

RESEARCH ARTICLE

Diagnostic Value of Neuron-Specific Enolase (NSE) and Cancer Antigen 15-3 (CA 15-3) in the Diagnosis of Pleural Effusions

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Abstract

Introduction: Pleural effusion diagnosis plays an important role in determining treatment strategies. The aim of this study was to determine the diagnostic capacity of tumor markers CA 15-3 and NSE solely or in combination in differentiating the nature of pleural fluid. **Methods and Materials:** In this cross-sectional study we evaluated 93 patients with pleural effusions (44 malignant and 49 benign). NSE and CA 15-3 serum and pleural levels were measured simultaneously using immunoenzyme assay kits. Diagnosis was established on the basis of cytological study. **Results:** Sensitivity and specificity of CA 15-3 serum and pleural level measurement were 70.4%, 49.0%, and 79.5% and 49.0%, respectively. Serum NSE levels had 75.0% sensitivity and 69.4% specificity while the respective pleural figures were 75.0% and 73.5%. The combination of NSE and CA 15-3 serum and pleural levels had the highest sensitivity (93.2%), although combined serum levels had the lowest sensitivity (47.7%). With an accuracy of 74.2%, pleural levels of NSE had the highest diagnostic potential. **Conclusion:** Measuring NSE and CA 15-3 tumor markers is a suitable approach to distinguish the nature of pleural effusions, with NSE pleural levels demonstrating the highest diagnostic accuracy.

Keywords: Pleural effusion- neuron-specific enolase- cancer antigen 15-3- diagnostic

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Introduction

Pleural effusion is a common complication of systemic or localized diseases and affects about 3000 people per million populations each year. It is a common manifestation of various malignancies (Cox and Katlic, 2015; Haridas, Suraj, Rajagopal, James and Chetambath, 2014). It is significantly important to correctly differentiate between benign and malignant pleural effusions. A malignant pleural effusion represents an advanced malignancy disease which is associated with high mortality; while benign cases have a high chance to be cured if an accurate diagnosis can be made and appropriate treatment given (Azimi, Rezadoost, Nadoushan and Davati, 2012; Nam, 2014). Pleural fluid aspiration and cytological examination is currently the main diagnostic method for determining pleural fluid characteristics which at ideal settings has a sensitivity of 60% (Sriram et al., 2011). Although the presence of tumor cells in pleural effusion is a diagnostic marker of malignant pleural effusion, the probability of finding them is low. It is more likely to find the factors secreted by tumor in the pleural fluid or serum (M Li et al., 2014; Xu, Yu, Zhan and Zhang, 2014).

Neuron-Specific Enolase (NSE) is the γ -subunit of the glycolytic enolase enzyme which is secreted from neuroendocrine cells as well as neurogenic tumors. NSE

has been recognized as a biomarker for small cell lung carcinoma (Ravibabu, Barman, and Rajmohan, 2015; Tiseo et al., 2008). Cancer Antigen 15-3 (CA 15-3) is another tumor marker that is widely used for diagnosis and prognosis of malignancies such as breast cancer (Li, Chen, Su, Song, and Gong, 2014; Lucarelli et al., 2014). The diagnostic values of these two tumor markers in distinguishing the nature of pleural effusion have been investigated in some studies and mixing results have been reported. This study assessed the diagnostic values of these two tumor markers in differentiating the nature of the pleural fluid.

Materials and Methods

Study Participants

In this cross-sectional investigation, 93 patients admitted to the pulmonology ward in Kashan Shahid Beheshti Hospital during fall and winter of 2013-2014, whose pleural effusions were proven through clinical examinations and imaging findings such as chest X-ray or chest CT scanning, were evaluated. Patients who received anticoagulants, had platelet counts less than 50000 per milliliter or had an INR value greater than 2 were excluded from the study. Patients were allocated by a nonrandom process and all patients were entered into the study after

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providing a written informed consent to participate.

Pleural Effusion and Blood Sampling

Ultrasound guidance was used to mark an appropriate site where fluid was most likely to be obtained. After skin disinfection and local anesthesia, a needle was introduced into the intercostal space parallel to the upper border of the rib below the chosen intercostal space. Following catheter placement into the pleural space 10 and 20 mL samples of the pleural fluid were separately withdrawn in pre-heparinized syringes and the samples were sent to the laboratory for cytological examination and measuring the tumor markers levels. Simultaneous 5 mL blood samples from a peripheral vein were obtained for the measurement of the serum levels of the tumor markers. All samples were immediately centrifuged at 3000 rpm and were then stored at -20°C until being used.

