Cirrhosis Originally Diagnosed as Nonalcoholic Steatohepatitis

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CASE

A 52-year-old white woman with a presumptive diagnosis of cirrhosis attributed to nonalcoholic steatohepatitis was referred for a liver transplant consultation. The patient reported increased abdominal discomfort, swelling, and lower extremity edema beginning 7 years earlier, as well as intermittent nausea, weakness, and confusion which had increased in frequency during the previous year. While undergoing a cholesteotomy for gallstones 2 years previously, the patient was noted to have ascites and a cirrhotic-appearing liver. Results of routine laboratory tests and an abdominal ultrasound performed at that time were consistent with cirrhosis.

The patient, a married housewife with 1 adult son, had a physical appearance that suggested chronic illness but was in no acute distress. She denied any history of drug or alcohol use and reported never having received a blood transfusion. She had no history of childhood liver disease and was unaware of any family history of gastrointestinal malignancy or liver disease. The patient did report, however, that 2 of her 7 siblings died from unknown causes as infants, her father died while in a coma of unknown etiology, her mother died from diabetic complications, and a third sibling died of a gynecologic cancer. The patient’s medical history was remarkable for obesity, gastroesophageal reflux disease, recurrent urinary tract infections, and arthritis. Physical examination was unremarkable, with the exception of mildly icteric sclera, spider nevi on the anterior chest wall, and bilateral lower extremity edema. Moderately decreased breath sounds in her lower left lung were also noted. Chest x-rays and spirometry were unremarkable.

Laboratory data were consistent with end-stage liver disease and also revealed mild anemia as well as thrombocytopenia and chronic kidney disease. Although at the time of her referral to our institution the patient had a presumptive diagnosis of cirrhosis related to nonalcoholic steatohepatitis, we investigated other causes for her liver failure.

The patient tested positive for hepatitis A IgG and negative for hepatitis A IgM antibodies, indicating a remote infection with hepatitis A virus. Hepatitis B and C virus serological tests were all negative. Serological markers were negative for antinuclear, anti–neutrophil cytoplasmic, antimitochondrial, and anti–smooth muscle antibodies. Hemochromatosis genetic tests by PCR for the C282Y, H63D, and S65C point mutations were negative. Other laboratory results are shown in Table 1.

DISCUSSION

Cirrhosis of the liver is a gradually worsening condition that leads to progressive hepatic fibrosis characterized by distortion of the hepatic architecture and formation of regenerative nodules. Determination of the etiology of cirrhosis is important for making treatment decisions, counseling family members, and predicting complications. The 2 most common causes of cirrhosis in the US are alcoholic liver disease and chronic hepatitis C infection. Other less common causes include hepatitis B infection, biliary disease, autoimmune liver disease, and metabolic causes such as hereditary hemochromatosis, nonalcoholic steatohepatitis, Wilson disease, and α₁-antitrypsin (A1AT) deficiency. Patient history and physical examination, as well as laboratory and radiologic findings, can often provide an etiologic diagnosis, thereby eliminating the need for a liver biopsy.

PATIENT DIAGNOSIS

At the time of this report the patient was waiting to receive a combined liver-kidney transplant. Because of this patient’s remarkably low plasma A1AT concentration (Table 1), we also performed A1AT phenotyping, which indicated Z homozygosity (Pi*ZZ). A1AT-deficient patients with the ZZ phenotype have a significantly increased risk of developing pulmonary disease, so at the time of this report the patient was undergoing close monitoring for pulmonary changes (1). She has also been referred to a genetic counselor because her son must carry at least 1 defective A1AT Z allele.
A1AT DEFICIENCY

A1AT deficiency is a relatively common condition that is characterized by decreased concentrations of plasma A1AT. Patients with this disorder are predisposed to chronic obstructive pulmonary disease, and in many cases they also develop chronic liver disease (1). The prevalence of severe A1AT deficiency in the US is about 1 in 2000 to 5000 individuals, so that approximately 100 000 Americans are severely A1AT deficient (2, 3). Despite this frequency, A1AT deficiency is persistently underrecognized, with A1AT-deficient patients seeing an average of 3 different physicians and experiencing a mean delay of 7.2 years between initial symptoms and diagnosis (3).

