D-dimer levels in pleural effusions

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Summary

Introduction: D-dimer is a degradation product of cross-linked fibrin. We hypothesized that hemorrhagic pleural effusions would have greater D-dimer levels than non-hemorrhagic pleural effusions, and that persistently bloody effusions would be distinguishable from thoracentesis-induced bloody effusions by the D-dimer level. Methods: Forty pleural effusions were studied. D-dimer levels (measured by ELISA), red blood cell (RBC) count, white blood cell (WBC) count, lactate dehydrogenase (LDH), and protein level was measured for each effusion. Ten effusions, five non-bloody, and five bloody were studied for each of the following disease states: parapneumonic effusion, congestive heart failure, post-coronary artery bypass grafting, and lung cancer. Results: No significant difference of the D-dimer level was noted between bloody and non-bloody effusions of different disease states ($P = 0.286$). There was no significant difference in the median D-dimer levels between all the bloody and all the non-bloody effusions ($P = 0.88$). There was no significant difference ($P = 0.51$) in D-dimer levels between five diseases groups when the bloody and non-bloody fluids were combined. The D-dimer levels did not correlate with the RBC count ($r = 0.11, P = 0.48$), WBC count ($r = 0.13, P = 0.53$), LDH ($r = 0.01, P = 0.93$), or protein levels ($r = -0.01, P = 0.93$) in any of the groups. Conclusion: Measurement of pleural fluid D-dimer levels does not distinguish persistently bloody effusions from non-bloody effusions, and does not aid in narrowing the differential diagnosis of an effusion.

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Introduction

D-dimer is a degradation product of cross-linked fibrin. It has been suggested that the D-dimer assay
of cerebral spinal fluid samples can be used to accurately differentiate subarachnoid hemorrhage from traumatic lumbar puncture in that the D-dimer levels are higher with subarachnoid hemorrhage. Therefore, we hypothesized that hemorrhagic pleural effusions would have higher D-dimer levels than non-hemorrhagic pleural effusions, and that persistently bloody effusions would be distinguishable from thoracentesis-induced bloody effusions by the D-dimer level.

**Methods**

Saint Thomas Hospital is a tertiary-care medical center. Since September 1, 1997, we have maintained a database on all patients who undergo ultrasonically guided thoracentesis and who sign an informed consent approved by the Institutional Review Board. Every patient (both outpatients and inpatients) who undergoes ultrasound-assisted thoracentesis in Saint Thomas Hospital and signs the consent form is prospectively entered. We conducted a retrospective pilot study to examine whether hemorrhagic pleural effusions have higher D-dimer levels than non-hemorrhagic pleural effusions, and whether persistently bloody effusions are distinguishable from thoracentesis-induced bloody effusions by the D-dimer level. The etiology of the pleural effusion, the lactate dehydrogenase (LDH) and total protein levels, red blood cell (RBC), and white blood cell (WBC) counts of the pleural fluid were recorded. Pleural fluids from 40 patients were studied. The pleural samples were collected between January 2003 and January 2004. We selected pleural fluids such that we would have 10 pleural fluids, five non-bloody, and five bloody from each of the following disease states: parapneumonic effusion, congestive heart failure, post-coronary artery bypass grafting (CABG), and malignant effusion due to lung cancer. A bloody pleural fluid was identified by its gross appearance as recorded by the technician. A pleural effusion was said to be caused by congestive heart failure if the patient had symptoms and signs of congestive heart failure that improved with appropriate therapy. A pleural effusion was said to be malignant if the patient had either a pleural fluid cytologic examination or a pleural biopsy specimen that was positive for malignancy, or if the patient had known metastatic malignancy with no other explanation for the pleural effusion. A pleural effusion was labeled post-CABG when it occurred within the first few months after CABG and had no other obvious explanation (e.g. congestive heart failure, chylothorax, or infection). A parapneumonic effusion was an effusion associated with bacterial pneumonia, lung abscess, or bronchiectasis. The samples for the D-dimer determinations were collected in vacutainer tubes containing 3.8% citrate, centrifuged (2500g for 15 min), and the supernatants were frozen at 0 °C D-dimer levels were measured by enzyme-linked immunosorbent assay (ELISA) (TintElize, Biopool, Sweden). The normal value of the TintElize D-dimer assay is 39 ng/mL with a standard deviation of 12. The TintElize test measures D-dimer antigen in the range 75–5000 ng/mL. The Biopool TintElize D-dimer utilizes the double antibody principle. Plasma sample or standard containing D-dimer is added to a microtest well, which is coated with a monoclonal antibody, MA-8D3, against D-dimer.

