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# DIAGNOSTIC IMPORTANCE OF TUMOR MARKERS, NEURON SPECIFIC ENOLASE (NSE), CARCINO-EMBRYONIC ANTIGEN (CEA) AND CANCER ANTIGEN 15-3 (CA 15-3), IN SERUM AND PLEURAL EFFUSIONS OF PATIENTS WITH MALIGNANT PULMONARY DISEASES

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## ABSTRACT

The tumor markers such as Neuron Specific Enolase (NSE), carcino-embryonic antigen (CEA), cytokeratin fragment 21-1 (CYFRA 21-1), cancer antigen 15-3 (CA 15-3) and Tissue Polypeptide Specific antigen (TPS) are considered diagnostically useful in the assessment of lung cancer with different sensitivities and specificities in variable settings. In this regard, the present study describes the results of determination of the tumor markers NSE, CA 15-3 and CEA in blood and pleural fluid of patients with pleural effusions, and to evaluate their usefulness as a non-invasive method for differentiating between benign and malignant conditions. Sixty nine (n = 69) patients during the period, February 2006 to March 2009, were included in the study. Thirty eight (n = 38) out of 69 patients were grouped as malignant cases, sub-classified as NSCLC, SCLC, Breast cancer etc, where as n = 31 were found to have benign lung disease (tuberculosis). All 31 patients with tuberculosis pleurisy were classified as patients with benign condition and used as comparative group. Blood and pleural fluid samples were analyzed for NSE, CA 15-3, and CEA using electrochemiluminescence (ECL) technology on automated immunoassay analyzer according to manufacturer's advice. Comparative study was performed individually for each malignant condition such as SCLC, NSCLC, breast carcinoma, and others (such as lymphoma and mesothelioma). In pleural fluid, NSE levels were noted to be significantly raised ($P< 0.001$) when compared with CEA and moderately raised ($P< 0.01$) when compared with CA 15-3 in SCLC group of patients. However, in NSCLC group, elevation pattern of NSE was moderately significant ($P< 0.01$) when compared with CEA and non-significant when compared with CA 15-3. Furthermore, levels of NSE observed to be low or in same range when compared with CA 15-3 and CEA in breast cancer and other malignant conditions. CA 15-3 was found to be significantly elevated in NSCLC with moderate capacity ($P <0.01$), with strong capacity in breast ($P< 0.0001$) and with mild capacity in other malignant conditions ($P<0.05$) when compared with both NSE and CEA. Similar pattern of elevation and moderate significance was noted in serum levels of NSE, CA 15-3 and CEA in SCLC, NSCLC and breast malignancy patients.

In conclusion NSE seems to be the most significant marker in pleural fluid for SCLC patients since it exhibited three fold elevations in its levels when compared with serum levels in same group of patients, followed by its moderate significance for NSCLC patients. CA 15-3, on the other hand, was found to be highly significant marker for breast cancer and moderately significant marker for NSCLC and other malignant conditions.

**Key words:** Neuron specific enolase (NSE), carcino-embryonic antigen (CEA), cancer antigen 15-3 (CA 15-3), pleural effusions, malignant pulmonary diseases.

## INTRODUCTION

A majority of malignant conditions lead to pleural effusion during the course of the disease progression (Wagner *et al.,* 2007). It is well documented that one of the major malignant disease, which is most often associated with pleural effusion is lung cancer, and accounts for up to 30% of all cases of malignant pleural effusions (Ghayumi *et al.*, 2005; Lyubimova *et al.*, 2002; Molina *et al*., 2008; Mumbarker *et al.*, 2006; Wagner *et al.*, 2007). In clinical and diagnostic setting, the evaluation of etiology of pleural effusion is frequently a dilemma, especially when differentiation is imperative between malignant and benign pleural effusions (Esscher *et al.*, 1985; Ghayumi *et al.*, 2005; Lee and Chang, 2005; Wagner *et al.*, 2007). It is reported that such differentiations is a necessacity for better treatment and prognosis (Light, 2001; Niwa et al., 1997; Wagner *et al*., 2007). Statistics for the year 2000 for the United States shows that the annual number of new cases of pleural effusion is over 1,000,000, approximately 200,000 of which are found to be associated with a malignant disease (ATS, 2000; Lodden Kemper, 1998; Wagner *et al.,* 2007). Attempts have been made in last two decades to improve the identification of malignant cases by assessing the usefulness of biological tumor markers (Ghayumi *et al.,* 2005; Lee and Chang, 2005; Lyubimova *et al.,* 2002; Miedouge *et al.,* 1999; Molina *et al*., 2008; Wagner *et al.,* 2007). These tumor markers are

