

ARTICLE

Diagnostic Value of Tumor Markers for Differentiating Malignant and Benign Pleural Effusions of Iranian Patients

Sied Mohammad Ali GHAYUMI,¹ Samrad MEHRABI,¹ Mehrnossh DOROUDCHI,² Abbas GHADERI^{2,3}

Departments of ¹Internal Medicine, ²Immunology, and ³Shiraz Institute for Cancer Research, Shiraz University of Medical Sciences, Shiraz, Iran

In order to evaluate the diagnostic yield of tumor markers in differentiating malignant and benign pleural effusions, we carried out a prospective study in a group of Iranian people. Pleural and serum levels of carcinoembryonic antigen (CEA), carbohydrate antigen 15-3 (CA 15-3), neuron-specific enolase (NSE) and cancer antigen 125 (CA 125) were assayed prospectively in patients with pleural effusion (40 malignant and 37 benign). The highest sensitivity was

obtained with a combination of CA 15-3 in serum, and CA 15-3 and CEA in pleural fluid (80%), also with combination of CA 15-3 in serum, and CA 15-3, NSE and CEA in pleural fluid (80%). The highest specificity was obtained with combination of CA 15-3 in serum, and CA 15-3 and NSE in pleural fluid (100%), and also with combination of CA 15-3 in serum, and CA 15-3, NSE and CEA in pleural fluid (100%). (Pathology Oncology Research Vol 11, No 4, 236–241)

Key words: pleural effusion, tumor markers

Introduction

Pleural effusion (PE) represents a common and challenging diagnostic problem of pulmonary medicine with diverse and non-similar etiologies. One of the most common etiology of PE is malignancy, among which lung and breast cancer metastases correspond to a great number of cases.^{1,2} However, other infectious and non-infectious diseases contribute to this clinical manifestation, too. Marel et al. have suggested that the distribution of causes responsible for pleural effusions is geographically different.²

Differentiation of malignant and non-malignant pleural effusion is of great importance; however, current methods are either insufficient or invasive.^{3,4} Cytological methods usually detect only 50–60% of malignant pleural effusions, and pleural needle biopsy adds only 7% diagnostic value to this method.⁵ The two invasive methods, thoracoscopy and thoracotomy, have a great sensitivity, but in addition to being expensive, they impose physical and mental stress to the patient.⁶ Recent investigations have focused on the detection

and use of reliable tumor markers as a less invasive replacement method,⁷ however, the results have been inconclusive.⁸ The most widely studied and recommended tumor marker is CEA, with sensitivity of less than 50%^{2,9,10} and specificity around 90% in the pleural fluid.¹¹ It is worth mentioning that different sensitivities and specificities have been reported by using different cut-off levels and in different settings.¹⁰⁻¹² Other widely used tumor markers include CA 125, a high-molecular-weight glycoprotein; CA 15-3, a glycoprotein found in normal and malignant breast, ovary, lung and pancreatic tissues; NSE, an enolase isoenzyme of the neurons; MCA, a mucin-like glycoprotein found in many adenocarcinomas, and CYFRA 21.

In this study the underlying diseases in 77 consecutive cases with pleural effusion were investigated. The level of CEA, CA 15-3, CA 125 and NSE was investigated in sera and pleural effusions of the patients with benign or malignant pleural effusion.

Materials and Methods

Patients

In a prospective study, 77 consecutive patients with pleural effusion were included who were referred to the Pulmonary Division of Internal Medicine Department of Faghihi Hospital in Shiraz, Iran. The underlying diseases were

Received: June 6, 2005; accepted: Oct 21, 2005

Corresponding: Dr. Sied Mohammad Ali GHAYUMI, Department of Internal Medicine, Shiraz University of Medical Sciences, Shiraz, Iran. Tel.: 0098 711 230 3687, fax: 0098 711 230 4952, e-mail: ghayyoumim@sums.ac.ir

diagnosed by using clinical signs and symptoms, physical examination, chest X-ray, CT-scan (when needed), biochemical, cytological and bacteriological analysis of pleural fluid, and when needed pleural biopsy and thoracoscopy.

