PLEURAL CYFRA 21-1 AND CA 15-3 IN DIFFERENTIATION OF MALIGNANT FROM BENIGN PLEURAL EFFUSIONS

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Abstract:Objective: The aim of this study was to evaluate the individual and combined diagnostic values of CYFRA 21-1 and CA15-3 in pleural fluid for differentiation between malignant and benign pleural effusions. **Subjects and Methods:** Twenty patients with malignant pleural effusion (17 with primary lung cancer and 3 with breast cancer) were included, in addition to 20 diseased controls with benign pleural effusion (10 with congestive heart failure, 7 with parapneumonic effusion and 3 with tuberculosis). Following radiological investigations, thoracocentesis and pleural fluid examination, pleural CA 15-3 was assessed by chemiluminescence immune assay and pleural CYFRA 21-1 by enzyme-linked immunosorbent assay. **Results:** Results of the present study revealed a high sensitivity 95% and specificity 90% of CYFRA 21-1 for diagnosis of malignant pleural effusion. Combining CYFRA 21-1 and CA 15-3 did not improve diagnostic performance than that of CYFRA 21-1 used individually. **Conclusion:** CYFRA 21-1 is a non-invasive reliable marker for differentiating pleural effusions of malignant from benign causes. Its high diagnostic performance will help detections of cases possibly missed by routine cytology. This high performance did not benefit from the adjuvant use of CA 15-3.

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1. Introduction

Pleural effusion is a vexing problem in clinical practice, especially in terms of differentiation between malignant (MPE) from benign pleural effusion (BPE), due to the significant difference in the treatment and prognosis involved. Most common causes of transudative effusions include congestive heart failure and hypoalbuminemic states, while those of exudative effusions involve malignancy, infection, and tuberculosis. Malignant pleural effusion accounts for 42 to 77% of cases[(1)].The majority of neoplasms can cause pleural effusion during their progression. Lung cancer accounts for up to 30% of all cases of malig-nant pleural effusion followed by breast cancer and lymphomas[(2)].Although most malignant effusions occur among patients with known cancers, effusion can be the first indication for the presence of malignancy in third of patients. This explains the importance of diagnosis of MPE[(3)]. Pleural fluid cytology findings are positive only in 60% of cases, while thoracoscopy will establish the diagnosis in approximately 95% of cases. The latter, however, is an expensive, invasive and potentially traumatizing interventional procedure, hence the necessity for less invasive discriminatory markers with comparable diagnostic performance[(4)].

CYFRA 21-1 is a cytokeratin-19 fragment, an acid-type cytoplasmic protein, with a molecular weight of 40 kD, expressed in epithelial cells. Following cell death, it is released in serum in the form of soluble fragments. CYFRA 21-1 is a potential diagnostic marker for MPE as it is found not only in serum but also in the pleural fluid[(5)]. In the course of searching for diagnostic tools for MPE, several combinations of markers have been studied including neuron specific enolase, CYFRA21-1, CA15-3,CA19-9 and CA125[(6)]. Since CYFRA21-1 and CA 15-3 have a high diagnostic performances for lung and breast cancers, respectively,their combined assay in pleural fluid provides a promising combination for diagnosis of MPE[(7)].

2. Subjects and Methods:

I- Subjects:

This study was conducted on 20 patients with MPE (group A) and 20 patients with BPE (group B) as disease controls. They were selected from the Oncology and Chest Departments, Ain Shams University Hospitals. Group A MPE were secondary to primary lung cancer (n = 17) and breast cancer (n = 3).These included 13 males and 7 females. Their ages ranged from 35 to 69 years, with median and interquartile range (IQR)of 55.5 (48-59). The effusions were considered to be malignant when malignant cells were encountered on cytological examination of the pleural fluid or in pleural biopsy. Group B included 15 males and 5 females, aged 25 to 70 years, with a median and IQR of 51.5 (41- 62.25) years. Causes of BPE included congestive heart failure (n = 10), parapneumonic pleural effusion (n= 7) and

tuberculosis (n= 3). BPE was chiefly diagnosed by clinical manifestations and laboratory examinations. Tuberculous effusion was diagnosed if one of the following criteria were met: (i) radiological and clinical evidence of tuberculous effusion with positive acid-fast bacilli (AFB) in sputum; (ii) identification of AFB in pleural fluid or biopsy specimen cultures and (iii) presence of caseous granulomas in pleural biopsy tissue. Parapneumonic pleural effusion was diagnosed by the presence of acute fever with purulent sputum, pulmonary infiltrate, leucocytosis, neutrophilia and identification of microorganisms in the pleural fluid. Congestive heart failure was determined by cardio-megaly and pulmonary venous congestion on the radiograph, peripheral edema, hepatomegaly, bilateral pleural transudate and findings on echocardiograph. Informed consent was taken from all participants in this study.

