Diagnostic Utility of Combined CEA, CA15-3 and CA125 Biomarkers and Cytomorphology in Suspicious and Malignant Serosal Fluid

Zahra Rahemi1, Abdolreza Javadi1,2, Behrang Kazeminejad1, Abdolali Ebrahimi1, Hooman Vosough2, Afsoon Taghavi4, Shahriar Dabiri5*

1. Department of Pathology, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Imam Hossein Central Medical Laboratory, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Department of Pathology, Modarres Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4. Department of Pathology, Shahid Beheshti University of Medical Sciences, Tehran, Iran
5. Department of Pathology, Afzalipour Medical School, Kerman, Iran

KEYWORDS

Body fluids, CA125, CA15-3, CEA, Chemiluminescence

Scan to discover online

ABSTRACT

Background & Objective: Early detection of malignancies in the serous fluids has been remained an issue. A classic diagnostic tool for the ascites and pleural effusions is cytologic study (morphology) with approximately 98% specificity for the detection of cancer cells. This study aimed to evaluate the diagnostic value of three complementary markers in the serosal fluids of patients with malignant cytology and suspected cases.

Methods: Seventy two patients with serosal effusion treated in three teaching hospitals were studied. The cases underwent a diagnostic workup to determine the pleural effusion malignancy and etiologies. Complementary markers, including CEA, CA15-3, and CA125 were measured in serosal fluids of three categories of benign, suspicious, and malignant. The study was carried out by Chemiluminescence immunoalayzer. The morphologies were re-evaluated by a consulting Cytopathologist.

Results: Of 72 serosal fluid specimens, 41 (56.9%) were related to pleural effusion and 31 (43.1%) were related to ascites. The sensitivity of CEA, CA125, and CA15-3 biomarkers were 64, 84, and 68%, respectively, and the specificity of each test was 100, 86, and 96%, respectively. This was statistically achieved for the combination of the area of markers below the curve (AUC), 0.93 and 90% sensitivity and 91% specificity.

Conclusion: The results suggest that complementary CA125, CA15-3, and CEA markers assayed with well-developed immunoassay method might be useful in the differentiation between malignant and benign effusions while combined with conventional cytology. CA125 yielded a significant correlation between cytomorphology and biomarkers based on the correlation coefficient analysis.

Introduction

Serosal effusion is one of the complications of a large number of diseases (1). The presence of serous fluid effusion, including pleural effusion and ascites, is a common and challenging diagnostic issue caused by different with etiologies varied form neoplastic such as metastatic involvement to non-neoplastic of infectious and non-infectious agents. The most important goal of choosing the treatment strategies is to differentiate the causes of malignant from non-malignant effusions (1). Many of the existing diagnostic methods are inadequate and invasive. Initial diagnosis is based on the thoraco-abdominal centesis, cytology, cell blocking, and biochemical assay of fluids (2). One of the most common diagnostic tools for the serous fluids is the detection of malignant cells using conventional cytology, which has a specificity of about 98% but a maximum sensitivity of 60% (3). In a study, cytology showed a clinical sensitivity of 57% and specificity of 89% for the detection of malignant cells in the effusion samples (4). Different studies and meta-analysis on the patients with unknown origin serosal effusions reported diagnostic accuracy of carcinoma embryonic antigen (CEA), CA15-3, and CA19-9. The results highlighted that applying one biomarker alone for the
malignant effusion cannot be advised. The definite role of
the combined tumor markers by conventional
immunoassay has been reported controversial (5-8).

On the other hand, a systemic review of 21 articles
indicated diagnostic value of combination of CA15-3,
CA19-9 and CYFRA-21-1 in pleural effusions (7), and
also emphasized on utility of CA15-3 for diagnosis of
malignant pleural effusions (9). Moreover, elevated
levels of both CEA and CA19-9 in peritoneal cavity
(11) and peritoneal washing fluid in gastric and
pancreatic carcinoma cases undergoing surgical
treatment might signalize advanced stage of the illness
(12).