macromolecules produced by neoplastic cells or whose production is increased in the presence of malignant condition (Malkin *et al.*, 1992; Wagner *et al.*, 2007). It is thoroughly reported in literature that these markers can be detected in various biological specimens such as blood, serous liquid, and tissue samples (Ferrer, 2000; Ghayumi *et al.*, 2005; Lee and Chang, 2005; Wagner *et al*., 2007). Various studies have reported the diagnostic usefulness of a number of tumor markers such as Neuron Specific Enolase (NSE), carcino-embryonic antigen (CEA), cytokeratin fragment 21-1 (CYFRA 21-1), cancer antigen 15-3 (CA 15-3) and Tissue Polypeptide Specific antigen (TPS) in lung cancer (Buccheri and Ferrigno, 2001; Esscher *et al.*, 1985; Fenton and Richardson, 1995; Ghayumi *et al.*, 2005; Gross, 1999; Lee and Chang, 2005; Molina *et al*., 2008; Mumbarker *et al.*, 2006; Porcel *et al.*, 2004; Satoh et al., 1995) with different sensitivities and specificities in variable settings (Braga, 1990; Dejsomritrutai *et al.*, 2001; Esscher *et al*., 1985; Ghayumi *et al.*, 2005; Malkin *et al*., 1992).

The present study describes the results of determination of the tumor markers NSE, CA 15-3 and CEA in blood and pleural fluid of patients with pleural effusions to evaluate their usefulness as a non-invasive method of differentiating between benign and malignant conditions.

## MATERIALS AND METHODS

Protocols described by Wagner *et al.*, (2007) were followed to execute various steps in the present study. The patients were composed of sixty nine (n = 69) consecutive cases referred to the department of Biochemistry, Liaquat National Hospital and Medical College and department of Pathology, Govt Layri General Hospital, Karachi between February 2006 to March 2009 for the investigation of pleural fluids and effusions were included in the study. The demographic data (age, gender, and profession), smoking habits of patients as well as the aspect of the pleural fluid, respiratory symptoms, information on past/present diseases, complimentary examinations, and recent/present use of medication were collected as per protocols detailed earlier (Wagner *et al.*, 2007). The criteria detailed by Light (2000), were considered for assessment of exudates and transudates such that the effusion was considered malignant when the cytopathologic study of the pleural fluid indicated malignancy. The pleural effusion was considered benign when the cytopathologic study of the pleural fluid was found negative for malignancy. Furthermore the pleural fluids was noted as benign if its accumulation was accompanied by parapneumonia or empyema (Light, 1995; as noted in Wagner *et al*., 2007), tuberculosis, congestive heart failure or liver diseases.

### Collection of blood and pleural fluids/effusions.

Thirty eight (n = 38) out of 70 patients were grouped as malignant cases where as 32 were found to have benign lung disease (n = 31 with tuberculosis and n = 1 with pneumonic effusion). However, for a single group clarity, only 31 patients with tuberculosis pleurisy were classified as patients with benign condition and used as comparative group. Blood samples and pleural fluids were simultaneously collected to determine the levels of tumor markers. All samples which were sent to laboratory for biochemical investigations were stored in aliquots of 1.5 to 2.0 ml at -25$^{O}$C until further use.

### Determination of tumor markers.

The electrochemiluminescence (ECL) technology was used to determine the levels of NSE, CA 15-3, and CEA on automated immunoassay analyzer Elecsys 2010 (Roche Diagnostics, Basel) according to manufacturer's advice. The values established for the respective markers in the serum were; CEA less than 4.6 ng/ml (non-smokers), less than 10.0 ng/ml (smokers); CA 15-3 less than or upto 40 ng/ml (Wagner *et al.*, 2007) and NSE less than or equal to 15 ng/ml.

### Statistical Analysis:

All data were expressed as mean ± standard deviations (SD). Statistical analysis was performed using statistical software (SPSS ver 13 Chicago, IL). Differences between the two groups were evaluated using the student "*t*" test and Pearson's correlation and statistical significance level was at least $P < 0.05$.