The A1AT enzyme, a member of the serine protease–inhibitor family, is produced in the liver and diffuses into the lungs, where its main function is to protect the lungs against proteolytic damage from neutrophil elastase (4). Individuals with serum A1AT protein concentrations below the protective threshold of 11 μmol/L have been shown to have increased risk for developing early-onset emphysema (2). In most cases, severe deficiency of this circulating protein occurs as a result of the inheritance of variant deficiency alleles arising in the A1AT gene, serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 (SERPINA1) (1). Variations in the A1AT gene are not the only causes of A1AT deficiency, however; secondary causes of A1AT deficiency include liver damage and conditions such as protein-losing enteropathies (4).

The A1AT enzyme, SERPINA1, is heterogeneous, with approximately 100 different alleles identified to date (5). The most common wild-type SERPINA1 gene sequence, designated as the M allele, and several variants identified by isoelectric focusing, including M1, M2, G, X, C, and D alleles, are associated with concentrations of the A1AT protein that are within the reference interval (approximately 20–40 μmol/L) (1, 4). A large number of variations within this gene, however, are associated with decreased concentrations or activity of the A1AT protein.

One of the most common A1AT deficiency alleles is the Z variation, which consists of a glutamate-to-lysine substitution at codon 342 (E342K) and is classified as both an A1AT deficiency and dysfunctional allele (4, 5). Although this mutation is associated with a normal rate of synthesis of A1AT, the conformational instability of the mutant A1AT Z protein leads to polymerization and accumulation of A1AT molecules within the hepatocytes (6). This effect leads to liver damage that ultimately progresses to cirrhosis in 30% to 40% of patients older than 50 years (1). Furthermore, because the small portion of A1AT protein that is secreted (only approximately 15%) is also defective in its ability to inhibit elastase, patients who are Z homozygous usually present with severe pulmonary disease (1, 4). Thus pulmonary emphysema is the most frequent cause of disability and early death among affected persons (1). Approximately 8 million Americans (1% to 3% of the white population) carry at least one copy of this most common severe deficiency allele (3, 7).

Another relatively common variation, the S allele, is also associated with decreased concentrations of circulating A1AT. This S mutation, a substitution of glutamate at codon 264 (E264V) (2), is associated with intracellular degradation of the A1AT protein; thus individuals homozygous for this variation

### Table 1. Selected patient laboratory results with corresponding reference intervals.

<table>
<thead>
<tr>
<th>Result</th>
<th>Reference interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L</td>
<td>114–144 (11.4–14.4)</td>
</tr>
<tr>
<td>Platelet count, ×10^9/L</td>
<td>150–400</td>
</tr>
<tr>
<td>Blood urea nitrogen, mmol/L (mg/dL)</td>
<td>2.9–8.9 (8–25)</td>
</tr>
<tr>
<td>Creatinine, μmol/L (mg/dL)</td>
<td>30.5–76.3 (0.4–1.0)</td>
</tr>
<tr>
<td>Aspartate aminotransferase, U/L</td>
<td>15–41</td>
</tr>
<tr>
<td>Alanine aminotransferase, U/L</td>
<td>&lt;34</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/L</td>
<td>32–91</td>
</tr>
<tr>
<td>γ-Glutamyl-transferase, U/L</td>
<td>7–50</td>
</tr>
<tr>
<td>Total bilirubin, μmol/L (mg/dL)</td>
<td>5.13–20.5 (0.3–1.2)</td>
</tr>
<tr>
<td>Plasma albumin, g/L</td>
<td>35–48</td>
</tr>
<tr>
<td>Prothrombin time (PT)</td>
<td>10.8–13.1 s</td>
</tr>
<tr>
<td>Activated partial thromboplastin time, s</td>
<td>25.0–39.0</td>
</tr>
<tr>
<td>A1AT, μmol/L (mg/dL)</td>
<td>15.6–39.2 (85–213)</td>
</tr>
</tbody>
</table>
have approximately 60% to 80% of normal concentrations of circulating A1AT concentrations (7, 8). These A1AT concentrations are usually sufficient to prevent pulmonary manifestations of A1AT deficiency in the absence of other risk factors (e.g., smoking) (1). No association appears to exist between the S allele and liver disease. The presence of a Z allele, however, is associated with further decreases in A1AT concentrations and increased risk for the development of chronic obstructive pulmonary disease (4). Rarely, A1AT-deficient individuals may have a null A1AT variant, which is associated with no A1AT protein synthesis and a significantly increased risk of developing A1AT deficiency–associated pulmonary disease.

