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Regular Article

# Pleural ELFA D-dimer assay: A surrogate marker for malignant pleural effusion

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ABSTRACT

*Background:* Malignant pleural effusion is associated with enhanced fibrinolysis. However, no data are available concerning the precise role of pleural D-dimer assay in pleural effusion. We therefore assessed the role of pleural D-dimer assay in predicting malignant pleural effusion.
*Patients and Methods:* A prospective laboratory investigation was conducted in a tertiary care teaching hospital. The study included consecutive patients with pleural effusion who presented at the Pulmonary Department between November 2009 and May 2010. Blood and pleural D-dimer levels were measured by Enzyme Linked Fluorescent assay (ELFA). The results were correlated with the clinical, laboratory, and radiological findings, and with the final diagnosis of the pleural fluid.
*Results:* A total of 103 patients with pleural effusion were included in the study. The Pleural ELFA D-dimer results were found to be positively correlated with pleural etiology of malignancy ($p = 0.0001$). Pleural etiology was also correlated with pleural LDH, pleural protein, pleural PH, pleural glucose, pleural and blood CRP, but not with ADA. In a binary logistic regression, only the pleural ELFA D-dimer assay was a significant predictor of the malignant pleural effusion (odds ratio 1.007; 95% confidence interval 1.002-1.012; $p = 0.007$). The area under the receiver operating characteristics curve for malignancy was 0.79. A D-dimer level of 146 mg/ml had a sensitivity of 82% and a specificity of 74%.
*Conclusions:* We found high D-dimer levels among malignant pleural effusion. D-dimer might be useful as a simple, noninvasive, surrogate marker for malignant pleural effusion.



## Introduction

Pleural effusion has many etiologies including malignancy, infection, congestive heart failure and collagen vascular diseases. [1] Although the pathophysiological mechanisms involved in pleural effusion are known, it is difficult to differentiate between malignant and non-malignant causes of effusion. Several studies have shown that patients with pleural effusion due to malignancy exhibit decreased fibrinolysis. [1–3]

It has been hypothesised that pleural effusion causes activation of the normal mesothelial cells, which in turn is followed by activation of a coagulation cascade and the inhibition of fibrinolysis into the pleural space. [4–6] The accumulations of fibrin into the pleural cavity may serve as a reliable marker for determining the cause of the pleural effusion.

The aim of the present study was to determine if the pleural D-dimer examined by ELFA (Enzyme-Linked Fluorescent Assay) could predict the etiology of the fluid.

## Patients and methods

### Study population

Patients with pleural effusion who presented at the Pulmonary Department between November 2009 and May 2010 were eligible for the study. The diagnosis of pleural effusion was based on chest x-ray or computed tomography (CT). The study was approved by the Ethics Committee of Meir Medical Center.

Patients younger than 18 years were excluded, as were patients who had received anticoagulation treatment or who had primary coagulopathy, nephrotic syndrome, surgery, or infection in the month preceding the study, severe liver or renal disease (prothrombin time [PT] international normalized ratio [INR] above 1.6 or creatinine above 3.0 mg/dl, respectively), a history of deep vein thrombosis in the last year, or acute coronary syndrome.

### Study protocol

Pleural fluid and blood were collected from each patient for biochemical, cytological, and microbiological analysis prior to any therapy. The clinicians who performed the laboratory studies were blinded to the etiology of the pleural effusion.

*Abbreviations:* aPTT, partial thromboplastin time; CHF, congestive heart failure; CRP, C-reactive protein; ELFA, Enzyme Linked Fluorescent assay; LDH, lactate dehydrogenase; OR, odds ratio; PT, prothrombin time; ROC, Receiver operating curve; WBC, white blood cells.

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All pleural samples were assessed for cell count, biochemical parameters including, pH, CRP (C reactive protein), and D-dimer, cytologic examination, and microbiological culture. Blood samples underwent the same testing, except for pH.

The diagnoses were divided into five categories based on the underlying disease/procedure:

1. The diagnosis of malignant effusion was made when malignant cells were found on cytologic examination or in a biopsy specimen. Patients with negative cytologic findings were further examined with closed pleural biopsy, and those with negative findings on closed biopsy underwent video-assisted thoracoscopic surgery.
2. The pleural effusion was considered due to infection if it was associated with acute febrile illness with purulent sputum, pulmonary infiltrate, and responsiveness to antibiotic treatment, or if the microorganism was identified in the pleural fluid in the absence of any other cause of the pleural effusion.
3. The pleural effusion was attributed to CHF(congestive heart failure) in patients in whom CHF was diagnosed based on findings of an enlarged heart, pulmonary venous congestion on the radiograph, and peripheral edema, with response to CHF treatment and the absence of malignancy or pulmonary infiltrates associated with an inflammatory process.
4. The diagnosis of postcardiotomy or postsurgery (Dressler's syndrome) pleural effusion was made when the pleural fluid developed after injury to the heart, the patient responded to treatment with anti-inflammatory agents or corticosteroids, and CHF, pulmonary embolism, and pneumonia were ruled out.
5. All other fluids were considered idiopathic.