## RESULTS

Of the 69 patients, 38 (55.10%) were diagnosed with malignant pleural effusion, 31 (44.90%) with benign effusion of tuberculosis. The gender base distribution was 34.80% males (n = 24) and 20.30% females (n= 14) in malignant group with mean age of 61.29 ± 20.16 yrs whereas benign group includes 31.90% males (n = 22) and 13.00% females (n =9) with mean age 54.20 ± 15.16 yrs (Table 1). The etiologic diagnoses of the cases of pleural effusion are summarized in Table 2.

**Table 1.** Distribution of the malignant and benign groups by age and gender.

| Variables | Malignant pleural effusion (n = 38, 55.10%) | Benign pleural effusions (n = 31, 44.90%) |
|---|---|---|
| Age (years) | 61.29 ± 20.16[a] | 54.20 ± 15.16[a] |
| Gender = Males | 34.80% (n = 24) | 31.90% (n = 22) |
| = Females | 20.30% (n = 14) | 13.00 (n = 9) |

[a]Data presented as mean ± standard deviation. Percent distribution was calculated as per total number of patients (n = 69)

**Table 2.** Etiological distribution of the cases of malignant and benign pleural effusion.

| **Causes** | **N** |
|---|---|
| Malignant | 38 |
| **I-secondary to carcinoma** | |
| a) Lung | 24 (divided into SCLC, NSCLC) |
| i) SCLC | 19 |
| ii) NSCLC | 5 |
| b) Breast | 6 |
| c) other malignancies | 3 (neck, oral, gastric) |
| **II-secondary to other tumor types** | |
| a) Lymphoma | 3 |
| b) Mesothelioma | 1 |
| c) Melanoma | 1 |
| **III-Tuberculosis** | 31 |

**Table 3.** Mean ± standard deviation (ng/ml) of tumor markers NSE, CA 15-3 and CEA obtained in the serum and pleural fluid of the patients with malignant or benign pleural effusion.

| **Markers** | Malignant | | Benign | |
|---|---|---|---|---|
| | **Pleural fluids n = 38** | **Serum n = 38** | **Pleural fluid n = 31** | **Serum n = 31** |
| NSE | 60.30 ± 15.10 | 20.10 ± 5.15 | 9.00 ± 2.10 | 3.00 ± 0.80 |
| CA 15-3 | 59.11 ± 16.35 | 31.14 ± 8.66 | 11.26 ± 3.50 | 10.16 ± 2.45 |
| CEA | 25.60 ± 13.21 | 5.50 ± 1.45 | 1.20 ± 0.95 | 1.20 ± 0.90 |

**Table 4.** Mean ± standard deviation (ng/ml) of the markers NSE, CA 15-3 and CEA in pleural fluids of patients with various malignant conditions.

| **Markers** | **SCLC** n = 19 | **NSCLC** n = 5 | **Breast cancer** n = 6 | **Others**[*] n = 8 |
|---|---|---|---|---|
| NSE | 102.20 ± 25.10[a] | 50.10 ± 14.20 | 23.10 ± 4.85 | 21.10 ± 6.18 |
| CA 15-3 | 79.00 ± 18.60[a,e] | 89.35 ± 15.60[b] | 101.15 ± 22.25[c] | 56.55 ± 10.62[d] |
| CEA | 29.50 ± 11.51 | 40.66 ± 21.54 | 50.21 ± 14.90 | 28.26 ± 8.35 |

[*]Other malignant conditions include neck, oral, gastric, lymphoma, mesothelioma, melanoma.
[a]Significant at P< 0.001 when compared with CEA only
[b]Significant at P< 0.01 when compared with NSE and CEA
[c]Significance at P< 0.0001 when compared with CEA and NSE
[d]Significance at P< 0.05 when compared with CEA and NSE
[e]Significance at P< 0.05 when compared with NSE only

**Table 5**. Mean ± standard deviation (ng/ml) of the markers NSE, CA 15-3 and CEA in serum of patients with various malignant conditions.

| **Markers** | **SCLC** n = 19 | **NSCLC** n = 5 | **Breast cancer** n = 6 | **Others*** n = 8 |
|---|---|---|---|---|
| NSE | 49.20 ± 11.40[a] | 25.35 ± 14.62 | 15.37 ± 6.20 | 10.70 ± 7.60 |
| CA 15-3 | 30.40 ± 10.10[a,e] | 46.20 ± 13.35[b] | 80.30 ± 20.71[c] | 23.15 ± 8.92[d] |
| CEA | 9.26 ± 2.10 | 11.60 ± 3.20 | 13.10 ± 6.6 | 8.29 ± 2.56 |

