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Pleural fluid tumour markers in malignant pleural effusion with inconclusive cytologic results

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ABSTRACT

Background The presence of tumour cells in pleural fluid or tissue defines an effusion as malignant. Cytology analysis of the pleural fluid has about 60% diagnostic sensitivity. Several tests have been proposed to improve diagnosis—among them, the concentrations of tumour markers in pleural fluid. We evaluated whether the concentrations of tumour markers in pleural fluid could improve the diagnosis of malignant pleural effusion (MPE) when cytology is doubtful.

Methods Lymphocytic pleural fluids secondary to tuberculosis or malignancy from 156 outpatients were submitted for cytology and tumour marker quantification [carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3), carbohydrate antigen 19-9 (CA19-9), cancer antigen 72-4 (CA72-4), cancer antigen 125 (CA125), and CYFRA 21-1). One-way analysis of variance, the Student t-test or Mann–Whitney test, and receiver operating characteristic curves were used in the statistical analysis.

Results Concentrations of the tumour markers CEA, CA15-3, CA125, and CYFRA 21-1 were higher in MPEs than they were in the benign effusions ($p < 0.001$), regardless of cytology results. The markers CA19-9 and CA72-4 did not discriminate malignant from benign effusions. When comparing the concentrations of tumour markers in MPEs having positive, suspicious, or negative cytology with concentrations in benign effusions, we observed higher levels of CEA, CA15-3, CYFRA 21-1, and CA125 in malignant effusions with positive cytology ($p = 0.003$, $p = 0.001$, $p = 0.002$, and $p = 0.001$ respectively). In pleural fluid, only CA125 was higher in MPEs with suspicious or negative cytology ($p = 0.001$) than in benign effusions.

Conclusions Given high specificity and a sensitivity of about 60%, the concentrations of tumour markers in pleural effusions could be evaluated in cases of inconclusive cytology in patients with a high pre-test chance of malignancy or a history of cancer.

Key Words Pleural effusion, cytology, tumour markers

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INTRODUCTION

Approximately 10 million new cases of cancer are diagnosed every year and 6 million people die from the disease[1]. Pleural metastases, which can occur during the evolution of all neoplasm types, are frequent in lung and breast cancers and are generally associated with diminished survival expectancy[2,3].

Approximately 20% of all pleural effusions are caused by neoplastic processes. However, in some patients, pleural effusion is the first manifestation of cancer. In about 7%–15% of cases, the primary site of the metastatic tumour might not be identified even after extensive diagnostic investigation[2].

The presence of tumour cells in pleural fluid or tissue defines the effusion as malignant. Although pleural fluid cytology is more sensitive than closed pleural biopsy, its sensitivity of 50%–60% is still insufficient for making clinical decisions, and usually, the diagnosis is made after the use of more-invasive techniques such as thoracoscopy or thoracotomy.

It is well known that most malignant pleural effusions (MPEs) are related to invasion by tumour cells. However, in

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some cases, nonspecific inflammatory effusion secondary to subpleural intraparenchymal lung tumour development, lymphatic vessel obstruction, or immune-mediated inflammation can explain the failure of a cytology examination to provide a diagnosis[4]. In the latter scenario, the availability of rapid and reliable proof of malignancy by less-invasive procedures is a constant goal, especially in the case of patients who have a prior history of cancer and who develop pleural effusion during follow-up.

Tumour markers are macromolecules which, in high concentrations, are frequently associated with the presence or growth of malignant neoplasms. Quantification of tumour markers in pleural fluid has been suggested in three special situations:

- As a supplementary method for diagnosing MPE
- For the early detection of pleural metastasis
- As tool to assist in identifying a primary tumour site

Controversy with respect to the general usefulness of the technique remains[5–11]. Quantification of tumour markers in pleural fluid could be indicated in cases of undetermined causes in the presence of a strong clinical suspicion of malignancy or when cytology results were inconclusive in patients with a prior history of cancer.

