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RESEARCH ARTICLE

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Clinical value of jointly detection pleural fluid Midkine, pleural fluid adenosine deaminase, and pleural fluid carbohydrate antigen 125 in the identification of nonsmall cell lung cancer-associated malignant pleural effusion

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Background: Midkine (MK) level has been shown to be elevated in serum of patients with nonsmall cell lung cancer (NSCLC). However, the diagnostic value of MK in pleural effusion in NSCLC has not been well validated and established.

Methods: Samples of NSCLC-associated malignant pleural effusions (MPE) and benign effusions (BPE) were collected. The pleural fluid MK (pMK), pleural fluid adenosine deaminase (pADA), pleural fluid lactate dehydrogenase (pLDH), pleural fluid glucose (pGLU), pleural fluid ferritin (pFER), pleural fluid CA199 (pCA199), pleural fluid CA125 (pCA125), pleural effusion white cell count (pWBC), and pleural effusion red cell count (pRBC) were analyzed, and the clinical data of each group were collected for statistical analysis.

Result: The level of pMK, pCA125, pMK + pCA125, and pMK + pCA125 + pADA in the MPE was significantly higher than the BPE group (P = .003, .000, .000, .000). The pADA level in the BPE was significantly higher than the MPE group (P = .003). It showed that the area under the ROC curve (AUC) (0.816) of jointly detection pMK, pCA125, and pADA was significantly higher than other markers for the diagnosis of MPE. Therefore, joint detection of pMK + pCA125 + pADA suggested that the sensitivity, specificity, and AUC was 82.54%, 74.19% at the cutoff 0.47 and diagnostic performance was higher than others.

Conclusion: Joint detection of pMK + pCA125 + pADA can be used as a good indicator for the identification of MPE of NSCLC.

KEYWORDS

adenosine deaminase, malignant pleural effusion, Midkine, nonsmall cell lung cancer, pleural fluid carbohydrate antigen 125

1 | INTRODUCTION

Pleural effusion is a common clinical symptom and can be caused by a variety of diseases especially common in cancers. The malignant pleural effusion (MPE) caused by lung cancer is the most frequent in various cancers and can be the first presentation under many circumstances. Lung cancer is the world's second major cancer, with a high degree of invasion and metastasis, is one of the important causes of cancer-related deaths. Nonsmall cell lung cancer (NSCLC) includes squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, accounting for 85% of all lung cancer.[1] And the treatment effect is poor, 5-year survival rate of <15%.[2-4] Therefore, there is an urgent

need for some means of differential diagnosis of pleural effusion to distinguish between benign and malignant, to achieve better prognosis. Cytological examination can distinguish the nature of pleural effusion as a basis for diagnosis, but unfortunately, the positive rate is only between 11% and 78%.[5] Thoracoscopy can improve the diagnostic efficacy, that the sensitivity can reach about 90%.[6] However, this invasive inspection due to cost, risk, and technical requirements and other factors cannot be widely used in clinical practice.

Nowadays, the conventional test markers of pleural effusion including pleural fluid adenosine deaminase (pADA), pleural fluid lactate dehydrogenase (pLDH), pleural fluid glucose (pGLU), pleural fluid ferritin (pFER), pleural fluid carbohydrate antigen 19-9 (pCA199), pleural fluid carbohydrate antigen 125 (pCA125), pleural effusion white cell count (pWBC), pleural effusion red cell count (pRBC), but those markers all have a low sensitivity and specificity. In recent years, many researchers are looking for reliable biomarkers that can differentiate MPE.

Midkine (MK) is a heparin-binding growth factor, has angiogenic activity and can signal through ALK receptors,[7,8] and can prevent cell apoptosis and enhance cell viability and migration.[9] In recent years, it has been shown to be highly expressed in most cancers and play a central role in carcinogenesis, and is closely related to its aggressiveness.[9,10] Thus, MK has become a new marker that is expected to be used in the diagnosis and prognosis of multiple cancers.[11,12] X Xia's study showed that MK in the serum and urine from NSCLC patients had a high expression, can be used to determine the prognosis.[11]

In this study, we aim to explore the clinical role of MK joint with other routine markers in the differential diag of carbohydrate antigen 19nosis of malignant pleural effusion caused by NSCLC and the possibility of impact on tumor metastasis, to guide proper treatment and achieve a better prognosis.

