Pleural Protein Capillary Electrophoresis for the Separation of Transudates and Exudates

To the Editor:
Categorization of pleural effusions as transudates or exudates assists diagnostic and therapeutics decisions. To meet the criteria of Light et al. (1) for exudates, an effusion must have at least one of the following: a ratio of pleural fluid (PF) protein to serum protein >0.5, a ratio of PF to serum lactic dehydrogenase (LD) >0.6, and PF LD more than two-thirds the upper limit of normal for serum LD. Numerous studies have examined the diagnostic accuracy of these criteria, which misdiagnose 10–30% of transudates as exudates (2,3).

Recently in this Journal, Chen and Lam (4) reported that qualitative protein zone electrophoresis is more sensitive (100% vs 95%) and specific (50% vs 38%) than the criteria of Light et al. (1) in a study of 51 patient samples (8 transudates and 43 exudates). Moreover, when quantitative analysis was performed, the PF α2-globulin:albumin ratio at the best cutoff point (0.28) showed a sensitivity and specificity of 85% and 80%, respectively.

To determine whether protein capillary electrophoresis rather than protein zone electrophoresis meets the accuracy of the criteria of Light et al. (1), we prospectively studied 116 adult patients with pleural effusions over a 1-year period. On the basis of predetermined clinical criteria (2–4), there were 29 transudates (25 heart failure, 3 liver cirrhosis, 1 hypoalbuminemia) and 87 exudates (30 malignant, 26 parapneumonic, 19 tuberculous, and 12 miscellaneous). LD and protein in both PF and serum were measured on a selective discrete multichannel analyzer (Hitachi 917). Protein capillary electrophoresis of PF was performed with a Paragon CZE 2000 (Beckman).

By the Student t-test, no differences were found between transudates and exudates in the mean percentages of PF α1, β1, and γ-globulin fractions. In contrast, albumin, α2-globulins, and the α2-globulin:albumin ratio were significantly different between transudates and exudates. We therefore used a nonparametric ROC analysis (SPSS 9.0 statistical software) where test thresholds were selected for the highest overall diagnostic accuracy. Table 1 shows the diagnostic accuracy of the different tests for identifying exudative pleural effusions compared with the performance of the criteria of Light et al. (1). After we excluded PF albumin for its low accuracy, the confidence intervals suggest that no differences exist among the remaining tests. We analyzed the misclassified effusions for each test. Three malignant exudates were misclassified as transudates by the criteria of Light et al. (of which two were correctly classified by the alternative tests). There was a good explanation for two of the “transudates” cytologically confirmed to be malignant in the face of atelectasis and heart failure, but the third patient died prematurely, precluding evaluation of potential causes. Notably, 16 and 12 exudates were falsely classified by the PF α2-globulins and the PF α2-globulin:albumin ratio, respectively, including 7 malignant effusions for which no alternative cause could be determined. Thus, we feel that these alternative criteria may provide clinicians false reassurance when evaluating patients with “transudative” effusions.

To recommend new tests on the basis of their higher specificity compared with the criteria of Light et al. fails to recognize that multiple tests combined in “or” rules [e.g., the criteria of Light et al. (1)] always have a higher specificity but lower specificity compared with noncombination single tests when each of the test components of the combination and the new single test have similar discriminative properties (5). We believe that the criteria of Light et al. (1) continue to be the most practical method of separating exudates from transudates.

Table 1. Accuracy for tests that identify exudates.

<table>
<thead>
<tr>
<th>Test</th>
<th>n</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
<th>PPV, % (95% CI)</th>
<th>NPV, % (95% CI)</th>
<th>PLR</th>
<th>NLR</th>
<th>OR (95% CI)</th>
<th>AUC, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light et al. (1)</td>
<td>115</td>
<td>96.6 (88.0–100)</td>
<td>71.4 (61.4–81.5)</td>
<td>91.3 (85.0–97.6)</td>
<td>87.0 (71.0–100)</td>
<td>3.38</td>
<td>0.05</td>
<td>70.0</td>
<td>(17.0–287.8)</td>
</tr>
<tr>
<td>PF albumin &lt;53%</td>
<td>116</td>
<td>67.8 (49.1–86.5)</td>
<td>55.2 (44.1–66.2)</td>
<td>81.9 (72.4–91.5)</td>
<td>36.4 (21.0–51.7)</td>
<td>1.51</td>
<td>0.58</td>
<td>2.6</td>
<td>37</td>
</tr>
<tr>
<td>PF α2-globulins &gt;7.5%</td>
<td>116</td>
<td>81.6 (65.8–97.4)</td>
<td>86.2 (78.4–94.0)</td>
<td>94.7 (88.9–100)</td>
<td>61.0 (44.8–77.1)</td>
<td>5.92</td>
<td>0.21</td>
<td>27.7</td>
<td>89</td>
</tr>
<tr>
<td>PF α2:albumin ratio &gt;0.14</td>
<td>116</td>
<td>86.2 (71.9–100)</td>
<td>82.8 (74.2–91.3)</td>
<td>93.8 (87.8–99.7)</td>
<td>66.7 (49.9–83.5)</td>
<td>5.00</td>
<td>0.17</td>
<td>30.0</td>
<td>86</td>
</tr>
</tbody>
</table>

