mune disease). Of the explanations proposed for these differences by the various investigators, the lower recovery of the second-generation assay experienced by Garg et al. (1) in 1998 is not consistent with 1998 data from the Tacrolimus International Proficiency Testing Scheme (coordinator, Dr. D.W. Holt, St George’s Hospital Medical School, London, UK), from which can be calculated a positive bias and mean recovery of 114% (range, 102.1–121.7%) in 20 samples to which 3–28 μg/L tacrolimus was added and a similar overestimate relative to the results reported by the small number of centers using HPLC/mass spectrometry. A bias in assay calibrators (3) or differences in the contribution of tacrolimus metabolites (3, 4) may be a more likely explanation for the differences between the first- and second-generation assay results. Given the inherent variability in assay performance and tacrolimus pharmacokinetics, we still doubt whether a difference of 1–2 μg/L in assay results would have major practical impact on management by the realistic clinician. This is true both early after transplantation, when tacrolimus trough concentrations usually exceed 10 μg/L (but are subject to variability because of alterations in graft function, drug dosage, and coadministered medication), and later in clinically stable patients, when tacrolimus concentrations are often below 10 μg/L (and pharmacokinetic variability is lower but still subject to the influence of food intake) (6). In this lower range, where the increased sensitivity of the second-generation assay is advantageous, the CVs in tacrolimus measurements will span the differences of 1–2 μg/L between assay results.

Applying the concept of functional sensitivity (analyte concentration at 20% interassay CV) to the second-generation tacrolimus assay, Schambeck et al. (2) have defined a value of 3.1 μg/L for single measurements and recommend the use of two replicates at such concentrations. However, it is difficult to justify duplicate measurements realistically in terms of cost-benefit, the inherent variability in biological determinants of drug concentrations referred to above, or the use of concentrations as an adjunct to indicators of graft function and clinical condition in regulating dosage. The functional sensitivity also may not necessarily equate with the practical lower limit of quantification of the assay in routine use, particularly because two mode 1 calibrators (tacrolimus-free samples) are used to adjust the calibration curve in every assay. Thus, in defining a practical lower limit for routine assay of tacrolimus, it could be argued that intraassay variability (i.e., in relation to the mode 1 or tacrolimus-free calibrators run on the same carousel) may be of greater relevance than the corresponding interassay variability used in determining functional sensitivity. Within an assay, and like Schambeck et al. (2), we reported that tacrolimus concentrations of 1–2 μg/L blood are distinguishable from tacrolimus-free samples at high significance and without overlap (3). Moreover, recent data from the Tacrolimus International Proficiency Testing Scheme (coordinator, Dr. D.W. Holt) reported a CV of 17.1% on a sample containing 3.0 μg/L circulated to 184 centers and a CV of 21.7% on a corresponding sample of 2.0 μg/L analyzed at 172 centers. In addition to reporting a minimum detection limit of ~1.5 μg/L (3), our own routine experience using low concentrations of tacrolimus has been acquired from 179 interassay measurements of control material containing 3 μg/L (obtained over 11 months with multiple operators and calibration curves). Despite a CV of 30.9%, acceptable results ranged from 1.1 to 4.5 μg/L when limits of 3 SD were used (outside which we routinely and immediately recalibrate the assay). Our additional experience with the second-generation assay over 28 months and >9500 samples is that results ≥2.0 μg/L have been obtained on known tacrolimus-free blood samples on only three occasions. This concentration (2 μg/L) is used as our minimum quantifiable concentration.

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J. Michael Tredger*  
Colleen D. Gilkes  
Christopher E. Gonde

Institute of Liver Studies  
King’s College Hospital  
and School of Medicine  
Denmark Hill, London SE5 9RS, UK

*Address correspondence to this author at: Institute of Liver Studies, Guy’s, King’s, St. Thomas’ School of Medicine at Denmark Hill, Bessemer Road, London SE5 9PJ, UK. Fax 44-171-346-3760; e-mail michael.tredger@kcl.ac.uk.