**Statistical methods**

All data are expressed as mean ± SD unless otherwise stated. Because the pleural fluid D-dimer, protein, LDH, RBC, and WBC counts were not normally distributed, Kruskal–Wallis one-way analysis of variance on ranks was used for comparing the D-dimer levels among groups, and Dunn’s method was used to perform multiple comparison procedures. Correlations were analyzed with the Spearman rank order correlation since the data was not normally distributed. Data were analyzed using SigmaStat v2.03 statistical software package (Jandel Scientific, San Rafael, CA, USA). Differences in results were considered significant when \( P < 0.05 \).
Results

D-dimer levels were at detectable levels in all the pleural fluids (ranged between 357 and 3179 ng/mL) (Fig. 1). One-way analysis of variance showed that the D-dimer levels between the eight groups were not significantly different ($P = 0.286$). Comparison of the D-dimer levels in each group between bloody vs. non-bloody effusions by $t$-test (Table 1) showed a significant difference in only malignant effusion group ($P = 0.002$). However, contrary to our hypothesis, non-bloody malignant effusions had higher D-dimer levels than bloody malignant effusions. There was no significant difference in the median D-dimer levels between all the bloody and all the non-bloody effusions ($P = 0.88$).

There was no significant difference ($P = 0.51$) in D-dimer levels by one-way analysis of variance between five diseases groups when the bloody and non-bloody fluids were combined.

We analyzed the data for all 40 patients to determine whether there was any relationship between the pleural fluid D-dimer levels and other characteristics of the pleural fluid. There was no correlation between D-dimer levels and the RBC counts ($r = 0.11, P = 0.48$), WBC counts ($r = 0.13, P = 0.53$), LDH ($r = 0.01, P = 0.93$), or protein levels ($r = -0.01, P = 0.93$).

Discussion

When blood enters the pleural space, the coagulation process is activated and the blood clots. Subsequently there is a fibrinolytic reaction due to the large amounts of plasminogen and plasminogen activators present in the pleural space. The main plasminogen activators in the pleural space are tissue plasminogen activator and urokinase plasminogen activator. The plasminogen activators convert the inactive zymogen plasmin into active plasmin, which, in turn, enzymatically breaks down fibrin. D-dimer is the primary degradation product of cross-linked fibrin and therefore serves as a direct marker of ongoing coagulation with fibrinolysis. Increased levels of D-dimer are found in conditions that result in activation of the fibrinolytic system.

Lang and coworkers reported that D-dimer levels in cerebral spinal fluid samples accurately differentiated subarachnoid hemorrhage from traumatic lumbar puncture. In their study, the D-dimer assay was positive in all six patients with subarachnoid hemorrhage whereas the D-dimer assay was not positive in the patients with traumatic lumbar puncture.

<table>
<thead>
<tr>
<th>Groups</th>
<th>$n$, Variables</th>
<th>D-dimer (ng/mL)</th>
<th>RBCC, $\times 10^3$ (µL)</th>
<th>WBC, $\times 10^3$ (µL)</th>
<th>TP (g/dL)</th>
<th>LDH (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPE/NB</td>
<td>2539 (2108–2676)</td>
<td>940 (526–3237)</td>
<td>2750 (830–6831)</td>
<td>3.1 (2.8–3.6)</td>
<td>442 (108–667)</td>
<td></td>
</tr>
<tr>
<td>PPE/B</td>
<td>2441 (2120–3023)</td>
<td>52,500 (28,000–63,375)</td>
<td>2000 (1562–3500)</td>
<td>4.3 (3.3–5.2)</td>
<td>182 (102–483)</td>
<td></td>
</tr>
<tr>
<td>CHF-NB</td>
<td>2851 (2079–2956)</td>
<td>175 (18.2–116.2)</td>
<td>90 (48.5–281)</td>
<td>3 (2.6–3.5)</td>
<td>86 (65.5–119)</td>
<td></td>
</tr>
<tr>
<td>CHF-B</td>
<td>2694 (2566–2836)</td>
<td>2700 (1481–415,000)</td>
<td>1000 (325–3000)</td>
<td>3.2 (3–3.2)</td>
<td>160 (123–206)</td>
<td></td>
</tr>
<tr>
<td>CABG-NB</td>
<td>2857 (2628–2944)</td>
<td>945 (416–3506)</td>
<td>1285 (1122–2856)</td>
<td>4 (3.1–4.4)</td>
<td>136 (110–198)</td>
<td></td>
</tr>
<tr>
<td>CABG-B</td>
<td>2786 (2548–3080)</td>
<td>150,000 (65,625–251,000)</td>
<td>1000 (911–3875)</td>
<td>2.9 (2.6–3.3)</td>
<td>357 (210–687)</td>
<td></td>
</tr>
<tr>
<td>LC-NB</td>
<td>2979 (2753–3035)</td>
<td>525 (52.5–21,375)</td>
<td>530 (331–1125)</td>
<td>4.1 (3.7–4.7)</td>
<td>148 (126–289)</td>
<td></td>
</tr>
<tr>
<td>LC-B</td>
<td>2156 (1853–2418)</td>
<td>56,000 (4550–116,062)</td>
<td>400 (275–562)</td>
<td>4.1 (4.1–4.9)</td>
<td>251 (187–418)</td>
<td></td>
</tr>
</tbody>
</table>

$P$-values

Data are presented as median (quartile boundaries).

