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RESEARCH ARTICLE

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Clinical value of combined detection of reactive oxygen species modulator 1 and adenosine deaminase in pleural effusion in the identification of NSCLC associated malignant pleural effusions

Fan Zhang[1] | Junjun Wang[1] | Jiali Fu[1] | Lijuan Hu[1] | Xiaoyong Zheng[1] | Yumin Wang[1] | Bicheng Chen[2]

[1]Department of Laboratory Medicine, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

[2]Science and Education Department, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou, China

Correspondence
Bicheng Chen, Science and Education Department, The First Affiliated Hospital of Wenzhou Medical University, Wenzhou 325000, China.
Email: chenbicheng404@163.com

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Abstract

Background: Reactive oxygen species modulator 1 (ROMO1) is recognized to be involved in cell proliferation and is elevated in serum of various cancer patients. However, ROMO1 had little research in distinguishing between malignant pleural effusions (MPEs) and benign pleural effusions (BPEs).

Methods: Malignant pleural effusion samples from patients with non–small-cell lung cancer (NSCLC) and benign pleural effusion (BPE) samples containing tuberculous and inflammatory pleural effusions were collected. The samples were tested for ROMO1, pleural effusion adenosine deaminase (pADA), pleural effusion carbohydrate antigen (pCA125, pCA153, pCA199), pleural effusion ferritin (pFER), and pleural effusion lactate dehydrogenase (pLDH) levels, and the other relevant partial clinical data that were gathered were used to conduct statistical analysis.

Results: The ROMO1, pCA125, pCA199, pCA153, pADA + ROMO1, pCA153 + ROMO1, pCA125 + ROMO1, and pCA199 + ROMO1 levels in MPE were appreciably higher in comparison with BPE group (all $P = .000$). The concentration of pADA in MPE was markedly lower than BPE ($P = .000$). When the cutoff = 0.38, the sensitivity of combined detection of ROMO1 + pADA is 98.67% and the specificity is 70.73%, respectively, and the AUC (0.941) is the highest among other parameters.

Conclusion: The combined detection of ROMO1 + ADA in pleural effusion is an effective biomarker for identifying MPE caused by NSCLC.

KEYWORDS

adenosine deaminase, malignant pleural effusion, non–small-cell lung cancer, reactive oxygen species modulator 1

Zhang, Wang and Fu: Co-first authors.

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1 | INTRODUCTION

Pleural effusion is a clinical symptom often secondary to a variety of diseases, especially kinds of cancer and tuberculosis, known as typical malignant pleural effusion (MPE) and tuberculous pleural effusion (TPE) [1,2]; sometimes, it also happens after a lung infection. Among them, the most common source of the MPE is lung cancer. Some studies have shown that among 840 causes of MPE, which due to lung cancer accounted for about 37% of the total, most of them were lung adenocarcinoma (50%).[3]

Lung cancer is recognized as highly aggressive and metastatic, ranking second in the world in cancer incidence. Research shows that about 1.8 million sufferers are diagnosed with lung cancer and almost 1.6 million die of the disease every year [4]; moreover, it is also the principal cause of cancer death worldwide.[5] The non–small-cell lung cancer (NSCLC) is the commonest type of lung cancer and accounts for a largest proportion of the total (85%),[6] including squamous cell carcinoma, large cell lung cancer, and adenocarcinoma. The treatment effect is poor, and the 5-year survival rate is lower than 15%.[7] Because the clinical symptoms of lung cancer are usually more clinically relevant in the late stage, most patients are already in advanced stage at diagnosis and have a poor prognosis. Therefore, early diagnosis of lung cancer is a valuable way to improve the prognosis of patients. However, regular sputum cytology and chest imaging examinations did not show significant advantages.[8] Thoracoscopy is a good way to diagnose lung cancer with a sensitivity of up to 90%.[9] However, because it is an invasive examination, the patient's acceptance is low, the operation is complicated, and the risk is high, and it is not suitable as a method for screening and diagnosing lung cancer. On the other hand, pleural effusion is one of the clinical manifestations of lung cancer patients. Differential diagnosis of MPE is also one of the ways to diagnose lung cancer. Cytology is the golden standard for distinguishing the nature of pleural fluid; however, the positive rate is not high (11% ~ 78%).[10] For the past few years, scientific research personnel had been struggling to quest suitable pleural effusion markers to identify the properties of pleural effusion. Currently, commonly used markers in pleural fluid include such as pLDH, pFER, pCA199, pCA125, pCA153, pADA, but the above markers have the shortcomings such as the low sensitivity and/or specificity for detecting MPE.

