CI = confidence interval, MPE = malignant pleural effusion, ROC = receiver-operating characteristic.

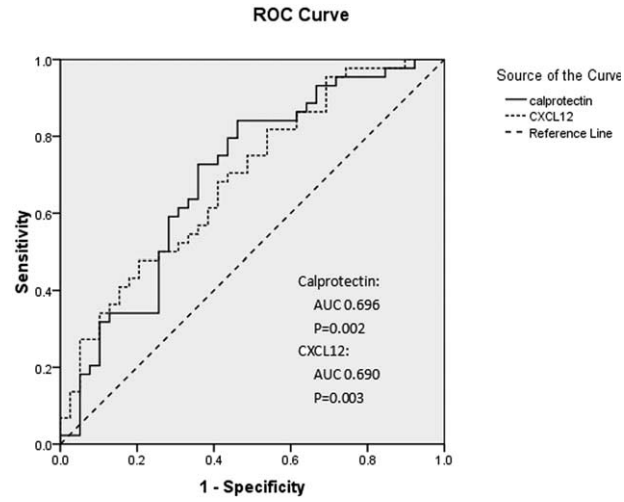

FIGURE 3. ROC curves of calprotectin and CXCL12 in patients with MPE and tuberculous PE. ROC curves of calprotectin and CXCL12 in patients with MPE and tuberculous PE. Line in black represented calprotectin, while dotted line in black represented CXCL12. The AUC of calprotectin and CXCL12 was 0.696 (95% CI 0.581–0.812, $P = 0.002$) and 0.690 (95% CI 0.0.577–0.803, $P = 0.003$), and the cut-off point was 421.72568 and 3.71092 ng/mL, respectively. AUC = area under the curve, CI = confidence interval, MPE = malignant pleural effusion, PE = pleural effusion, ROC = receiver operating characteristic.

Similarly, the univariate logistic regression of patients with MPE and tuberculous PE showed a significant association between MPE and the low levels of calprotectin and CXCL12, with an unadjusted OR of 0.162 ($P = 0.000$) and 0.259 ($P = 0.008$), as well as the same parameters aforementioned (Table 6). The multivariate logistic regression demonstrated that calprotectin ≤421.73 ng/mL and CXCL12 ≤3.71 ng/mL were also independent predictors apart from age (OR 0.164, $P = 0.009$; and OR 0.216, $P = 0.046$, respectively).

TABLE 3. Cut-off, Sensitivity, Specificity, Predictive Values, and Likelihood Ratios for Calprotectin and CXLC12 in Discriminating MPE From BPE

Group and Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR+	LR−
Calprotectin ≤500.19 ng/mL	64.10	71.43	60.98	74.07	2.24	0.50
CXCL12 ≤6.11 ng/mL	79.49	42.86	49.21	75.00	1.39	0.48
Calprotectin ≤500.19 ng/mL and CXCL12 ≤6.11 ng/mL	48.72	78.57	61.29	68.75	1.87	0.65

BPE = benign pleural effusion, LR+ = positive likelihood ratio, LR− = negative likelihood ratio, MPE = malignant pleural effusion, NPV = negative predicting value, PPV = positive predictive value.

TABLE 4. Cut-off, Sensitivity, Specificity, Predictive Values, and Likelihood Ratios for Calprotectin and CXCL12 in Discriminating MPE From Tuberculous PE

Group and Cut-off	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR^+	LR^-
Calprotectin ≤421.73 ng/mL	53.85	84.09	75.00	67.27	3.38	0.55
CXCL12 ≤3.71 ng/mL	46.15	81.82	69.23	63.16	2.54	0.66
Calprotectin ≤421.73 ng/mL and CXCL12 ≤3.71 ng/mL	25.64	95.45	83.33	59.15	5.64	0.78

LR+ = positive likelihood ratio, LR− = negative likelihood ratio, MPE = malignant pleural effusion, NPV = negative predicting value, PE = pleural effusion, PPV = positive predictive value.

TABLE 5. Univariate and Multivariate Logistic Regression Analysis of Calprotectin, CXCL12 and Other Selected Biomarkers and Demographic Characteristics in Patients With MPE and BPE

	Univariate Logistic Regression		Multivariate Logistic Regression	
Characteristics	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Calprotectin ≤500.19 ng/mL	0.224 (0.093–0.537)	0.001	0.324 (0.108–0.967)	0.043
CXCL12 ≤6.11 ng/mL	0.344 (0.134–0.881)	0.026	0.204 (0.050–0.830)	0.026
Sex (male)	0.865 (0.371–2.017)	0.738		
Age (y)	0.958 (0.935–0.983)	0.001	0.974 (0.942–1.008)	0.128
Smoking (%)	2.256 (0.924–5.507)	0.074	2.585 (0.867–7.708)	0.088
ESR (mm/h)	1.002 (0.990–1.015)	0.750		
Pleural fluid				
Protein (g/L)	1.079 (1.024–1.136)	0.004	1.055 (0.989–1.126)	0.210
GLU (mmol/L)	0.886 (0.736–1.066)	0.199	1.184 (0.909–1.542)	0.210

BPE = benign pleural effusion, ESR = erythrocyte sedimentation rate, GLU = glucose, MPE = malignant pleural effusion, OR = odds ratio.