A combination of complementary tumor markers,
including CEA, CA125, and CA15-3 with a defined
diagnostic cut-off point can be used as an adjunct to
diagnose suspicious patients in cytology, confirming
malignant and benign groups, and accelerate the
decision-making process. To select multiple tumor
markers, only complementary tumor-specific markers
should be used (13).

Based on the updated systematic reviews, the
present study used most reliable tumor markers from
multiple epitopes of specific tumor antigens in serous
fluids, applying finest automated Chemiluminescence
immunoassay. This method is a non-invasive method
and can initially replace invasive procedures, such as
thoracoscopy and laparoscopic biopsy with a clinical
sensitivity of about 95%. In this immunoassay method,
the protein antigen is labeled with fluorescein and then
reacted with specific antibody to the antigen (13).

In this study, diagnostic value indices, including
sensitivity, specificity, as well as predictive values of
tumor markers alone and in combination are evaluated
in these patient categories.

**Material and Methods**

The study was conducted on patients with invasive
carcinoma of the breast who had undergone surgery
between the years 2010 and 2015 in Rohani Hospital,
Babol, Iran. All the formalin-fixed paraffin blocks of
the tumors were available. The inclusion criteria were
presence of an invasive carcinoma component in the
available paraffin block along with normal breast
tissue in the tumor margin. Those cases diagnosed as
carcinoma in situ or presented with extensive tumor
necrosis in all slides were excluded from the study.
Clinicopathological data, including age, gender, tumor
stage, and grade, were retrieved from standard reports,
which were prepared according to the American Joint
Committee on Cancer (AJCC), 7th edition.

**Immunohistochemistry**

To highlight lymphatic and blood vessels, two 3-4
μm thick sections were prepared from each block (one
block per case), and IHC staining for D2-40 and CD31
(Dako, Glostrup, Denmark) was performed. Sections
were dewaxed at 60°C in an oven for about one hour,
and then they were put in xylol and rehydrated through

a descending concentration of ethanol. For antigen
retrieval, sections were microwaved for 15 minutes in
ethylenediaminetetraacetic acid (EDTA) buffer
(pH=9). Sections were left at room temperature for 15
minutes to cool down. They were washed in tris-
buffered saline (TBS) for five minutes and incubated in
3% H2O2 in dark humid condition. After that, they were
washed in TBS for five minutes. Sections were
incubated with primary antibody for 60 minutes at
room temperature and with secondary antibody for 30
minutes. Sites of binding were detected by a 10-minute
incubation with diaminobenzidine (DAB).

IHC and archived H&E slides were reviewed by
two pathologists, not knowing the pathology report.
Data were imported in SPSS 20 (SPSS Inc., Chicago,
Ill., USA); Chi-square, Fisher’s exact, and t tests were
performed for statistical analysis.

**Results**

The age range of the patients was 32 to 94 years old.
Participating patients were assigned into three benign,
malignant, and suspicious cytomorphology groups.
There were 12 males and 10 females with the mean age
of 73.73 ± 13.9 years in benign cytology group, 9 males
and 22 females with the mean age of 59 ± 13.34 years
in malignant cytology group, and 5 males and 14
females with the mean age of 59.84 ± 15.71 years
in suspicious cytology group. The age range in the
benign, malignant, and suspicious cytology groups was
43-94, 35-83, and 32-84 years, respectively. Although
the age did not represent a clinically significant
difference between the categories, it was statistically
significant ($P<0.001$), so that the lowest and the highest
age range belonged to the patients in the malignant and
benign cytology groups, respectively (Table 1).

Of 72 serosal fluids, 41 (56.9%) were related to
pleural effusion and 31 (43.1%) were related to
peritoneal effusions. Morphologically, 31 samples
(43.4%), 22 samples (30.6%), and 19 samples (26.4%)
were allocated in the benign, malignant, and suspicious
categories, respectively. Three groups were matched in
terms of sex, and there was no statistically significant
difference between the above groups in this regard
($P=0.103$).The mean levels of tumor markers in all
cytology groups are shown in Table 2. The mean levels
of CA15-3, CA125, and CEA tumor markers were
69.91 ± 93.88, 39 ± 395.44, and 157.17 ± 234.13,
respectively. The highest CA125, CEA, and CA15-3
levels were observed in the malignant cytology group,
which was significantly different from the negative
cytology group. Indeed, all markers exhibited a similar
pattern in all groups. The mean CA15-3 levels in the
malignant and benign cytology groups were 110.02 ±
105.83 and 11.79 ± 9.62, respectively, being
statistically significant ($P<0.001$).