All studied individuals were submitted to thorough history taking, proper clinical examination, chest X-ray and thoracocentesis, in addition to physical, chemical, bacteriological and cytological examination of pleural fluid.Pleural CA 15-3 was assessed by chemiluminescence immune assay and pleural CYFRA 21-1 by enzyme-linked immuno-sorbent assay (ELISA).

II- Samples:

Pleural fluid specimens were obtained by thoracocentesis with aseptic technique. Supernatant of the pleural fluid obtained by centrifugation at 3000g for 10 min was aliquoted and stored frozen at -20°C prior to assay.

III- Methods:

A- Assay of CA 15-3 by Chemiluminescence Immune Assay:

This was done on fully automated Immulite 2000 (Siemens Healthcare Diagnostics, USA) using instrument manufacturer's reagent. This is a two-step sequential chemiluminescent immunoassay using two monoclonal mouse antibodies, 115D8 and DF3 specific for CA 15-3, for capture and detection, respectively, and chemiluminescence as the detection signal.

B-Assay of CYFRA 21-1 by ELISA:

The assay was performed using the DRG CYFRA 21-1 ELISA Kit supplied by DRG International (DRG International, New York, USA). It consists of a solid phase ELISA based on the sandwich principle. The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a CYFRA 21-1 molecule. An aliquot of patient sample containing endogenous CYFRA 21-1 is incubated in the coated well with enzyme conjugate, which is an anti-CYFRA 21-1 monoclonal antibody conjugated with horseradish peroxidase. Following incubation, the unbound conjugate is washed off. The amount of bound peroxidase is proportional to the concentration of CYFRA 21-1 in the sample. Having added the substrate solution, the intensity of color developed is proportional to the concentration of CYFRA 21-1 in the patient sample, and is deduced from a calibration curve drawn from standard results obtained in the same run.

IV- Statistical Analysis:

Statistical analysis was done using SPSS software package (version 15.0, 2006, Echosoft Corporation, USA). Data were expressed descriptively as percentages for qualitative data and median and IQR for quantitative non- parametric data. Comparison between groups was done using Mann Whitney U test for quantitative non-parametric data. $p< 0.05$ was considered significant and $p< 0.01$ was considered highly significant. Ranked Spearman correlation coefficient was used in correlatingnon-parametric variables. The diagnostic performance of CA 15-3 and CYFRA 21-1 was evaluated in terms of diagnostic sensitivity, specificity, positive (PPV) and negative predictive values(NPV), and efficacy. The best possible cutoff was selected from the receiver operating characteristics (ROC) curve.

3. Results:

The descriptive data of the studied MPE patients (group A) and BPE patients (group B) are shown in Table (1) and Figures (1 and 2). Comparison of groups A and B(Table 2 and Figure 3) revealed a significantly higher CA15-3 ($z = -3.088$, $p< 0.01$) and CYFRA 21-1 ($z = -5.309$, $p< 0.001$)levels in group A than B. Correlation between CA 15-3 and CYFRA 21-1 (Figure 4)among group A subjects revealed a positive significant correlation ($r= 0.664$, $p<0.001$).

Study of the diagnostic performance of CA 15-3 and CYFRA 21-1 for differentiating MPE from BPE (Table 3 and Figure5), revealed that a best cutoff for CA15-3 level of 35 U/mL yielded a sensitivity, specificity, PPV, NPV and efficiency of 80%, 65%, 69.6%, 76.5% and 72.5% and a best cutoff for CYFRA 21-1 level of 45 ng /mL yielded 95 %,90 %, 90.5%, 94.7 %, and 92.5%.Combination of CYFRA 21-1 and CA 15-3 positivity (cases considered positive if either CYFRA 21-1 or CA 15-3 were positive) did not result in an additional benefit over the diagnostic performance of CYFRA 21-1 used individually.