Determination of etiology

A pleural fluid was considered malignant when malignant cells were detected in the cytological or biopsy specimen of the pleura. An effusion was considered parapneumonic if acute febrile condition with cough, sputum and pulmonary infiltration without malignancy was detected. The diagnosis of empyema was based on the detection of pus at thoracentesis, or a positive smear and/or positive culture of pleural fluid. Tuberculosis pleurisy was diagnosed by detection of granuloma in pleural biopsy specimens. Congestive heart failure (CHF) was diagnosed with an enlarged heart on chest X-ray and transudative effusion with good response to anti-failure therapy. Pulmonary embolism was considered in case of a lung perfusion scan with high probability for pulmonary embolus and clinically suspicious.

Samples

Eight ml venous blood and 10 ml pleural fluid were collected from each subject. The samples were centrifuged immediately at 1500xg, and then the serum and pleural fluid were kept at -20°C until use. Determination of tumor marker levels was performed by a non-competitive biotin-avidin-based sandwich ELISA assay (Can-Ag Diagnostics AB, Gothenburg, Sweden) according to the manufacturer's instructions. The evaluated markers included CEA, CA 15-3, NSE and CA 125. The cut-off levels of CEA, CA 15-3, NSE and CA 125 for differentiation of benign and malignant pleural effusions were 3.60 µg/ml and 2.95 µg/ml; 21.11 U/ml and 29.07 U/ml; 5.21 U/ml and 10.36 U/ml; 1196.67 µg/ml and 50.65 µg/ml in pleural fluids and sera, respectively.

Statistical analysis

Due to the lack of normal distribution among variables (Kolmogorov-Smirnov test), non-parametric tests were used for analysis of data. Mann-Whitney test was used to analyze the difference between groups. Correlation between tumor marker levels in sera and pleural fluids was detected by Pearson test. Receiver operating characteristic (ROC) curve was plotted for the tumor markers. The level of significance was considered <0.05.

Results

Among the 77 studied individuals 40 (51.9%) were diagnosed to have a malignant and 37 (48.1%) were diagnosed to have benign pleural effusion. Of the 40 malig-

Table 1. Etiology of pleural effusions

Cause	Number	Percent
<i>Malignant</i>		
Metastatic malignancy	11	14.3
Metastatic adenocarcinoma	8	10.4
Non-Hodgkin's lymphoma	7	9.1
Lung cancer	6	7.8
Breast cancer	2	2.6
Hodgkin's lymphoma	2	2.6
Thyroid carcinoma	2	2.6
Metastatic bone tumor	1	1.3
Multiple myeloma	1	1.3
<i>Benign</i>		
Parapneumonic effusion	9	11.7
CHF	9	11.7
Tuberculosis	7	9.1
Empyema	3	3.9
Traumatic pleural effusion	2	2.6
Hemothorax	2	2.6
Lupus pleuritis	2	2.6
Post-CABG	1	1.3
Pulmonary embolism	1	1.3
Nephrotic syndrome	1	1.3
Total	77	100

CHF: congestive heart failure, CABG: coronary artery bypass graft

nant cases, 22 (55%) were male and 18 (45%) female. The average age of this group was 49.7 years (range 12-77 years). Of the 37 benign cases, 26 (70.3%) were male and 11 (29.7%) female, with an average age of 51.6 years (range 16-88 years). Distribution of pleural effusion etiologies are shown in *Table 1*. Among pulmonary malignancies, 2 were squamous cell carcinoma, one adenocar-

Table 2. Serum and pleural fluid levels of CEA, CA 15-3, CA 125 and NSE in the studied patients (mean ± SEM)

Tumor marker	Malignant (n=40)	Benign (n=37)	P value
<i>CEA (µg/ml)</i>			
Serum	7.16 ± 1.78	3.54 ± 1.44	0.232
Pleural fluid	49.20 ± 14.96	4.63 ± 2.27	0.022
<i>CA 15-3 (U/ml)</i>			
Serum	80.56 ± 20.16	28.50 ± 2.72	0.006
Pleural fluid	154.80 ± 40.06	18.26 ± 4.63	0.0001
<i>CA 125 (U/ml)</i>			
Serum	584.17 ± 354.02	98.28 ± 24.63	0.878
Pleural fluid	2629.16 ± 458.80	1507.98 ± 355.34	0.980
<i>NSE (µg/ml)</i>			
Serum	10.14 ± 1.33	10.80 ± 1.97	0.446
Pleural fluid	22.36 ± 4.41	12.85 ± 4.87	0.001