### *Sample collection and D-dimer assays*

After informed consent was obtained, 4 ml of blood and 3 ml pleural effusion were collected in 3.2% buffered sodium citrate and centrifuged at 2000 g for 15 minutes within 4 hours of collection. All supernatant fluids were stored at −20 °C until assayed. All pleural samples were diluted 1:1000 before assay (due to the high D-dimer levels compared to plasma).

Plasma and pleural fibrin D-dimer was assayed by Vidas D-dimer Exclusion TM (ELFA-ELISA based technique, BioM'erieux, Lyon, France). The procedures were performed as recommended by kit/assay manufacturer. Specimen batch was assayed together with controls purchased from the manufacturer.

### *Data collection*

Data collected from all patients included age, sex, drug intake, and comorbid diseases. Hematology data including complete blood count, prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen levels, D-dimer, and C-reactive protein (CRP). Pleural data including pH, total protein, glucose, lactate dehydrogenase (LDH), white blood cell (WBC), adenosine de-aminase (ADA), and lymphocyte percentage in the pleural fluid were analyzed. Radiological and CT scan findings in patients who underwent CT scan were reviewed. Data concerning diagnostic procedures, including thoracocentesis and thoracoscopy for cytological and pathological diagnosis were collected.

### *Statistical analysis*

Results were given as mean ± standard deviation or frequencies according to type of parameter. Univariate analyses among diagnoses (3+ diagnoses)(pleural etiology) by D-dimer levels were calculated by one-way ANOVA with Bonferroni post hoc comparisons. Differences between the 2 groups (malignant vs. non-malignant) for continuous parameters were calculated using Student *t*-test. Pearson correlation coefficient (r) and its significance (p) were calculated between D-dimer level and the clinical variables and laboratory parameters.

In parallel, non-parametric tests were also performed because of wide dispersion of some of the data (Mann-Whitney, Kruskal-Wallis, Spearman correlation, each when appropriate). To determine if the D-dimer assay predicted the etiology of the pleural effusion, a series of binary logistic regression analyses were performed.

ROC (Receiver operating curve) Curves were presented to show sensitivity and specificity of D-dimer levels for malignancy. P values less than or equal to ≤0.05 were considered statistically significant. The statistical evaluation was carried out using the Statistical Package for the Social Sciences (SPSS).

## Results

### *Study population*

A total of 103 patients were included in the study. Table 1 summarizes the demographic and clinical characteristics of the study population. The mean age of the patients was 71 ± 16 years. Most pleural effusions were unilateral; only 5% were bilateral. The most common etiology was CHF (41.7%), with malignancy and infections the next most common at 26.2% and 24.3%, respectively. The most common co-morbid disease was ischemic heart disease (69%), followed by metabolic syndrome (47.6%).

### *Correlations of the study parameters with the diagnosis*

There were statistically significant differences between diagnoses of the fluid in LDH, protein, pH, and glucose (Table 2) with higher levels of pleural D-dimer in malignant cases compared to others (Fig. 1). Both pleural and blood CRP differed from infectious and malignant pleural effusions, with more significant correlations with infectious pleural effusions (10.2 ± 8.2 vs 2.8 ± 2.4 and 20.4 ± 14.1 vs 11.7 ± 7.7, in the pleural fluid and blood, respectively). The lowest CRP levels were noted in CHF; 0.9 ± 0.5 and 4.4 ± 4.5, in pleural fluid and blood, respectively.

The plasma D-dimer was not different between samples from malignant etiology compared to the other etiologies (Table 2).

**Table 1**
Clinical and demographic data of the study population (n = 103).

| | |
|---|---|
| Age (years) | 71.2 ± 16 |
| Sex M:F | 64:36 |
| Location, n (%) | |
| Right | 60 (58) |
| Left | 38 (37) |
| Bilateral | 5 (5) |
| Colour, n (%) | |
| Yellow | 72 (70) |
| Bloody | 28 (27) |
| Milky | 2 (3) |
| Diagnosis, n (%) | |
| Malignancy | 27 (26.2) |
| CHF | 43 (41.7) |
| Infection | 25 (24.3) |
| Dressler syndrome | 2 (2.0) |
| Idiopathic | 6 (5.8) |
| Thoracoscopy, n (%) | 11 (10.7) |
| Amount (ml) | 875 ± 909 |
| Co-morbid diseases, n (%) | |
| COPD | 18 (17) |
| Ischemic heart disease | 71 (69) |
| Renal disease | 22 (21.4) |
| Metabolic syndrome | 49 (47.6) |

CHF = Congestive heart failure, COPD = Chronic obstructive lung disease.