The mean CA125 levels in the malignant and
negative cytology groups were 456.47 ± 121.15 and
286.25 ± 166, respectively, being statistically significant
($P<0.001$). We observed that the mean level of the
above tumor markers in the suspicious cytology group was 422.32 ± 136.43. There was a statistically significant difference between benign and suspicious cytology groups ($P=0.008$). The analysis showed a mean level of CEA at 256.71 ± 260.38 and 26.6 ± 116.19 in the cytology malignant and benign groups, respectively, which was considered statistically significant ($P=0.001$) (Table 2).

### Table 1. Age and sex distribution of patients (Mean ± SD).

<table>
<thead>
<tr>
<th>Cytomorphology</th>
<th>Total</th>
<th>Benign</th>
<th>Malignant</th>
<th>Suspicious</th>
<th>P-value</th>
<th>Pairwise comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean ± SD</strong></td>
<td>63.72±15.48</td>
<td>73.73±13.9</td>
<td>59±13.34</td>
<td>59.84±19.71</td>
<td>0.001**</td>
<td>[p(1,2)=&lt;0.001], [p(1,3)=0.0080]</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>34(32.94)</td>
<td>77(43.94)</td>
<td>58(35.83)</td>
<td>62(32.84)</td>
<td>0.103*</td>
<td></td>
</tr>
<tr>
<td><strong>Male</strong></td>
<td>26(36.1%)</td>
<td>12(54.1%)</td>
<td>9(29%)</td>
<td>5(26.3%)</td>
<td>0.103*</td>
<td></td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td>46(63.9%)</td>
<td>10(45.5%)</td>
<td>22(71%)</td>
<td>14(73.7%)</td>
<td>0.103*</td>
<td></td>
</tr>
</tbody>
</table>

*:P-value is based on the chi-square test.

**:P-value is based on the ANOVA (multiple comparison corrections were carried out using the Bonferroni method in all the above analyses).

### Table 2. Serosal fluid levels of CA15-3, CA 125, and CEA in three cytology groups (Mean ± SEM).

<table>
<thead>
<tr>
<th>Tumor markers</th>
<th>Cytomorphology</th>
<th>CA15-3 (U/mL)**</th>
<th>CA125 (U/mL)</th>
<th>CEA (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total</strong></td>
<td>Benign (n=31)</td>
<td>69.91±93.88</td>
<td>26.6±116.91</td>
<td>256.71±260.38</td>
</tr>
<tr>
<td></td>
<td>Malignant (n=22)</td>
<td>11.79±69.62</td>
<td>26.05(1.2, 339.6)</td>
<td>152.4(0.2,550)</td>
</tr>
<tr>
<td></td>
<td>Suspicious (n=19)</td>
<td>110.02±105.83</td>
<td>456.47±121.15</td>
<td>145.97±221.91</td>
</tr>
<tr>
<td></td>
<td>P-value</td>
<td>&gt;0.001</td>
<td>&gt;0.001</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td></td>
<td>Pairwise comparison</td>
<td>[p(1,2)=&lt;0.001]</td>
<td>[p(1,2)=&lt;0.001], [p(1,2)=0.008], [p(1,2)=&lt;0.001]</td>
<td></td>
</tr>
</tbody>
</table>

*:P-value is based on the ANOVA (multiple comparison corrections were carried out using Bonferroni method in the above analyses).