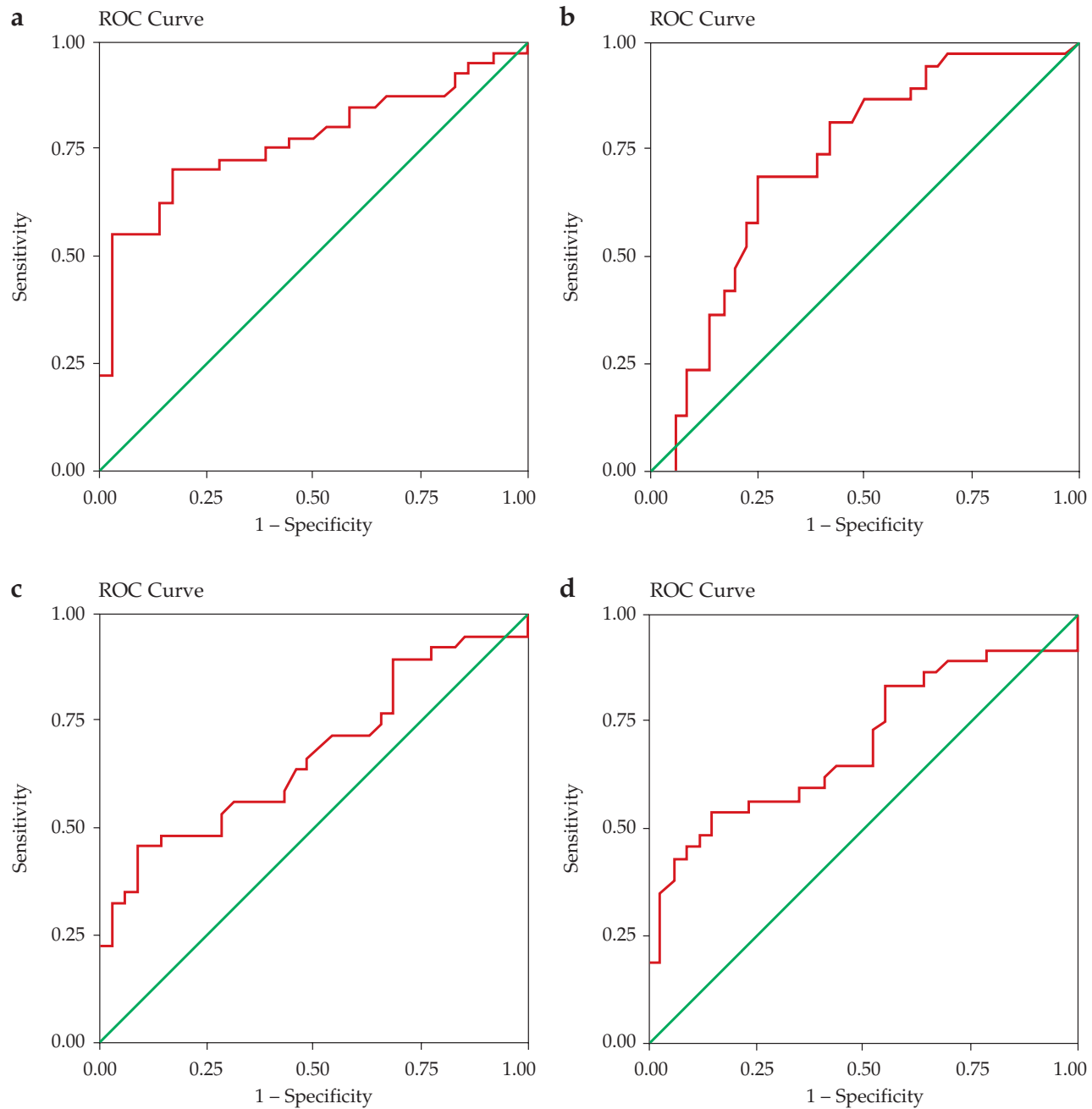


Figure 1. Receiver operating characteristic curve for CA15-3 (a), NSE (b) and CEA (c) in pleural fluid, and CA15-3 in serum (d) for distinguishing between malignant and benign pleural effusions

cinoma, one small cell carcinoma, one poorly differentiated carcinoma and one undiagnosed.

