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Clinical value of CD97 and CD55 levels in the differential diagnosis of tuberculous and malignant pleural effusions

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Abstract

This study evaluated the clinical levels of CD97 and CD55 for the differential diagnosis of pleural effusion.

Pleural effusion samples were collected from 106 patients (55 tuberculous pleural effusions [TPE] and 51 malignant pleural effusions [MPE]). CD97 and CD55 levels in pleural effusions were measured by enzyme-linked immunosorbent assay.

CD97 levels were significantly higher in the TPE group than in the MPE group ($P < .001$), while CD55 levels in the MPE group were significantly higher than the TPE group ($P < .001$). The sensitivity and specificity of CD97 testing for the differential diagnosis of TPE and MPE was 80.0% and 60.8%, respectively, while the sensitivity and specificity of CD55 testing for TPE and MPE was 88.2% and 85.5%, respectively. Furthermore, the sensitivity and specificity of combinatorial CD97 and CD55 testing for TPE and MPE was 90.0% and 87.5%, respectively. Moreover, CD97 and CD55 were negatively correlated in the MPE group ($r = -0.383$, $P = .005$), while no correlations were observed in the TPE group. CD97 or CD55 showed no correlations with other inflammatory cytokines (tumor necrosis factor α, interleukin 1β, erythrocyte sedimentation rate, and C-reactive protein) in both groups ($P > .05$).

CD97 and CD55 may be used as biological markers for the differential diagnosis of pleural effusion in clinical settings.

Abbreviations: GPCR = G-protein coupled receptor, IL-1β = interleukin 1β, MPE = malignant pleural effusion, TNF-α = tumor necrosis factor α, TPE = tuberculous pleural effusion.

Keywords: cluster of differentiation 55, cluster of differentiation 97, malignancy, pleural effusion, tuberculosis

1. Introduction

Pleural effusions arise from a variety of disease states or medications.[1] Evaluations of patients with pleural effusion are challenging because a differential diagnosis is broad and includes benign and life-threatening conditions.[2] The frequency of effusion depends on the clinical setting, but studies have shown that cancer, heart failure, and parapneumonic infections account for most cases.[3]

Fluid analysis of pleural fluid often provides diagnostic clues.[1] Protein and lactate dehydrogenase levels can determine whether pleural fluid represents a transudative or exudative effusion, and both serve as evaluation criteria for Light criteria.[4–7] Further testing is guided by clinical enquiry, and may include acid-fast bacillus testing for tuberculosis, and triglyceride, cholesterol, amylase, hematocrit, N-terminal pro-brain natriuretic peptide measurements. Currently, no pleural fluid markers for malignant disease are available for clinical use.[8–10]

Pleural malignant disease and tuberculous pleurisy are challenging in terms of definitive identification, yet they are important to exclude when faced with recurrent undiagnosed exudates.[2] Studies have found that pleural fluid cytology sensitivity is approximately 60% to 70%, therefore, a negative fluid cytology test does not exclude malignant disease.[4,11] For tuberculosis, pleural fluid culture sensitivity is consistently below 40%.[12] For patients with a reasonable functional status, a pleural biopsy is indicated, however, clinicians should clarify the cause before invasive therapeutic measures are implemented, as recommended by the British Thoracic Society.[4] Therefore, novel pleural fluid markers that differentially diagnose tuberculosis and other malignant pleural effusions, must be investigated.

The innate complement control system is part of the fundamental innate immune defense mechanism that targets

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cancer by exerting immune-surveillance.[13,14] Recently, complement functions have been implicated in the immunopathology of tuberculosis.[15,16] As one of the key membrane complement regulatory proteins, CD55 activates T and B cells by binding CD97, which is expressed on macrophages and granulocytes.[17] Therefore, we speculated that CD97 and CD55 may be potential biomarkers for tumors or infection. However, their role in pleural effusions was not reported, therefore we investigated the diagnostic utility of CD97 and CD55 in pleural fluid from patients with pleural effusions.