*Other malignant conditions include neck, oral, gastric, lymphoma, mesothelioma, melanoma.
[a]Significant at P< 0.0001 when compared with CEA only
[b]Significant at P< 0.01 when compared with NSE and CEA
[c]Significance at P< 0.0001 when compared with CEA and NSE
[d]Significance at P< 0.05 when compared with CEA and NSE
[e]Significance at P< 0.05 when compared with NSE onl

Table 3 describes the mean ± SD levels of tumor markers, NSE, CA 15-3 and CEA for the pleural fluids, both in malignant and benign groups. In the patients with malignant pleural effusions, cumulative levels of NSE and CEA were significantly higher in the pleural fluid than in the serum ($P < 0.001$). However, regarding CA 15-3, moderate significance ($P < 0.01$) was noted in difference between the serum and pleural fluid in same group.

In the cases of benign pleural effusion, NSE levels were moderately higher in the pleural fluid than in the serum ($P < 0.01$), whereas no significant difference was noted in the levels of CEA and CA 15-3.

To further elaborate the significance level of tumor markers in pleural fluids and serum, comparison was also performed separately for each malignant condition such as SCLC, NSCLC, breast carcinoma etc (Table 4, 5). In pleural fluid, NSE levels were noted to be significantly raised (P< 0.001) when compared with CEA and moderately raised (P< 0.01) when compared with CA 15-3 in SCLC group of patients. In NSCLC group, elevation pattern of NSE was moderately significant (P< 0.01) when compared with CEA and non-significant when compared with CA 15-3. However levels of NSE observed to be low or in same range when compared with CA 15-3 and CEA in breast cancer and other malignant conditions. CA 15-3 was found to be significantly elevated in NSCLC (P <0.01), breast (P< 0.0001) and other malignant conditions (P<0.05) when compared with both NSE and CEA. Furthermore, although much lower in concentrations, but also similar pattern of elevation and moderate significance was noted in serum levels of NSE, CA 15-3 and CEA in SCLC, NSCLC and breast malignancy patients.

In present study NSE seems to be the most significant marker in pleural fluid for SCLC patients since it exhibited three fold elevations in its levels when compared with serum levels in same group of patients, followed by its moderate significance for NSCLC patients. However, CA 15-3 was found to be the moderately significant marker for NSCLC and other malignant conditions, and highly significant for breast cancer.

## DISCUSSION

It has been constantly argued that differential diagnosis between benign and malignant pleural effusion represents a distinctive challenge (Esscher *et al.*, 1985; Ghayumi *et al.*, 2005; Lee and Chang, 2005; Lyubimova *et al.*, 2002; Sahn, 1997; Wagner *et al.*, 2007). Among the potential factors responsible for this difficulty are the inadequate collection method, laboratory manipulation of the pleural fluid, as well as an insufficient volume of pleural material (Romero *et al.*, 1996; Wagner *et al.*, 2007). During last two decades, attempts have been made by scientists, researchers, in collaboration with clinicians and oncologists, to explore other scientific and diagnostic tools, such as determination of tumor markers, to establish their potential usefulness as an alternate method; more efficient, less invasive and economical, for identifying as well as differentiating malignant and benign pleural effusions (Esscher *et al.*, 1985; Lee and Chang, 2005; Molina *et al.*, 2008; Wagner *et al.*, 2007). In this regard, several tumor markers including NSE, CEA, CA 15-3, CYFRA 21-1, TPS have been studied and explored for malignant lung disease such as NSCLC, SCLC and benign conditions such as tuberculosis (Fernandes *et al.*, 1996; Gross, 1999; Lyubimova *et al.*, 2002; Mumbarkar *et al.*, 2006; Wagner *et al.*, 2007). However, it was pointed out that comparisons among such studies to draw a definite conclusion is difficult due to differences in the numbers and types of markers evaluated, uniformity in the laboratory technology used in various studies and establishment of cut-off points to determine specificity/sensitivity, in addition to the detection of markers in benign diseases and the prevalence of the tumor type in the groups studied (Wagner *et al.*, 2007). To overcome such lacunae, several studies have been by utilizing a combination of more than one marker to increase their sensitivity in detecting malignant conditions (Hillas *et al.*, 2008; Porcel *et al.*, 2004; Romero *et al.*, 1996; Shitrit *et al.*, 2007; Villena *et al.*, 2003;

Wagner *et al.,* 2007) and especially those related to pulmonary area. In a significant study documented earlier (Wagner *et al.,* 2007), a combination of tumor markers CEA, CYFRA21-1, and CA 15-3 were chosen because of the observation that the majority of the such studies conducted earlier have at least two of these in their marker panel, suggesting that their inclusion would increase the diagnostic sensitivity and specificity in the detection of malignant pleural effusion (Ferrer *et al.,* 1999; Porcel *et al.,* 2004; Shitrit *et al*., 2005; Trape *et al.,* 2004; Wagner *et al.,* 2007).