Cytological Assesment

Cytologic preparations were made by direct smearing of fluid sediment or by cytocentrifugation. The smears were either fixed in 95% ethanol or air-dried. Fixed smears were stained by the Papanicolaou technique, with hematoxylin and eosin, and air-dried smears were stained with the Romanowsky technique. Red blood cells in bloody smears were lysed by fixing in Carnoy solution for 3-5 min. A Ficoll- Hypaque solution was used to separate red blood cells from nucleated cells in a markedly bloody specimen. The cell blocks obtained by centrifugation were fixed in formalin and processed as an issue sample and cell block sections were stained with hematoxylin and eosin (Kashani et al., 2013; Nikzad, Kashani, Kabir-Salmani, Akimoto and Iwashita, 2013).

Tumor Markers Measurement

Serum and pleural levels of NSE and CA 15-3 were measured using immunoenzyme assay kits manufactured by DiaMetra incorporation, Italy. Malignant effusions were diagnosed if malignant cells or tissues were found in the pleural fluid or biopsies. Pleural effusions were classified as malignant if the effusions occurred in a patient with a known active malignancy until another cause was identified.

Statistical analysis

Results from this study were analyzed using SPSS software version 16 (Hosseini, Moniri, Goli, and Kashani, 2016; Sharif et al., 2016). Nonparametric tests were used for statistical analysis because the data did not follow a normal distribution. Mann-Whitney U test was used for comparisons of differences between the two groups. To find the optimal diagnostic cut-off points of the studied tumor markers Receiver operating characteristic (ROC) curve was plotted. The results are reported as percentages or mean ± standard deviation. The significance level of p was less than 0.05.

Results

Of the all 93 studied patients, 64 (68.8%) were men. The mean age of the studied patients was 70.1±16.8. 44

Table 1. Causes of Pleural Effusion

| Causes | Number | Percent |
|--------------------------|--------|---------|
| Malignant | | |
| Lung cancer | 12 | 12.9 |
| Colon cancer | 8 | 8.6 |
| Unknown origin | 4 | 4.3 |
| Breast cancer | 3 | 3.2 |
| Lymphoma | 3 | 3.2 |
| Bladder cancer | 3 | 3.2 |
| Prostat cancer | 3 | 3.2 |
| Gastric cancer | 2 | 2.2 |
| Pancreas cancer | 2 | 2.2 |
| Soft tissue malignancy | 2 | 2.2 |
| Hepatic cancer | 1 | 1.1 |
| Parotid cancer | 1 | 1.1 |
| Benign | | |
| Congestive heart failure | 13 | 14.0 |
| Trauma | 10 | 10.8 |
| Renal disease | 9 | 9.7 |
| Empyema | 8 | 8.6 |
| Tuberculosis | 3 | 3.2 |
| Rheumatologic disease | 2 | 2.2 |
| Pulmonary embolism | 2 | 2.2 |
| Hepatic cirrhosis | 2 | 2.2 |
| Total | 93 | 100.0 |

effusions (47.3%) were reported malignant and of these 26 (59.1%) were men. The mean age of patients with malignant effusions was 66.6±16.6 years versus 73.4±16.5 years in patients with a benign pleural effusion. Pleural effusion causes on the basis of its nature are shown in Table 1.

The average levels of the studied tumor markers were significantly higher in cases of malignant effusions compared to the benign ones. The mean levels of the measured tumor markers in the serum and pleural fluids of patients are shown in Table 2.

ROC curve analysis (Figure 1) showed that the largest

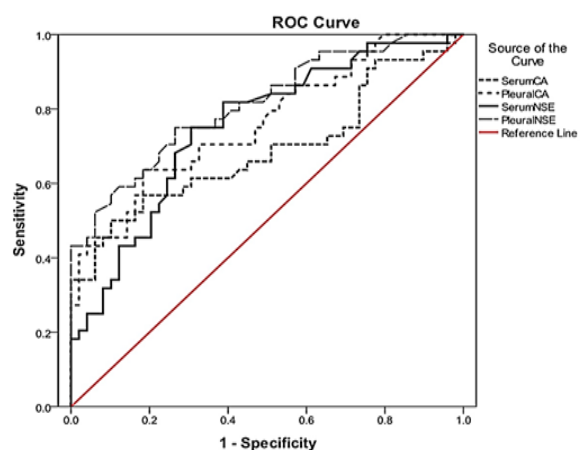


Figure 1. Receiver Operating Characteristics (ROC) Curves for All the Investigated Parameters