Reactive oxygen species modulator 1 (ROMO1) is a novel protein selected from human complementary DNA (cDNA) library.[11] Reactive oxygen species modulator 1 can induce the production of intracellular reactive oxygen species (ROS), while ROS can promote cell proliferation,[12] many studies had shown that the level of ROMO1 is up-regulated in many cancers [13,14]; during the past several years, increasingly articles on the expression levels of ROMO1 in tissues and various body fluids and various The relationship between the diseases,[15,16] and other studies have shown that ROMO1 is also related to the prognosis and drug resistance of cancer.[17,18]

The aim of our research was to inquire into the feasibility of using ROMO1 and several conventional biomarkers in the differential diagnosis of MPE derived from NSCLC, and the correlation between several others clinical indicators of NSCLC patients.

2 | MATERIALS AND METHODS

The pleural fluid samples were gathered from 116 inpatients in the First Affiliated Hospital of Wenzhou Medical University, China, from August 2018 to March 2019.

2.1 | Sample selection and grouping

The transudates specimens were excluded by detecting the specific gravity and protein levels of the pleural effusion. Then through pathological cytology, including X-ray, CT, MRI, and other imaging studies and X-Pert, bacterial culture and T-spot distinguish between tuberculous pleural effusion, NSCLC-induced MPE, and infectious pleural effusion. Firstly, patients with lung diseases were screened by imaging examination. In the bacterial culture, M. tuberculosis or T-spot-positive pleural effusion samples were considered to be tuberculous pleural effusions. Samples which tumor cells detected by pathological cytology were considered to be malignant pleural effusions, exudative pleural effusions with negative examination and the patients which had pneumonia manifestation were considered to be infectious pleural effusions, and finally the above judgments were confirmed by the comprehensive diagnosis when the patient leave hospital. Tuberculous pleural effusion and infectious pleural effusion are collectively referred to as BPE. Finally, the clinical information on MPE specimens was recorded, including histological types, gender, age, cigarette smoking, the situation of distant metastasis, and implicated in lymph node. The pathological basis of diagnosis of NSCLC is the World Health Organization/International Lung Cancer Research Organization lung cancer histology classification standard. Eliminate conditions included coronary heart disease, cholelithiasis, hyperlipidemia, hepatic diseases, and several central nervous system diseases. In the end, we got 75 MPE samples consisting of 26 women and 49 men, between the ages of 23 and 89 (65.72 ± 10.94), and according to histological type, it is divided into 11 squamous cell carcinomas, 51 adenocarcinomas, and 7 unclassified. 41 BPE samples consist of 13 women and 28 men, aged between 16 and 86 (48.15 ± 20.01). Chi-square test revealed no pronounced differences in sexuality between the two teams.

2.2 | Collection and detection of pleural effusion

We gathered about 10 mL pleural fluid from above patients by conventional thoracentesis and centrifuged in 4°C, 1200 r/min for 10 minutes, and the supernatant was separated and then stored at −20°C. The level of pFER, pCA153, pCA125, and pCA199 was tested using DXI800 Immunoassay analyzer (Beckman). The pADA and pLDH contents were detected with the Beckman Coulter AU5800 Clinical Chemistry Analyzer (Beckman). The level of ROMO1 was measured by ELISA kit (USCN Life Science Inc.), intra-assay

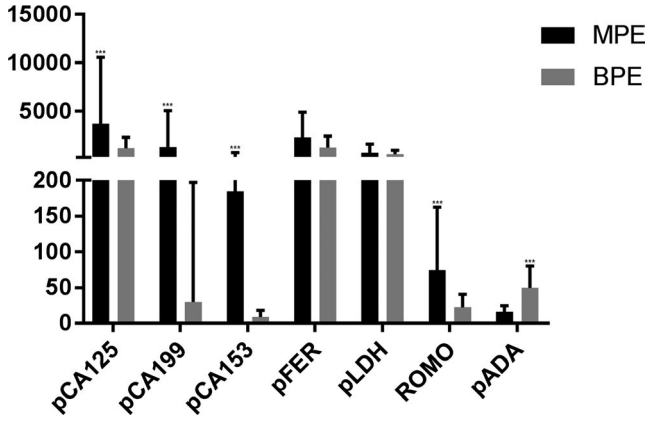

FIGURE 1 Comparison of the parameters of the two groups of pleural effusion showed that the levels of ROMO1, pCA125, pCA199, and pCA153 in the MPE group were significantly higher than those in BPE. In addition, the pADA level of the BPE group also showed a significant increase compared with the MPE.
***$P < .001$

CV <10%, inter-assay CV <12%. The study was approved by the Institutional Ethics Review Board of the First Affiliated Hospital of Wenzhou Medical University. All of the relevant patients have signed an informed consent form to take part in our study.

