The Role of Serum and Pleural Fluid Levels of Cyfra-21-1, Carcinoembryonic Antigen and Neuron Specific Enolase in Clinical Evaluation of Pleural Effusions for Malignancy

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Summary

The aim of this study was to investigate the diagnostic potential of serum and pleural fluid determinations of Cyfra 21-1, CEA and NSE in clinical evaluation of pleural effusions for their possible malignant origin.
Cyfra 21-1, CEA and NSE were quantified by radioimmuassay kits in sera and pleural fluid samples of 36 patients with malignant pleural effusions and 20 with benign ones.
The mean serum and pleural levels of Cyfra 21-1 (13.65±29.03 and 41.16±31.91 ng/ml), CEA (25.19±43.43 and 51.51±92.11 ng/ml) and NSE (20.91±33.77 and 17.97±36.66 ng/ml) in the patients with malignant pleural effusions were significantly higher than those of the control group (Cyfra 21-1: 1.30±4.18 and 7.61±12.52 ng/ml; CEA: 2.94±3.96 and 2.63±1.7 ng/ml and NSE: 8.73±6.07 and 6.69±6.33 ng/ml). The sensitivities of pleural Cyfra 21-1, CEA and NSE determination in diagnosis of malignant effusions were 61.1 %, 50 % and 13.8 % respectively and those of serum levels were 52.7 %, 36.1 % and 25 % respectively. When they were analyzed together and associated with cytological examination, discriminative diagnostic potential increased.
These results have demonstrated that determination of serum and pleural levels of Cyfra 21-1 and CEA together with cytological examination may help to reveal malignant origin of a pleural effusion.
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Key Words: Cyfra 21-1, CEA, NSE, malignant pleural effusion, tumor marker

Özet

Plevral Effüzyonların Malignite Açısından Klinik Değerlendirilmesinde Serum ve Plevra Sıvısı Cyfra 21-1, Karsinoembriyonik Antijen ve Nöronspesifik Enolaz Düzeylerinin Rolü

Bu çalışmanın amacı plevral effüzyonların olası malignite kaynağı açısından klinik değerlendirilmesinde serumda ve plevra sıvısında Cyfra 21-1, CEA ve NSE ölçümlerinin değerini araştırmaktı.
36 malign ve 20 benign plevral effüzyonlu hastada, radyoimmünoassay kitleriyle plevra sıvısı ve serum Cyfra 21-1, CEA ve NSE ölçümleri yapıldı.
Malign plevral effüzyonlu grupta Cyfra 21-1 (serum: 13.65±29.03; plevra: 40.10±33.05 ng/ml), CEA (serum: 25.19±43.43; plevra: 51.51±92.11 ng/ml) ve NSE'nin (serum: 20.91±33.77; plevra: 17.92±36.66 ng/ml) ortalama serum ve plevra sıvısı düzeyleri, benign plevral effüzyonlu gruba göre (Plevra sıvısı; Cyfra 21-1: 11.71±25.10, CEA: 2.63±1.7, NSE: 6.69±6.33 ve Serum; Cyfra 21-1: 1.30±4.18, CEA: 2.94±3.962, NSE: 8.73±6.07 ng/ml) istatistiksel olarak farklıydı. Malign plevral effüzyonların tanınmasında plevra sıvısı Cyfra 21-1'in sensitivitesi % 61.1, CEA'nin % 50 ve NSE'ın % 13.8; serum ölçümlerinin sensitiviteleri ise sırasıyla % 52.7, % 36.1 ve % 25 bulundu. Her üç tümör belirleyicisi birlikte değerlendirilip veya plevra sıvısı sitolojik incelemesiyle kombine edildiğinde tanısal değerler yükseldi.
Bu sonuçlar sitolojik incelemeyle beraber değerlendirildiğinde plevral Cyfra 21-1 ve CEA ölçümlerinin bir plevral effüzyonun malign kaynaklı olduğunun belirlenmesinde yardımcı olabileceğini göstermiştir.
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Key Words: Cyfra 21-1, CEA, NSE, malign plevral effüzyon, tümör belirleyicisi

Introduction

Pleural effusion is an important problem in daily medical practice that cannot sometimes be solved despite intensive and invasive, clinical and laboratory procedures. Furthermore, it may especially be difficult to distinguish malignant effusions from tuberculous pleurisy in endemic countries for tuberculosis. In this respect, various tumor markers have been investigated for differential diagnosis of malignant pleural effusion (1-12). Quantification of neuron specific enolase (NSE) and carcinoembryonic antigen (CEA) in serum and pleural fluid has been shown to