2 | MATERIALS AND METHODS

Pleural effusion specimens were prospectively collected from 125 patients from the First Affiliated Hospital of Wenzhou Medical College, China, from March 2016 to October 2017, 63 were shown to have MPE from NSCLC diagnosed by histologic and immunohistologic analyses. The other 62 patients had benign pleural effusion (BPE) resulting from a disease such as tuberculosis, pneumonia or other lung infections. The MPE specimens consisted of 30 men and 33 women, aged between 36-91 years (67.16 ± 11.60). The BPE specimens consisted of 44 men and 18 women, aged between 21-96 years (60.55 ± 18.14). Statistical analysis showed no significant differences in age and gender composition among 3 groups ($P > .05$). In addition, MPE specimens in accordance with the clinical variables, including age, gender, the status of lymph node metastasis and distant metastasis, cigarette smoking, and the histological type. These cases were confirmed by X-ray, chest CT, pathology, pleural biopsy, fiberoptic bronchoscopy, pleural fluid cytology, and so on. The criteria used for the histopathologic diagnosis of NSCLC were the World Health Organization/International Association for the Study of Lung Cancer lung cancer histologic classification standards. Exclusion criteria included hyperlipidemia, coronary heart disease, central nervous system diseases such as intracranial tumors or Alzheimer disease, cholelithiasis, and liver diseases.

Pleural fluid (10 mL) was collected from patients by routine thoracentesis, centrifuged (4°C, 603.72 *g*, 10 minutes) after routine testing, and then the separated supernatant was refrigerated at −20°C. This study was approved by the Institutional Ethics Review Board of the First Affiliated Hospital of Wenzhou Medical College, and all patients provided written informed consent to participate in this study.

Level of carbohydrate antigen 19.9 (CA19.9), carbohydrate antigen 125 (CA125), and ferritin (FER) in pleural fluid was detected by DXI800 Immunoassay analyzer (Beckman, Brea, CA, USA). ADA, LDH, and GLU in pleural fluid were determined by Beckman Coulter AU5800 Clinical Chemistry Analyzer (Beckman). The white blood cell (WBC) and red blood cell (RBC) counts were detected by Niu Bao's counting board. The levels of MK were determined by ELISA kit (USCN Life Science Inc, Wuhan, China).

The Shapiro–Wilk test was used to evaluate the distribution of data. A comparison between the 2 groups was performed using the Mann-Whitney *U* test or *t* test. Differences between categorical variables were tested with the chi-square test. A value of $P < .05$ was statistically significant. Receiver operating characteristic curves (ROC) was analyzed, and the area under the ROC curve (AUC) was used to evaluate the ability. All statistical analyses were performed with SPSS 23.0 (Statistical Package for the Social Sciences Corporation, Chicago, IL, USA) and MedCalc (MedCalc Software, Ostend, Belgium).

3 | RESULTS

All of the parameters were not normally distributed as determined by the Shapiro-Wilk test. The results were further analyzed by the Mann-Whitney *U* test. The pMK, pCA125, pMK + pCA125 level and pMK + pCA125 + pADA level in the MPE were significantly higher than the BPE group (Mann-Whitney $U = 1362.000$, 1126.000, 929.000, 719.000 $P = .003$, .000, .000, .000). The pADA level in the BPE was significantly higher than the MPE group (Mann-Whitney $U = 1349.000$, $P = .003$). The pLDH, pGLU, pWBC, pRBC, pFER, pCA199 in the MPE showed no significant difference when compared with the BPE group (see Figure 1 and Table 1).