The mean level of different tumor markers in serum and pleural fluid of malignant and benign cases are shown in Table 2. A high correlation was observed between CA 15-3 level in serum and pleural fluid ($r=0.58$, $P=0.0001$), and a relatively good correlation was observed between CEA level in serum and pleural fluid ($r=0.28$, $P=0.026$). However, no significant correlation was observed in levels of

NSE and CA 125 between serum and pleural fluid ($r=0.14$ and $r=0.04$, respectively).

Comparison of the tumor marker levels revealed a statistically significant difference in the CA 15-3 serum level between malignant and benign diseases ($P=0.006$). For the other tumor markers, no significant difference between the sera of patients with benign and malignant disease was observed. The level of CA 15-3, NSE and CEA in malignant and benign pleural fluids showed statistically signifi-

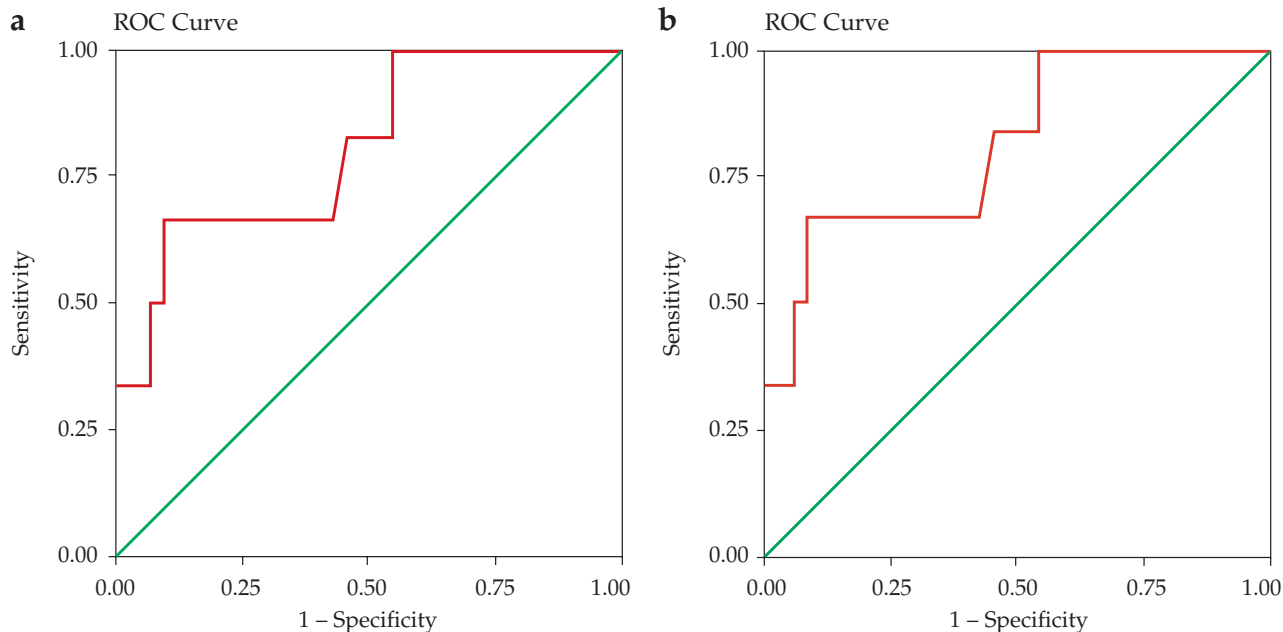


Figure 2. Receiver operating characteristic curve for CA15-3 in serum for distinguishing between pleural effusions due to lung cancer and other causes of pleural effusion (a), and between lung cancer and other malignant causes of pleural effusion (b)

cant differences with P values less than 0.0001, 0.001 and 0.022, respectively.

Analysis of ROC curves (Figure 1) of the tumor markers in the pleural fluid revealed the highest area under the curve for CA 15-3 (0.78, 95% CI=0.67-0.88), followed by NSE (0.73, 95% CI=0.60-0.84) and CEA (0.66, 95% CI=0.53-0.78). Analysis of ROC curve (Figure 2) for CA 15-3 in the serum of patients with malignant pleural effusion due to lung cancer, compared to the other causes of pleural effusion (0.81, 95% CI=0.62-1.0), showed a larger area under the curve with cut-off level of ≥ 41.89 U/ml ($P < 0.017$). However, the area under the curve for CA 15-3 in sera of lung cancer patients did not show any difference compared to other malignant causes (0.61, 95% CI=0.6-0.82).

The sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of the tumor markers were also determined both individually and in combination. Table 3 shows the aforementioned values for serum and pleural fluid of the studied subjects. The highest (100%) specificity and highest (100%) PPV could be obtained by detection of CA 15-3 in serum plus NSE and CA 15-3 in the pleural fluid. Additional detection of CEA in the pleural fluid did not add any value to this level of specificity or PPV. The highest (80%) sensitivity could be obtained by detection of CA 15-3 in serum and CA 15-3 and CEA in the pleural fluid of the patients. The highest (81.3%) NPV and the highest (89%) accuracy could be obtained by detection of CA 15-3 in serum plus CA 15-3, NSE and CEA in the pleural fluid. Among the individual-

ly detected tumor markers, the most sensitive (70%) and accurate (76%) one was CA 15-3, while the most specific (85.3%) tumor marker was CEA.

Discussion

In the current study CA 15-3 level in serum showed 59.5% sensitivity and 63.6% specificity with a significant difference between malignant and benign conditions. Serum CA 15-3, which is known to be specifically elevated in breast cancer, has been reported to have a 86% sensitivity and 67% specificity in differentiating malignant and benign pleural effusions.¹¹ The sensitivity and specificity of CA 15-3 detection in pleural fluids of our patients (i.e. 70% and 83.3%, respectively) are comparable with those in the reports of other investigators,^{11,13} and higher than those in some other reports.^{14,15} The observed variations in the calculated sensitivities and specificities might be due to differences in the selected cut-off values, sample sizes and the etiology of pleural effusions in different settings.

In the present study we did not observe significant difference between serum CEA level of malignant and benign diseases. This is contrary to the results of Ferrer et al. who suggested that CEA was the best serum tumor marker of their patients.⁹ In addition, Marel et al. and Hernandez et al. reported a significant difference between serum CEA level of patients with malignant pleural effusion compared to those with benign pleural effusion.^{2,16} It has been suggested that in patients with empyema and parapneumonic

effusion elevated serum levels of CEA can be detected.¹⁷⁻²⁰ Elevated level of CEA in CHF has also been reported.²¹ In addition, the elevation of CEA in tuberculosis patients is a rare event.^{10,22} Lower proportion of tuberculosis to parapneumonic/empyema patients in our study compared to those in the study of Hernandez et al. (9.1/27.3 vs. 16.2/28.5) may explain the lack of difference between malignant and benign condition in our setting. Moreover, comparison of our malignant group with that of Marel et al. reveals that 39% of their patients had lung cancer with malignant or paramalignant effusions, while only 7.8% of our patients had lung cancer. It is therefore possible that the etiology of malignant condition might affect the results. Such an effect has been shown by the observation that pleural effusions secondary to a metastatic malignancy have higher levels of CEA in pleural fluid and serum than those related to malignant mesothelioma.^{23,24}

Contrary to the serum, the levels of 3 tumor markers, namely CEA, CA 15-3 and NSE, were significantly elevated in malignant pleural fluids of our patients. Among them the highest specificity was found for CEA and the highest sensitivity was found for CA 15-3, however, due to the both high sensitivity and high specificity of CA 15-3, the highest accuracy was found by measuring this marker in pleural fluid. The latter is in accordance with the results of Alatas et al.,¹¹ although we observed a higher specificity of CEA due to the higher cut-off value in our study compared to their study (3.6 ng/L vs. 3 ng/L). Alatas et al.¹¹ did not observe any difference between NSE level in malignant and benign conditions, however, Kuralay et al. reported a much higher sensitivity and specificity of NSE in

pleural fluid despite a higher cut-off level than ours (8.7 ng/L vs. 5.21 ng/L).²⁵ Therefore, besides cut-off level, other factors might interfere with the observed specificities and sensitivities.

