Efficacy of the Detection of the $\alpha_1$-Antitrypsin "Z" Deficiency Variant by Routine Serum Protein Electrophoresis

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Abstract

Deficiency of $\alpha_1$-antitrypsin (AAT) is a common, potentially life-threatening genetic disorder.1 Estimates have placed the prevalence of AAT deficiency as approximately 1 in 2,500 to 3,000 people. There are potentially 100,000 affected people in the United States, most of whom remain undiagnosed.2-4 AAT is the most abundant circulating protease inhibitor in the body. Absence of sufficient protease inhibitor results in the degradation of the alveolar structural protein elastin and progressive pulmonary damage. Some AAT protein variants, such as the Z gene product, are improperly processed during synthesis, forming aggregates in the liver, resulting in liver disease.5

The AAT gene locus is extremely polymorphic. In addition to the native M allele variant, which exhibits full antiproteolytic activity, more than 100 variants have been identified to date. The Z and S alleles account for the majority of the deleterious mutations in patients with AAT deficiency, with the Z mutation being the more severe. Approximately 1 in 3,000 people in the United States is homozygous for ZZ and is at high risk for the development of emphysema or hepatic disease.4,6 The estimated prevalence of MZ carriers is thought to be approximately 1 in 35, and these carriers are typically thought not to be susceptible to disease.3,7

Pulmonary symptoms related to AAT disease often do not develop until the fourth decade of life, whereas hepatic disease may be noted in infancy or early childhood. Identification of people susceptible to AAT deficiency-related pulmonary and hepatic damage is crucial. Disease progression can be significantly slowed by minimizing exposure to aggravating environmental agents and by using enzyme replacement therapy. Frequently, diagnosis occurs several years after the onset of symptoms.8 Although widespread screening for AAT
deficiency has not been implemented, evaluation of targeted populations has been suggested.9

A number of methods can be used to detect AAT deficiency.10 The most common method is by protein phenotyping using isoelectric focusing (IEF). Quantitative protein concentrations can be measured directly by nephelometry or immunoturbidimetry. AAT is an acute phase reactant; therefore, measuring AAT concentrations alone can be misleading at times because increased and/or decreased AAT concentrations can be caused by conditions unrelated to AAT deficiency.11 Genotyping assays are also available for the most common deficiency-associated alleles such as S and Z, but these assays can miss significant numbers of at-risk people with other alleles.12,13

Serum protein electrophoresis (SPEP) is a commonly used technique to evaluate patients for potential lymphocyte malignancies such as multiple myeloma.14,15 Although these populations are generally not considered to be at higher risk for AAT deficiency or to have a higher prevalence of deleterious AAT alleles, the widespread use of SPEP provides an opportunity for incidental identification of people at risk for AAT deficiency. The AAT protein is the most abundant protein in the SPEP α1-globulin fraction.14,16 AAT deficiency was originally observed more than 40 years ago in patients with emphysema in whom extremely low α1-globulin protein fractions were shown by SPEP.17 Modern automated electrophoretic techniques, such as capillary gel electrophoresis, while having similar sensitivity to traditional agarose gel electrophoresis for the detection of M proteins, exhibit improved precision in protein fraction measurements.18,19

Materials and Methods

In this study, we evaluated the Sebia CAPILLARYS (Norcross, GA) capillary gel electrophoresis platform for identifying AAT deficiency using serum samples with established AAT enzyme concentrations and phenotypes. A total of 90 surplus samples (47 MM, 24 MZ, and 19 ZZ) submitted to ARUP Laboratories (Salt Lake City, UT) for AAT enzyme concentrations and phenotyping by IEF were used in this study. All samples were collected in accordance with guidelines established by the University of Utah Institutional Review Board, Salt Lake City. IEF was performed as previously described.13 Stained band patterns were visually examined to determine the AAT phenotype by comparison with control samples of known phenotypes. Serum concentrations of AAT were determined by using a Roche P Module immunoturbidimetric assay (Roche Diagnostics, Indianapolis, IN) (coefficient of variation [CV], 2.7% for the low [0.074 g/dL] and 2.4% for the high [0.165 g/dL] control materials).