The ROC analysis shows that the diagnostic sensitivities of pMK, pGLU, pWBC, pFER, pCA125, pCA199, pMK + pCA125, pMK + pCA125 + pADA for MPE were 58.73%, 68.25%, 66.67%, 73.02%, 85.71%, 31.75%, 79.37%, 82.54%, respectively. The specificities of those markers were 74.19%, 48.39%, 51.61%, 45.16%, 54.84%, 88.71%, 64.52%, 74.19%, respectively, for the diagnosis of MPE. And the AUCs of those parameters were 0.651, 0.546, 0.554, 0.551, 0.712, 0.587, 0.762, 0.816, which found that the AUC (0.816) for joint detection of pMK, pCA125 and pADA (cutoff = 0.47) was significantly higher than the other markers, and the diagnostic

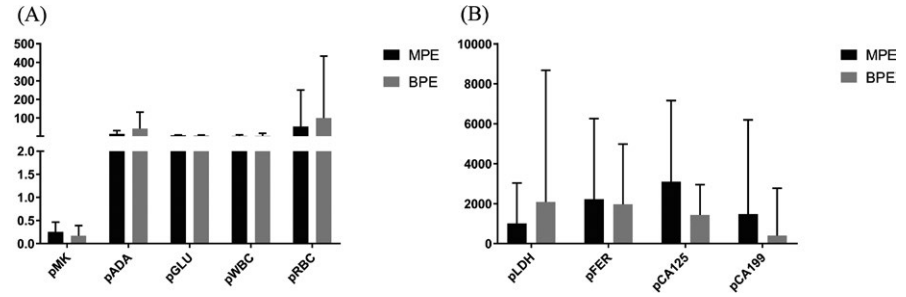

FIGURE 1 Comparison of the parameters in pleural effusion from both groups. A, The pMK level in the malignant pleural effusion (MPE) group was significantly higher than which in the benign pleural effusion (BPE) group. The pADA level in the BPE group was obviously higher than witch in the MPE group. B, The pleural fluid CA125 level in MPE was markedly higher than that in the BPE group. pADA, pleural fluid adenosine deaminase; pMK, pleural fluid Midkine

TABLE 1 Comparison of the parameters in pleural effusion in the 2 groups

	BPE	MPE	Mann-Whitney *U* test	*P*
pMK (ng/mL)	0.1 (0.02-1.52)	0.16 (0.01-0.85)	1362.000	.003
pADA (U/mL)	18.5 (0.96-517)	11 (3-96)	1349.000	.003
pLDH (U/mL)	489.5 (76-43 154)	402 (133-10 461)	1804.000	.462
pGLU (mmol/L)	5.755 (0.02-13.36)	6.15 (0.03-15.16)	1775.000	.379
pWBC (10^3/μL)	1.075 (0.03-100.8)	1.38 (0.09-47.04)	1741.000	.295
pRBC (10^3/μL)	6.18 (0.01-1770)	3.76 (0.11-1450)	1834.500	.558
pFER (μg/L)	950.65 (111-15 000)	1218.1 (90-27 326)	1755.000	.328
pCA125 (U/mL)	941.05 (2.2-6743.4)	2185 (127.6-26 464.8)	1126.000	.000
pCA199 (U/mL)	3.195 (0.8-18 690)	8.8 (0.8-20 220)	1613.500	.093
pMK + pCA125	0.39 (0.23-0.92)	0.55 (0.25-1)	929.000	.000
pMK + pCA125 + pADA	0.35 (0-0.99)	0.64 (0.09-1)	719.000	.000

BPE, benign pleural effusion; MPE, malignant pleural effusions; pADA, pleural fluid adenosine deaminase; pFER, pleural fluid ferritin; pGLU, pleural fluid glucose; pLDH, pleural fluid lactate dehydrogenase; pMK, pleural fluid Midkine; pWBC, pleural effusion white cell count.
Median (min-max) in the parameters in the table.

sensitivity (82.54%) was obviously higher than pMK (58.73%) and pMK + pCA125 (79.37%), specificity (74.19%) was the same as pMK (74.19%; see Figure 2 and Table 2).