4. Discussion:

Pleural effusions are common complications of a wide varietyof diseases.Thoracoscopy is the MPE gold diagnostic standard with a diagnostic sensitivity of 93–97%.A wide range of markers have been proposed for the detection of MPE, with no existing one yet having sufficient diagnostic accuracy in discriminating

MPE from BPE. One of the promising tumor markers is CYFRA 21-1[4].

In our study, the median value of CA 15-3 in pleural fluid was significantly higher among patients with MPE than patients with BPE which agrees with **David *et al.* (2005)**[8] and **Li *et al.* (2007)**[6]. These results could be explained by CA 15-3 over-expression on the cell surfaces of malignant glandular cells, and increasing amounts being shed into pleural fluid[9].As regards CYFRA21-1,our study revealed that its median value in pleural fluid was significantly higher among patients with MPE those with BPE. Many studies, as **David *et al.* (2005)**[8], **Li *et al.* (2007)**[6]**,Liang *et al.*(2008)**[7]and **Huang *et al.*(2010)**[10]**,**supported our results. This finding could be attributed to increased cytokeratin solubility which results frommodification at the amino and carboxyl terminals of keratin (phosphorylation, glycosylation andtransglutamination) occurring during transformation of normalcells into malignant cells. Furthermore, higher CYFRA 21-1 levels in MPE may be due to proteolytic degradation ofkeratin during cell lysis, abnormal mitosis and tumor necrosis. Thus, CYFRA 21-1 spills over from cells undergoing proliferation and apoptosis[10].Moreover, both markers (CA 15-3 and CYFRA 21-1) were highly significantly correlated in MPEgroup ($r = 0.664$, $p < 0.001$). This may be explained by common mechanism of release by spilling over from proliferating cells. This correlation was also proved in the study of **Li *et al.* (2007)**[6].

CA 15-3 showed a diagnostic accuracy of 72.5% for differentiating MPE from BPE with sensitivity and specificity of 80% and 65%, respectively. The resultsof **Li and his colleagues (2007)**[6](n= 62) showed a sensitivity of 62.5% and specificity of 73.3 %and results of **Antonangelo*et al.* (2009)**[11](n=78) revealed a sensitivity of 64.4% and specificity of 89.5% of CA 15-3 in diagnosing MPE. The lower specificity reported in our study was due to the overlap in CA 15-3 results between MPE and BPE groups, possibly underlined by our fewer number of study subjects.As regards CYFRA 21-1, asensitivity of 95%, specificity of 90%, and accuracy of 92.5% were demonstrated in our study for diagnosis of MPE. This is in accordance with most published values as those in the studies of **Alatas*et al.*(2001)**[12](n= 74), **Li *et al.*(2007)**[6](n = 62) and **Huang *et al.*(2010)**[10](n =134) which revealed a sensitivity and specificity of 91% and 90%,84.4 and 90.0% and 80.5% and 92.5%, respectively, for diagnosis of MPE. However, other studies reported lower sensitivities of CYFRA 21-1 for diagnosing MPE. **David et al (2005)**[8] (n=116) reported a sensitivity of 59.1% and specificity of 80.5%. At a cutoff of 70 ng/mL, **Antonangelo*et al.* (2010)**[9]**(**n=175)reported a sensitivity of 46% and specificity of 94% for differentiating MPE from BPE. This lower sensitivity can be explained either by their high chosen cutoff level which achieved higher specificity at the expense of sensitivity or by the variation in the types of primary tumors causing MPE included in their studies. The etiologies of MPE in their studies were lung, breast, colorectal, ovarian, renal, lymphoproliferative and prostatic cancers.