For our present study also, we have determined three markers, NSE, CA 15-3 and CEA in patients with lung, breast and related cancers as well as benign pulmonary conditions to establish their potential usefulness in detecting malignant pleural effusions and differentiating it from benign ones. In the study, NSE was noted to be the most significant markers in pleural effusions from SCLC patients when compared with CEA and CA 15-3 and exhibited three fold raise in its concentrations when compared with the serum levels in same group of patients. It also exhibited moderate significance for pleural fluid for NSCLC patients when compared with other two markers. Another tumor marker, CA 15-3 also showed moderate significance for SCLC, NSCLC and other malignant conditions when compared with NSE in pleural effusions and serum. However, it also manifested high significance for breast cancer related pleural effusions, surpassing both NSE and CEA. CEA, on the other hand, although showed raised concentration in pleural fluids than serum, but elevation was moderate as compared to NSE and CA 15-3, and also devoid of any specific pattern of elevation in all malignant conditions.

For the three markers studied, there were statistically significant differences between the serum and pleural fluid samples, as well as between their concentration levels in malignant and benign forms of pleural effusion. These results are in agreement with what has been reported in other studies (Molina *et al.,* 2008; Mumbarkar *et al.,* 2006; Porcel *et al.,* 2004; Romero *et al.,* 1996; Villena *et al.,* 2003; Salama *et al.,* 1998; Wagner *et al.,* 2007). It was documented that the use of the tumor marker combinations such as NSE, CEA, CA 15-3 or CYFRA 21-1 presents significantly greater sensitivity for the identification of malignancy than did the cytopathological study (Esscher *et al.,* 1985; Ghayumi *et al.,* 2005; Trape *et al*., 2008).

Regarding the studies carried out for individual markers, Kuralay *et al* (2000) reported a much higher sensitivity and specificity for NSE in pleural fluid, however Alatas *et al*., (2001) did not observe any difference between NSE level in malignant and benign conditions. Therefore, besides cut-off level, it was believed that other factors might interfere with the observed specificities and sensitivities. Furthermore, elevated pleural fluid levels of NSE were found in 75% of cytology-positive SCLC patients at diagnosis (Esscher *et al.,* 1985). This percentage is almost equivalent to positive rate in serum NSE levels in SCLC patients, which was noted to be in the range of 65-94% (Carney *et al.,* 1982). Moreover, elevated pleural fluid levels of NSE were found only in two of 37 carcinomatous pleural effusions in NSCLC, in two of 39 tuberculous pleural effusions and in none of ten cytology-negative SCLC pleural effusions at diagnosis (Esscher *et al.,* 1985). Raised levels of NSE were also seen in serum lung cancer patients as well as in pleural effusion with lung cancer patients (Mumbarkar *et al.,* 2006). In a related study, pleural fluid's NSE in SCLC was reported to be significantly higher than in NSCLC and consequently demonstrated its usefulness in pleural fluid to distinguish SCLC from NSCLC (Lee and Chang, 2005; Shimokata *et al.,* 1989). As regard CEA, it was found to be a moderately significant tumor marker in pleural fluid, however not in accordance with previous reports (Pastor *et al.,* 1997; Villena *et al*., 1996).

As stated earlier various combinations of tumor markers, especially CEA and NSE increase their efficacy. Therefore Menard *et al.,* (1993) reported that the determination of NSE in pleural fluid improved the sensitivity of CEA alone. Furthermore, in pleural fluid, the determinations of both CEA and NSE were rather superior to those of all three: CEA, NSE, and CYFRA 21–1 (Menard *et al.,* 1993). Interestingly concurrent measurements of CYFRA 21–1 and CEA in serum increased both sensitivity and specificity vs CEA alone, whereas the addition of NSE to CEA determination, in addition to CYFRA 21–1, increased sensitivity.