2.3 | Statistical analysis

Shapiro-Wilk test is used to assess the distribution of the data we have obtained. Comparisons between the two teams were made using t test or Mann-Whitney *U* test. The chi-square test was used to evaluate the difference between the categorical variables. $P < .05$ was statistical significance. Analyze the receiver operating characteristic curves (ROC), and the area under the ROC curve (AUC) was used to assess the performance of the subject. All of the above statistical analyses were processing by MedCalc (MedCalc Software) and SPSS 23.0 (Statistical Package for the Social Sciences Corporation).

3 | RESULTS

3.1 | Differences in parameters between MPE and BPE groups

The data distribution of all parameters was detected by Shapiro-Wilk test, and as a result, all the laboratory test data were proved as non-normal distribution; therefore, the data were subsequently analyzed using Mann-Whitney *U* test. The concentration of ROMO1, pCA125, pCA199, pCA153, pADA + ROMO1, pCA153 + ROMO1, pCA125 + ROMO1, and pCA199 + ROMO1 in the MPE was obviously higher compared with BPE team (Mann-Whitney $U = 828.000$, 823.000, 590.500, 472.500, 235.000, 413.000, 665.000, 617.000, The *P* value of all the above items is .000). The pADA concentration of BPE team was distinctly higher in comparison with the other group (Mann-Whitney *U* test = 494.000, $P = .000$). The pFER and pLDH values of the MPE group show no significant differences comparison between the BPE group ($P = .226, .749$) (see Figure 1 and Table 1).

3.2 | Diagnostic efficacy of various parameters for MPE

The ROC curve shows the sensitivities of ROMO1, pCA125, pCA199, pCA153, pADA, pADA + ROMO1, pCA153 + ROMO1, pCA125 + ROMO1, and pCA199 + ROMO1 for MPE were 52%, 71.83%, 63.01%, 67.61%, 96%, 98.67%, 66.2%, 50.7%, and 56.16%, respectively, and the specificities of those markers for the diagnosis of MPE were 82.93%, 58.97%, 94.87%, 94.87%, 70.73%, 70.73%, 92.31%, 97.44%, and 89.74%. The AUC of those parameters was, respectively, 0.706, 0.720, 0.754, 0.831, 0.09, 0.941, 0.842, 0.762, and 0.777. We found that when combined assay ROMO1 and pADA, the AUC (0.941) was the highest among whole parameters, when the cutoff value is 0.38 the sensitivity (98.67%) was higher than other indicators, and the specificity (70.73%) was the same as pADA (see Figure 2 and Table 2).

TABLE 1 Comparison of the parameters in pleural effusion in the MPE and BPE groups

	BPE	MPE	Mann-Whitney *U* test	*P*
ROMO1 (ng/mL)	16.58 (1.43-67.21)	41.34 (5.17-334.24)	828.000	.000
pCA125 (U/mL)	917.80 (3.30-4590.9)	1784.40 (30.30-51210.00)	823.000	.000
pCA199 (U/mL)	2.30 (0.80-1050.50)	15.20 (0.80-20210.00)	590.500	.000
pCA153 (U/mL)	6.00 (2.40-53.00)	23.60 (2.40-3221.20)	472.500	.000
pADA (U/mL)	48.80 (6.80-120.10)	13.70 (2.70-56.90)	494.000	.000
pFER (μg/L)	897.40 (367.60-6899.00)	1328.60 (51.40-12527.00)	911.000	.226
pLDH (U/mL)	404.00 (127.00-1819.00)	382.00 (34.20-5663.00)	1390.000	.749
pADA + ROMO1	0.06 (0.00-0.98)	0.89 (0.07-1.00)	235.000	.000
pCA153 + ROMO1	0.38 (0.28-0.93)	0.89 (0.34-1.00)	413.000	.000
pCA125 + ROMO1	0.5 (0.32-0.92)	0.75 (0.33-1.00)	665.000	.000
pCA199 + ROMO1	0.48 (0.39-0.98)	0.71 (0.41-1.00)	617.000	.000

Note: Median (min-max) in the parameters in the table.

