RESEARCH ARTICLE

CEA, AFP, CA125, CA153 and CA199 in Malignant Pleural Effusions Predict the Cause

Xin-Feng Wang[1,2,3], Yan-Hua Wu[3], Mao-Shui Wang[3]*, Yun-Shan Wang[1,2,4]*

Abstract

Determination of the cause of malignant pleural effusions is important for treatment and management, especially in cases of unknown primaries. There are limited biomarkers available for prediction of the cause of malignant pleural effusion in clinical practice. Hence, we evaluated pleural levels of five tumor biomarkers (CEA, AFP, CA125, CA153 and CA199) in predicting the cause of malignant pleural effusion in a retrospective study. Kruskal-Wallis or Mann-Whitney U tests were carried out to compare levels of tumor markers in pleural effusion among different forms of neoplasia - lung squamous cell carcinoma, adenocarcinoma, or small cell carcinoma, mesothelioma, breast cancer, lymphoma/leukemia and miscellaneous. Receiver operator characteristic analysis was performed to evaluate sensitivity and specificity of biomarkers. The Kruskal-Wallis test showed significant differences in levels of pleural effusion CEA ($P<0.01$), AFP ($P<0.01$), CA153 ($P<0.01$) and CA199 ($P<0.01$), but not CA125 ($P>0.05$), among the seven groups. Receiver operator characteristic analysis showed that, compared with other four tumor markers, CA153 was the best biomarker in diagnosing malignant pleural effusions of lung adenocarcinoma (area under curve (AUC): 0.838 (95% confidence interval: 0.787, 0.888); cut-off value: 10.2U/ml; sensitivity: 73.2% (64.4-80.8)%, specificity: 85.2% (77.8-90.8)%), lung squamous cell carcinoma (AUC: 0.716 (0.652, 0.780); cut-off value: 14.2U/ml; sensitivity: 57.6% (50.7-64.3)%, specificity: 91.2% (76.3-98.0)%), and small-cell lung cancer (AUC: 0.812 (0.740, 0.884); cut-off value: 9.7U/ml; sensitivity: 61.5% (55.0-67.8)%, specificity: 94.1% (71.2-99.0)%); CEA was the best biomarker in diagnosing MPEs of mesothelioma (AUC: 0.726 (0.593, 0.858); cut-off value: 1.43ng/ml; sensitivity: 83.7% (78.3-88.2)%, specificity: 61.1% (35.8-82.6)%) and lymphoma/leukemia (AUC: 0.923 (0.872, 0.974); cut-off value: 1.71ng/ml; sensitivity: 82.8% (77.4-87.3)%, specificity: 92.3% (63.9-98.7)%). Thus CA153 and CEA appear to be good biomarkers in diagnosing different causes of malignant pleural effusion. Our findings implied that the two tumor markers may improve the diagnosis and treatment for effusions of unknown primaries.

Keywords: Diagnosis - discrimination - malignant pleural effusion - tumor markers - unknown primary tumor

Asian Pac J Cancer Prev, **15 (1)**, 363-368

Introduction

In China, malignant disease involving the pleura is the second leading cause of pleural effusions (PEs) after tuberculosis. A study showed that, at Beijing, 49.6% of 668 PEs were due to Mycobacterium tuberculosis infection, malignancy accounting for 24.7% (Sun, 2011). Currently, Lung cancer and breast cancer account for 50-65% of malignant pleural effusions (MPEs). Lymphomas, tumors of the genitourinary tract and gastrointestinal tract account for a further 25%. Causes of unknown primary (CUP) are responsible for 7-15% of all malignant pleural effusions (Roberts et al., 2010).

To determine the cause of MPE is important for treating and managing MPE. Firstly, the most important factor influencing the life expectancy in patients with MPEs is the source of the tumor. The shortest survival time is observed in MPEs secondary to lung cancer and the longest in ovarian cancer, while MPEs due to an unknown primary have an intermediate survival time (Sears et al., 1987; van de Molengraft et al., 1989; Abbruzzese et al., 1994; Bonnefoi et al., 1996; Heffner et al., 2000), so determining the cause of MPE is useful to guess the survival time. Second, the main reason to determine the cause of MPE is to decide whether systemic chemotherapy is indicated. Systemic chemotherapy is effective at least in MPE patients with small cell carcinoma, breast carcinoma or lymphoma (Roberts et al., 2010). For differentiating causes of MPEs, especially CUP, light microscopy, immunocytochemistry, molecular profiling, endoscopy, and imaging have been evaluated by others, limitations of these common techniques are high cost, low sensitivity and selective application. Accordingly, economic rapid and accurate techniques for differentiating causes of MPE are urgently required.