The relationship between expression level of MK in clinical variables was summarized in Table 3. No statistically significant difference was seen between level of MK and age ($P = .981$), gender ($P = .175$), cigarette smoking status ($P = .094$), the histological type ($P = .714$), the status of lymph node metastasis ($P = .113$) and distant metastasis ($P = .564$).

4 | DISCUSSION

In China, cancer (especially lung cancer) and tuberculosis are the main cause of pleural effusion. However, the prognosis and treatment approaches are evidently different, and it is extremely important for the differential diagnosis of BPE and MPE. The routine test item such as histopathology and cytology was the gold-standard methods, but an important limitation was those methods probably lacked of sensitivity. So, the diagnosis of BPE and MPE is still a clinical problem. In searching for new biological marker, we examined the expression of MK and other routine markers in pleural effusions.

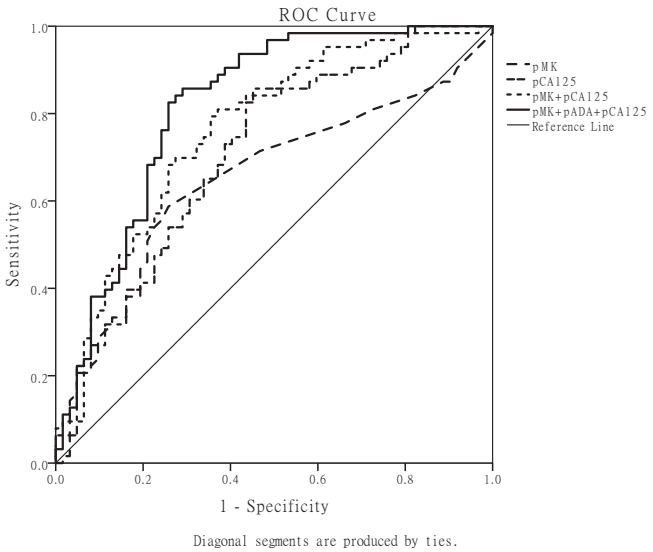

FIGURE 2 ROC curve of parameters for the diagnosis of MPE. At the cutoff value = 0.47, the sensitivity and specificity of joint detection of pMK, pADA, and pCA125 were 82.54%, 74.19% for the diagnosis of MPE, and the AUC (0.816) was the highest among all the other markers tested. AUC, area under the ROC curve; MPE, malignant pleural effusions; pADA, pleural fluid adenosine deaminase; pMK, pleural fluid Midkine

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

	AUC	95% confidence Interval	*P*	Cutoff	Sensitivity (%)	Specificity (%)
pMK (ng/mL)	0.651	0.553-0.750	.004	0.12	58.73	74.19
pADA (U/mL)	0.345	0.245-0.446	.003	13	73.02	62.9
pLDH (U/mL)	0.462	0.359-0.565	.462	468	63.49	53.23
pGLU (mmol/L)	0.546	0.444-0.647	.379	5.57	68.25	48.39
pWBC (10^3/μL)	0.554	0.452-0.656	.295	1.1	66.67	51.61
pRBC (10^3/μL)	0.470	0.368-0.571	.558	23.04	79.37	32.26
pFER (μg/L)	0.551	0.449-0.653	.328	748.3	73.02	45.16
pCA125 (U/mL)	0.712	0.621-0.802	.000	1073.3	85.71	54.84
pCA199 (U/mL)	0.587	0.486-0.688	.094	46.5	31.75	88.71
pMK + pCA125	0.762	0.678-0.846	.000	0.42	79.37	64.52
pMK + pCA125 + pADA	0.816	0.739-0.892	.000	0.47	82.54	74.19

AUC, area under the ROC curve; MPE, malignant pleural effusions; pADA, pleural fluid adenosine deaminase; pFER, pleural fluid ferritin; pGLU, pleural fluid glucose; pLDH, pleural fluid lactate dehydrogenase; pMK, pleural fluid Midkine; pWBC, pleural effusion white cell count.