References
5. Heffner JE. Evaluating diagnostic tests in the...

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Drs. Lam and Chen respond:

To the Editor:

We read with interest the Letter to the Editor by Porcel et al., who followed the direction we took in our previous article (1). As we suggested, the authors carried out a study using protein capillary electrophoresis to further examine the diagnostic accuracy of electrophoresis in the separation of transudates and exudates. Given the small sample size of our original study, we hesitated to draw the conclusion that protein zone electrophoresis (PZE) is more sensitive and specific than the criteria of Light et al. (2), but we were strongly moved by our results to encourage further evaluation. Under the constraint of the sample size, we stated in our conclusion that “there is a good agreement between the results obtained with the PZE and the criteria of Light et al. (1) [Ref. (2) in this reply]. In some cases, PZE also provides additional information for the diagnostic separation of exudates from transudates”.

In their Letter, Porcel et al. indicate that the 95% confidence interval of the odds ratio, using the criteria of Light et al., was 17.0–287.8, and those of the pleural fluid α2-globulins and the pleural fluid α2-globulins:albumin ratio were 8.5–90.9 and 9.6–93.8, respectively. There were significant overlaps of the three confidence intervals, and the authors rightly conclude that these results show no difference between the tests. More importantly, the area under the ROC curve for pleural fluid α2-globulins was 0.89 with a 95% confidence interval of 0.81–0.96. This is already sufficiently good for a routine diagnostic test. In summary, the authors provide data that supports the use of protein electrophoresis for the diagnostic separation of exudates and transudates.

The authors further their discussion by examining the incidence of misdiagnosis of exudates as transudates, but they fail to consider the incidence of misdiagnosis of transudates as exudates, an error with serious consequences. That two of the three exudates misclassified by the criteria of Light et al. as transudates were correctly classified by the alternative criteria points out the inherent insufficiency of the former criteria to reveal underlying pathology when patients with heart failure are involved (3). The data of Porcel and others will allow us to further improve the interpretation of protein electrophoreograms to minimize the clinical consequences of misdiagnosis.

We also wish to comment on the authors’ general statement that “multiple tests combined in ‘or’ rules [e.g., the criteria of Light et al. (1)] are alternative criteria points out the inherent insufficiency of the former criteria to reveal underlying pathology when patients with heart failure are involved (3). The data of Porcel and others will allow us to further improve the interpretation of protein electrophoreograms to minimize the clinical consequences of misdiagnosis.” We suggest that, by adjusting the cutoff value, one can increase sensitivity while sacrificing specificity, depending on the clinical need for screening or diagnosis.

References


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Correction of Positive Bias of the Roche Tina-quant II Hemoglobin A1c (HbA1c) Assay at Low HbA1c Percentages

To the Editor:

Diabetes mellitus is a serious health problem in the United States, and much of the morbidity and mortality associated with diabetes mellitus can be ameliorated by proper blood glucose control (1). Consequently, serious effort has been put forth to increase the accurate and timely monitoring of blood glucose concentrations. Hemoglobin A1c (HbA1c) is a biomarker that provides a snapshot of long-term glucose control in diabetes. The Core Laboratory for Clinical Studies at Washington University School of Medicine is a Secondary Reference Laboratory (SRL no. 4) of the National Glycohemoglobin Standardization Program (NGSP) (2). We routinely measure HbA1c percentages from whole blood samples by the HbA1c Tinaquant® II method (Roche Diagnostics Corporation) on a Roche Hitachi 917 Analyzer. The Tina-quant II HbA1c assay is an NGSP-certified method (3) that quantifies the total hemoglobin mass (g/dL) spectrophotometrically and the HbA1c mass (g/dL) by turbidimetric immunoinhibition.

During the course of a method comparison of the Tina-quant II assay with an HPLC method (Bio-Rad Variant1™), we observed a positive bias at HbA1c percentages below ~5.5% (data not shown). Addition-