Several tumor markers are produced by the limited

*[1]Institute of Basic Medicine, Shandong Academy of Medical Sciences, [2]School of Medical and Life Sciences, Jinan University, [3]Department of Lab Medicine, Shandong Provincial Chest Hospital, [4]Medical Research and Laboratory Diagnostic Center, Jinan Central Hospital Affiliated to Shandong University, Jinan, China *For correspondence: wangmaoshui@gmail.com, wys20739@126.com*

Table 1. Characteristics of Patients with Difference Causes of MPEs

	Lung adenocarcinoma	Lung squamous cancer cell	Breast carcinoma	Mesothelioma	Small-cell lung cancer	Lymphoma /leukemia	Miscellaneous tumors
Cases	128(60.0%)	34(13.5%)	11(4.4%)	18(7.2%)	17(6.8%)	13(5.2%)	30(12.0%)
Age (Mean±SD)	59.64±13.54	65.24±10.02	51.73±8.73	53.61±13.43	59.94±11.50	44.13±18.70	54.67±13.04
Sex (male)	70(54.7%)	30(88.2%)	0(0%)	8(44.4%)	12(70.6%)	7(53.8%)	20(66.7%)
CEA(ng/ml)	375.2±36.9	104.3±46.3	109.4±80.0	11.6±7.1	31.7±16.6	0.9±0.3	302.3±210.9
AFP(ng/ml)	2.12±0.11	1.61±0.19	1.75±0.27	2.55±1.10	1.48±0.17	1.14±0.22	6.72±2.94
CA125(U/ml)	2168.6±145.3	1421.0±202.9	1293.0±256.7	2020.2±411.0	2531.6±125.2	1917.3±366.6	2706.6±1046.8
CA153(U/ml)	138.6±19.3	8.7±1.3	113.7±90.3	21.3±6.7	5.3±0.8	8.2±4.0	45.4±15.0
CA199(U/ml)	516.6±72.4	74.4±59.2	86.8±77.3	181.5±116.5	16.3±11.0	4.2±1.8	354.6±142.7

Data were expressed as mean ± SEM; CEA, carcinoembryonic antigen; AFP, alpha-fetoprotein; CA125, cancer antigen 125; CA153, cancer antigen 153; CA199, cancer antigen 199

organ and have the advantage for the determination of the origin. Some markers were classified as oncofetal antigens, such as carcinoembryonic antigen (CEA), alpha-fetoprotein (AFP). Some markers classified as tumor associated antigens are produced by various organs and are not specific to cancer but associated with cancer, such as, cancer antigen (CA) 125, CA153, CA199 (Watanabe, 1996; Malati, 2007). Now, levels of pleural effusion CEA, AFP, CA125, CA153 and CA199 have been assayed in many studies focusing on differentiating benign and malignant pleural effusions (Botte et al., 1990; Cascinu et al., 1997; Porcel et al., 2004; Shitrit et al., 2005; Gaspar et al., 2008; Liang et al., 2008). But there is as yet no evidence that PE tumor markers were employed in predicting the cause of MPE.

In this study, we aimed to measure levels of five PE tumor markers in patients with different causes of MPEs and evaluated their diagnostic values in differentiation of different causes of MPEs.

Materials and Methods

Subjects

This study has been conducted by the Department of Lab Medicine, Shandong Provincial Chest Hospital, Jinan, Shandong Province. The study protocol was approved by the Ethical Committee of the Shandong Provincial Chest Hospital. Written informed consent was not required because of the retrospective nature of the investigation. Between August 2010 and June 2013, patients who accepted PE tumor markers measurements were enrolled in this retrospective study. Subsequently, the patients were included if they met the following criteria: the examinations of PE or biopsy specimens revealed underlying malignancy; primary tumors were confirmed histologically.

Sample collection and analysis

All PE samples were collected before any treatment was initiated within 24 h after hospitalization. PEs were centrifuged at 4°C 1200 r/min for 15min, and the supernatants were processed for analyzing CEA, AFP, CA125, CA153 and CA199 on UniCel DxI 800 immunoassay system (Beckman Coulter, Brea, CA).