**:Units are based on the ADVIA Centaur® assay chart (Version 1.0.CU Test Definitions,078D0022-22 Rev. A, 2010-03).

The Spearman correlation coefficient was calculated to investigate the association between tumor markers and cytological results. As mentioned earlier, the highest CA15, CEA, and CA15-3 levels were observed in the malignant cytology group, as presented in the above Table. In other words, the correlation coefficient of all three tumor markers revealed that the cytological results were more likely to be malignant with increasing tumor marker levels, CA125 showed higher correlation coefficient than the other two tumor markers ($r=0.345$, $P=0.003$) (Table 3).

The etiologies of cytologically malignant effusions and the sites of origin are demonstrated in Table 4.

### Table 3. Correlation coefficients and relationships between tumor markers with cytomorphological results.

<table>
<thead>
<tr>
<th></th>
<th>CA15-3</th>
<th>CA125</th>
<th>CEA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cytology Spearman correlation coefficient</strong></td>
<td>0.26*</td>
<td>0.345**</td>
<td>0.311**</td>
</tr>
<tr>
<td><strong>P-value</strong></td>
<td>0.028</td>
<td>0.003</td>
<td>0.075</td>
</tr>
</tbody>
</table>

### Table 4. Distribution of Malignant Serosal fluids by confirmed etiology.

<table>
<thead>
<tr>
<th>Causes</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignant</td>
<td>31</td>
</tr>
<tr>
<td>Secondary to adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>6</td>
</tr>
<tr>
<td>Breast</td>
<td>7</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>7</td>
</tr>
<tr>
<td>Ovary</td>
<td>8</td>
</tr>
<tr>
<td>Other tumor types</td>
<td>3</td>
</tr>
</tbody>
</table>
In order to determine the sensitivity and the specificity of the tumor markers levels in cancer diagnosis (positive cytological result), ROC curves were plotted for each tumor marker separately, as well as for a combination of all three tumor markers (Figures 1–4).

**Fig. 1.** ROC curve for CA15-3.

**Fig. 2.** ROC curve for CA125.

**Fig. 3.** ROC curve for CEA.
Accordingly, the best cut-off point and the sensitivity and specificity values were calculated for each tumor marker value (Table 4).

The best cut-off point is actually the number that has the highest accuracy in distinguishing cancer patients from those of non-cancer. These cut-off points were 39.55 u/mL for CA15-3, 486.00 u/mL for CA125, and 6.90 ng/mL for CEA. In the meantime, if we use the information on the combination of three complementary markers to predict the risk of cancer, this figure would be 0.38. The areas under the curve (AUC) for all three tumor markers consists of CA15-3, CA125 and CEA were 0.85 ± 0.054, 0.85 ± 0.058, and 0.75 ± 0.071, respectively. Also, if we use the information on the combination of three tumor markers to predict the risk of cancer, the AUC curve would be 0.93 ± 0.039. The closer this number to 1, the better the differentiatiation and accuracy power of the test will be. These results revealed that CA15-3, CA125, and CEA markers levels were respectively 85%, 85%, and 75%, higher in the randomly selected positive cytology (malignant) group as compared to the negative cytology (benign) group. The above probability for the combination of three tumor markers would be 93%. The most efficient predictive tumor marker is the one with the highest sensitivity and specificity value. As can be seen, all three tumor markers produced the same results; however, it seems that the combination of three tumor markers yields much more reliable predictive value.

According to the AUC criterion, one of the best criteria for determining the predictive power, the use of information on the combination of three tumor markers is the best method to predict the risk of cancer. The resulting cut-off point (0.38) for the combination of three tumor markers showed the highest specificity (91%) and sensitivity (90%). The sensitivity value indicated that 90% of the patients were diagnosed correctly, and the specificity value indicated that 91% of benign cases were diagnosed correctly. The closer the sensitivity and specificity values to 100, the higher the accuracy and differentiation power of the test will be (Table 5).

Table 5. Diagnostic value of single tumor marker and the combination of complementary triple markers.