CA 15-3, which is best known for breast cancer, exhibited 59.5% sensitivity and 63.6% specificity in a study reported earlier with a significant difference between malignant and benign conditions of patients (Ghayumi *et al.,* 2005). Serum CA 15-3 has been reported earlier to have 86% sensitivity and 67% specificity in differentiating malignant and benign pleural effusions (Ghayumi *et al.,* 2005). Moreover, an evaluation study conducted earlier regarding the sensitivity and specificity of CA 15-3 in pleural fluids of lung cancer patients were found to be in accordance other investigators, however exhibited a much higher pattern than reported in few later reports (Ghayumi *et al.,* 2005; Romero *et al.,* 1996; Villena *et al.,* 1996).

It was suggested that the best combination of tumor markers could be obtained by measurement of CA 15-3 in serum and pleural fluid in addition to NSE in pleural fluid that can brought 100% specificity and 100% PPV with 76.5% sensitivity (Ghayumi *et al.,* 2005).Additionally, a higher sensitivity (80%) with the same level of specificity (100%) could be obtained by additional measurement of CEA in pleural fluid (Ghayumi *et al.,* 2005). In coincidence with our study, highly significant difference have not been observed between serum CEA level of malignant and benign diseases, however this has not been the case in pleural effusion studies that exhibited raised values in

malignant pleurisy. The results observed by Ferrer *et al.* (1999), therefore, suggested that CEA was the best serum tumor marker in lung cancer patients. In addition, Marel *et al.,* (1995) and Hernandez *et al.* (2002) reported a significant difference between serum CEA level of patients with malignant pleural effusion compared to those with benign pleural effusion. It has been suggested in few studies that in patients with empyema and parapneumonic effusion, elevated serum levels of CEA can be detected (Garcia-Pachon *et al.,* 1993; Mckenna *et al.,* 1980; Pinto *et al.,* 1992; Romero *et al.,* 1986; Whiteside and Dekker, 1979). Similarly, elevated level of CEA in CHF has also been reported (Whiteside and Dekker, 1979).

In conclusion and reviewing the related studies in cases of suspected malignancy and inconclusive initial findings, tumor marker level or concentration of a combination of tumor markers should be determined prior to the performance of invasive procedures. The present study represents the investigation of potential usefulness of tumor markers in pleural effusions to distinctively differentiate malignant disease from benign conditions. Our findings demonstrate that determining pleural fluid levels of a combination of tumor markers, such as NSE and CA 15-3 in addition to CEA, is useful in differentiating between benign and malignant pleural effusions.

**REFERENCES**

Alataş, F., O. Alataş, M. Metintaş, O. Colak, E. Harmanci and S. Demir (2001). 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.

Buccheri, G. and D. Ferrigno (2001). Lung tumor markers of cytokeratin origin: an overview. *Lung Cancer*; 34 suppl 2:S65–9.

Braga, A.C.O. (1990). Estudo do antígeno carcinoembrionário no diagnóstico dos derrames pleurais [dissertação]. Rio de Janeiro: Universidade Federal Fluminense,

Carney, D.N., P.J. Marangos, D.C. Ihde, P.A. Bunn, M.H. Jr, Cohen, J.D. Minna and A.F. Gazdar (1982). Serum neuron-specific enolase: a marker for disease extent and response to therapy of small-cell lung cancer. *Lancet*; i:583-85

Dejsomritrutai, W., S. Senawong and B. Promkiamon (2001). Diagnostic utility of CYFRA 21-1 in malignant pleural effusion. *Respirology*; 6:213–6.

Esscher, T., L. Steinholtz, J.E. Bergh, K. Nilsson and S. Pahlman (1985). Neurone specific enolase: a useful diagnostic serum marker for small cell carcinoma of the lung. *Thorax,* 40: 85-90

Fenton, K.N. and J.D. Richardson (1995). Diagnosis and management of malignant pleural effusions. *Am J Surg*; 170:69–74.

Ferrer, J.S. (2000). Marcadores tumorales em líquido pleural. *Arch Bronconeumo.,* 36(6): 295–7.

Ferrer, J., M.A. Villarino, G. Encabo, E. Felip, B. Bermejo, S. Vilà and R. Orriols (1999). 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(08): 1488–95.

Fernández, C., J.M. Arriero, A. Espasa, A. Candela, C. Martín and J. Sánchez-Paya (1996). CEA, CYFRA 21-1 in serum and pleural fluid of patients with pleural effusions. *Eur Respir J.,* 9: 17-23, 1996

Garcia-Pachon, E., I. Padilla Navas, M. Querol and J. Custardoy (1993). The behavior of carcinoembryonic antigen in pleural empyema. *Med Clin.,* 101: 237-238.