**DIAGNOSIS AND MANAGEMENT**

Indications for the possible presence of A1AT deficiency include early-onset emphysema (before the age of 45 years), emphysema in a nonsmoker, emphysema with predominant basilar changes, unexplained chronic liver disease, and family history of liver or lung disease (1). A critical aspect of clinical management is the identification of specific genetic variations and disease-associated risks. Also important is minimizing exposure to environmental risk factors, most importantly cigarette smoke and other respiratory irritants. Once a genetic etiology is recognized, other at-risk family members should be identified and their disease or carrier status determined (4). Genetic counseling is recommended in these cases (1).

Although a low serum A1AT concentration (<15–18 µmol/L (<80–100 mg/dL)) suggests the diagnosis of A1AT deficiency, genotyping or phenotyping should be used to identify cases in which low concentrations of A1AT are attributable to a genetic cause (4, 8, 9). Phenotyping by isoelectric focusing assay is considered the gold standard for diagnosing A1AT deficiency, according to the American Thoracic Society/European Respiratory Society Statement (1). Although isoelectric focusing phenotyping assays can be used to simultaneously identify many different alleles, these assays are time-consuming and technically demanding. Recently published algorithms from large reference laboratories recommend the use of genotyping by melting-curve analysis as a front-line test, in conjunction with serum A1AT quantification (4, 8). Although these A1AT genotyping assays require less technical skill, they are designed to detect only the most common deficiency alleles, S and Z. The proposed algorithms, therefore, suggest the use of reflex testing by phenotyping when S/Z genotyping produces discordant results (e.g., a low serum A1AT concentration without an observable S or Z allele). Genotyping by direct sequencing of the coding regions of the SERPINA1 gene may be considered in cases in which the presence of a null allele or another uncommon allele is suspected but not identified by either S/Z genotyping or phenotyping assays (9).

Potentially, the most important principle in the management of A1AT deficiency is the avoidance of cigarette smoking and other respiratory irritants, because long-term exposures to smoke, dust, and fumes have been shown to markedly accelerate the destructive lung disease associated with A1AT deficiency (1). Specific therapeutic options for A1AT deficiency include infusion of purified A1AT from pooled human plasma to increase the circulating concentration of A1AT protein in serum above the protective threshold. This approach is believed to be safe and may augment survival by slowing the decline of lung function (10). Long-term studies of the effectiveness of this expensive treatment are not available, however, so cost and efficacy must be taken into account when considering the use of this therapy. Gene-targeted therapies using adeno-associated virus containing the SERPINA1 gene may be treatment options in the future, but currently their applications are limited because they do not repair liver tissue damaged by the accumulation of dysfunctional protein (11). Unfortunately, at this time no specific medical therapy for this hepatic disease is available, but liver transplantation is curative (5).
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References