Statistical analysis

Statistical analysis was carried out using SPSS 17.0 software and MedCalc Version 8.0.1.0. Data were expressed as mean ±standard error of the mean (SEM). Non-Parametric tests were used since tumor markers data were skewed distributed as determined by the Kolmogorov-Smirnov test. Comparisons of data between different groups were performed using Kruskal–Wallis test or Mann-Whitney U test. Receiver operator characteristic (ROC) analysis was performed to evaluate sensitivity and specificity of biomarker (s), a cut-off point was determined as the value of the parameter that maximized the sum of specificity and sensitivity. positive and negative likelihood ratio were also determined. A P value <0.05 was considered statistically significant.

Results

Of 286 PEs studied, 35 were excluded due to unknown primary origin, and the remaining 251 PEs were included in our study. All patients were hospitalised. The study population had a mean age of 58.25±13.77 years (range 5 to 89 years), and 58.6% were male. Table 1 presents the mean age, sex and other characteristics of every evaluated group. It showed that lung adenocarcinoma (128 cases, 60.0%) was the main cause of malignant pleural effusions, followed by lung squamous cell carcinoma (34 cases, 13.5%), miscellaneous tumors (30 cases, 12.0%), mesothelioma (18 cases, 7.2%), small-cell lung cancer (17 cases, 6.8%), lymphoma/leukemia (13 cases, 5.2%) and breast cancer (11 cases, 4.4%).

Kruskal-Wallis test showed significant difference in levels of PE CEA (χ^2=87.37, P<0.01), AFP (χ^2=18.32, P<0.01), CA153 (χ^2=95.957, P<0.01) and CA199 (χ^2=37.53, P<0.01) among the seven groups, except CA125 (χ^2=7.68, P>0.05).

CEA, AFP, CA153 and CA199 in MPEs

The levels of CEA, AFP, CA153 and CA199 in PE of patients with different causes are presented in Table 1. Mann–Whitney U test for differences between the studied groups in PE tumor markers (CEA, AFP, CA153 and CA199) were performed, and P values were presented in Table 2 for each tumor markers. Some biomarkers just existed statistical difference between two groups. Such as, levels of PE AFP in small-cell lung cancer patients (1.48±0.17ng/ml), just existed statistical difference (P<0.05) with the lung adenocarcinoma group (2.12±0.11ng/ml) and showed no difference with other

Table 2. Results (*P* values) of Mann–Whitney U Test for Differences between the Studied Groups in Tumor Markers

		Lung squamous cell carcinoma	Breast cancer	Mesothelioma	Small-cell lung cancer	Lymphoma /leukemia	Miscellaneous tumors
Lung adenocarcinoma	CEA	0	0.007	0	0	0	0
	AFP	0.012	0.402	0.052	0.033	0.003	0.938
	CA153	0	0.049	0	0	0	0
	CA199	0.01	0.048	0.026	0	0	0.019
Lung squamous cell carcinoma	CEA		0.99	0.018	0.047	0	0.044
	AFP		0.544	0.81	0.905	0.114	0.134
	CA153		0.011	0.538	0.019	0.048	0.647
	CA199		0.802	0.985	0.208	0.007	0.798
Breast cancer	CEA			0.044	0.1	0	0.103
	AFP			0.492	0.458	0.072	0.571
	CA153			0.225	0.002	0.009	0.106
	CA199			0.822	0.145	0.015	0.965
Mesothelioma	CEA				0.287	0.025	0.383
	AFP				0.909	0.082	0.201
	CA153				0.069	0.066	0.848
	CA199				0.306	0.025	0.865
Small-cell lung cancer	CEA					0	0.782
	AFP					0.198	0.163
	CA153					0.558	0.18
	CA199					0.096	0.207
Lymphoma/leukemia	CEA						0.002
	AFP						0.009
	CA153						0.096
	CA199						0.008

CEA, carcinoembryonic antigen; AFP, alpha-fetoprotein; CA125, cancer antigen 125; CA153, cancer antigen 153; CA199, cancer antigen 199