Markers most routinely evaluated in the laboratory include carcinoembryonic antigen (CEA); cancer antigens 15-3 (CA15-3), 72-4 (CA72-4), and 125 (CA125); carbohydrate antigen 19-9 (CA19-9); and CYFRA 21-1. The diagnostic accuracy of those markers has been evaluated in serum and pleural fluid, but little is known about their behavior and variability in MPEs with positive, negative, or suspicious cytology. It would be clinically important if the concentrations of tumour markers could discriminate the cause of an effusion when the cytology exam is inconclusive, especially given that thoracentesis with biochemical and cytologic analyses of the pleural fluid is the first diagnostic approach. The main objective of the present study was therefore to evaluate whether the concentrations of tumour markers in pleural fluid can improve the diagnosis of MPEs when cytology is inconclusive.

METHODS

Our prospective study, which was approved by the ethics committee of our institution, enrolled 156 outpatients between March 2011 and November 2012. All patients had lymphocytic pleural effusion secondary to cancer or tuberculosis and were being followed at the Pleural Disease Service of the University of São Paulo. Patients underwent routine investigation by diagnostic thoracentesis, followed by pleural biopsy or video thoracoscopy, or both, when necessary.

Samples of pleural fluid were collected into EDTA tubes for quantification of tumour markers. After centrifugation for cell removal (1200 rpm for 15 minutes), the supernatants were stored at –80°C until analysis. Before analysis, the samples were re-centrifuged to avoid any possible matrix effect. An Elecsys 2010 immunoassay system (Roche Diagnostics, Mannheim, Germany) was used to quantitate the tumour markers.

The diagnosis of MPE was based on a combination of tumour cells being found in pleural tissue or fluid and the clinical history and follow-up of the patients. The primary tumour site was determined by radiologic, histologic, and immunohistochemical evaluation.

The cytology examination was performed by two experience cytologists who prepared and stained slides of fresh pleural fluid samples. The results classified the exudates as positive for malignancy, negative, or suspicious. For the comparative analysis, samples from patients with tuberculosis were included as a benign pleural effusion group, because such effusions are also lymphocytic exudates.

The diagnosis of tuberculosis took into account the patient's clinical history, together with one or more of these factors: positive acid-fast bacilli smear or culture for *Mycobacterium tuberculosis* in sputum, pleural fluid, or tissue; pleural biopsy demonstrating a chronic granulomatous process with caseous necrosis; and lymphocytic pleural effusion with a high adenosine deaminase level and satisfactory response to specific treatment after 6 months' follow-up[12].

Statistical Analysis

The statistical analyses were carried out using the SigmaStat (version 3.5 for Windows: Systat Software, Chicago, IL, U.S.A.) and SPSS Statistics (version 17.0 for Windows: SPSS, Chicago, IL, U.S.A.) software applications.

Concentrations of pleural fluid biomarkers are expressed as means with 1st and 3rd quartiles. Nonparametric comparisons were performed because the tumour marker data showed skewed distribution when assessed by a Kolmogorov–Smirnov test. Comparisons between the malignant and benign groups were performed using a Kruskal–Wallis test or Mann–Whitney U-test. Comparisons of the cytologic assessment groups (positive, negative, suspicious) of pleural fluid tumour markers with the benign group used a one-way analysis of variance followed by a Tukey test for multiple comparisons when differences between the samples were observed.

Receiver operator characteristic analysis was performed to evaluate the sensitivity and specificity of the tumour markers; the value that maximized the sum of the specificity and the sensitivity was chosen as a cut-off point. Any p value less than 0.05 was considered statistically significant.

RESULTS

Of the 156 enrolled patients, 114 had MPE [42 men (36.8%), 72 women (63.2%); mean age: 58.4 ± 14.8 years], and 42 had pleural tuberculosis [27 men (64.3%), 15 women (35.7%); mean age: 36.5 ± 16.7 years]. Table I shows the primary neoplastic sites.

Of the 114 patients with MPE, 65 (57%) had positive cytology; 34 (29.8%), suspicious cytology; and 15 (13.2%), negative cytology. Breast and lung were the most common primary tumour sites, and adenocarcinoma was the predominant histologic type.

Table II shows the cut-off values and the estimated sensitivities, specificities, positive predictive values, and negative predictive values for CEA, CA15-3, CA19-9, CA72-4, CA125, and CYFRA 21-1.

The concentrations of the tumour markers CEA, CA15-3, CA125, and CYFRA 21-1 were higher in MPEs than in benign effusions ($p < 0.001$), regardless of the cytology results. The CA19-9 and CA72-4 markers did not discriminate the malignant from the benign groups ($p > 0.05$, Table III).