Comparing the diagnostic performance of the two markers studied individually at their best cutoff level, CYFRA 21-1 gave a much better diagnostic accuracy than CA 15-3 (92.5% versus 72.5%). These findings are in accordance with **Li and his coworkers(2007)**[6] who conducted their research on 32 patients with MPE from advanced lung cancer and 30 patients with BPE with five tumor markersmeasured in the pleural fluidincluding CYFRA 21-1, CA15-3, CA19-9, neuron-specific enolase and CA125. They concluded that CYFRA 21-1 was the tumor marker with the highest sensitivity (84.4%), specificity (90.9%), and accuracy (87.1%). The combined use of both of CYFRA 21-1 and CA 15-3 in their study did not add to the performance of CYFRA 21-1 used alone. However, **Alatas and his colleagues (2001)**[12]reported that when CA 15-3 and CYFRA 21-1 were combined, the sensitivity increased to 100% and specificity decreased to 83%. Comparable results could be achieved in our study by using CYFRA 21-1 alone at a lower cutoff value of 35 ng/mL instead of our chosen 45 ng/mL, where the sensitivity, specificity and accuracy would have been 100%, 85% and 92.5%.

In conclusion, our data suggest that CYFRA 21-1 is a non-invasive reliable marker for differentiating MPE from BPE. The use of CYFRA21-1 as a single marker had the same performance as its combination with CA15-3. Addition of CYFRA 21-1 to the current standard tests for diagnosis of MPE could be helpful for identification of MPE patients that might have been missed by routine cytology. Based on very high detection rates of CYFRA 21-1, negative CYFRA21-1 patients mightbe alleviated from proceeding to unnecessary thoracoscopy. The benefit of combining tumor markers for increasing diagnostic performance needs further large-scale studies in clinical practice with trial of different markers. Further studies are needed to determine the value of tumor markers in pleural fluid for judging prognosis and efficacy of therapy.

Table (1): Descriptive Statistics of the Demographic and Pleural Malignant (group A) and Benign Examination Data of the Studied (group B) Pleural Effusion Patients

	Group A (n = 20)	**Group B (n = 20)**
Sex:		
Males	13 (65%)	15 (75%)
Females	7 (35%)	5 (25%)
Age (years)*	55.5 (48-59)	51.5 (41-62)
Causes of effusion	Lung cancer 17 (85%) Breast cancer 3 (15%)	CHF 10 (50%) Parapneumonic 7 (35%) Tuberculosis 3 (15%)
Pleural fluid appearance:		
Bloody	10 (50%)	0 (0%)
Clear straw colored	0 (0%)	15 (75%)
Turbid	10 (50%)	5 (25%)
Gram stain positive	0 (0%)	4 (20%)
ZN stain positive	0 (0%)	1 (5%)
Positive Culture	0 (0%)	6 (30%)
Protein (fluid/serum ratio)*	0.74 (0.65-0.83)	0.35 (0.27-0.47)
LDH (fluid/serum ratio)*	1.25 (0-1.5)	0.45 (0.3-0.88)
Glucose (mg/dL)*	37.5 (33.5-42.3)	102.5 (42.3-167)
TLC / mL*	3000 (2000-4650)	490 (350-570)
Predominant cells:		
Mononuclear cells	20 (100%)	16 (80%)
Polymorphs	0 (0%)	4 (20%)
CA 15-3 (U/mL)*	75.5 (35-100)	30 (25-51.3)
CYFRA 21-1 (ng/mL)*	185 (122.5-237.5)	17.5 (10-28.8)

* Median (interquartile range)
CHF: congestive heart failure; ZN: ZeihlNeelsen stain.

Table (2): Statistical Comparison of CA 15-3 and CYFRA 21-1 Levels between MPE patients (group A) and BPE patients (group B) using Mann Whitney U test

	Group A	Group B	Z	P
CA 15-3 (U/mL)	75.5 (35-100)	30 (25-51.3)	-3.088	< 0.01
CYFRA 21-1 (ng/mL)	185 (122.5-237.5)	17.5 (10-28.8)	-5.309	< 0.001

p< 0.01and p< 0.001: Highly significant.