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be helpful in clinical evaluation of malignant pleural effusions (3-5, 8-10, 12). However it has not been reached a satisfactory sensitivity level in most of the studies. Recently, serum fragment of cytokeratin subunit 19, referred to as Cyfra 21-1, has been accepted as a new tumor marker for lung cancers particularly for squamous cell carcinoma (9-17).
In the current study, we aimed to determine whether simultaneous quantification of pleural and serum levels of Cyfra 21-1, CEA and NSE could help to predict malignant origin of pleural effusions.

Materials and Methods

Thirty-six patients with pleural effusions related to histologically proven malignancies and 20 patients with benign pleural effusions were enrolled to the study. Of the malignancy group, 20 had "true" malignant pleural effusion proven by either pleural fluid cytology or pleural biopsy while remaining 16 patients had at least two consecutive negative cytological examinations for malignancy. Eight out of 36 malignant pleural effusions were due to extrathoracic tumors. Characteristics of both groups were shown on Table I.
A simultaneous blood and pleural fluid samples were taken from patients with thoracic tumors, benign effusions and the patient with stomach cancer at the time of diagnosis of the diseases and from the other 7 patients with extrathoracic tumors at the time of diagnosis of pleural effusions during their follow-up after appropriate therapy for malignancy.
The blood and pleural fluid samples were centrifuged to separate serum from blood and to discard the cell pellet from pleural fluid and stored at -40 °C until tested.
All three markers were quantified by using radioimmunoassay kits. (for Cyfra 21-1, Centocor Inc. USA; for CEA, RADIM, Roma, Italy; for NSE, AB Santeg Medical, Bromma, Sweden) Since our control group consisting of benign pleural effusions was not large and diverse enough to define a cut-off level, we used the pathologic cut-off levels in serum and pleural fluid for malignancy defined in previous studies as the levels, which had given 95 % specifity (2, 4, 8, 11). The pleural cut-off levels for CEA and NSE were accepted as 20 and 25 ng/ml and we used 12.5 and 15 ng/ml levels as serum cut-off values for CEA and NSE respectively. For serum and pleural Cyfra 21-1 evaluation, we used the cut-off levels of 3.3 ng/ml and 20 ng/ml respectively.
The pleural and serum values of Cyfra 21-1, CEA and NSE in the study and control groups were expressed as mean ± standard deviation and compared by using Mann-Whitney U test. Specificity, sensitivity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy (DA: the sum of true positive and negative results in the whole study and control group) of the tests were calculated in respect to cut-off levels defined above.

Results

The mean pleural and serum levels of all three markers in the study and control groups were shown on Table II. Both mean serum and pleural fluid quantifications of Cyfra 21-1, CEA and NSE were higher in malignancy group than benign one.
In the malignant group, 19 of 36 patients had elevated serum Cyfra 21-1 levels while in 22 of them, pleural cyfra 21-1 levels were higher than the cut-off value. Similarly in the benign group, only one of 20 patients with parapneumonic effusion had higher serum Cyfra 21-1 levels than the cut-off value (one false positive) but 2 of the patients with tuberculous pleurisy had elevated pleural Cyfra 21-1 values (2 false positives).
In the malignant group, 13 of the 36 patients had elevated serum and 18 had elevated pleural CEA levels. In the benign group serum level of CEA was higher than the cut-off value in only one patient with tuberculous pleurisy (one false positive) while pleural fluid CEA levels were always lower than the cut-off value. (no false positive)
Nine of the 36 patients had elevated serum and 5 had elevated pleural fluid NSE levels in the malignant group. In the benign group NSE serum and pleural fluid levels were higher than the cut-off values in 3 (two tuberculous pleurisy and one hepatic vein thrombosis) and 1 (tuberculous pleurisy) of the patients (false positives) respectively.
The discriminative parameters of specificity, sensitivity, PPV, NPV and DA with regard to the diagnosis of malignant origin of pleural effusions were calculated to establish the validity of these tumor markers in predicting malignancy origin of a pleural effusion (Table III).
When each marker is taken alone, pleural Cyfra 21-1 determination has the highest sensitivity (61.1 %) with the specificity of 90 %. In association with pleural fluid cytological examination, sensitivity of pleural Cyfra 21-1 increased to 72.2 %. Similarly, the sensitivity of pleural fluid NSE and CEA elevated from 13.8 % and 50 % to 55.5 % and 63.8 % respectively. When only malignant pleural effusions whose pathological examination of pleura or pleural fluid were positive for malignancy were taken into account, DA of the markers increased (Table IV).
The utility of the markers were also evaluated just for thoracic tumors (Table V). The sensitivities of pleural Cyfra 21-1, CEA and NSE level for thoracic tumors were 60.7 %, 46.4 % and 14.2 % respectively. They increased to 71.4 %, 64.2% and 60.7 % respectively when they were combined with cytological examination of which sensitivity was 60.7 %. The sensitivity of serum Cyfra 21-1 (64.2 %) was higher than that of pleural one for thoracic tumors and it increased to 82.1 % adjunct to cytological examination. Diagnosis of a malignant pleural effusion related to a thoracic tumor was best made by combined analysis of pleural Cyfra 21-1 plus serum Cyfra 21-1 quantification (sensitivity: 85.7 %, specificity: % 85, PPV: 88.8 %, NPV: 80.9 % and DA: 85.4 %).