<table>
<thead>
<tr>
<th>Tumor marker</th>
<th>The mean ± standard deviation of AUC</th>
<th>95% confidence interval</th>
<th>P-value</th>
<th>Cut-off point</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Youden Index*</th>
<th>Positive Predictive Value (PPV)</th>
<th>Negative Predictive Value (NPV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA15-3</td>
<td>0.85±0.054</td>
<td>0.74-0.96</td>
<td>&lt;0.001</td>
<td>39.50</td>
<td>64%</td>
<td>100%</td>
<td>64%</td>
<td>73.3%</td>
<td>85.7%</td>
</tr>
<tr>
<td>CA125</td>
<td>0.85±0.058</td>
<td>0.73-0.96</td>
<td>&lt;0.001</td>
<td>486.00</td>
<td>84%</td>
<td>86%</td>
<td>70%</td>
<td>80.8%</td>
<td>75%</td>
</tr>
<tr>
<td>CEA</td>
<td>0.75±0.071</td>
<td>0.62-0.90</td>
<td>0.002</td>
<td>6.20</td>
<td>68%</td>
<td>96%</td>
<td>63%</td>
<td>67.7%</td>
<td>95.5%</td>
</tr>
<tr>
<td>CA15-3 + CA125 + CEA</td>
<td>0.93±0.039</td>
<td>0.86-0.1</td>
<td>&lt;0.001</td>
<td>0.38</td>
<td>90%</td>
<td>91%</td>
<td>81%</td>
<td>87%</td>
<td>93.3%</td>
</tr>
</tbody>
</table>

*: Youden's J statistic (also called the Youden's index). The maximum distance between the ROC curve and the baseline model. PPV: positive predictive value. NPV: negative predictive value. AUC: area under the ROC curve
Discussion

One of the less invasive methods for examination of the diffusion of pleural and abdominal serous fluids is fluid puncture (TAP). Conventional cytology is one of the crucial methods for examination of serosal fluid by which presence and origin of the malignancies are evaluated, however, it shows low clinical sensitivity for diagnosis of malignant lesions (approximately 60%) (2, 15).

The conventional cytology alone is associated with a low clinical sensitivity and is usually unable to determine the primary source of the malignancy in serosal fluids. The gold standard for specifying the type and origin of malignancies is studying of pleural biopsy, laparoscopy biopsy or open surgery.

There have been numerous studies aiming at improving none-invasive diagnostic methods, one of which is the study of biomarkers in serum serous fluids.

Article reviews and meta-analysis of the pleural fluids of CEA, CA125, CA19-9, and CA15-3, showed that the combined use of the mentioned tumor markers along with morphology was helpful (16-18). However, poor sensitivity value was reported (5).

Purell et al., (2004) in a comprehensive study on 416 patients showed that malignant pleural fluid contains significantly higher levels of tumor markers in the effusion samples. The combination of four tumor markers (CEA>50 ng/mL, CA125>2800 u/mL, CA15-3>75 u/mL, and CYFRA 21.1>175 ng/mL) yielded a clinical sensitivity of 54%. They suggested that panel analysis of complementary tumor markers could be used as an adjunct to the cytological diagnosis of malignancy (15).

In another study on 77 pleural fluid samples, Mehrabi et al., (2005) reported the highest specificity value (100%) for a combination of serum CA15-3 and CA15-3, CEA and NSE in pleural fluids and the highest sensitivity (80%) for a combination of serum CA15.3 and CA15-3, NSE, and CEA in pleural fluids by ELISA method (19).

In a study on 74 serous fluid samples, Kornke et al., (2009) showed that combination of two or more tumor markers could be effective in enhancing the diagnostic value. Overall, pleural fluid tumor markers outperformed the serum markers in detecting the etiology of pleural effusion (20).

Another meta-analysis done by Liang et al., (2008) revealed the same results with the combination of complementary tumor markers, for example AUC for CEA/CA125 was reported as 0.86 and 0-0.93 for CEA/CYFRA 21-1 in pleural fluid (21).

The application of complementary biomarkers may improve diagnostic accuracy. Ferrer et al., (1999) achieved 65% sensitivity and 100% specificity for a combination of CEA, CA125, and CA15-3 assessment in a serosal fluid (22).