Table 3. ROC Analysis for Tumor Markers in Diagnosing Different Causes of MPEs

	CA153			CA199			AFP			CEA		
	AUC	*P*	95%CI	AUC	*P*	95%CI	AUC	*P*	95%CI	AUC	*P*	95%CI
Lung adenocarcinoma	0.838	0	0.787,0.888	0.698	0	0.633,0.763	0.614	0.002	0.544,0.684	0.811	0	0.758,0.864
Lung squamous cell carcinoma	0.716	0	0.652,0.780	0.589	0.095	0.499,0.679	0.59	0.093	0.492,0.687	0.574	0.164	0.491,0.658
Breast cancer	0.514	0.878	0.364,0.664	0.438	0.485	0.316,0.560	0.484	0.86	0.324,0.644	0.446	0.544	0.321,0.570
Mesothelioma	0.635	0.056	0.513,0.758	0.572	0.311	0.437,0.707	0.588	0.216	0.451,0.724	0.726	0.001	0.593,0.858
Small-cell lung cancer	0.812	0	0.740,0.884	0.681	0.012	0.580,0.783	0.602	0.162	0.473,0.730	0.693	0.008	0.588,0.798
Lymphoma/leukemia	0.803	0	0.687,0.920	0.801	0	0.690,0.912	0.723	0.007	0.565,0.882	0.923	0	0.872,0.974

groups (all $P>0.05$). Some biomarkers existed statistical difference between three, four or more groups, such as, levels of PE CA199 in patients with mesothelioma (181.5±116.5U/ml) showed statistical difference ($P<0.05$) with lung adenocarcinoma group (516.6±72.4U/ml) and lymphoma/leukemia group (4.2±1.8U/ml), and no statistical difference with other groups (all $P>0.05$); levels of PE CEA in patients with breast cancer (109.4±80.0ng/ml) just had statistical difference ($P<0.05$) with lung adenocarcinoma group (375.2±36.9 ng/ml), mesothelioma group (11.6±7.1 ng/ml), and lymphoma/leukemia group (0.9±0.3 ng/ml); levels of PE CA199 in lymphoma/leukemia group (4.2±1.8 U/ml) had statistical difference ($P<0.05$) with lung adenocarcinoma group (516.6±72.4 U/ml), lung squamous cell carcinoma group (74.4±59.2 U/ml), breast cancer group (86.8±77.3 U/ml), mesothelioma group (181.5±116.5 U/ml), and miscellaneous tumors group (354.6±142.7 U/ml).

Excitingly, levels of PE CEA in lung adenocarcinoma group (375.2±36.9 ng/ml) had statistical difference (all $P<0.05$) with other six groups, including lung squamous cell carcinoma group (104.3±46.3 ng/ml), breast cancer group (109.4±80.0 ng/ml), mesothelioma group (11.6±7.1 ng/ml) small-cell lung cancer group (31.7±16.6 ng/ml) lymphoma/leukemia group (0.9±0.3 ng/ml) and miscellaneous tumors group (302.3±210.9 ng/ml); levels of PE CEA in lymphoma/leukemia group also had statistical difference (all $P<0.05$) with other six groups; level of PE CA153 in lung adenocarcinoma group (138.6±19.3 U/ml) was statistically different (all $P<0.05$) from other groups, such as, lung squamous cell carcinoma group (8.7±1.3 U/ml), breast cancer group (113.7±90.3 U/ml), mesothelioma group (21.3±6.7 U/ml), small-cell lung cancer group (5.3±0.8 U/ml) lymphoma/leukemia group (8.2±4.0 U/ml) and miscellaneous tumors group (45.4±15.0 U/ml); It also showed significant difference (all $P<0.05$) in levels of PE CA199 between lung adenocarcinoma group (516.6±72.4 U/ml) and the remaining groups, such as, lung squamous cell carcinoma group (74.4±59.2 U/ml), breast cancer group (86.8±77.3 U/ml), mesothelioma group (181.5±116.5 U/ml), small-cell lung cancer group (16.3±11.0 U/ml), lymphoma/leukemia group (4.2±1.8 U/ml) and miscellaneous tumors group (354.6±142.7 U/ml).