When the concentrations of tumour markers in MPEs with positive ($n = 65$), suspicious ($n = 34$), or negative ($n = 15$) cytology and in benign effusions ($n = 42$) were compared, levels of CEA, CA15-3, CYFRA 21-1, and CA125 were higher in malignant effusions with positive cytology ($p = 0.003$, $p = 0.001$, $p = 0.002$, and $p = 0.001$ respectively). Only the CA125 concentration was higher in MPEs with suspicious or negative cytology ($p = 0.001$) than in benign effusions (Table IV). None of the other tumour markers discriminated MPEs with negative or suspicious cytology from benign effusions.

TABLE I Site of the primary tumour in cases of malignant pleural effusion

Site	(*n*)
Breast	47
Lung	43
Lymph system	4
Ovary	5
Colon or rectum	3
Bone	3
Other[a]	9
TOTAL	114

[a] Thymus ($n = 2$), kidney ($n = 2$), lung [mesothelioma ($n = 2$)], stomach ($n = 1$), pancreas ($n = 1$), skin [melanoma ($n = 1$)].

DISCUSSION

Our study demonstrates that concentrations of CEA, CA15-3, CA125, and CYFRA 21-1 are higher in MPEs than in benign effusions.

The concentration of CA125 in MPEs, regardless of cytology (positive, suspicious, negative), was higher than it was in benign effusions. Only this marker differentiated MPEs with negative or suspicious cytology from benign effusions. None of other tumour markers contributed toward discriminating MPEs with inconclusive cytology.

Previous studies have demonstrated the clinical utility of tumour markers in the differential diagnosis of pleural diseases[5–11,13], but depending on tumour markers in pleural fluid for diagnosing MPE is controversial. However, most authors felt that quantification of a panel of tumour markers could improve the cytologic diagnosis and should be considered in selected cases of inconclusive diagnosis of pleural effusions.

Also controversial is the usefulness of tumour markers in MPE when the site of the primary tumour is unknown. However, combined with clinical and radiologic findings, the concentrations of tumour markers in serum or pleural fluid can be useful in determining the origin of a tumour[14].

In our study, CEA provided the greatest diagnostic specificity for MPE (97.5%) at a cut-off point of 5.2 ng/mL (sensitivity: 65.1%). At a cut-off point of 345.65 U/mL, CA125 concentration provided a diagnostic sensitivity of 68.1% (specificity: 83.5%). Similar results were described by Ferrer *et al.*[15], who quantified tumour markers in samples of pleural fluid and serum from patients with MPEs and benign pleural effusions. Those authors observed no significant

TABLE II Tumour markers in pleural fluid[a] during the diagnosis of malignant pleural effusions

Marker	Cut-off value	AUC	Sensitivity (%)	Specificity (%)	Predictive value (%)	
					Positive	Negative
Carcinoembryonic antigen	≥5.2 ng/mL	0.775	65.09 (55.22–74.10)	97.50 (86.84–99.94)	98.57 (92.30–99.96)	51.32 (39.57–62.96)
Cancer antigen 15-3	≥29.69 U/mL	0.733	57.14 (47.45–66.45)	90.48 (77.38–97.34)	94.12 (85.62–98.37)	44.19 (33.48–55.30)
Carbohydrate antigen 19-9	≥13.1 U/mL	0.577	40.74 (31.38–50.62)	77.50 (61.55–89.16)	83.02 (70.20–91.93)	32.63 (23.36–43.02)
Cancer antigen 72-4	≥7.25 U/mL	0.603	48.54 (38.56–58.60)	82.50 (67.22–92.66)	87.72 (76.32–94.92)	38.37 (28.08–49.49)
Cancer antigen 125	≥345.65 U/mL	0.846	68.14 (58.71–76.59)	83.33 (68.64–93.03)	91.67 (83.58–96.58)	49.30 (37.22–61.44)
CYFRA 21-1	≥52.87 U/mL	0.697	53.76 (43.12–64.16)	79.49 (63.54–90.70)	86.21 (74.62–93.85)	41.89 (30.51–53.94)

[a] Values expressed as medians, with interquartile range in parentheses.
AUC = area under curve.

differences in the concentrations of tumour markers in serum or pleural fluid, except for CA125, which was higher in pleural fluid. However, for 100% specificity, the maximum sensitivity was 40% with the combination of CYFRA 21-1, CEA, and CA125. The association of tumour markers with cytology increased the diagnostic sensitivity to 81% from 55.8%. The authors suggested that quantification of CYFRA 21-1, CEA, and CA125 in pleural fluid combined with cytology should be considered for diagnosing MPE.