Table (3): Diagnostic Performance of CA 15-3 and CYFRA 21-1 in discrimination of malignant from benign pleural effusions

Best cutoff	Sensitivity	Specificity	PPV	NPV	Efficiency
CA 15-3 (35 U/mL)	80%	65%	69.6%	76.5%	72.5%
CYFRA 21-1 (45 ng/mL)	95%	90%	90.5%	94.7%	92.5%
CYFRA 21-1 and CA 15-3	95%	90%	90.5%	94.7%	92.5%

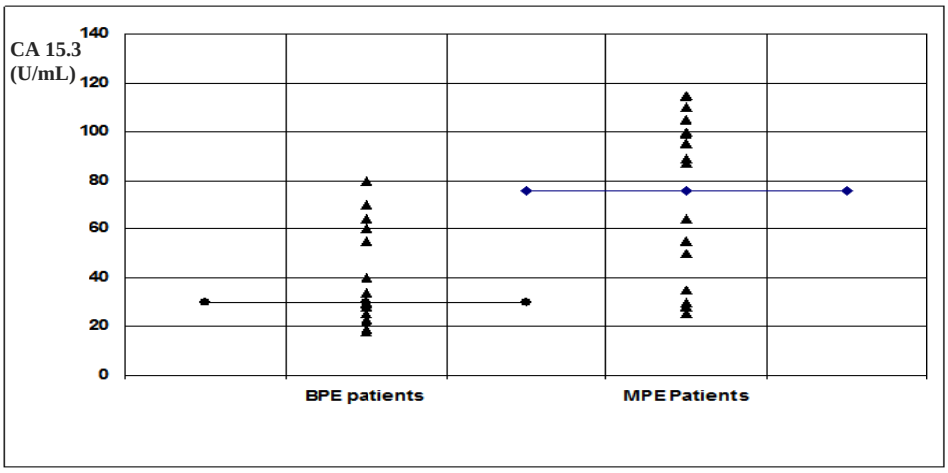

Figure (1): Scatterdiagram showing the distribution of results of CA15-3 among patients with benign (BPE) and malignant (MPE) effusions.

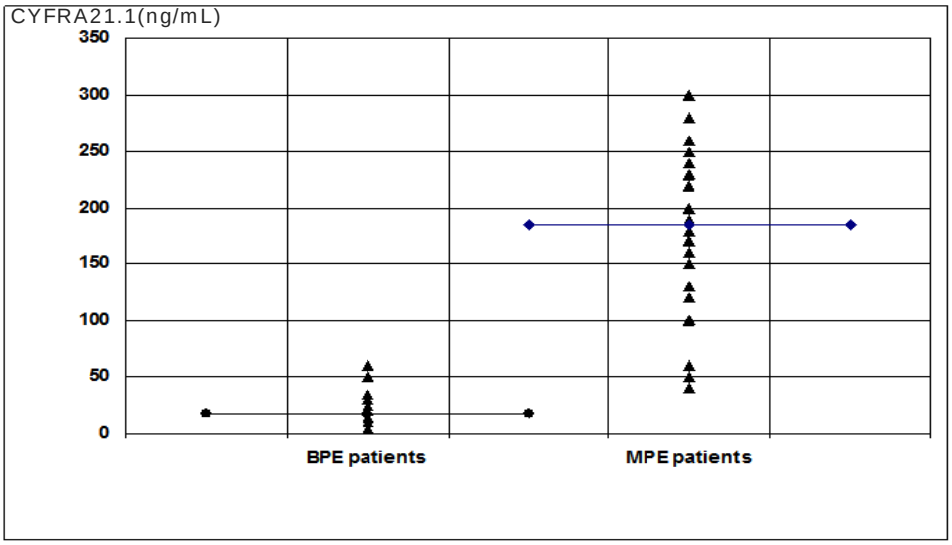

Figure (2): Scatterdiagram showing the distribution of results of CYFRA21-1 among patients with benign (BPE) and malignant (MPE) effusions.

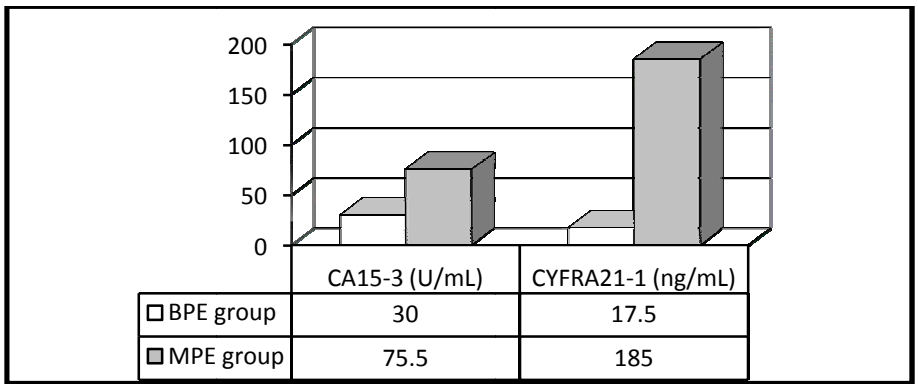

Figure (3): Comparison between benign (BPE) and malignant (MPE) effusion groups as regards median values of CA15-3 and CYFRA21-1.