A CAPILLARYS 2 (Sebia) system was used to separate serum proteins by using routine capillary electrophoresis according to the manufacturer’s instructions. The Sebia Phoresis software (version 5.4.1) and imaging system were used to determine the percentage of total observed protein density attributable to the α1-globulin fraction, which appears as a single broad peak in this technique. The α1-globulin densities were determined by using user-defined region boundaries and software-defined baseline absorbance. The CV for the α1-globulin fraction control material, which represented 4% of the total SPEP protein, was less than 7%. The concentration of total protein in the sample was determined by the Beckman Coulter LX-20 instrument (Beckman Coulter, Fullerton, CA) (CVs of the 3 levels of control material were all <3%). The total protein concentration was multiplied by the relative SPEP α1-globulin percentage of total density to yield the final α1-globulin peak concentration. All descriptive statistics were generated by using Excel (Microsoft, Redmond, WA) or EP Evaluator (David Rhoads Associates, Kennett Square, PA).

Results

Comparison of serum AAT concentrations by turbidimetric assay exhibited moderate correlation with the α1-globulin protein fraction as determined by methods commonly used for routine SPEP analysis [Figure 1]. The precision of the measured α1-globulin fraction in control for

![Correlation between measured α1-antitrypsin (AAT) protein concentration and measured α1-globulin fraction. The total measured AAT protein concentration was determined by turbidimetric immunoassay (Roche), and serum protein electrophoresis analysis was used to measure total α1-globulin protein. Deming regression best-fit analysis is shown. The linear best fit is described by [AAT] = 0.44[α1-globulin protein] + 0.002. The regression line r² is 0.67, and the S_{yx} is 0.024 g/L. Specimens that exhibited measured AAT concentrations below the AAT concentration assay detection limit of 0.030 g/L were omitted from this figure.](image-url)
all material capillaries in this study (CV, <7%) was slightly better than in a previous evaluation of the CAPILLARYS system (<11%). 19 The measured α₁-globulin protein concentrations from SPEP analysis was approximately twice the measured AAT serum concentration.

The distribution of the measured α₁-globulin protein concentrations for different AAT phenotypes is shown in Figure 21. As expected, Z variants resulted in lower observed α₁-globulin concentrations. Various α₁-globulin protein concentration cutoffs were used to quantify the ability of SPEP analysis to distinguish different phenotypes. For the MM samples, the observed α₁-globulin fractions by SPEP ranged from 0.21 to 0.86 g/dL (median, 0.30 g/dL), the MZ phenotypes ranged from 0.14 to 0.44 g/dL (median, 0.24 g/dL), and the ZZ phenotypes ranged from 0.12 to 0.30 g/dL (median, 0.19 g/dL). The lack of MM samples with α₁-globulin fractions of less than 0.21 g/dL suggested that this may be an appropriate cutoff for excluding the MM phenotype. The cutoff of less than 0.21 g/dL resulted in an 84.2% sensitivity for the ZZ phenotype. However, 7 (29%) of the 24 MZ heterozygote phenotypes also exhibited α₁-globulin fractions below this cutoff. Reducing the α₁-globulin fraction concentration cutoff to less than 0.15 g/dL reduced the number of MZ phenotypes detected to 1 (4%) but also reduced the fraction of ZZ samples with α₁-globulin fractions detected to 6 (32%) of 19 samples.

Discussion

The speed and precision of capillary gel electrophoresis have resulted in this method becoming an increasingly utilized alternative to traditional agarose gel electrophoretic methods for the quantitative measurement of serum proteins. Although there is variation in published estimates of AAT protein phenotype prevalence and ZZ phenotypes for each cutoff using the estimated prevalence of the MZ phenotype. A single MM phenotype sample with a measured α₁-globulin fraction of 0.86 g/dL was omitted from this figure.