Diagnostic performance of tumor markers in differentiation of different causes of MPEs

The capacity of tumor markers to differentiate

Table 4. CA153 and CEA as Candidate Tumor Markers in Diagnosing the Cause of MPE

	Tumor marker	Cut-off	Sensitivity (95% CI)	Specificity (95% CI)	Positive likelihood ratio	Negative likelihood ratio
Lung adenocarcinoma	CA153	10.2 U/ml	73.2(64.4-80.8)	85.2(77.8-90.8)	4.93	0.32
Lung squamous cell carcinoma	CA153	14.2 U/ml	57.6(50.7-64.3)	91.2(76.3-98.0)	6.53	0.46
Mesothelioma	CEA	1.43 ng/ml	83.7(78.3-88.2)	61.1(35.8-82.6)	2.15	0.27
Small-cell lung cancer	CA153	9.7 U/ml	61.5(55.0-67.8)	94.1(71.2-99.0)	10.46	0.41
Lymphoma/leukemia	CEA	1.71ng/ml	82.8(77.4-87.3)	92.3(63.9-98.7)	10.76	0.19

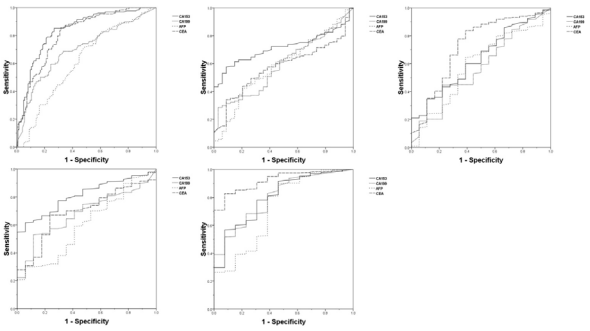

Figure 1. ROC Curves Showing Discrimination Between Malignant Pleural Effusion Patients with Lung Adenocarcinoma Vs Other Causes (Top Left) (CA153, AUC: 0.838 (95%CI: 0.787, 0.888); CA199, AUC: 0.698 (95%CI: 0.633, 0.763); AFP, AUC: 0.614 (95%CI: 0.544, 0.684); CEA, AUC: 0.811 (95%CI: 0.758, 0.864)); lung squamous cell carcinoma vs other causes (top middle) (CA153, AUC: 0.716 (95%CI: 0.652, 0.780); CA199, AUC: 0.589 (95%CI: 0.499, 0.679); AFP, AUC: 0.590 (95%CI: 0.492.0.687); CEA, AUC: 0.574 (95%CI: 0.491, 0.658)); Mesothelioma vs other causes (top right) (CA153, AUC: O.635 (95%CI: 0.513, 0.758); CA199, AUC: 0.572 (95%CI: 0.437, 0.707); AFP, AUC: 0.588 (95%CI: 0.451, 0.724); CEA, AUC: 0.726 (95%CI: 0.593, 0.858)); Small-cell lung cancer vs other causes (bottom left) (CA153, AUC: 0.812 (95%CI: 0.740, 0.884); CA199, AUC: 0.681 (95%CI: 0.580, 0.783); AFP, AUC: 0.602 (95%CI: 0.473, 0.730); CEA, AUC: 0.693 (95%CI: 0.588, 0.798)); Lymphoma/leukemia vs other causes (bottom middle) (CA153, AUC: 0.803 (95%CI: 0.687, 0.920); CA199, AUC: 0.801 (95%CI: 0.690, 0.912); AFP, AUC: 0.723 (95%CI: 0.565, 0.882); CEA, AUC: 0.923 (95%CI: 0.872, 0.974))

causes of MPEs was assessed with receiver operating characteristic curve analysis. The area under curve (AUC) when tumor markers were used to differentiate causes of MPEs was presented in Table 3. Cut-off values, positive and negative likelihood ratio (P/NLR) were determined (Table 4). It showed that, compared with other tumor markers, CA153 was the best biomarker in diagnosing pleural effusion of lung adenocarcinoma (AUC: 0.838, 95%confidence interval (CI): 0.787, 0.888; cut-off value: 10.2U/ml; sensitivity: 73.2% (64.4-80.8)%, specificity: 85.2% (77.8-90.8)%, PLR: 4.93, NLR: 0.32), lung squamous cell carcinoma (AUC: 0.716, 95%CI: 0.652, 0.780; cut-off value: 14.2U/ml; sensitivity: 57.6% (50.7-64.3)%, specificity: 91.2% (76.3-98.0)%, PLR: 6.53, NLR: 0.46), and small-cell lung cancer (AUC: 0.812, 95%CI: 0.740, 0.884; cut-off value: 9.7 U/ml; sensitivity: 61.5% (55.0-67.8)%, specificity: 94.1% (71.2-99.0)%, PLR: 10.46, NLR: 0.41); CEA was the best biomarker in diagnosing pleural effusion of mesothelioma (AUC: 0.726, 95%CI: 0.593, 0.858; cut-off value: 1.43 ng/ml; sensitivity: 83.7% (78.3-88.2)%, specificity: 61.1% (35.8-82.6)%, PLR: 2.15, NLR: 0.27) and lymphoma/leukemia (AUC: 0.923, 95%CI: 0.872, 0.974; cut-off value: 1.71ng/ml; sensitivity: 82.8% (77.4-87.3)%, specificity: 92.3% (63.9 -98.7)%, PLR: 10.76, NLR: 0.19) among the four biomarkers (Figure 1). Meanwhile, data implied that these tumor markers weren't accurate in diferentiating pleural effusion of breast cancer from other causes.