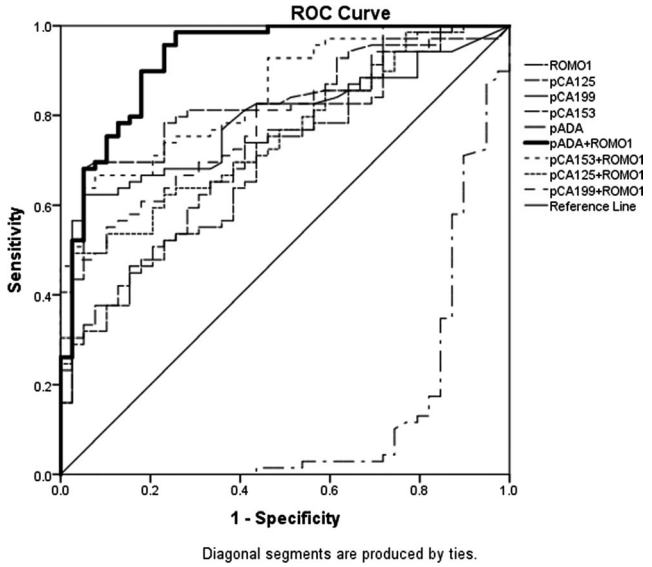

FIGURE 2 ROC curve for diagnosing various parameters of the MPE. When the cutoff value is 0.38, the sensitivity and specificity of the combined detection of ROMO1 and pADA are 98.67% and 70.73%, respectively, and the AUC is the highest among all other parameters

3.3 | Relevancy between ROMO1 and clinical information of NSCLC patients

We investigated age, gender, lymph node involvement, distant metastasis, tumor histological types, and smoking status in patients with NSCLC related to ROMO1 expression levels. Their relationship is summarized in Table 3. It shows that in our study, the expression level of ROMO1 in pleural effusions of NSCLC patients was not significantly different from age range (P = .051), gender (P = .229), smoking status (P = .185), tumor histology type (P = .065), and lymph node involvement (P = .905), but was associated with distant metastasis of tumors (P = .033). Then, the ROC curve shows that its AUC is 0.667, and the cutoff value = 34.1 ng/mL, and the sensitivity and specificity are 63.16% and 83.3%, respectively (see Figure 3).

4 | DISCUSSION

In China, pleural fluid is a clinically relevant symptom. The major diseases which cause of this symptom are carcinoma of lungs (especially NSCLC) and tuberculosis. The treatment and prognosis of the two diseases are quite different, and some studies show that lung cancer-related hydrothorax was related to the prognosis of the patient.[19] Thus, the identification of the nature of exudative pleural effusion is very important. Conventional histopathology and pleural cytology are the gold criteria for distinguishing between malignant and benign pleural effusions, but its low sensitivity limits its clinical utility. Therefore, the problem of how to improve the differentiate of pleural effusion was still researchable. In this study, we examined routine markers for pleural effusion and a less well-documented marker, ROMO1, to explore new markers that could be used to distinguish the nature of pleural effusions.

Reactive oxygen species modulator 1 is a membrane protein discovered in 2006, located in the mitochondria, capable of controlling the production of its intracellular ROS,[11] thereby affecting cell proliferation.[12] Studies have shown that the concentration of ROMO1 in serum of lung cancer patients is significantly higher than normal people.[14] In addition, cells lacking ROMO1 are more sensitive to the effects of apoptosis.[20] To date, many studies have shown that ROMO1 expression is specifically elevated to a variety of diseases accompanied with high oxidative stress and inflammation.[15,21]

Adenosine deaminase is known as an important enzyme which could catalyze the conversion to adenosine to inosine and is widely distributed among humans. ADA high activity is associated with T lymphocyte subsets involved in tuberculosis-induced inflammatory responses.[22,23] To date, many studies have shown the potential for ADA levels in effusions to diagnose tuberculous serous effusions, including pleural effusions, peritoneal effusion, and pericardial effusion.[24] In addition, some researchers have discovered that pleural effusion can be used as one of the indicators for distinguishing between NSCLC-related MPEs.[25]

The AUC forecasts the veracity of the diagnostic test. The diagnostic value was poor if AUC is between 0.5 and 0.7, and the diagnostic value is higher when AUC > 0.8. In our study, when the cutoff

TABLE 2 The AUC, cutoff value, sensitivity, and specificity of parameters for the diagnosis of MPE

	AUC	95% confidence interval	*P*	Cutoff	Sensitivity (%)	Specificity (%)
ROMO1 (ng/mL)	0.706	0.598-0.814	.001	36	52	82.93
pCA125 (U/mL)	0.720	0.613-0.827	.001	1001.9	71.83	58.97
pCA199 (U/mL)	0.754	0.654-0.853	.000	5.8	63.01	94.87
pCA153 (U/mL)	0.831	0.749-0.913	.000	11.7	67.61	94.87
pADA (U/mL)	0.090	0.009-0.170	.000	27.7	96	70.73
pADA + ROMO1	0.941	0.884-0.998	.000	0.38	98.67	70.73
pCA153 + ROMO1	0.842	0.763-0.921	.000	0.6	66.20	92.31
pCA125 + ROMO1	0.762	0.665-0.860	.000	0.73	50.70	97.44
pCA199 + ROMO1	0.777	0.682-0.872	.000	0.63	56.16	89.74