Table 1

Summary of the Percentage of Protein Phenotype Samples With Observed α₁-Globulin Protein Fraction Concentration Below Each Cutoff

<table>
<thead>
<tr>
<th>α₁-Globulin Concentration Cutoff (g/dL)</th>
<th>No. (%) of Samples Below Cutoff</th>
<th>Estimated Positive Predictive Value in General Population</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MM (n = 47)</td>
<td>MZ (n = 24)</td>
</tr>
<tr>
<td>0.31</td>
<td>24 (51)</td>
<td>18 (75)</td>
</tr>
<tr>
<td>0.25</td>
<td>9 (17)</td>
<td>13 (54)</td>
</tr>
<tr>
<td>0.22</td>
<td>1 (2)</td>
<td>8 (33)</td>
</tr>
<tr>
<td>0.21</td>
<td>0 (0)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>0.20</td>
<td>0 (0)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>0.17</td>
<td>0 (0)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>0.15</td>
<td>0 (0)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

*The estimated positive predictive value of a positive test result was calculated using a phenotype frequency prevalence of 1 in 3,000 for the ZZ phenotype and 1 in 35 for MZ phenotype (see “Discussion”). In the positive predictive value column, the number of samples with measured α₁-globulin protein levels below the cutoff in which a single ZZ phenotype sample would be expected to be present is also shown.
the ZZ phenotype and a 1 in 35 MZ heterozygote phenotype prevalence were used for this analysis. It follows that for US patients undergoing routine SPEP analysis, a population of 30,000 specimens would contain, on average, 10 ZZ phenotype and approximately 857 MZ phenotype samples. For an α₁-globulin fraction cutoff of less than 0.21 g/dL, this method detected 16 (84%) of 19 ZZ phenotype samples. No MM phenotypes were observed with α₁-globulin fraction cutoffs of less than 0.21 g/dL. However, because 7 (29%) of 24 MZ heterozygous samples had α₁-globulin fractions of less than 0.21 g/dL, there would be approximately 250 MZ heterozygous patients in this population who would have α₁-globulin fractions below this cutoff. Thus, on average, by using the α₁-globulin fraction cutoff of less than 0.21 g/dL, approximately 1 of every 31 samples identified would represent a ZZ phenotype sample (specifically, 8.4 ZZ phenotype samples out of a total of 258.6 MZ plus ZZ phenotype samples). By using the more stringent cutoff of less than 0.15 g/dL, the estimated positive predictive value improved greatly, with 1 in 12 samples below the cutoff representing a ZZ phenotype. This improvement in positive predictive value occurs at the expense of missing approximately two thirds of the ZZ patients.

The number of affected patients at risk for AAT deficiency identified by this approach may prove to be even higher in clinical practice than what is estimated herein. There are significant numbers of patients who have rare deficiency alleles, other than the Z allele, that also result in greatly reduced AAT concentrations when paired with an additional deficiency allele. Although the prevalence of AAT deficiency in samples submitted for SPEP is believed to be similar to what has been observed in the general population, this has not been established by experimental procedures. Any increase in the prevalence of the ZZ phenotype in sample populations typically submitted for SPEP analysis may result in an increase in positive predictive value for detection of people at risk for developing AAT deficiency. Finally, the approach outlined herein could be used with different ZZ and MZ phenotype prevalence estimates to calculate the positive predictive value of these SPEP α₁-globulin fraction cutoffs in different ethnic or geographic populations.

Our data suggest that further evaluation for AAT deficiency of people with low α₁-globulin levels by capillary gel SPEP is warranted. Further studies are needed to determine potential appropriate quantitative α₁-globulin fraction cutoffs for other electrophoretic methods. The benefits of the use of lower α₁-globulin cutoffs, which exhibit increased positive predictive values, should be carefully weighed against reduction in the frequency of detection of ZZ phenotype samples. Capillary gel SPEP may not be suitable for general population screening for AAT deficiency. However, the estimated 1 in 31 chance of detecting a ZZ phenotype for any routine SPEP test exhibiting an α₁-globulin fraction of less than 0.21 g/dL may justify the expense needed for further investigation of AAT deficiency by protein phenotyping or genetic testing. Quantitative SPEP α₁-globulin concentration cutoffs could be used to develop footnotes to alert clinicians of potential AAT deficiency or to establish reflexive testing protocols when low α₁-globulin levels are “incidentally” observed and, thereby, offer an opportunity for earlier clinical intervention in disease.

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