Gross, J.L. (1999). Marcadores tumorais séricos em pacientes com carcinoma de pulmão não pequenas células [dissertação]. *Evaluation of serum and pleural levels of the tumor markers CEA, CYFRA21-1 and CA 15-3 in patients with pleural effusion,* p 191 São Paulo: Faculdade de Medicina, Universidade de São Paulo.

Gyayumi, S.M.A., S. Mehrabi, M. Doroudchi and A. Ghaderi (2005). Diagnostic value of tumor markers for differentiating malignant and benign pleural effusions of Iranian Patients. *Pathol. Oncol. Res*., 11: 236-241

Hernandez, L., A. Espasa, C. Fernandez, A. Candela, C. Martin and S. Romero (2002). CEA and CA 549 in serum and pleural fluid of patients with pleural effusion. *Lung Cancer,* 36: 83-89.

Pinto, M.M., L.H. Bernstein, R.A. Rudolph, D.A. Brogan and M. Rosman (1992). Diagnostic efficiency of carcinoembryonic antigen and CA125 in the cytological evaluation of effusions. *Arch Pathol Lab Med*., 116: 626- 631.

Hillas, G., C. Moschos, K. Dimakou, F. Vlastos, S. Avgeropoulou, I. Christakopoulou, A. Rasidakis and P. Bakakos (2008). Carcinoembryonic antigen, neuron-specific enolase and cytokeratin fragment 19 (CYFRA 21-1) levels in induced sputum of lung cancer patients. *Scand J Clin Lab Invest*., 8:1-6.

Kuralay, F., Z. Tokgoz and A. Comlekci (2000). Diagnostic usefulness of tumor marker levels in pleural effusions of malignant and benign origin. *Clin Chim Acta*., 300: 43-55.

Lee, J.H. and J.H. Chang (2005). Diagnostic utility of serum and pleural fluid CEA, NSE and CYFRA in patients with effusions from primary lung cancer. *Chest,* 128: 2298-2303.

Light, R.W. (1995). A new classification of parapneumonic effusions and empyema. *Chest;* 108:299–301.

Light RW. 2001. Pleural effusion related to metastatic malignancies. In: *Light RW, editor. Pleural Diseases.* 4th ed. Philadelphia: Lippincott; p.108–34.

Light, R.W. (2002). Pleural effusion. *N Engl J Med.,* 346: 1971-7

Loddenkemper, R. (1998). Thoracoscopy-state of the art. *Eur Respir J.*, 11(1): 213–21.

American Thoracic Society (2000). Management of malignant pleural effusion. *Am J Respir Crit Care Med.,* 162: 1987–2001.

Lyubimova, N.V., T.N. Yagya, A.G. Chuchalin and N.E. Kushlinskii (2002). Diagnostic value of tumor markers CYFRA 21-1 and NSE in analysis of Pleural fluid. *Bull. Exp Biol Med.,* 133: 552-555.

Malkin, A. (1992). Tumor Makers. In: (Tannock I, Hill R, editors). In: *The basic science of oncology.*2nd ed. Baskerville: McGraw- Hill;p.196–206.

Marel, M., B. Stastny, L. Melinova, E. Svandova and W. Light Richards (1995). Diagnosis of pleural effusions. Experience with clinical studies, 1986 to 1990. *Chest*; 107: 1598-1603.

McKenna, J.M., A.J. Chandrasekhar and R.E. Henkin (1980). Diagnostic value of carcinoembryonic antigen in exudative pleural effusions. *Chest*, 78: 587-590.

Menard, O., B. Dousset, C. Jacob and Y. Martinet (1993). Improvement of the diagnosis of the cause of a pleural effusion in patients with lung cancer by simultaneous quantification of carcinoembryonic antigen (CEA) and neuron-specific enolase (NSE) pleural levels. *Eur J Cancer*, 13: 1806–1809.

Miédougé, M., P. Rouzaud, G. Salama, M.C. Pujazon, C. Vincent, M.A. Mauduyt, J. Reyre, P. Carles and G. Serre (1999). Evaluation of seven tumour markers in pleural fluid for the diagnosis of malignant effusions. *Br J Cancer*. 81(6): 1059-65.

Molina, R., J.M. Auge, J.M. Escudero, R. Marrades, N. Vinolas, E. Carcereny, J. Ramirez and X. Filella (2008). Mucins CA 125, CA 19.9, CA 15-3 and TAG-72.3 as tumor marker in patients with lung cancer: comparison with CYFRA 21-1, CEA, SCC and NSE. *Tumor Biol.*, 29: 371-380.