Discussion

The differential diagnosis between benign, especially tuberculous pleurisy, and malignant effusions represents a critical clinical problem. Cytological analysis is the unique method to identify malignant cells in a pleural effusion, however it is not sensitive enough (40 to 60%). In the case of uncertainty, blind or thoracoscopic-guided biopsy

should be used. The latter is highly sensitive, but, unfortunately, it is also invasive.

Cytological examination of pleural fluid has 100 % specificity but its sensitivity is low due to low content of malignant cells in the pleural fluid. Furthermore paramalign effusions may occur in some medical conditions associated with primer lung tumor including mediastinal lymphatic vessel obstruction, parapneumonic effusion, atelectasis with transudative effusion due to obstructing bronchial lesion, superior vena cava obstruction, pulmonary embolism, hypoalbuminemia and congestive heart failure (19).

Pleural fluid level of CEA is elevated in 45-55% of the malignant pleural effusions while simultaneous serum quantification reveals elevated levels in only 30-40 % of the patients (1, 3-5, 9, 20-22). This diversity in sensitivities is due to differences in the chosen cut-off levels and characteristics of the populations in different studies.

NSE is the neuronal form of the glycolytic enzyme enolase, which is found in extracts of brain tissue, neuroendocrine cells and neuroendocrine tumors including small cell carcinoma (23). Serum level of NSE is elevated in 50-70 % of the patients with small cell carcinoma while elevated pleural NSE level in patients with small cell carcinoma may be seen in 70-75 % of the patients whose pleural effusions are due to small cell carcinoma (4, 8-10). Low diagnostic yield of serum and pleural fluid NSE determination in our study may be due to small small cell carcinoma number (only 2 patients) among malignant cases.

The tumor marker assay Cyfra 21-1 was developed in 1992 to measure a fragment of cytokeratin 19 with a molecular weight of 30.000 Daltons. Cyfra 21-1 is valuable in lung tumors particularly in squamous cell carcinoma and it also appears to be the most useful of all known markers for muscle-invasive bladder carcinoma. The sensitivity of serum Cyfra 21-1 quantification is 16-36 % for squamous cell carcinoma, % 57-68 for small cell carcinoma, % 27-42 for adenocarcinoma of lung and % 30-34 for undifferentiated large cell carcinoma (9, 11, 20, 21) Rastel et al. and Ebert et al. have recommended that the cut-off value for serum Cyfra 21-1 evaluation would be 3.3 ng/ml in two multicenter studies separately (13, 15). Satoh et al. also recommended that an optimal cut-off value for pleural fluid Cyfra 21-1 determination was almost 6-fold higher than the serum cut-off value (2). Therefore, we used the level of 20ng/ml as a cut-off value for pleural Cyfra 21-1 evaluation.