2. Materials and methods

2.1. Patients

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the ethical committee of First Hospital of Jilin University (Project approval No; 18K042–002). All participants gave their signed informed consent.

In total, double-blind trial was conducted in the Department of respiratory wards at First Hospital of Jilin University. One hundred six patients with primary pleural effusion were included between April 2017 and November 2018 if they met inclusion and exclusion criteria. Patient ages ranged between 17 and 89 years old. Of these, 55 patients had TPE, while 51 had MPE. Patient pleural effusion was performed according to Light criteria for exudative pleural effusion. All patients were diagnosed with tuberculosis or malignant tumors through a pleural effusion cytology specimen, using thoracoscopy.

Table 1 shows patient groups, which were divided into samples from TPE and MPE patients.

Exclusion criteria: patients who had a prior similar pleural effusion; patients who had a definite diagnosis before or had received treatment for pleural effusion; patients first segment of fluid could not be accessed; patients who had serious or other related diseases which could affect CD97 and CD55 expression, and patients lacking complete data.

2.2. Pleural fluid collection

Initial pleural effusions were stored at 4 °C, centrifuged at 1500 rpm for 5 minutes and the supernatant removed. The supernatant was centrifuged again at 4000 rpm for 10 minutes. Then, an aliquot (1 mL) was stored at –80 °C.

Table 1

Baseline characteristics and inflammatory marker levels in serum and pleural effusions from TPE and MPE patients.

Groups	TPE (n = 55)	MPE (n = 51)	*P*-value
Gender: Male/Female	34/21	28/23	$P > .05$
Age	48.81 ± 18.71	62.82 ± 10.90	$P > .05$
IL-1β, pg/L	3300.67 ± 1642.23	2242.21 ± 1073.3	$P = .01$
TNF-α, pg/mL	385.64 ± 128.84	255.98 ± 160.97	$P < .01$
CRP, mg/L	84.98 ± 36.11	19.62 ± 14.81	$P < .01$
ESR, mm/h	49.25 ± 20.45	30.20 ± 18.89	$P < .01$

Primary lesions in the MPE group included 45 cases of lung cancer, 2 breast cancer cases, 2 esophageal cancer cases, 1 pleural mesothelioma, and 1 lymphoma case.

CRP = C-reactive protein, ESR = erythrocyte sedimentation rate, IL-1β = interleukin 1β, MPE = malignant pleural effusion, TNF-α = tumor necrosis factor, TPE = tuberculous pleural effusion.

2.3. Quantification of biomarkers and inflammatory factors

CD97, CD55, tumor necrosis factor α (TNF-α), and interleukin 1β (IL-1β) levels were measured using a commercially available enzyme linked immunosorbent assay (USCN, China), according to manufacturer's instructions. All samples were encrypted and blindly analyzed with respect to patient diagnoses.

2.4. Statistical analysis

All analyses were performed using SPSS 23.0 software (SPSS, Inc., Chicago, IL). A P-value $< .05$ was accepted as statistically significant. To define the best cut-off point for biomarkers, a receiver operating characteristic (ROC) curve was used, which representing the relationship between sensitivity and specificity were compared with a nonparametric approach. A cutoff was estimated in the learning cohort by maximizing the Youden index (J = sensitivity + specificity – 1).[18] Pearson linear correlations were used to determine correlations between various parameters.

3. Results

3.1. CD97 and CD55 levels in pleural effusion samples

The median concentration of CD97 was significantly higher in TPE (161.80 ± 66.46 ng/mL) than the MPE (111.97 ± 52.20 ng/mL) ($P < .001$) group (Fig. 1A). Also, the median concentration of CD55 was significantly higher in the MPE group (116.22 ± 40.46 ng/mL), when compared with the TPE group (64.21 ± 19.89 ng/mL) ($P < .001$) (Fig. 1B).