Discussion

To determine the cause of malignant pleural effusion (MPE) is important for treating and managing MPEs. In this study, we measured levels of five PE tumor biomarkers (CEA, AFP, CA125, CA153 and CA199) for differentiating causes of MPEs. Our data showed that significant difference existed in levels of PE CEA (P<0.01), AFP (P<0.01), CA153 (P<0.01) and CA199 (P<0.01) among the seven groups of MPE patients, except CA125 (P>0.05). ROC analysis showed that, CA153 was the best biomarker in diagnosing MPEs of lung adenocarcinoma, lung squamous cell carcinoma, and small-cell lung cancer; CEA was the best biomarker in diagnosing MPEs of mesothelioma and lymphoma/leukemia. These data implied that PE CA153 and CEA can be used to predict the cause of MPE, the two markers would improve to find primary tumor in CUP cases.

The new clinical practice guidelines published by ESMO for the diagnosis, treatment and follow-up of cancers of unknown primary site set out what you need to know in order to manage these patients (Pavlidis et al., 2010). They point out that it is essential to differentiate clinical and pathological subsets of CUP. Light microscopy is the traditional method for MPE diagnosis, but an adequate sample of tumor tissue is essential. Immunocytochemistry is an adjunct to the cytological diagnosis of metastatic carcinomas in MPE (Pomjanski et al., 2005; Zhu et al., 2007; Bocking et al., 2009; Elstrand et al., 2009; Liu et al., 2012). Molecular profiling is very useful (Davidson et al., 2007; Cheng et al., 2008; Jiang et al., 2008; Pu et al., 2008; Liu et al., 2012), it may aid in the diagnosis of the putative primary tumor site in some patients. However, their impact on patient outcome via administration of primary site-specific therapy remains questionable and unproven in prospective trials. Endoscopy is sometimes useful, but not in all patients. Its use should be guided by specific symptoms or signs. For example, ENT panendoscopy (cervical node involvement), bronchoscopy (positive chest X-ray or CT scan with a cough). In terms of imaging, a routine chest radiograph is part of the initial evaluation of the patient with CUP. CT scan and MRI are useful, especially in the detection of primary breast tumors. A recent meta-analysis

(Kwee et al., 2009) showed that, overall, FDG-PET/CT was able to detect 37% of primary tumors in patients with CUP, with both sensitivity and specificity of 84%. On the other hand, it should be realized that FDG-PET/CT is an expensive examination, and false-positive FDG-PET/CT findings may result in unnecessary additional invasive diagnostic procedures, which have associated morbidities and costs (Kwee et al., 2008). Based on these problems, tumor markers may be the efficient, cost-effective diagnostic alternative method in differnetiating causes of MPEs, including CUP.

Now, there was very little evidence about the use of tumor markers in the diagnosis of primary tumors in CUP patients. Patients with CUP should have serum human chorionic gonadotropin β (β-HCG), AFP and prostate-specific antigen (PSA) tested (in men) to exclude treatable extragonadal germ-cell tumors and to identify metastatic prostate cancer amenable to endocrine treatment (Losa Gaspa et al., 2002; Tsukushi et al., 2006; Destombe et al., 2007). High levels of serum thyroglobulin in CUP patients with bone metastases suggest an occult thyroid cancer. Serum CA153 and CA125 could be of some help, i.e. in isolated axillary node adenocarcinomas and in peritoneal papillary adenocarcinomatosis, respectively. In all other cases, routine evaluation of commonly used epithelial serum tumor markers (CEA, CA199, CA 153, CA125) has no proven prognostic or diagnostic value, and non-specific elevations of multiple markers occurs in the majority of CUP patients (Panza et al., 1987; Pavlidis et al., 1994). Currently, there are few studies on PE tumor markers in detection of CUP, meanwhile, few tumor markers are evaluated in diagnosing different causes of MPEs. So, this retrospective study was performed and aimed to compare levels of PE tumor markers between different causes of MPEs. ROC analysis showed the good performance of tumor markers in differentiation of MPEs. According to our data, PE tumor marker offered an efficient, cost-effective diagnostic alternative method in differential diagnosis of causes of MPEs.