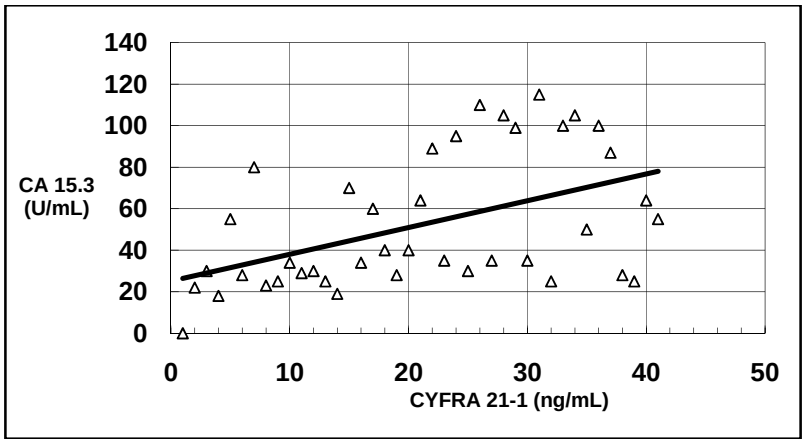

Figure (4): Correlation between CA 15-3 and CYFRA 21-1in malignant pleural effusion group (r= 0.664, *p*<0.001).

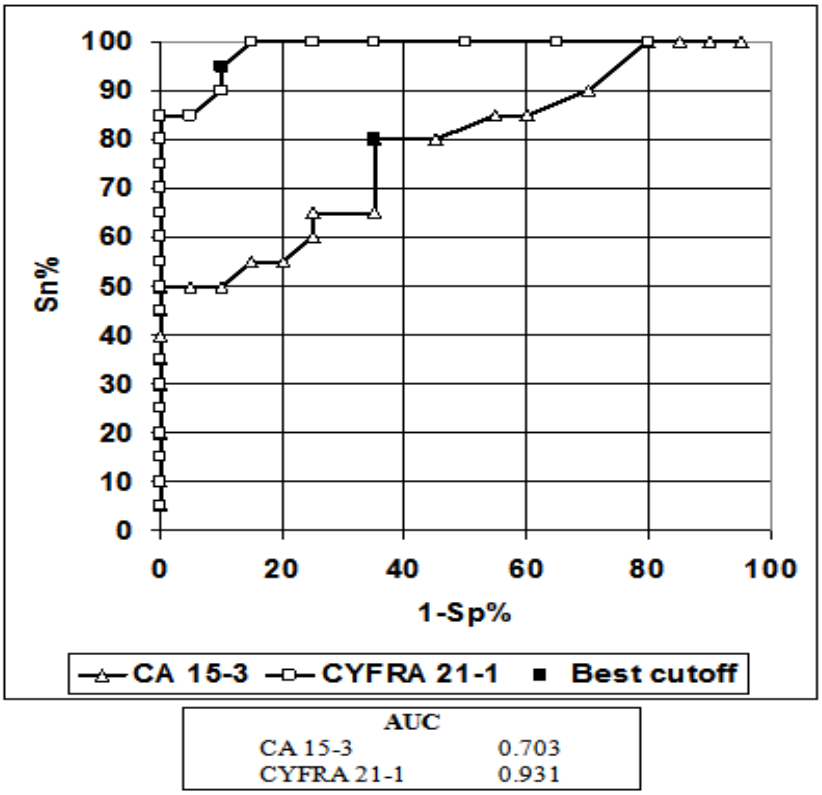

Figure (5):ROC curve analysis showing the diagnostic performance of CA 15-3 and CYFRA 21-1 for discriminating MPE from BPE.

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