3.2. Diagnostic value of CD97 and CD55 measurements

ROC curve analysis results revealed that the area under the curve (AUC) for CD97 was 0.724, between the TPE and MPE groups (Fig. 2). The diagnostic sensitivity and specificity of the pleural fluid CD97 test, at an optimal cut-off value of 105.26 ng/mL, was 80.0% and 60.8%, respectively.

Moreover, CD55 revealed an AUC of 0.917, between the TPE and MPE groups. The diagnostic sensitivity and specificity of the pleural fluid CD55 test, at an optimal cut-off value of 79.05 ng/mL, was 88.2% and 85.5%, respectively (Fig. 3).

The combined measurements for CD97 and CD55 revealed an AUC of 0.922, between the TPE and MPE groups. The diagnostic sensitivity and specificity of the combined measurements for CD97 and CD55 was 92.7% and 80.4%, respectively (Fig. 4).

3.3. Correlations between CD97, CD55, and inflammation markers in pleural effusion

The results of correlation analysis revealed that IL-β concentrations were positively correlated with TNF-α levels in the pleural effusion from TPE patients ($r = 0.766$, $P < .01$) or from MPE patients ($r = 0.568$, $P < .01$). The levels of CD55 and CD97 has no significant correlation with TNF-α, IL-1β, erythrocyte sedimentation rate, and C-reactive protein levels both in TPE group ($P > .05$) and MPE groups ($P > .05$). CD97 levels were negatively correlated with CD55 in the MPE group ($r = –0.383$, $P = .005$), while there was no correlations between CD97 levels and CD55 levels in the TPE group ($P > .05$).

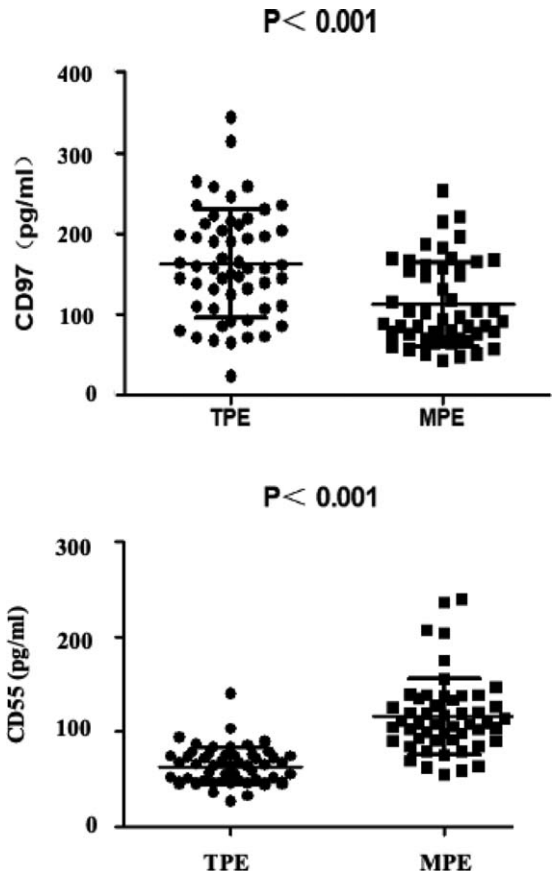

Figure 1. CD97 and CD55 levels in pleural effusion samples from TPE and MPE groups. MPE = malignant pleural effusion, TPE = tuberculous pleural effusion.