TABLE 6. Univariate and Multivariate Logistic Regression Analysis of Calprotectin, CXCL12, and Other Selected Biomarkers and Demographic Characteristics in Patients With MPE and Tuberculous PE

	Univariate Logistic Regression		Multivariate Logistic Regression	
Characteristics	**OR (95% CI)**	***P***	**OR (95% CI)**	***P***
Calprotectin ≤421.73 ng/mL	0.162 (0.058–0.452)	0.000	0.164 (0.042–0.638)	0.009
CXCL12 ≤3.71 ng/mL	0.259 (0.096–0.699)	0.008	0.216 (0.048–0.973)	0.046
Sex (male)	0.889 (0.364–2.171)	0.797		
Age (y)	0.952 (0.926–0.978)	0.000	0.952 (0.912–0.994)	0.024
Smoking (%)	3.095 (1.177–8.137)	0.022	2.544 (0.707–9.156)	0.153
ESR (mm/h)	1.003 (0.990–1.017)	0.669		
Pleural fluid				
Protein (g/L)	1.104 (1.038–1.174)	0.002	1.074 (0.988–1.167)	0.093
GLU (mmol/L)	0.864 (0.697–1.071)	0.182	1.186 (0.873–1.611)	0.275

ESR = erythrocyte sedimentation rate, GLU = glucose, MPE = malignant pleural effusion, OR = odds ratio, PE = pleural effusion.

DISCUSSION

Our study found that concentrations of calprotectin and CXCL12 were both significantly lower in patients with MPE than BPE and tuberculous PE, and the combination of these 2 biomarkers showed a high specificity in predicting MPE.

Sánchez-Otero et al[24] and Kohmo et al,[25] respectively, compared calprotectin and CXCL12 in MPE with that in BPE and tuberculous PE. They demonstrated that calprotectin and CXCL12 level was significantly increased in nonmalignant pleural fluid compared with malignant pleural fluid, which supported the measurement of both biomarkers in pleural effusion as a possible noninvasive strategy for the differential diagnosis of MPE. However, studies regarding the utility of combination of calprotectin and CXCL12 in discrimination between MPE and BPE are still scarce, to date. In our study, we measured both calprotectin and CXCL12 in pleural fluid, and the results showed, for the first time, that the combination of these 2 biomarkers greatly improved the diagnostic specificity in predicting MPE.

Our study further demonstrated the significant lower levels of calprotectin and CXCL12 in MPE, but the sensitivity and specificity were lower compared with that reported by Sánchez-Otero et al[24] and Kohmo et al,[25] which we think may result from the different diagnosis criteria of tuberculous PE, cut-off points, and patient races. In our study, in addition to the direct evidence of TB, such as *M. tuberculosis* and caseating granulomas, we also made a diagnosis of tuberculous PE if the patients presented remarkable response to anti-TB treatment. In other words, some patients already received anti-TB treatment before a definite diagnosis was made, and unquestionably the anti-TB treatment may decrease the overall calprotectin and CXCL12 concentration in BPE and result in lower cut-off points, which eventually decreased the differences of calprotectin levels between MPE and BPE, and affected AUC, sensitivity, and specificity in our study.

High sensitivity and specificity are both important for biomarkers in the diagnosis of malignant disease, because any biomarkers are only initial screening methods, but not the final diagnostic methods. Although our study showed relatively low sensitivity of calprotecin and CXCL12 compared with previous studies,[24,25] it never means that calprotectin and CXCL12 have no clinical values in differential diagnosis in MPE. Among the 39 patients with MPE in our study, first pleural cytology test identified 18 patients with a sensitivity of 46.15%, which is lower than that of calprotectin and CXCL12, but similar to the combination of calprotectin and CXCL12.

Moreover, the addition of calprotectin $\leq$500.19 ng/mL and CXCL12 $\leq$6.11 ng/mL further identified 11 and 16 from the 21 firstly cytology-negative MPE patients, respectively. Therefore, to some extent, measuring calprotectin and CXCL12 levels in PE may decrease the necessity and hazard of further invasive procedures, which we think is the highlight and clinical significance of these 2 novel tumor markers. However, clinical application of calprotectin and CXCL12 should also be cautious due to the inconsistent sensitivity, specificity, and cut-off points reported in different studies, and the unknown cost-effect that need future investigations. We suggest clinicians consider the effects of drugs and races of patients, and establish the best cut-off points in accordance with the local conditions to maximize the efficacy of calprotectin and CXCL12.