Table 2. The Mean Serum and Pleural Levels of NSE and CA 15-3 on The Basis of Pleural Fluid Nature (Mean±SD)

| Tumor marker | Malignant (n=44) | Benign (n=49) | P value |
|-----------------|------------------|---------------|---------|
| Serum CA 15-3 | 58.7±80.5 | 17.9±11.4 | 0.002 |
| Pleural CA 15-3 | 87.4±197.2 | 9.4±8.1 | <0.001 |
| Serum NSE | 39.9±41.1 | 14.5±18.9 | <0.001 |
| Pleural NSE | 24.9±35.1 | 4.0±3.9 | <0.001 |

* CA-125, (U/ml); NSE, (µg/ml)

area under the curve belongs to the pleural levels of NSE (0.81, 95% CI= 0.72-0.9) and the least area under the curve represents the serum levels of CA 15-3 (0.69, 95% CI= 0.58-0.8).

Of the studied markers, the pleural level of NSE is the most accurate which is associated with a high sensitivity and specificity. Among all four studied markers the lowest accuracy belongs to the serum level of CA 15-3, which has the lowest sensitivity and specificity as well. The diagnostic value of the combination of these two tumor markers was also calculated. If at least two factors had levels above the cut-off point, the results would be considered positive. Results are shown in Table 3.

Discussion

Because of the significant differences in treatment and prognosis, the distinction between malignant and benign pleural effusions is important but challenging. About 12 to 24% of all pleural effusions are malignant (Heffner, 2008). Malignant pleural effusions usually occur in patients already known to have cancer but in one third of the cases, pleural effusion is the first manifestation of cancer (Froudarakis, 2008; Yamamuro et al., 2007). Thoracocentesis is a safe and simple procedure to obtain the pleural fluid. In the past two decades, due to non-invasiveness, there has been growing interest in the use of tumor markers for the diagnosis of malignant pleural effusions.

In this study it was found that malignant pleural effusions can be diagnosed using the serum and pleural levels of NSE. In one study by Alam et al which comprised 33 patients with pleural effusions due to Small Cell Lung Carcinoma (SCLC) and Non-Small Cell Lung Carcinoma (NSCLC) and 30 other patients with a benign pleural

effusion caused by tuberculosis, it was found that the mean serum and pleural levels of NSE was significantly higher in malignant effusions compared with the benign ones. Despite the fact that NSE is usually produced and secreted by SCLC cells, higher serum and pleural NSE levels have also been reported with NSCLC compared to the benign conditions (Alam, Baig, Mahmood, Asghar, and Ali, 2010). Lin et al., (2010) investigating tumor markers levels in 62 patients with malignant pleural effusion and 48 patients with benign pleural effusion found that the levels of NSE in serum and pleural effusion in malignant groups were significantly higher. In another study performed by Liu et al 99 patients with malignant pleural effusion caused by lung cancer (SCLC, adenocarcinoma and squamous cell carcinoma) were compared with 37 patients with benign pleural effusion and 35 healthy persons. They found that the levels of NSE in serum and pleural effusion of all the malignant groups were significantly higher than those in the benign groups. No significant differences were seen between the benign ones and the healthy ones (Liu, Yu, and Lin, 2006) 17. Similar results have been obtained from other studies (Lee and Chang, 2005; Menard, Dousset, Jacob, and Martinet, 1993; Wang, Zhang, Gu, Ma, and Jia, 2002). Patients with malignant pleural effusions with non-pulmonary origins were also enrolled in our study. Based on the present study's results and past findings, it can be concluded that measuring serum and pleural NSE levels is a useful test not only in diagnosing malignant pleural effusions due to primary lung tumors, but also for malignant effusions secondary to extra pulmonary cancers.

In present study, using cut-off point of 11.3ng/ml for serum NSE the sensitivity and the specificity were 75% and 69.4%, respectively. Also, the sensitivity and the specificity of pleural NSE were 75% and 73.5% respectively using the cut-off point of 5ng/ml. In the study by Lee et al., (2005) using the cut-off point of 20ng/ml for pleural NSE the sensitivity and the specificity were 36% and 94% respectively. In Ghayumi et al., (2005) study, considering a cut-off point of 5.21ng/ml for pleural NSE the overall sensitivity, specificity, positive predictive value and negative predictive value were as follows: 68.4%, 75%, 74.3% and 69.2% respectively. For serum NSE with the cut-off point of 10.36, sensitivity and specificity were 38.9% and 63.6% respectively. Positive predictive value was 51.1% and negative predictive value was 46.2% . Kuralay et al., (2000) using the cut-off point of 8.7ng/ml