NB: non-bloody; B: bloody; PPE: parapneumonic effusion; CHF: cardiac heart failure; CABG: post-cardiac bypass surgery; LC: lung cancer.
negative in control groups of 14 patients with hemorrhagic cerebral spinal fluid secondary to traumatic lumbar puncture and in 20 patients with normal cerebral spinal fluid.

We hypothesized that hemorrhagic pleural effusions would have higher D-dimer levels than non-hemorrhagic pleural effusions, and that persistently bloody effusions would be distinguishable from thoracentesis-induced bloody effusions by the D-dimer level. However, this study showed that the D-dimer levels are not significantly different between bloody and non-bloody effusions from patients with parapneumonic effusion, congestive heart failure, post-cardiac bypass surgery, and lung cancer. The only significant difference observed was in the lung cancer group. However, the mean D-dimer level in non-bloody malignant effusions was higher than that in the bloody effusions, which was opposite to our hypothesis. It has been previously suggested that there is hyperfibrinolysis in malignant pleural effusions, and this is mainly due to excessive concentrations of tissue plasminogen activator and urokinase plasminogen activator. Nevertheless, the D-dimer levels were not higher in malignant than in benign effusions.

Only two previous investigations have evaluated the role of D-dimer measurement in the differential diagnosis of pleural effusions. Lu et al. reported that D-dimer levels measured by ELISA were significantly higher in pleural fluid from patients with tuberculous pleuritis and empyema than those in pleural fluid from patients with malignant pleural effusions. Moreover, D-dimer levels were positively correlated with LDH in pleural fluid ($r = 0.4168$, $P < 0.01$). In contrast, Philip-Joet et al. reported no significant difference in the mean pleural fluid D-dimer levels, between different etiologies including 10 empyema, nine tuberculosis, 31 cancer, seven cardiac failure, and three patients with undetermined etiology. The present study is in agreement with that of Philip-Joet et al. and although the numbers of patients were small in each disease category, there was no hint that the D-dimer levels would ever be useful in the differential diagnosis of pleural effusions. These data including that of the present study suggest that pleural D-dimer levels may depend on various other factors such as inflammation. It seems like the degree of inflammation has more effect on the pleural effusion D-dimer levels than ongoing bleeding or the clotting balance. Therefore, we were not able to prove our hypothesis that bloody effusions would have higher D-dimer levels than non-bloody effusions.

In the present study, the D-dimer levels were higher than the normal upper limit for serum in all pleural effusions suggesting strong fibrinolytic activity. Abnormal fibrinolysis occurs when the balance between activators and inhibitors is disturbed. Increased production of plasminogen activator inhibitors is partially responsible for fibrin deposition within the pleural space of patients with parapneumonic effusions, whereas overexpression of plasminogen activators leads to excessive fibrinolysis that is observed in patients with malignant effusions. Alemán et al. found that plasminogen activity and the levels of plasminogen activators were significantly lower in malignant effusions than other types of effusions. The concentrations of plasminogen activator inhibitor 2 in empyema and complicated parapneumonic effusions exceeded those in tuberculous effusions. In addition, the pleural fluid levels of tissue plasminogen activators were higher in patients with tuberculous or malignant effusions than other patients. Although existent data support the importance of pleural injury as the cause of increased intrapleural fibrinolytic activity in exudative pleural effusions, the mechanism to explain the increased pleural D-dimer levels in patients with heart failure remains unclear.

There have been several previous studies that measured D-dimer levels in pleural fluid in conjunction with many other measurements and mainly as a marker of fibrinolysis or inflammation. Agrenius et al. found that D-dimer levels were higher than normal concentrations in chronic malignant effusions ($62.7 \pm 25.5 \mu g/ml$), and decreased 6 h after the intrapleural instillation of quinacrine ($12.2 \pm 7.9 \mu g/ml$). They suggested that an activated fibrinolytic system with a chronic malignant pleural effusion is partially deactivated after intrapleural quinacrine instillation. Rodriguez-Panadero et al. reported similar findings in a study designed to show that impairment in fibrin formation or increased intrapleural fibrinolysis would lead to failure of pleurodesis. In their study, fibrinolytic activity (as assessed by D-dimer levels) showed a clear decline 24 h after talc poudrage in patients with a good pleurodesis outcome in contrast to those with a poor outcome and to those in the control group who had no significant change in the D-dimer levels with time.

In conclusion, the present study demonstrates that blood in pleural fluid is not associated with a significant increase in the pleural fluid D-dimer levels. Measurement of pleural fluid D-dimer levels does not distinguish persistently bloody effusions from non-bloody effusions. Moreover, measurement of pleural fluid D-dimer levels does not aid in narrowing the differential diagnosis of an effusion.
References