Calprotectin and CXCL12 are well known as proinflammatory proteins and are mainly found in inflammatory diseases. Johne et al[27] found that S100A8 and S100A9 were released during the activation and turnover of leukocytes, and studies have documented that S100 proteins perform a broad range of physiological and pathological functions; such as bowel and joint inflammation, neurodegeneration, and metastatic growth in cancer, but in particular, the S100A8 and S100A9 play an important role in leukocyte trafficking and arachidonic acid metabolism via activation of the innate immunity pathway mediated by Toll-like receptors, which made them ideal markers for mycobacterial infection.[17,28,29] CXCL12 was reported as a highly efficacious and highly potent mononuclear cell attractant, which acted on lymphocytes and monocytes, but not on neutrophils, as trafficking of lymphocytes through tissues to inflammation during immune surveillance via G-protein-coupled receptors.[30] Thus, the decreased amount of S100A8, S100A9, and CXCL12 in MPE is expectable, as is demonstrated by Rodríguez-Piñeiro et al that S100A8 and S100A9 were differentially expressed in MPE and BPE (S100A8: 0.28 ± 0.12 vs 1.88 ± 0.88; S100A9: 0.27 ± 0.23 vs 1.96 ± 0.66), and by Kohmo et al that CXCL12 level in pleural fluid was significantly higher in TB pleurisy than malignant effusion (4456 ± 1013 vs 2741 ± 1264 pg/mL; $P < 0.001$). However, we could not conclude whether their levels are lower in normal persons compared with patients with malignant tumors due to the lack of relevant studies and the insufficient knowledge of their exact roles, which requires future investigations.

Some studies reported that calprotectin was associated with poorer prognosis in different cancers, such as prostate cancer and kidney cancer.[31,32] Meanwhile, CXCL12 has been recently shown to be important in migration and metastasis of solid tumors including lung cancer.[18] In our study, univariate logistic regression corroborated that calprotectin and CXCL12 levels were both relevant to the risk of the malignant origin of the effusion. Moreover, multivariate logistic regression showed that both calprotectin and CXCL12 were independent predictors of MPE, which further verified the conclusions of the study conducted by Sánchez-Otero et al.[24]

Carcinoembryonic antigen is one of the classic tumor markers and is usually elevated in serum in diverse tissue-originated carcinoma. A large number of studies have explored the role of CEA in differential diagnosis of PE, and a recent meta-analysis of 15 studies demonstrated that CEA in PE exhibit a sensitivity of 45.9% and a specificity of 97.0% in the diagnosis of malignant PE, which was concluded as a useful noninvasive method to diagnose malignant PE.[33] In our study, pleural CEA was measured in some patients (26 patients with MPE and 25 patients with BPE) based on the determination of the relevant attending physician; thus we roughly compared the pleural CEA levels in MPE and BPE to discuss the potential differential role of CEA. Even though, our study showed the sensitivity and specificity of CEA levels greater than 5 mmol/L was 82.61% and 100%, but it should be interpreted cautiously due to the small samples (51 patients) and this is the reason why we did not include CEA in the multivariate analysis.

Our study showed that the pleural proteins in MPE were significantly lower than that in BPE, but we also found that the plasma proteins in MPE were significantly lower, which were not shown in the manuscript. Therefore, lower pleural proteins resulted from lower plasma proteins in MPE. Malnutrition is recognized as a common problem in cancer patients as an important component of adverse outcomes, including increased morbidity and mortality and decreased quality of life.[34,35] Studies reported that at the time of diagnosis, 60% of patients with lung cancer have already experienced a significant weight loss.[36,37] Plasma protein level can be used to evaluate the nutritious conditions in patients with cancers, and patients with advanced cancers usually have decreased plasma protein concentration, which is due to decreased synthesis and increased protein turnover.[36,37] Thus, decreased serum and pleural protein levels in MPE are expectable. However, in patients with TB, malnutrition is usually reported as a risk factor of predisposition, not a concomitant,[38–40] and the underlying mechanisms are still unknown. Therefore, our results may have some suggestive significance, and further studies are eagerly awaited.

Some limitations are present in our study. First, the number of patients was low, especially in the different etiologies of PE included. Second, our study was a single-center analysis, and the patients were recruited from a unique respiratory department. Thus, more large-scale studies must be performed to further determine the value and applicability of calprotectin and CXCL12 in discriminating MPE from BPE in clinical settings.

CONCLUSIONS

Calprotectin and CXCL12 in pleural fluid are informative diagnostic biomarkers for predicting patients with MPE, and could be good complements to cytological methods.

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