By calculating ROC curves we observed the highest area under curve for CA 15-3, which points to the usefulness of this tumor marker in diagnosis of malignant pleural effusions. This marker was also powerful in differentiating lung cancer from other etiologies of pleural effusion in our patients, however, it was not powerful in differentiating between malignancies. The best combination of tumor markers which revealed 100% specificity and 100% PPV with 76.5% sensitivity could be obtained by measurement of CA 15-3 in serum and pleural fluid plus NSE in pleural fluid. A higher sensitivity (80%) with the same level of specificity (100%) could be obtained by additional measurement of CEA in pleural fluid. The observed specificity is the maximum attainable specificity with which the sensitivity (77%) is still higher than that of the combination of cytology / CEA / CA 549 with 97% specificity,¹⁶ and that of CYFRA 21 / CEA / CA 125 (65.1%) with the same level of specificity.⁹ To date the only method having 100% sensitivity and specificity in malignant pleural fluid is thoracoscopy,²⁶ and no combination of tumor markers has reached this level of accuracy. One of the highest reported specificity (97%) and sensitivity (100%) of tumor markers in malignant pleural fluid is combination of MCA / CA 125 / CA 19.9 in pleural fluid.²⁵ The same report assigns sensitivity and specificity of 95% to CA 125, which did not show any difference between malignant and benign cases in our study. Therefore, it seems that the value of any combination

Table 3. Characteristics of tumor markers in patients with pleural effusion

	Cut-off	Sensitivity %	Specificity %	PPV %	NPV %	Accuracy %
<i>Serum</i>						
CA 15-3	29.07	59.5	63.6	64.7	58.3	61
NSE	10.36	38.9	63.6	51.1	46.2	46
CEA	2.95	47.2	81.8	42.6	26.1	37
CA 125	50.65	50.0	45.5	53.0	47.2	50
<i>Pleural fluid</i>						
CA 15-3	21.11	70.0	83.3	82.4	71.4	76
NSE	5.21	68.4	75.0	74.3	69.2	72
CEA	3.60	47.4	85.3	78.3	59.2	65
CA 125	1196.67	48.6	70.6	42	34.5	39
CA 15-3 + NSE		57.5	91.9	88.5	66.7	74
CA 15-3 in S and PF		71.4	86.4	87	70.4	78
CA 15-3 in S + NSE in PF		76.2	77.3	76.3	77.3	77
CA 15-3 in S + CA 15-3 and CEA in PF		80.0	88.9	88.9	80.0	84
CA 15-3 in S + CA 15-3 and NSE in PF		76.5	100.0	100.0	77.8	87
CA 15-3 in S + CA 15-3, NSE and CEA in PF		80.0	100.0	100.0	81.3	89

PPV: positive predictive value, NPV: negative predictive value, S: serum, PF: pleural fluid

of tumor markers differs in various settings which have different etiologies with different rates in malignant and benign pleural effusions. Accordingly, we suggest the diagnostic usefulness of CA 15-3 / NSE / CEA combination in Southern Iranian patients with pleural effusion.

Acknowledgement

Financial support from Shiraz University of Medical Sciences and Shiraz Institute for Cancer Research is greatly appreciated.