TABLE 3 Comparison of ROMO1 expression levels and various clinical data in MPE group

Clinical variables	Number	ROMO1 (ng/mL)	Mann-Whitney U test	*P*
Ages (y)				
>60	53	43.37 (5.17-334.24)	415.500	.051
≤60	22	24.85 (5.68-109.96)		
Gender				
Male	49	40.04 (5.68-334.24)	529.000	.229
Female	26	46.63 (5.17-320.19)		
Cigarette smoking status				
Smoker	37	40.04 (5.17-334.24)	578.000	.185
Non-smoker	38	43.05 (5.81-320.19)		
Histological type				
Squamous cell carcinoma	11	22.21 (5.17-77.29)	202.500	.065
Adenocarcinoma	57	42.63 (5.68-334.24)		
Lymph node metastasis				
Positive	45	41.34 (5.17-334.24)	664.000	.905
Negative	30	38.37 (5.81-320.19)		
Distant metastases				
Positive	57	43.98 (5.17-320.19)	341.500	.033
Negative	18	21.22 (10.8-334.24)		

Note: Median (min-max) in the parameters in the table.

value was 0.38, the diagnostic sensitivity and specificity of combined assay of ROMO1 and pADA in pleural effusion were 98.67% and 70.73%, respectively. The AUC of differentiate MPE from pleural effusion was 0.941, which was evidently higher than other indicators. At the same time, we studied the correlation between some clinical information such as age, gender, distant metastasis, lymph node metastasis, and the level of ROMO1 in pleural effusion, and found that the level of ROMO1 has a certain relationship between the distant metastasis of the patient's tumor.

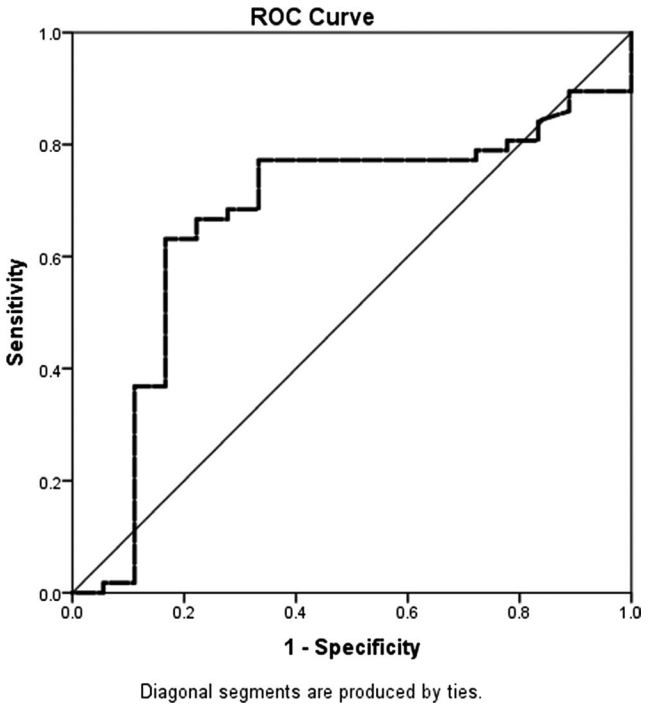

FIGURE 3 ROC curve of pleural effusion ROMO1 level to determine whether there is distant metastasis of tumor in NSCLC patients. When the cutoff value is 34.1 ng/mL, the sensitivity and specificity of ROMO1 is 63.16% and 83.3%, respectively

However, the AUC result between distant metastasis and ROMO1 level is not high, which may be related to the small number of clinical samples; this is a potential limitation of our research. On the other hand, the detection of our research projects suggests significant differences between the two groups, and the results were in line with the research of others. We will conduct a larger sample study in the future to confirm our conclusion and conduct deeper research.

In summary, the combined detection of ROMO1 + pADA in pleural effusion has a high diagnostic efficiency for distinguishing between NSCLC-related MPEs or BPEs, which could be used as a good biomarker for early diagnosis of NSCLC-associated MPE and also could be a guidance before the invasive diagnosis performing. At the same time, there may be some suggestive effects on the distant metastasis of NSCLC patients, which needs further research in the future.

ORCID

Fan Zhang https://orcid.org/0000-0002-7177-7218
Yumin Wang https://orcid.org/0000-0001-8243-3659

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