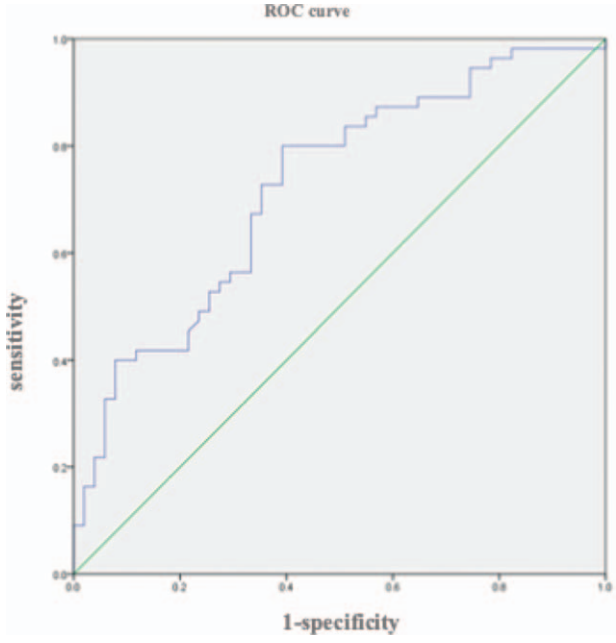

Figure 2. ROC curve analysis of CD97 levels for TPE diagnostics. ROC = receiver operating characteristic, TPE = tuberculous pleural effusion.

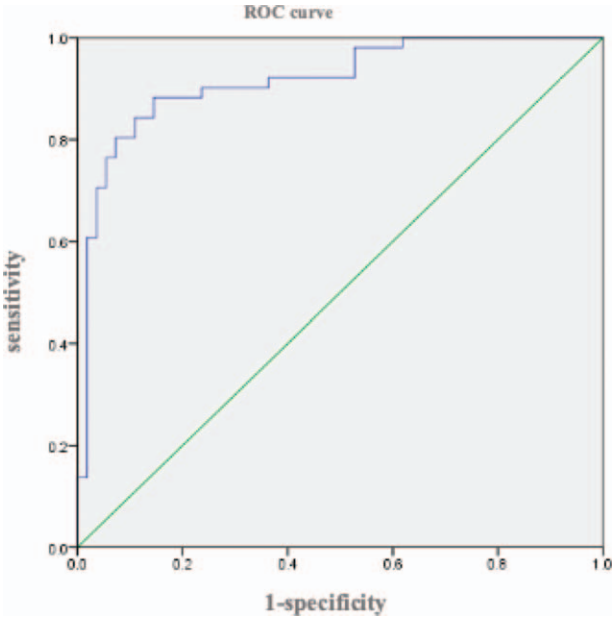

Figure 3. ROC curve analysis of CD55 levels for TPE diagnostics. ROC = receiver operating characteristic, TPE = tuberculous pleural effusion.

4. Discussion

This study showed that both CD97 and CD55 have the potential to be diagnostic markers for TPE and MPE. Similarly, when combined, both biomarkers had higher sensitivity and specificity for differential diagnostics. To the best of our knowledge, no previous data connects CD97 and CD55 with TPE or MPE, therefore, this is the first study to analyze the 2 biomarkers in pleural effusions.

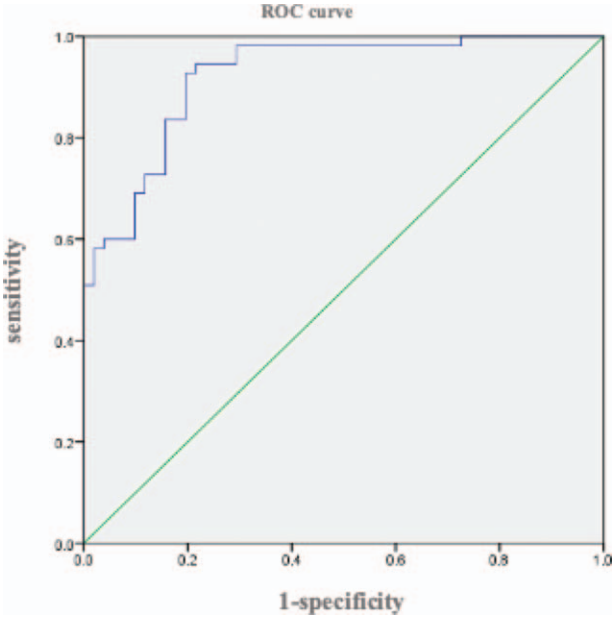

Figure 4. ROC curve analysis of combined CD97 and CD55 levels for TPE diagnostics. ROC = receiver operating characteristic, TPE = tuberculous pleural effusion.