References

1. Chernow B, Sahn SA: Carcinomatous involvement of the pleura: an analysis of 96 patients. *Am J Med* 63: 695-702, 1977
2. Marel M, Stastny B, Melinova L, et al: Diagnosis of pleural effusions. Experience with clinical studies, 1986 to 1990. *Chest* 107: 1598-1603, 1995
3. Maskell NA, Butland RJ: Pleural Diseases Group, Standards of Care Committee, British Thoracic Society. BTS guidelines for the investigation of a unilateral pleural effusion in adults. *Thorax* 58 (Suppl 2): 8-17, 2003
4. Loddenkemper R: Medical thoracoscopy. In: *Textbook of Pleural Diseases*. (Eds: Light RW, Gray Lee, YC), 2003, pp 498-512
5. Prakash UB, Reiman HM: Comparison of needle biopsy with cytologic analysis for the evaluation of pleural effusion: analysis of 414 cases. *Mayo Clin Proc* 60:158-164, 1985
6. Johanson WW: Cancer, 1985; Light RW, Approach to the patient. In: *Pleural Diseases*. (Ed: Light RW), 3rd ed, William & Wilkins, Baltimore, 1995, pp 75-82
7. Porcel JM, Vives M, Esquerda A, et al: 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: 1757-1763, 2004
8. Richard WL: Tumor markers in undiagnosed pleural effusions. *Chest* 126: 1721-1722, 2004
9. Ferrer J, Villarino MA, Encabo G, et al: Diagnostic utility of CYFRA 21-1, carcinoembryonic antigen, CA 125, neuron specific enolase, and squamous cell antigen level determinations in the serum and pleural fluid of patients with pleural effusions. *Cancer* 86: 1488-1495, 1999
10. Garcia-Pachon E, Padilla-Navas I, Dosda MD, et al: Elevated level of carcinoembryonic antigen in nonmalignant pleural effusions. *Chest* 111: 643-647, 1997
11. Alatas F, Alatas O, Metintas M, et al: Diagnostic value of CEA, CA 15-3, CA 19-9, CYFRA 21-1, NSE and TSA assay in pleural effusions. *Lung Cancer* 31: 9-16, 2001
12. Ryu JS, Lee HJ, Cho JH, et al: The implication of elevated carcinoembryonic antigen level in pleural fluid of patients with non-malignant pleural effusion. *Respirology* 8: 487-491, 2003
13. Villena V, Lopez-Encuentra A, Echave-Sustaeta, et al: Diagnostic value of CA 72-4, carcinoembryonic antigen, CA 15-3, and CA 19-9 assay in pleural fluid. A study of 207 patients. *Cancer* 78: 736-740, 1996
14. Shimokata K, Totani Y, Nakanishi K, et al: Diagnostic value of cancer antigen 15-3 (CA15-3) detected by monoclonal antibodies (115D8 and DF3) in exudative pleural effusions. *Eur Respir J* 4: 341-344, 1988
15. Romero S, Fernandez C, Arriero JM, et al: CEA, CA 15-3 and CYFRA 21-1 in serum and pleural fluid of patients with pleural effusions. *Eur Respir J* 9: 17-23, 1996
16. Hernandez L, Espasa A, Fernandez C, et al: CEA and CA 549 in serum and pleural fluid of patients with pleural effusion. *Lung Cancer* 36: 83-89, 2002
17. Pinto MM, Bernstein LH, Rudolph RA, et al: Diagnostic efficiency of carcinoembryonic antigen and CA125 in the cytological evaluation of effusions. *Arch Pathol Lab Med* 116: 626-631, 1992
18. McKenna JM, Chandrasekhar AJ, Henkin RE: Diagnostic value of carcinoembryonic antigen in exudative pleural effusions. *Chest* 78: 587-590, 1980
19. Romero Candreira S, Hernandez Blasco L, Senent Espanol C, et al: Clinical usefulness of tumor markers in the diagnosis of pleural effusions. Carcinoembryonic antigen, alpha-fetoprotein and orosomucoid. *Med Clin* 86: 439-443, 1986
20. Garcia-Pachon E, Padilla Navas I, Querol M, Custardoy J. The behavior of carcinoembryonic antigen in pleural empyema. *Med Clin* 101: 237-238, 1993
21. Whiteside TL, Dekker A: Diagnostic significance of carcinoembryonic antigen levels in serous effusions. Correlation with cytology. *Acta Cytol* 23: 443-448, 1979
22. Niwa Y, Kishimoto H, Shimokata K: Carcinomatous and tuberculous pleural effusions. Comparison of tumor markers. *Chest* 87: 351-355, 1985
23. Faravelli B, Nosenzo M, Razzetti A, et al: The role of concurrent determinations of pleural fluid and tissue carcinoembryonic antigen in the distinction of malignant mesothelioma from metastatic pleural malignancies. *Eur J Cancer Clin Oncol* 21: 1083-1087, 1985
24. Mezger J, Calavrezos A, Drings P, et al: Value of serum and effusion fluid CEA levels for distinguishing between diffuse malignant mesothelioma and carcinomatous pleural metastases. *Lung* 172: 183-184, 1994
25. Kuralay F, Tokgoz Z, Comlekci A: Diagnostic usefulness of tumor marker levels in pleural effusions of malignant and benign origin. *Clin Chim Acta* 300: 43-55, 2000
26. Fenton KN, Richardson JD: Diagnosis and management of malignant pleural effusions. *Am J Surg* 170: 69-74, 1995