Also deserving of comment is the increase in CA125 concentration often observed in benign or malignant effusions, considering that this protein is synthesized by normal and malignant cells of varying origin[16]. Elevated CA125 requires a cautious clinical interpretation in patients with serosal involvement.

Miralles *et al.*[17] analyzed the prevalence of increases in serum CA125 in a population of patients presenting at a general hospital on 4 different days. Increased CA125 was found in 16% of 380 randomly selected patients. In that sample, 9 (14.7%) had heart failure; 11 (18%), lung disease; 7 (11.4%). hepatic cirrhosis; 6 (10%), intra-abdominal non-hepatic disease; 17 (27.8%), prior surgery; and 2 (3%) miscellaneous conditions. By contrast, an increase in CA125 was observed in only 9 patients (14.7%) with malignancy. Notably, effusions with increased concentrations of CA125 were observed in 34 patients (55.7%). The authors suggested that their findings support the opinion that CA125 lacks utility as a marker of malignancy.

The diagnosis of MPE is based fundamentally on the finding of tumour cells in pleural fluid or tissue. However, depending on the tumour's histologic type and degree of pleural invasiveness, tumour cells might not be detected in pleural fluid or tissue obtained by closed biopsy[3,18]. Biopsies guided by video thoracoscopy provide a diagnosis in about 90% of cases, but this procedure is not always possible when patients with advanced disease are in unstable clinical condition[3]. In such cases, the ability to make the diagnosis using samples of pleural fluid is highly advantageous, because thoracentesis is a mildly invasive and well-tolerated procedure. Another positive factor to consider is the rapid availability of laboratory results, allowing for better decision-making about a therapeutic approach in these patients.

Nevertheless, the limited sensitivity of pleural fluid cytology has engendered a constant search for complementary methods that will improve the reliability of diagnoses, particularly in inconclusive cases. In a recent study, we used cells from fresh pleural fluid to demonstrate the usefulness of fluorescence *in situ* hybridization for the

TABLE III Tumour markers in pleural fluid[a] in cases of malignant and benign pleural effusions

Marker	Case group		*p* Value
	Malignant (*n*=114)	Benign (*n*=42)	
Carcinoembryonic antigen (ng/mL)	14.0 (2.6–139.4)	1.3 (1.0–2.2)	≤0.001
Cancer antigen 15-3 (U/mL)	41.0 (14.0–190.0)	13.5 (10.2–21.6)	≤0.001
Carbohydrate antigen 19-9 (U/mL)	7.9 (2.1–38.0)	5.9 (1.8–12.0)	0.152
Cancer antigen 72-4 (U/mL)	7.1 (3.9–58.5)	6.8 (6.1–7.2)	0.059
Cancer antigen 125 (U/mL)	796.0 (258.0–1831.0)	86.5 (22.3–305.2)	≤0.001
CYFRA 21-1 (U/mL)	57.8 (18.7–271.3)	19.5 (10.3–47.4)	≤0.001

[a] Values expressed as medians, with interquartile range in parentheses.

TABLE IV Tumour markers in pleural fluid[a] by cytology results in cases of malignant and benign pleural effusions