The literature review demonstrated that there were no independent tumor markers with excellent sensitivity and specificity value, as well as high diagnostic value in distinguishing between benign and malignant fluids.

Based on the earlier studies, a combined evaluation of complementary tumor markers is one of the methods that can increase the diagnostic value of biomarkers (19, 20).

In the present study, the CEA, CA125, and CA15-3 markers levels of 72 samples collected from three teaching hospitals in the categories of benign, suspicious, and malignant serous fluids (pleural fluid and ascites) were analyzed by 4th generation Chemiluminescence immunoassay in Imam Hossein Core Laboratory.

According to the ROC statistical analysis, AUC achieved as much as 85%, 85%, and 75%, for CEA, CA125, and CA15-3 tumor markers, respectively; it means that 85-73% of patients randomly selected from the malignant group demonstrated significantly higher tumor markers than the benign group.

The sensitivity values of these tumor markers were 64%, 84%, and 68% for CEA, CA125, and CA15-3 respectively; also, the specificity was 100%, 86%, and 96%, respectively.

In our analysis, AUC, sensitivity, and specificity of the combination of the above three tumor markers were calculated as 93%, 90%, and 91%, respectively. Some studies, such as the one carried out by Kernke et al., showed the AUC of CEA, CA125, CYFRA 21-1, and NSE tumor markers was 89% (20).

Previous studies revealed lower diagnostic values for the serum measurement equal to 0.65. Overall, serosal fluid markers were superior to the serum markers in determining the pleural fluid etiology (20). However, no concomitant serum assay was performed in the present study.

On the other hand, the serum specimen of our patients was not usually available in practical use in cytopathology laboratories. However, it was possible to assay complementary biomarkers along with cytomorphology on a single fluid specimen.

Results of the present study also showed that the diagnostic sensitivity of a single tumor marker, especially in a combination, was more than the clinical sensitivity of conventional cytology. The increased amount in the sensitivity and specificity of complementary tumor markers in the serosal fluid was 26% and 5%, respectively.

In the present study, the Youden indices for CEA, CA125, CA15-3, and the combination of these tumor markers were 63%, 70%, 64%, and 81%, respectively. The Youden index is a useful parameter enabling a comparative analysis of data found by various authors when single values of sensitivity and specificity are reported. The diagnostic yields of the three most accurate markers CEA, CA125, and CA15-3 calculated...
Conclusion

The present study demonstrated that non-invasive techniques such as complementary biomarkers assay with well-developed methods (4th generations of immunoassay) in combination with conventional cytology may lead to an acceptable increase in the diagnostic power of fluid cytology which was in line with previous studies.

The advanced technology for ultra-sensitive detection of tumor markers can be used instead of conventional immunoassay methods (enzyme-linked immunosorbent assay and radio-immunoassay). The electrochemical devices are rapid, simple, and alternative methodologies for determination of the fluid tumor markers. The limitation of this study was that, the reference ranges and cut-off values of these markers differ in pleural and peritoneal fluids. It can be interpreted by different metabolism, half-life, and turnover of the markers in the serosal fluids. On the other side, there are no unanimous cut-off points and reference ranges of these analyses in the body fluids that may be due to immunoassay heterogeneity and complexities. Many physiological conditions such as renal and hepatic functions can directly affect the levels of these biomarkers in fluids. In the future, multivariate statistical analysis, and novel immunoassay methods such as multiplex immunoassay, SERS-based multiplex immunoassay, and reverse phase protein array may significantly improve the diagnostic accuracy of fluid cytology examination for the comprehensive tumor marker panels.

Acknowledgements

We acknowledge the cytologic consultation performed by Dr. Shahriar Dabiri, at the Department of Pathology, Medical University, Kerman, Iran.

Conflict of Interest

No conflicts of interest were declared in relation to this study.

References


12. Crepaldi-Filho R, Palma RT, Marcelo Franchini Giusti MF, de Assis Galveo Bueno M, da Silva PSL, Waisberg J. Levels of carcinoembryonic antigen and CA 19-9 in the sera and peritoneal washing of patients undergoing...


How to Cite This Article