This study has demonstrated that it may be possible, at least to some degree, to distinguish malignancy associated pleural effusions from benign ones by pleural quantification of Cyfra 21-1, CEA, NSE and various combinations of these markers. As shown on tables 3 and 5, a diagnostic accuracy greater than that of pathological examination could be yielded only by combined analysis of serum and pleural Cyfra 21-1 and pleural Cyfra 21-1 and CEA quantification. A single test never gave a better result than pathology. However, when they analyzed together with pathological examination, serum and pleural Cyfra 21-1 and pleural CEA levels resulted in a better diagnostic yield than that of pathology. When only true malignant pleural effusions were considered, serum and pleural Cyfra 21-1 and pleural CEA levels gave a satisfactory diagnostic accuracy (Table IV). But, we suppose that this fact cannot solve clinical problem because they were already proven malignant pleural effusions. Since we aimed to establish relevance of a pleural effusion to a malignant tumor, not to diagnose a

Table I: Characteristics of patients in study and control groups.

Study Groups	Number	Positive Pathology	Mean Age	Sex M/F
Malignancy Group	36	20	51.08	20
Thoracic Tumors	28	18		
Squamous cell carcinoma	12	7		
Adenocarcinoma of lung	10	6		
Malignant mesothelioma	3	3		
Small cell carcinoma	2	1		
Hodgkin's disease	1	-		
Extratoracic Tumors				
Breast carcinoma	3	1		
Colonic adenocarcinoma	2	1		
Acute myeloblastic leukaemia	1			
Non-Hodgkin lymphoma	2			
Adenocarcinoma of stomach	1	1		
Benign Group	20		29.33	15/5
Tuberculous Pleurisy	13			
Parapneumonic Effusion	4			
Congestive Heart Failure	2			
Hepatic Vein Thrombosis	1			

malignancy, by the tumor marker quantification, we initially enrolled to the study not only pathologically proven malignant pleural effusions but also those of accepted as paramalignant ones. Thereafter, true malignant effusions were evaluated separately and the tumor marker quantifications were combined with cytological analyses for all cases.

In association with pleural fluid cytological examination, various combinations of Cyfra 21-1, CEA and NSE quantification were improved discriminative diagnostic values of most of them, because of specificity of the cytological examination is 100 %(Table III).

According to the results of this study, when each of these markers is used as a single test, evaluation of pleural effusions for malignancy is best made by using pleural Cyfra 21-1 determination (sensitivity: 61.1 %; specificity: 90 %; PPV: 91.6 %; NPV: 56.2% and DA: 71.4 %). When only thoracic tumors are considered, serum Cyfra 21-1 has higher sensitivity alone (64.2 %) and in association with pleural fluid cytological examination (82.1 %). It is also noteworthy that pleural CEA determination has 100 % specificity for malignant pleural effusions and none of three malignant mesothelioma patients has elevated serum or pleural CEA levels latter of which is consistent with previous reports (4-6,22).

If it is available to use two tumor markers to distinguish

Table II: Mean serum and pleural levels of all three markers in patients with malignancy related and benign pleural effusions.

Parameter		Benign Effusions	Malignancy Related Effusions	Significance
Cyfra 21-1	S	1.30±4.18	13.65±29.03	p<0.0001
	P	7.61±12.52	41.16±31.91	p<0.0001
CEA	S	2.94±3.96	25.19±43.43	p<0.001
	P	2.63±1.70	51.51±92.11	p<0.0005
NSE	S	8.73±6.07	20.91±33.77	p<0.0005
	p	6.69±6.33	17.97±36.66	p<0.01

s: Serum; p: Pleural fluid;

Table III: Discriminative diagnostic values of pleural and serum quantification of Cyfra 21-1, CEA, NSE, and various combined analyses of them.

Parameter	Sensitivity %	Specifity %	PPV %	NPV %	DA %
s/ Cyfra	52.7(72.2)	95	95(96.3)	52.7(65.5)	67.8(80.4)
s/ CEA	36.1(61.1)	95	92.8 (95.6)	45.2(57.6)	57.1(73.2)
s/ NSE	25(58.3)	85	75(87.5)	38.6(53.1)	46.4(67.8)
p/ Cyfra	61.1(72.2)	90	91.6(92.8)	56.2(64.3)	71.4(78.6)
p/ CEA	50(63.8)	100	100(100)	50(60.6)	66.6(76.8)
p/ NSE	13.8(55.5)	95	83.3(95.2)	38(54.2)	42.8(69.6)
p+s/ Cyfra	80.5(86.1)	85	90.6(91.1)	68(77.2)	80.7(85.7)
p/ Cyfra+CEA	86.1(88.8)	90	93.9(94.1)	78.2(81.8)	87.5(89.2)
Pathology	55.5	100	100	55.5	71.4

The numbers in parentheses show the values when combined with cytological examination. s: serum, p: pleural fluid

Table IV: Discriminative diagnostic values of pleural and serum quantification of Cyfra 21-1, CEA, NSE, and various combined analyses of them for only pathologically proven malignant pleural effusions.