The results of this study must be interpreted with caution because of its retrospective nature. Since our hospital was a tuberculosis referral hospital in China, the disease spectrum in this study was not similar like other reports (Cellerin et al., 2008; Roberts et al., 2010), the disease spectrum can directly affect PE tumor markers' diagnostic parameters (AUC, sensitivity and specificity) in differentiating causes of MPEs. Meanwhile, in some groups (such as, breast cancer, mesothelioma, small-cell lung cancer and lymphoma/leukemia), number of evaluated cases were small. Hence, a future approach will be to analyze levels of PE tumor markers in a prospective larger cohort of samples to ascertain their levels in MPE patients and therefore to validate their suitability as candidate biomarkers in differentiating MPEs.

In conclusion, we have demonstrated that CA153 and CEA can be used to differentiating causes of MPEs, such as, lung adenocarcinoma, lung squamous cell carcinoma, small-cell lung cancer, mesothelioma and lymphoma/leukemia. We believe our findings would aid to find the primary tumor in CUP cases. In the future, a prospective larger cohort study needs to be performed to ascertain levels of PE tumor markers in MPE patients and therefore to validate their suitability as candidate biomarkers in differentiating causes of MPEs.

Acknowledgements

This work was supported in part by a grant from the Health Department of Shandong Province (NO. 2011HZ085); in part by a grant from the Science and Technology Department of Jinan (NO. 201303043).

References

Abbruzzese JL, Abbruzzese MC, Hess KR, et al (1994). Unknown primary carcinoma: natural history and prognostic factors in 657 consecutive patients. *J Clin Oncol*, **12**, 1272-80.

Bocking A, Pomjansky N, Buckstegge B, et al (2009). Immunocytochemical identification of carcinomas of unknown primaries on fine-needle-aspiration-biopsies. *Pathologe*, **30**, 158-60.

Bonnefoi H, Smith IE (1996). How should cancer presenting as a malignant pleural effusion be managed? *Br J Cancer*, **74**, 832-5.

Botte G, Laferrere L, Etchepare S, et al (1990). Diagnostic value of tumor markers in pleural effusions. *Medicina*, **50**, 213-6.

Cascinu S, Del Ferro E, Barbanti I, et al (1997). Tumor markers in the diagnosis of malignant serous effusions. *Am J Clin Oncol*, **20**, 247-50.

Cellerin L, Marcq M, Sagan C, et al (2008). Malignant pleural effusion as the presenting site of cancer: comparison with metastatic pleural effusions from known cancers. *Rev Mal Respir*, **25**, 1104-9.

Cheng M, Chen Y, Yu X, et al (2008). Diagnostic utility of LunX mRNA in peripheral blood and pleural fluid in patients with primary non-small cell lung cancer. *BMC Cancer*, **8**, 156.

Davidson B, Baekelandt M, Shih IeM (2007). MUC4 is upregulated in ovarian carcinoma effusions and differentiates carcinoma cells from mesothelial cells. *Diagn Cytopathol*, **35**, 756-60.

Destombe C, Botton E, Le Gal G, et al (2007). Investigations for bone metastasis from an unknown primary. *Joint Bone Spine*, **74**, 85-9.

Elstrand MB, Kleinberg L, Kohn EC, et al (2009). Expression and clinical role of antiapoptotic proteins of the bag, heat shock, and Bcl-2 families in effusions, primary tumors, and solid metastases in ovarian carcinoma. *Int J Gynecol Pathol*, **28**, 211-21.

Gaspar MJ, De Miguel J, Garcia Diaz JD, et al (2008). Clinical utility of a combination of tumour markers in the diagnosis of malignant pleural effusions. *Anticancer Res*, **28**, 2947-52.

Heffner JE, Nietert PJ, Barbieri C (2000). Pleural fluid pH as a predictor of survival for patients with malignant pleural effusions. *Chest*, **117**, 79-86.