CD97 is a member of the G-protein coupled receptor (GPCR) family, and is expressed in inflammatory, endothelial and smooth muscle cells, and bone marrow-derived mesenchymal stem cells (MSC).[19,20] Functionally, CD97 plays a role in angiogenesis, tumor differentiation and cell invasion by interacting with its ligand CD55, but CD97 function is not entirely understood.[21] High CD97 level are observed in immune cells where the molecule regulates cell adhesion and trafficking.[22] CD97 is also up-regulated and/or biochemically modified in several malignancies.[23] Consistently, CD97 has been shown to promote tumor growth and metastatic spread in mouse models of colorectal, gastric, thyroid, and pancreatic cancer; whereas CD97-silencing regulates migration and invasion of tumor cells in vitro.[24–27] In this study, the median concentration of CD97 was significantly higher in TPE than in MPE, but there were no correlations with inflammatory factors (TNF-α, IL-1β, erythrocyte sedimentation rate, and C-reactive protein). This indicated that increasing CD97 levels during pleural effusion may be inconsistent with inflammatory responses. The higher levels of CD97 recorded in our TPE group may suggest that CD97 or another GPCR is involved in immune response regulation after tuberculosis infection. However, the function of CD97 and associated immune mechanisms are not entirely understood, therefore, further in vitro analyses are required.

CD55 (decay accelerating factor, DAF) is a glycosylphosphatidylinositol-anchored membrane inhibitor of complement.[28] DAF inhibits complement activation by interfering with C3/C5 convertases, in both classical and alternative pathways.[29] CD55 is expressed on the plasma membrane of all blood cells, and almost all other cell types in contact with plasma component proteins.[30] Physiologically, CD55 expression protects host cells from pathogenic microorganisms.[31] As one of the key membrane bound complement regulatory proteins, CD55 is crucial for cancer progression, including lung cancer.[17,32–35] Our findings show that median CD55 levels in the MPE group were significantly higher than the TPE group, and levels were also inconsistent with other inflammatory factors. These data agree with other studies showing that CD55 contributes to the progression of various cancers.[17,32–35] CD55 may also affect the progress of malignant pleural effusions through complement regulation, however, this conclusion must be confirmed in larger, sample size studies. Pearson correlation analyses showed that CD97 was negatively correlated with CD55 in the MPE group, which does not agree with other studies, for example, Wu et al,[36] showed that CD97 expression was associated with its ligand CD55 in pancreatic cancer, and He et al,[37] also concluded the same for pancreatic cancer. Karpus et al[38] observed in mice, that down-regulation of both CD97 subunits occurred within minutes after first contact with CD55, correlating with increased plasma levels of soluble CD97. These authors concluded that CD55 down-regulated CD97 surface expression on circulating leukocytes by a process that required physical force.[38] This study revealed mechanistic insights into the regulation of GPCR CD97 and its ligand CD55 in body fluids, which may also explain our experimental results. But further confirmation is required.

5. Conclusions

In summary, CD97 and CD55 can be used as biological markers for the differential diagnosis of pleural effusion in the clinic. Equally, combining both biomarkers generated higher sensitivity and specificity for differential diagnostics. The mechanisms behind CD97 and CD55 increase in MPE and TPE requires greater study in the future.

Author contributions

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Methodology: Jinying Gao.
Resources: Ying Liu.
Writing – original draft: Chunyan Wang, Jing Jie.
Writing – review & editing: Jinying Gao, Ying Liu, Lei Song.

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