Protein Zone Electrophoresis of Pleural Effusion: The Diagnostic Separation of Transudates and Exudates

To the Editor:

The cause of a pleural effusion is not always easily determined. Invasive procedures such as pleural biopsy are indicated only in patients with exudative pleural effusions. Therefore, a frequent early step in the evaluation of pleural effusions is to classify them as transudates or exudates.

The diagnostic criteria developed by Light et al. (1) characterize pleural
exudates as having at least one of the following: pleural fluid/serum total protein ratio >0.5; pleural fluid/serum lactate dehydrogenase (LDH) ratio >0.6, and pleural fluid LDH more than two-thirds of the upper reference limit of serum LDH. However, the results produced by Light et al. (1) are not always reproducible (2), and the low specificity of the criteria of Light et al. may lead to unwarranted invasive intervention in up to 20–30% of patients with transudates (3).

One report (4) described the use of protein electrophoresis (by Tiselius U-tube) to study patterns of protein in pleural fluid in disease but did not address the role of protein zone electrophoresis (PZE) in the differentiation of exudate from transudate. We postulate that low-molecular weight molecules such as albumin ($M_r$ 66 400), $\alpha_1$-antitrypsin ($M_r$ 54 000), and transferrin ($M_r$ 76 500) pass through the pleura to enter the pleural spaces in transudative pleural effusion, whereas high-molecular weight molecules such as $\alpha_2$-macroglobulin ($M_r$ 725 000), haptoglobin ($M_r$ 400 000), immunoglobulin (IgG, $M_r$ 150 000; IgA, $M_r$ 160 000; IgM, $M_r$ 950 000), and $\beta$-lipoprotein ($M_r$ 250 000) do so only when capillary permeability increases in exudative pleural effusion formation.

We tested our hypothesis in patients who presented with pleural effusion in the Princess Margaret Hospital during 1997–1998. No selection criteria were set with respect to the type of disease, age, or sex. Routinely, pleural fluid was aspirated for cytology, bacterial culture, mycobacterial culture, total protein, and LDH measurements. Pleural biopsy was performed if relevant pathology was suspected. Diagnosis was done by the respiratory physicians.

Pleural fluid and blood samples were from specimens sent to the laboratory. Specimens were received on the day of collection. Whenever possible, paired samples of pleural fluid and blood collected on the same date were used. If same-date specimens were unavailable, blood samples taken within 24 h before or after the pleural fluid collection were accepted (3). Blood and pleural fluid samples were centrifuged, and the supernatant was separated into aliquots and kept at $-80^\circ$C until analysis.

The diagnosis of tuberculous pleuritis required either the identification of Mycobacterium tuberculosis by culture or biopsy or the presence of caseous granuloma. Malignant pleural effusion was diagnosed when malignant tissue in the pleural cavity was shown by pleural biopsy or cytology. Effusion was considered parapneumonic when there was an acute febrile illness associated with pneumonia, lung abscess, or bronchiectasis in the absence of malignancy. Empyema was defined as the presence of purulent pleural fluid and positive bacterial culture associated with parapneumonic effusion. Congestive heart failure was diagnosed when all of the following criteria were satisfied: cardiomegaly, radiological evidence of congested lungs, peripheral edema, and re-

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**Table 1. Pleural effusions.**

<table>
<thead>
<tr>
<th>Exudate</th>
<th>Clinical criteria</th>
<th>PZE</th>
<th>Criteria of Light et al. (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignancy</td>
<td>23</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td>Tuberculosis</td>
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<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Pneumonia</td>
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<td>3</td>
<td>2</td>
</tr>
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<td>1</td>
</tr>
<tr>
<td>Cholangitis</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acute pancreatitis</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Parapneumonic disease</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Reactive effusion to chest infection</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Reactive effusion to subphrenic abscess</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
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<td>43</td>
<td>41</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Transudate</th>
<th>Clinical criteria</th>
<th>PZE</th>
<th>Criteria of Light et al. (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis</td>
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<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nephrotic syndrome</td>
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<td>1</td>
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<td>End-stage renal failure</td>
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<tr>
<td>Congestive heart failure</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Acute pulmonary edema</td>
<td>1</td>
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<td>0</td>
</tr>
<tr>
<td>Hypoalbuminemia related to malnutrition</td>
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<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>