Table 3. Characteristics of Tumor Markers in Patients with Pleural Effusion

| | Cut-off | Sensitivity% | Specificity% | PPV% | NPV% | Accuracy% |
|----------------------|---------|--------------|--------------|------|------|-----------|
| Serum CA 15-3 | 13.9 | 70.4 | 49.0 | 55.4 | 64.9 | 59.1 |
| Pleural CA 15-3 | 6.68 | 79.5 | 49.0 | 58.3 | 72.7 | 63.4 |
| Serum NSE | 11.3 | 75.0 | 69.4 | 68.7 | 75.5 | 72.0 |
| Pleural NSE | 5.0 | 75.0 | 73.5 | 71.7 | 76.6 | 74.2 |
| CA 15-3 (S+P) | | 68.2 | 63.3 | 62.5 | 68.9 | 65.6 |
| NSE (S+P) | | 59.1 | 89.8 | 83.9 | 71.0 | 75.3 |
| CA 15-3 (S)+ NSE (S) | | 47.7 | 85.7 | 75.0 | 64.6 | 67.7 |
| CA 15-3 (P)+ NSE (P) | | 61.4 | 73.7 | 77.1 | 70.7 | 73.1 |
| All markers | | 93.2 | 44.9 | 60.3 | 88 | 67.7 |

PPV, Positive Predictive Value; NPV, Negative Predictive Value; S, Serum; P, Pleural

reported a sensitivity of 100% and a specificity of 95%. Considering these results from the present and the past studies as a whole it is seen that with different cut-off points this test will have a high specificity, thus it can be used to rule out malignant effusions with an acceptable certainty.

Our results imply that the serum and the pleural levels of CA 15-3 have a significant association with the malignant nature of the pleural effusions. Shitrit et al assessing 44 cases of malignant effusions and comparing them with 72 cases of non-malignant effusions found that the pleural levels of CA 15-3 in the malignant cases is significantly higher than the controls (Shitrit, Zingerman, Shitrit, Shlomi, and Kramer, 2005). In the study by Alatas et al., (2001) the Pleural and the serum levels of CA 15-3 were assayed in 44 patients with malignant and 30 with benign effusions and CA 15-3 was shown to higher in malignant ones and there was a statistical association between CA 15-3 and the nature of the effusions. Similar results have been observed by other studies (Ghosh et al., 2013; Hernández et al., 2002; Miedouge et al., 1999; Romero et al., 1996).

In this study considering the cut-off point of 13.9ng/ml for serum CA 15-3 the calculated sensitivity and specificity were 70.4% and 49% respectively. Likewise using the cut-off points of 6.68ng/ml for pleural CA 15-3 the sensitivity and the specificity were found to be 79.5% and 49% respectively. Alatas et al., (2001) considering the cut-off point of 20ng/ml for serum CA 15-3 have reported a sensitivity of 86% and a specificity of 67%. Also in the cut-off point of 14ng/ml for pleural CA 15-3 the calculated sensitivity and specificity were 80 and 93% respectively. Two separate studies have reported the sensitivities of 48 and 97% and the specificities of 100 and 97% respectively for the diagnosis of malignant effusions (Gaspar, De Miguel, Díaz, and Diez, 2008; Shimokata et al., 1988). According to the results of the present study it seems that this factor has a good diagnostic accuracy for the distinction between the malignant and the benign pleural effusions.

Our results showed that the co-measurement of these two factors will change the diagnostic accuracy. Previous studies have shown that measuring the combination of these factors will enhance the sensitivity and the specificity of the diagnosis (Alataş et al., 2001; Gaspar et al., 2008; Miedouge et al., 1999). Co-measurement of all 4 factors compared to measuring only the serum or the pleural levels of NSE decreased the accuracy but increased its sensitivity. It appears that the co-measurement of the studied factors does not improve the diagnosis of the pleural effusion nature.

The current study indicated that measuring NSE and CA 15-3 tumor markers is a suitable method to distinguish the nature of the pleural effusion and NSE pleural levels had the highest diagnostic accuracy.

Ethical approval

This article does not contain any studies with animals performed by any of the authors. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional

and national research committee and with the 1964 Helsinki declaration and its later amendments.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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Compliance with Ethical Standards: There is no financial support for the current study.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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