Mumbarkar, P.P., A.S. Raste and M.S. Ghadge (2006). Significance of tumor markers in lung cancer. *Ind J Clin Biochem.,* 21: 173-176.

Niwa, Y., H. Kishimoto and K. Shimokata (1997). Carcinomatous and tuberculous pleural effusions. Comparison of tumor markers. *Chest.,* 87: 351-355.

Pastor, A., R. Menéndez, M.J. Cremades, V. Pastor, R. Llopis and J. Aznar (1997). Diagnostic value of SCC, CEA and CYFRA 21–1 in lung cancer: a Bayesian analysis. *Eur Respir J*., 10: 603–609.

Porcel, J.M., M. Vives, A. Esquerda, A. Salud, B. Pérez and F. Rodríguez-Panadero (2004). Use of a panel of tumor markers (carcinoembryonic antigen, câncer antigen 125, carbohydrate antigen 15-3, and cytokeratin 19 fragments) in pleural fluid for the differential diagnosis of benign and malignant effusions. *Chest.,* 126(6): 1757–63.

Romero, S., C. Fernandez, J.M. Arriero, A. Espasa, A. Candela, C. Martin and J. Sanchez-Paya (1996). CEA, CA 15-3 and Cyfra 21-1 in serum and pleural fluid of patients with pleural effusion. *Eur Respir J*; 9:17–23.

Romero, C.S., B.L. Hernández, E.C. Senent, R.A. García, Z.C. Zaragoza, B.J. Serralta and S.C. Martín (1986). Clinical usefulness of tumor markers in the diagnosis of pleural effusions. Carcinoembryonic antigen, alpha-fetoprotein and orosomucoid. *Med Clin.,* 86: 439-443.

Salama, G., M. Miédougé, P. Rouzaud, M.A. Mauduyt, M.C. Pujazon, C. Vincent, P., Carles and G. Serre (1998). Evaluation of pleural CYFRA 21-1 and carcinoembryonic antigen in the diagnosis of malignant pleural effusions. *Br J Cancer*, 77: 272-476

Sahn, S.A. (1997). Pleural diseases related to metastatic malignancies. *Eur Respir J.*, 10: 1907–13.

Satoh, H., M. Sumi, H. Yagyu, H. Ishikawa, T. Suyama, T. Naitoh, T. Saitoh and S. Hasegawa (1995). Clinical evaluation of CYFRA 21–1 in malignant pleural fluids. *Oncology.*, 52: 211–214.

Shimokata, K., Y. Totani, K. Nakanishi, M. Yamamoto, Y.H. Hasegawa, H. Kawatsu, H. Sasou, T. Murate, S. Ichiyama and T. Kurasawa (1988). 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.

Shimokata, K., Y. Niwa, M. Yamamoto, H. Sasou and M. Morishita (1989). Pleural fluid neuron-specific enolase. A useful diagnostic marker for small cell lung cancer pleurisy. *Chest*, 95; 602-603.

Shitrit, D., B. Zingerman, A.B. Shitrit, D. Shlomi and M.R. Kramer (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. *The Oncologist.,* 10(7): 501–7.

Trape J, Molina R, Sant F. 2004. Clinical evaluation of the simultaneous determination of tumor markers in fluid and serum and their ratio in the differential diagnosis of serous effusions. *Tumor Biol*; 25:276–81.

Villena V, López-Encuentra A, Echave-Sustaeta J, Martín-Escribano P, Ortuño-de-Solo B, Estenoz-Alfaro J. 1996. 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.

Villena, V., A. López-Encuentra, J. Echave-Sustaeta, P. Martin- Escribano, B. Ortuño-de Solo and J. Estenoz-Alfaro (2003). Diagnostic value of CA 549 in pleural fluid. Comparison with CEA, CA 15.3 and CA 72.4. *Lung Cancer*, 40(3): 289–94.

Wagner, I.C., M.J.B. Guimarães, L.K.N. da Silva, F.M. de Melo and M.T.C. Muniz (2007). Evaluation of serum and pleural levels of the tumor markers CEA, CYFRA21-1 and CA 15-3 in patients with pleural effusion. *J Bras Pneumol.*, 33(2): 185-191

Whiteside, T.L. and A. Dekker (1979). Diagnostic significance of carcinoembryonic antigen levels in serous effusions. Correlation with cytology. *Acta Cytol.*, 23: 443-448.

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