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**Fig. 1. Typical electrophoretic patterns in pleural fluid (P) and paired serum (S) samples for three patients (1–3).**

Albumin, $\alpha_1$, and transferrin bands are present in lane P-1 in a transudate. $\alpha_2$, $\beta$-lipoprotein, and immunoglobulin bands are present in lane P-2, and $\alpha_2$ and paraprotein bands are present in lane P-3 in an exudate.
sponse to treatment for congestive heart failure. Renal failure was diagnosed when urea and creatinine were increased in the presence of clinical evidence of fluid overload and an absence of purulent sputum, malignancy, or pulmonary infiltrates. Nephrotic syndrome was classified when the patient had proteinuria >3.5 g/24 h, edema, and hypoalbuminemia. Liver cirrhosis was diagnosed by clinical and laboratory evidence of hepatic damage with portal hypertension or hypoalbuminemia. Other causes of hypoalbuminemia were determined when serum albumin was <30 g/L in the absence of proteinuria and histologically confirmed liver cirrhosis.

Pleural fluid and serum were analyzed in paired samples by PZE (Beckman Coulter Paragon® SPE kit). Serum was diluted 1:5 (one volume of serum plus four volumes of barbitral buffer, as recommended by the manufacturer) and pleural fluid 1:3 (one volume of serum plus two volumes of barbitral buffer). When any one of the α-2, β-lipoprotein, and γ bands are present in the electrophoretogram, the fluid is classified as an exudate. When only albumin, α1-antitrypsin, and transferrin are present in the electrophoretogram, the fluid is classified as a transudate.

According to our criteria, 51 cases had definitive clinical diagnoses. Among them, 43 were exudative conditions and 8 were transudative conditions (Table 1).

In most transudates, only low-molecular weight molecules were present, and no high-molecular weight molecules were present (Fig. 1, lane P-1), whereas typical exudates (lanes P-2 and P-3) contained α-2, β-lipoprotein, and γ bands.

The criteria of Light et al. (1) correctly classified 41 exudates (95%) and 3 transudates (38%), whereas PZE correctly classified 43 exudates (100%) and 4 transudates (50%).

The criteria of Light et al. (1) failed to identify an exudate attributable to pneumonia. PZE clearly shows an α-2 band and a γ band. The patient had a pleural/serum total protein ratio of 0.33, a pleural fluid LDH of 99 U/L, and a pleural/serum LDH ratio of 0.33. In another case diagnosed as parapneumonia, PZE showed an α-2 band and a γ band. However, the patient had a pleural/serum total protein ratio of 0.38, a pleural fluid LDH of 79 U/L, and a pleural/serum LDH ratio of 0.31.

In four transudates diagnosed by clinical criteria, protein zone electrophoregrams had characteristics of exudates. Three of these four transudates were classified as exudates by both the criteria of Light et al. (1) and PZE. They included one patient suffering from acute pulmonary edema and two patients suffering from end-stage renal failure. In the remaining case of nephrotic syndrome, the criteria of Light et al. classified it as a transudate (pleural/serum total protein ratio, 0.33; pleural/serum LDH ratio, 0.18; and pleural fluid LDH, 47 U/L), but the PZE classified it as an exudate (α-2 and γ bands present). The patient also suffered from systemic lupus erythematosus, which probably complicated the influence of nephrotic syndrome in pleural fluid formation. It is suspected that this patient also suffered from pleuritis. Apparently, our stringent clinical criteria for transudative pleural effusion still underestimated the degree of exudative activity in the pleura.