TABLE 3 Comparison of the expression level of MK in MPE group and association with the clinical variables

Clinical variables	Number	MK (ng/mL)	Mann-Whitney *U* test	*P*
Ages (y)				
≥60	46	0.155 (0.01-0.85)	389.500	.981
<60	17	0.16 (0.02-0.68)		
Gender				
Male	30	0.125 (0.01-0.85)	396.500	.175
Female	33	0.16 (0.04-0.73)		
Cigarette smoking status				
Smoker	18	0.115 (0.02-0.62)	295.000	.094
Nonsmoker	45	0.17 (0.01-0.85)		
Histological type				
Squamous cell carcinoma	4	0.135 (0.1-0.31)	105.000	.714
Adeno carcinoma	59	0.16 (0.01-0.85)		
Lymph node metastasis				
Positive	34	0.18 (0.01-0.85)	378.000	.113
Negative	29	0.15 (0.04-0.66)		
Distant metastases				
Positive	38	0.15 (0.01-0.85)	434.000	.564
Negative	25	0.16 (0.04-0.73)		

MK, Midkine; MPE, malignant pleural effusions.
Median (min-max) in the parameters in the table.

Midkine, a growth factor, at the beginning was identified as the product of a retinoic acid responsive gene expressed during embryogenesis. MK is involved in the proliferation, invasion, and metastasis of tumor cells in cancer and has obvious regulation effect on tumor neovascularization.[13,14] In a variety of cancer, the expression was higher than normal tissue.[9,10] Recent years, enzyme immunoassays have been developed to detect this protein, and some reports were shown that an increase in the serum and urinary in patients with many kinds of cancer.[10,11,15]

Cancer Antigen-125 (CA125) is a glycoprotein which is expressed on the surfaces of ovarian cancer cells, which has been widely used as a primary screening index of human ovarian cancer. Subsequently, research shows that human mesothelial cells were also demonstrated to secrete CA125. However, the specificity of CA125 in the diagnosis of cancers remains controversial. Research by Li, X reported that CA125 was expressed in lung cancer tissues, with the expression level closely related to the degree of differentiation of the tumor.[16]

Adenosine deaminase (ADA) is widely distributed in various tissues of the body, with the highest content in the thymus, spleen and other lymphoid tissues. It can convert adenosine to inosine and therefore has some influence on the activity of lymphocytes.[17] The level of ADA in BPE has implications for the diagnosis of many diseases, especially pleural tuberculosis. However, the role of ADA in malignant pleural effusion is not clear. Some studies have shown that pleural effusion ADA can participate in the differential diagnosis of malignant pleural effusion and BPE.[18]

The size of AUC shows that the veracity of diagnostic tests. It has a lower diagnostic value when AUC is from 0.5 to 0.7 and has the higher diagnostic value when AUC > 0.8. In our study we found that at the cutoff 0.47, the sensitivity and specificity of the joint detection of pMK, pCA125 and pADA were 82.54% and 74.19% for the diagnosis of MPE and the AUC 0.816, giving it a significantly higher diagnostic value for MPE than the other studies indicators. The results of joint detection of pMK, pCA125, and pADA indicated that diagnostic performance was significantly higher than that of pMK or the joint detection of pMK and pCA125.

In our study, the results revealed that there was no evident relationship between the expression of MK in NSCLC-associated malignant pleural effusion and the clinical variables as age, gender, cigarette smoking status, the histological type, the status of lymph node metastasis or distant metastasis.

In summary, the joint detection of pMK, pCA125, and pADA has a high diagnostic efficacy for differential diagnosis of NSCLC-associated MPE, and hence, it is an important marker to determine the nature of pleural effusion, which can play a certain role in guiding suitable clinical treatment and the prognosis evaluation. Thus, joint detection of pMK, pCA125, and pADA has better diagnostic performance than pMK or the joint detection of pMK and pCA125, and all the 3 markers were simple and noninvasive, could be used in routine screening programs. Our study provides a new idea of noninvasive examination for the clinical diagnosis of NSCLC.

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