Marker	Malignant cases			Benign cases Tuberculosis (*n*=42)	*p* Value
	Positive cytology (*n*=65)	Suspicious cytology (*n*=34)	Negative cytology (*n*=15)		
Carcinoembryonic antigen (ng/mL)	24.0 (2.7–307.2)	8.2 (1.6–90.0)	1.4 (0.7–28.8)	1.3 (1.0–2.2)[b]	0.025
Cancer antigen 15-3 (U/mL)	105.6 (17.6–275.0)	21.5 (13.1–97.1)	15.4 (11.0–89.7)	13.5 (10.2–22.0)[b]	0.002
Carbohydrate antigen 19-9 (U/mL)	10.3 (2.8–51.6)	7.1 (3.6–27.0)	2.8 (0.6–13.1)	5.9 (1.8–11.7)	0.356
Cancer antigen 72-4 (U/mL)	13.3 (4.0–99.4)	6.5 (4.0–9.0)	6.3 (2.2–7.5)	6.8 (6.1–7.2)	0.145
Cancer antigen 125 (U/mL)	1138.0 (325.0–2271.0)	561.0 (194.0–1452.0)	414.0 (117.8–813.0)	87.0 (22.3–305.0)[c]	0.001
CYFRA 21-1 (U/mL)	76.0 (32.7–309.4)	45.0 (15.0–157.0)	21.0 (8.0–125.0)	19.5 (10.3–47.4)[b]	0.024

[a] Values expressed as medians, with interquartile range in parentheses.
[b] Significant difference between benign pleural effusions and malignant pleural effusions with positive cytology.
[c] Significant difference between benign pleural effusions and malignant pleural effusions with positive, negative, or suspicious cytology.

diagnosis of MPE, because the presence of aneuploid cells is commonly associated with malignancy[19]. Those results demonstrated the high sensitivity and specificity of fluorescence *in situ* hybridization for diagnosing MPE.

A literature review found few studies exploring the diagnostic role of tumour markers in pleural fluid from patients with MPE and negative or atypical cytology. In a recent study by Hsieh *et al.*[20], the authors evaluated the role of tumour markers in cases of pleural effusion with negative cytology from patients with lung cancer. They compared the performance of HER2, CYFRA 21-1, and CEA in differentiating MPE with negative cytology from benign effusions and noted significant differences in all three tumour markers; however, the sensitivity for the markers was 12.1%, 30.3%, and 63.6% respectively. The combination of CEA and CYFRA 21-1 improved the sensitivity to 66.7%, suggesting that the concentrations of those markers are useful for identifying MPES when the cytology exam is negative or inconclusive.

Currently, no single tumour marker or group of tumour markers is sufficiently specific and sensitive in identifying a malignant cause of an effusion so as to be considered for a diagnostic algorithm.

The rationale for the present study was to verify whether a panel of tumour markers commonly used in clinical practice might have diagnostic value in the pleural fluid setting. A panel of biomarkers that could identify MPES when cytology is suspicious or negative, avoiding more-invasive procedures, would be of special value. Although the concentrations of CEA, CA 15-3, and CYFRA 21-1 were higher in patients with malignant than with benign effusions, those concentration differences were unable to identify MPES when cytology was suspicious or negative. In the present study, only CA125 differentiated malignant from para-malignant effusions. However, further studies in larger series are necessary to confirm our finding, because an increased concentration of CA125 can be present in multiple benign pathologies, especially those whose course results in serosal involvement.

In clinical laboratories, cytology examination of pleural fluid allows for a diagnosis of MPE by 4–6 hours after thoracentesis. From the clinical standpoint, especially for patients with prior history of cancer, pleural fluid cytology is the first laboratory approach for diagnosis. In cases with negative or suspicious cytology, complementary methods are often necessary to improve the diagnostic certainty, mainly for patients with advanced disease whose clinical condition does not allow for more invasive procedures.

The main limitations of the present study are the number of cases, the variety of primary tumour sites, the diversity of histologic subtypes, and the low number of MPES with negative cytology. Although CA125 was able to discriminate MPES from benign effusions when cytology was negative or suspicious, the low number of cases in the study means that it is not yet reasonable to recommend CA125 concentration as a diagnostic tool.

CONCLUSIONS

With high specificity and a sensitivity of about 60%, measurement of the concentrations of tumour markers in pleural effusions could potentially be diagnostically useful in patients with an indeterminate pleural effusion and a high pre-test chance of malignancy, or in patients with a prior history of cancer for whom the results of a cytology exam were inconclusive. A larger series of pleural effusions with negative cytology results has to be examined to confirm the panel of biomarkers that could best be used as an auxiliary method of diagnosing MPES.

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CONFLICT OF INTEREST DISCLOSURES

We have read and understood *Current Oncology*'s policy on disclosing conflicts of interest, and we declare that we have none.

AUTHOR AFFILIATIONS

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