To put qualitative analysis into quantitative assessment, we used a Beckman Coulter Appraise® Densitometer System to scan the electrophoretic gels. The relative area (as a percentage) of each protein fraction was obtained, and the ratio of the α-2 protein fraction to the albumin fraction was calculated. The data were divided into two separate groups according to the qualitative separation by PZE into exudates and transudates. There was a statistically significant difference between the α-2/albumin ratios of PZE-classified transudates and exudates (P = 0.0134, Mann–Whitney U-test).

By ROC curve analysis (MedCalc, Ver. 4.20), the optimal α-2/albumin cutoff ratio for exudate identification was 0.28. The area under the ROC curve was 0.84 (95% confidence interval, 0.70–0.93). At the optimal cutoff point, the diagnostic sensitivity and specificity were 85% (95% confidence interval, 70–94%) and 80% (95% confidence interval, 29–97%), respectively. At a cutoff value of 0.35, the diagnostic sensitivity and specificity were 70% (95% confidence interval, 54–83%) and 100% (95% confidence interval, 100–100%) respectively.

The accuracy and precision of densitometric scanning are affected by many factors: the densitometer, the wavelength used, the agarose slides, and the duration of electrophoresis, fixation, staining, and destaining. In addition, the protein concentration is not always proportional to its staining intensity because of the variations in staining specificity of different protein fractions. Another major shortcoming of densitometric scanning lies in the subjectiveness of manual adjustment of boundaries of protein zones. In this series, there is a negative deviation of albumin concentration obtained by densitometric scanning from that measured by bromocresol green method (Trace Scientific) of up to 56%. Sources of imprecision in protein fraction quantification by densitometry have been documented elsewhere (5, 6). An even more precise measure could be based on capillary electrophoresis or chromatography with protein detected by ultraviolet absorption (7).

In conclusion, there is a good agreement between the results obtained with the PZE and the criteria of Light et al. (1). In some cases, PZE also provides additional information for the diagnostic separation of exudates from transudates.

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References

Mo-Lung Chen1
Ching-Wan Lam2*

1 Department of Pathology
Princess Margaret Hospital
Hong Kong, China
2 Department of Chemical Pathology
The Chinese University of Hong Kong
Prince of Wales Hospital
Hong Kong, China

*Author for correspondence. Fax 852-2636-5090; e-mail ching-wanlam@cuhk.edu.hk.

Correction
In the article by J.T. Hindmarsh, L. Oliveras, and D.C. Greenway, entitled “Plasma Porphyrins in the Porphyrias” (Clin Chem 1999;45:1070–6), the HCl concentrations given on page 1071 under “Calibrators and controls” and “Extraction procedure” in Materials and Methods should be in mol/L, not mmol/L. The authors regret any confusion this may have caused.

Correction
In the article by R.H. Christenson, H. Vaidya, Y. Landt, R.S. Bauer, S.F. Green, F.A. Apple, et al., entitled “Standardization of Creatine Kinase-MB (CK-MB) Mass Assays: The Use of Recombinant CK-MB as a Reference Material” (Clin Chem 1999;45:1414–23), the first full sentence on page 1417 should read: “Based on this amino acid content, the absorbance of a 1 g/L CK-MB solution at 280 nm was estimated as 0.82, using a pathlength of 1 cm (12)” rather than “Based on this amino acid content, the molar absorptivity of CK-MB was estimated as 0.82 L/cm-mol (absorbance of 1 g/L solution of CK-MB at 280 nm using 1-cm pathlength) (12)” . The authors regret any confusion this may have caused.

Correction
In the article by L.G. Raisz, entitled “Physiology and Pathophysiology of Bone Remodeling”, which appeared in the Beckman Conference supplement (Clin Chem 1999;45:1353–8), the legends for Figs. 1 and 2 on page 1354 are reversed.