Commentary

Morten Dahl

It is estimated that there are at least 100,000 individuals with α₁-antitrypsin (A1AT) deficiency in the US, but only 6000 of these had been identified by 2006 (1). If the diagnosis rate increases in the future, prevention could become a promising alternative to treatment of the disease. Guidelines from the American Thoracic Society and European Respiratory Society already indicate which features should alert clinicians that a patient may be more likely to have A1AT deficiency. Snyder and Fantz report a case of A1AT deficiency identified in a patient with unexplained liver disease, a situation for which testing for A1AT deficiency is strongly recommended. Other situations for which testing for A1AT deficiency is strongly recommended include the presence of persistent airway obstruction or necrotizing panniculitis. Siblings of individuals with A1AT deficiency should also be tested. Whereas Snyder and Fantz report testing targeted to a certain patient type, Zorzetto and colleagues, whose report also appears in this issue of Clinical Chemistry, describe their large-scale screening for A1AT deficiency in 1399 Swiss individuals from the general population (2). Zorzetto et al. successfully identified one individual with severe ZZ A1AT deficiency and 154 individuals with SZ and MZ who had intermediate deficiencies. Testing for A1AT deficiency typically involves measurement of serum A1AT concentrations combined with genotyping for the common S and Z alleles by melting curve analysis (3), restriction-fragment length polymorphism PCR, or another assay. When serum A1AT and genotyping results are discordant, subsequent DNA sequencing or isoelectric focusing is often needed. Alternatively, direct sequencing can be performed on specimens with A1AT concentrations <1 g/L (4). Ideally the reports by Snyder and Fantz and Zorzetto et al. will help promote diagnosis in individuals with unrecognized A1AT deficiency so they may receive counseling and therapy when necessary.

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References

2. Zorzetto M, Russi E, Senn O, Imboden M, Ferrarotti I, Tinelli C, et al. SERPINA1 gene variants in individuals from the general population with reduced alpha1-antitrypsin concentrations. Clin Chem 2008.; [Please supply the correct reference for this article to be published in the same issue as this commentary]
Commentary

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Snyder and Fantz present an interesting case of α₁-antitrypsin (A1AT) deficiency caused by homozygosity for the Z-deficiency allele. Although A1AT deficiency is generally associated with the Z and/or S alleles, other deficiency alleles also occur. The clinical laboratory is faced with the challenge of detecting all clinically relevant alleles in a timely and cost-effective manner. Phenotyping by isoelectric-focusing electrophoresis has been used for many years to identify a wide range of alleles. Although commercial reagent sets are available, phenotyping remains technically demanding. In addition, commercial standards are available only for the S and Z alleles. Recently, DNA-based genotyping has generated interest as an alternative to phenotyping. Because most genotyping assays focus on detection of only the S and Z alleles, however, at least 2 groups of investigators have developed diagnostic algorithms for A1AT deficiency that include quantification, genotyping, and phenotyping (1, 2). The testing begins with measurement of A1AT concentration and genotyping for the S and Z alleles. An A1AT concentration that is lower than expected for the observed genotype suggests the presence of another deficiency allele, a result that requires reflex testing with a method that enables identification of numerous alleles. If phenotyping is selected, in-house comparison samples must be accumulated to permit accurate identification of rare deficiency alleles. Even theoretically, however, it is impossible to collect all potential phenotypes. Alternatively, DNA sequencing can be used to identify rare, deleterious alleles. Although more expensive, sequencing can detect null alleles, is not subject to inference by replacement therapy, and does not require a sample bank of unusual phenotypes. DNA sequencing may become an important component of diagnostic algorithms for A1AT deficiency (3, 4).

Despite efforts to raise awareness of this relatively common genetic disorder, A1AT deficiency remains underdiagnosed. Detailed recommendations for diagnostic testing for symptomatic individuals and asymptomatic individuals who are at increased risk for carrying deficiency alleles have been established (5). Clearly the clinical laboratory will continue to play a central role in the implementation of these recommendations and thus in the diagnosis of A1AT deficiency.

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References