Jiang B, Wu GP, Zhao YJ, et al (2008). Transcription expression and clinical significance of TTF-1 mRNA in pleural effusion of patients with lung cancer. *Diagn Cytopathol*, **36**, 849-54.

Kwee TC, Takahara T, Ochiai R, et al (2008). Diffusion-weighted whole-body imaging with background body signal suppression (DWIBS): features and potential applications in oncology. *Eur Radiol*, **18**, 1937-52.

Kwee T C, Kwee RM (2009). Combined FDG-PET/CT for the detection of unknown primary tumors: systematic review and meta-analysis. *Eur Radiol*, **19**, 731-44.

Liang QL, Shi HZ, Qin XJ, et al (2008). Diagnostic accuracy of tumour markers for malignant pleural effusion: a meta-

analysis. *Thorax*, **63**, 35-41.
Liu L, Cohen C, Siddiqui M T (2012). Thyroid transcription factor 1 and napsin a double staining in lung adenocarcinoma in pleural fluid. *Acta Cytol*, **56**, 425-30.
Losa Gaspa F, Germa JR, Albareda JM, et al (2002). Metastatic cancer presentation. Validation of a diagnostic algorithm with 221 consecutive patients. *Rev Clin Esp*, **202**, 313-9.
Malati T (2007). Tumour markers: An overview. *Indian J Clin Biochem*, **22**, 17-31.
Panza N, Lombardi G, De Rosa M, et al (1987). High serum thyroglobulin levels. Diagnostic indicators in patients with metastases from unknown primary sites. *Cancer*, **60**, 2233-6.
Pavlidis N, Kalef-Ezra J, Briassoulis E, et al (1994). Evaluation of six tumor markers in patients with carcinoma of unknown primary. *Med Pediatr Oncol*, **22**, 162-7.
Pavlidis N, Briasoulis E, Pentheroudakis G (2010). Cancers of unknown primary site: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*, **21**, v228-31.
Pomjanski N, Grote HJ, Doganay *P*, et al (2005). Immunocytochemical identification of carcinomas of unknown primary in serous effusions. *Diagn Cytopathol*, **33**, 309-15.
Porcel JM, Vives M, Esquerda A, et al (2004). Use of a panel of tumor markers (carcinoembryonic antigen, cancer antigen 125, carbohydrate antigen 15-3, and cytokeratin 19 fragments) in pleural fluid for the differential diagnosis of benign and malignant effusions. *Chest*, **126**, 1757-63.
Pu RT, Giordano TJ, Michael CW (2008). Utility of cytology microarray constructed from effusion cell blocks for immunomarker validation. *Cancer*, **114**, 300-6.
Roberts ME, Neville E, Berrisford RG, et al (2010). Management of a malignant pleural effusion: British Thoracic Society Pleural Disease Guideline 2010. *Thorax*, **65**, ii32-40.
Sears D, Hajdu SI (1987). The cytologic diagnosis of malignant neoplasms in pleural and peritoneal effusions. *Acta Cytol*, **31**, 85-97.
Shitrit D, Zingerman B, Shitrit AB, et al (2005). Diagnostic value of CYFRA 21-1, CEA, CA 19-9, CA 15-3, and CA 125 assays in pleural effusions: analysis of 116 cases and review of the literature. *Oncologist*, **10**, 501-7.
SUN Yongchang, HU Hong, XIA Guoguang, XU Wenbing, WANG Hongwu (2011). An invesigation on diagnosis of pleural effusions in some teaching hospitals at Beijing district. *Chin J Tube Resp Dise*, **34**, 233-.
Tsukushi S, Katagiri H, Kataoka T, et al (2006). Serum tumor markers in skeletal metastasis. *Jpn J Clin Oncol*, **36**, 439-44.
van de Molengraft FJ, Vooijs GP (1989). Survival of patients with malignancy-associated effusions. *Acta Cytol*, **33**, 911-6.
Watanabe N (1996). Organ specificity of tumor markers and its application for clinical diagnosis. *Nihon Rinsho*, **54**, 1592-6.
Zhu W, Michael CW (2007). WT1, monoclonal CEA, TTF1, and CA125 antibodies in the differential diagnosis of lung, breast, and ovarian adenocarcinomas in serous effusions. *Diagn Cytopathol*, **35**, 370-5.