Letters

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 electrophoregrams 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)] [Editor’s note: Light et al. is Ref. (2) in this reply.] always have a higher sensitivity 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”. 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


Drs. Lam and Chen respond:

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References


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 (SRIL no. 4) of the National Glycohemoglobin Standardization Program (NGSP) (2). We routinely measure HbA1c percentages from whole blood samples by the HbA1c Tina-quant® 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 immuno-inhibition.

During the course of a method comparison of the Tina-quant II assay with an HPLC method (Bio-Rad Variant™), we observed a positive bias at HbA1c percentages below ~5.5% (data not shown). Addition-
ally, we observed that our Level 1 quality-control (5.6% HbA1c) material had a long-term imprecision that was nearly one-half as variable as our Level 2 (10.3% HbA1c) material: CV = 1.7% vs 2.6%, respectively. These observations led to dilution linearity experiments for the individual component measurements in the Tina-quant II assay and revealed that although the hemoglobin measurement diluted linearly, the A1c mass component had a positive deviation from linearity at a HbA1c concentration of ~5.3% (Fig. 1A). When we inspected the cumulative NGSP monthly survey data and incorporated selected low-percentage HbA1c whole blood NGSP samples, we observed a positive bias at HbA1c percentages <5.5%. To correct this bias we fit a nonlinear function that was asymptotically linear in the limit:

$$\text{Corrected A1c} = 0.747 + 1.225 \times (\text{observed A1c mass} - 0.567) - 0.341 \times \sqrt{[(\text{observed A1c mass} - 0.567)^2 + 0.005]}$$

by nonlinear least-squares analysis to the A1c mass value in the dilution analysis (STATA Release 6, College Station, TX). This correction was then applied to every Tina-quant II A1c mass value, and the percentage of HbA1c was recalculated using the raw hemoglobin value. The resulting HbA1c percentage was finally corrected with a linear regression slope and intercept to agree with the NGSP. These data were plotted against the NGSP HPLC value (Fig. 1C), demonstrating an improved correlation at low HbA1c percentages.

The nonlinearity of the Tina-quant II assay compared with various HPLC methods has been noted previously (4). We report here one solution to correct this deviation. Although the observed bias at HbA1c values <6% has little clinical significance [these values are in the reference range (4–6% HbA1c), which is generally considered desirable], it is undesirable for certification efforts. Additionally, such bias may be confusing during method comparisons or when changing methodologies. It is not our intention to recommend this correction for routine laboratory testing because this action may require off-line data manipulation, change the status of the assay to that of “homebrew”, and have little effect on patient care. However, this cor-

Fig. 1. Identification and correction of positive bias at low HbA1c percentages.

(A), whole blood was diluted with plasma split from the same sample to create a diluted whole blood series. Diluted whole blood was assayed for hemoglobin (■) and A1c mass (●) using the Tina-quant II HbA1c assay. (B), percent HbA1c method comparison of the Tina-quant II (uncorrected data) vs the NGSP HPLC method. The inset shows expanded percent HbA1c axes to emphasize the positive bias at low HbA1c percentages. (C), percent HbA1c method comparison of the Tina-quant II vs the NGSP HPLC method. The Tina-quant II data have been corrected by application of Eq. 1 and subsequent slope and intercept adjustment.
rection method has improved the agreement of the Tina-quant II assay with the NGSP surveys and will allow greater likelihood of certification of candidate methods by reducing the apparent bias at low HbA1c percentages.

We wish to acknowledge the cooperation and thoughtful discussion provided by Dr. Randie Little of the University of Missouri, Columbia, MO.

References
2. NGSP Steering Committee. Implementation of the National Glycohemoglobin Standardization Program (NGSP). Diabetes 1997;46(Suppl 1): 151A.

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\( \alpha_1 \)-Antitrypsin Deficiency as a Result of Compound Heterozygosity for the Z and M_Heerlen Alleles

To the Editor:
The specificity of many common rapid molecular techniques for only one or a few mutations may produce confusion when a rare mutation is present. We describe a patient in whom the M_Heerlen allele of the AAT gene for the protease inhibitor \( \alpha_1 \)-antitrypsin (AAT) was missed by widely used molecular genotyping (1–4) and phenotyping methods for mutations in the AAT gene. The majority of these assays were developed to detect the S and Z mutations (Glu264→Val and Glu342→Lys, respectively), which are the most prevalent mutations in a Caucasian population (5).

A 40-year-old woman with a serum AAT of 0.3 g/L (reference interval, 0.8–2.0 g/L; Beckman Array), suffering from dyspnea and a familial history of emphysema was genotyped as PIMZ. However, isoelectric focusing (IEF) analysis (Blood Transfusion Service, Amsterdam, The Netherlands) revealed only the Z isof orm. Hence she was considered to be a ZZ phenotype, in agreement with her low serum AAT and her early emphysema. The only sibling/sister of the propositus was also affected by pulmonary dysfunction and was also genotyped as PIMZ with a ZZ phenotype. The sister's children had a normal phenotype, M; their genotype was MM. However, based on the phenotype of their mother, the children were expected to have at least a partial Z phenotype. We decided to check for the presence of other mutations in the AAT gene that may account for the difference in results between the two tests.

DNA sequence analysis (ABI PRISM 3700) of the amplicon generated by PCR amplification of the AAT gene that may account for the presence of the Z allele revealed the presence of the M_Heerlen mutation (Pro369→Leu), which was described previously by Hofker et al. (6). Hence, the patient's actual genotype was PIM_HeerlenZ. The M_Heerlen mutation is thought to encode for the synthesis of a misfolded protein that is degraded in the endoplasmic reticulum of hepatocytes without being secreted. This explains the absence of a M-like protein in IEF gels. The low serum AAT (0.3 g/L) is in fact the result of expression of only the Z allele. Both the M_Heerlen and Z alleles could be traced back to a previous generation of this family (Table 1); the M_Heerlen allele, but not the Z allele, also appeared in later generations. Both children of the propositus' sister carried the M_Heerlen allele and a nonmutated M allele, as verified by sequence analysis. Therefore, the father must have at least one nonmutated M allele. In all cases, serum AAT concentrations corresponded with expected protein expression of the respective genotypes, assuming that the M_Heerlen allele does not significantly contribute to serum AAT concentrations (Table 1). Because the presence of the M_Heerlen allele fully accounted for the described phenotypes, no efforts were undertaken to look for other mutations in the AAT gene.

Obviously, none of the routine tests [PCR-restriction fragment length polymorphism (RFLP) analysis and IEF] detected the cause of the partial

<table>
<thead>
<tr>
<th>Patient</th>
<th>Genotype determined by PCR-RFLP</th>
<th>Genotype determined by sequence analysis</th>
<th>Phenotype determined by IEF</th>
<th>Serum AAT, g/L (0.3–2.0)</th>
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</thead>
<tbody>
<tr>
<td>Paternal aunt</td>
<td>MM</td>
<td>MM_Heerlen</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Mother</td>
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<tr>
<td>Sister</td>
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<td>Z</td>
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<tr>
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<td>M_Heerlen</td>
<td>M</td>
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</tr>
<tr>
<td>Nephew</td>
<td>MM</td>
<td>M_Heerlen</td>
<td>M</td>
<td>0.9</td>
</tr>
</tbody>
</table>

a Both the DNA-based PCR-RFLP test as well as IEF yield improper results if the M_Heerlen allele is present. The presence of the M_Heerlen allele can be verified only by DNA sequence analysis. Despite the improper result, IEF yields an acceptable explanation for the partial AAT deficiency.

b Reference interval.

c ND, not determined.