Parameter	Sensitivity %	Specifity %	PPV %	NPV %	DA %
s/ Cyfra	75	95	93.7	79.1	85
s/ CEA	40	95	88.8	61.2	67.5
s/ NSE	25	85	62.5	53.1	55
p/ Cyfra	75	90	88.2	78.2	82.5
p/ CEA	65	100	100	74.1	82.5
p/ NSE	15	95	75	50	55
p+s/ Cyfra	95	85	86.3	94.4	90
p/ Cyfra+CEA	85	90	89.4	85.7	87.5

s: serum, p: pleural fluid

malignant pleural effusions from benign ones, simultaneous pleural Cyfra 21-1 and CEA quantification in association with cytological examination have also a considerable high diagnostic value (specificity: 90 %; sensitivity: 88.8 %; PPV: 94.1 %; NPV: 81.8 %, DA: 89.2). Similarly in thoracic tumors, by using two tumor markers, differentiation of malignancy associated pleural effusions is best made by serum plus pleural Cyfra 21-1 levels in combination with pathologic examination (specificity: 85 %; sensitivity: 92.8 %; PPV: 89.6 %; NPV: 89.4 %, DA: 89.4 %) and pleural Cyfra 21-1 plus pleural CEA levels (specificity: 90 %; sensitivity: 88 %; PPV: 92 %; NPV: 85 %). The other combinations of pleural and serum levels of all three markers did not alter discriminative diagnostic values.

Undoubtedly, diagnosis of a malign disease is only made by histopathologic examination. However, it needs sometimes to be known the possibility of malignancy of a pleural effusion particularly in distinguishing malignant effusions from tuberculous pleurisy. Despite new rapid and accurate diagnostic methods, response to antituberculous therapy still continues to be a diagnostic method in tuberculous pleurisy. Tumor marker evaluation may be helpful before using response to therapy to exclude malignancy especially in endemic countries for tuberculosis. Previous reports support this suggestion (2, 10, 12, 17, 20). Though, it should be always remembered that tumor markers especially Cyfra 21-1 and CEA may be elevated in some nonmalignant pulmonary diseases (26, 27)

In conclusion, we point out that, as a single test, either serum or pleural fluid quantification of Cyfra 21-1, CEA and NSE cannot reveal malignant potential of a pleural effusion with a satisfactory diagnostic accuracy. However analyses of pleural fluid Cyfra 21-1 combined with those of serum Cyfra 21-1 or pleural fluid CEA may help to establish "the possibility" of malignancy of a pleural effusion. By using these tests in selected cases the patients with high possibility of benign pleural effusions may be avoided from the risks of invasive procedures or patients with high possibility of malignant pleural effusions may be underwent to invasive diagnostic procedures earlier.

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Table V: Discriminative diagnostic values of pleural and serum quantification of Cyfra 21-1, CEA, NSE, and various combined analyses of them for only thoracic tumors.

Parameter	Sensitivity %	Specificity %	PPD %	NPD %	DA %
s/ Cyfra 21	64.2 (82.1)	95	94.2 (95.8)	65.5 (79.1)	77.1 (87.5)
s/ CEA	28.5 (64.2)	95	88.8 (94.7)	48.7 (65.5)	56.2 (77.1)
s/ NSE	28.5 (67.8)	85	72.7 (86.3)	45.9 (65.3)	52.1 (75)
p/ Cyfra 21	60.7 (71.4)	90	89.4 (90.9)	62.1 (69.2)	72.9 (79.1)
p/ CEA	46.4 (64.2)	100	100 (100)	57.1 (66.6)	68.7 (79.1)
p/ NSE	14.2 (60.7)	95	80 (94.8)	44.2 (62.5)	47.9 (75)
s, p/Cyfra 21	85.7 (92.8)	85	88.8 (89.6)	80.9 (89.4)	85.4 (89.5)
p/Cyfra 21, CEA	82.1 (89.2)	90	92 (92.5)	78.2 (78.2)	85.4 (89.4)
Pathology	60.7	100	100	64.5	77.1

s/: Serum, p/: Pleural fluid, the numbers in parentheses show the values when combined with cytological examination

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