Electrophoresis of Serum Protein to Detect $\alpha_1$-Antitrypsin Deficiency: Five Illustrative Cases

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We describe five cases of severe $\alpha_1$-antitrypsin (AAT) deficiency to illustrate the importance of visual inspection of electrophoretic patterns of serum proteins. In four patients the diagnosis of AAT deficiency was clinically unsuspected; in the other patient, the electrophoretic pattern was the first clue to confirm the diagnosis. Densitometric scanning of these patterns invariably overestimated the concentration of $\alpha_1$-globulin. By visually inspecting electrophoretic strips instead of relying on densitometry, clinical chemists can help detect AAT deficiency earlier.

Additional Keyphrases: $\alpha_1$-globulin  densitometry compared

$\alpha_1$-Antitrypsin (AAT), the major protease inhibitor in human serum, is synthesized by hepatocytes. The secretion rate of this glycoprotein into the bloodstream normally maintains its concentration in serum at about 1.5 to 2.0 g/L. In response to inflammatory processes, this concentration may rise considerably to counterbalance increased proteolytic activity at inflammation sites, thereby preventing tissue destruction. Although the M-type is by far the most common form of AAT in all populations studied, numerous genetic variants have been identified, some of which appear to be associated with the decrease in AAT concentrations in circulation. This decrease, which is pronounced for the Z-type phenotype and moderate for the S-type, is termed AAT deficiency (1–3). It varies remarkably in clinical manifestations, ranging from the absence of any noticeable damage to life-threatening illness, including severe neonatal hepatitis (eventually leading to cirrhosis in infants and children), early-onset lung emphysema, and cryptogenic cirrhosis in adults (4–6). The severity of the disease depends on the degree of AAT deficiency and other precipitating factors, the most notorious being cigarette smoke (5, 7, 8). Patients with AAT concentrations less than the threshold value of 0.7–0.8 g/L, i.e., primarily SZ heterozygotes or ZZ homozygotes, are generally considered at high risk for developing clinical manifestations. For SS, MZ, or MS phenotypes, on the other hand, AAT concentrations exceed 35% of normal, which seems to confer virtually the same degree of protection as for the “normal” MM phenotypes (1).

Given the tremendous impact of smoking on the life expectancy of persons at risk for developing lung emphysema, screening the general population for AAT deficiency might lead to the early establishment of effective preventive measures, such as stopping smoking or increasing the patient’s concentrations of AAT in serum—and hence in lung—by treatment with danazol or AAT replacement therapy (9, 10). In view of the rather low frequency of ZZ (1 to 3500) or SZ phenotypes (1 to 800) in U.S. Caucasians, as well as the expense of the methods of choice for determining AAT (immunochemical methods) and phenotyping (isoelectric focusing), some argue that mass-screening for AAT deficiency is unlikely to be cost-effective (1). However, because AAT normally represents more than 90% of the $\alpha_1$-globulin fraction in human serum, severe AAT deficiency can be detected by carefully scrutinizing the patterns produced by electrophoresis of serum proteins. The following five cases we report emphasize the role of the clinical pathologist in early detection of AAT deficiency, and illustrate the variability of the clinical manifestations of the disease.

Materials and Methods

For electrophoresis of serum proteins we used an Olympus Hite 200 apparatus (Olympus Optical Co., Hamburg, F.R.G.), with cellulose acetate and Ponceau S protein stain. Occasionally, we also examined results with the Beckman “Microzone” system (Beckman Instruments, Fullerton, CA) and a Beckman R 112 densitometer. We determined the concentrations of AAT with a Hyland laser-nephelometer and Hyland reagents (Hyland, Costa Mesa, CA). AAT phenotyping was by isoelectric focusing in polyacrylamide gels (11).

Case Histories

Patient 1

A 40-year-old woman admitted for the first time to our hospital for an acute attack of bronchial asthma had a longstanding history of recurrent asthmaticiform crises since childhood, with repeated hospitalizations. An allergic origin had been documented previously, and routine blood tests upon admission disclosed no unexpected findings. However, visual inspection of the patient’s serum proteins after electrophoresis revealed the almost complete absence of a distinct $\alpha_1$-globulin peak, despite a normal concentration of $\alpha_1$-antitrypsin (2.0 g/L) determined by densitometric scanning of the electrophoretic pattern (normal reference interval, 1.5–3.5 g/L). Profound AAT deficiency was confirmed by nephelometry, which demonstrated an AAT concentration <0.4 g/L (normal, 1.4–2.7 g/L). Chest roentgenogram and lung-function tests documented the presence of beginning emphysema at the lung bases.

Patient 2

A seven-week-old boy was hospitalized for prolonged icterus and hepatomegaly. Total serum bilirubin was 72 mg/L with a directly reacting bilirubin fraction of 52 mg/L. Liver enzyme activities were increased in serum: aspartate aminotransferase (EC 2.6.1.1) was 76 U/L (normal, 15–60), alanine aminotransferase (EC 2.6.1.2) was 87 U/L (5–28), alkaline phosphatase (EC 3.1.3.1) was 719 U/L (180–560) and $\gamma$-glutamyltransferase (GOT, EC 2.3.2.2) was 610 U/L (9–160). Bile-duct atresia was considered the most probable diagnosis until the almost complete absence of $\alpha_1$-globulin at visual examination of the protein electrophoresis pattern suggested AAT deficiency. This diagnosis was ultimately confirmed by nephelometry (AAT concentration, 0.5 g/L).
The $\alpha_1$-globulin concentration as estimated by densitometric scanning of the electrophoreogram, however, was 1.2 g/L. Microscopic examination of a liver-biopsy specimen revealed the presence of periodic acid-Schiff (PAS)-positive inclusions containing immunoreactive AAT. Isoelectric focusing established the presence of a SZ phenotype in the patient’s serum and in that of his mother, a heavy smoker, whereas his father and sister had MZ phenotypes.

Patient 3

A 76-year-old woman was transferred from a geriatric hospital because of cyanosis, polypnea, and fever. The abdominal examination revealed a tender right-upper quadrant. Concentrations of liver enzymes and serum bilirubin were normal except for the GGT activity of 56 U/L (normal, 5–29 U/L). Scintigraphic examination of the lungs showed multiple embolic sites and the patient was treated with heparin. An echographic exploration of the abdomen was suggestive for liver metastases. However, neither an abdominal computed tomodigraphy scan nor a liver scintigraphy supported this hypothesis. Although the $\alpha_1$-globulin concentration was estimated to be as much as 2 g/L by densitometric scanning, visual inspection of the electrophoretic strip indicated a very faint $\alpha_1$-globulin fraction, whereupon an AAT was ordered and a laparoscopy was performed. The AAT in serum was as low as 0.5 g/L, and the liver biopsy showed the presence of micronodular cirrhosis with PAS-positive hepatocellular inclusions, containing immunoreactive AAT. A ZZ phenotype was documented.

Patient 4

A 37-year-old woman was referred to the rheumatology clinic for further investigation after an ophthalmologic diagnosis of Sjögren’s syndrome. No physical or biological signs of collagen disease were present. A routine protein electrophoresis showed an almost complete absence of the $\alpha_1$-globulin peak. AAT concentrations in serum were as low as 0.4 g/L, although $\alpha_1$-globulin quantified by densitometry was estimated to be 1.5 g/L. So far, no early signs of the typical complications of AAT deficiency have been evident in this patient.

Patient 5

A 29-year-old man was referred to our hospital because of his progressively deteriorating renal function. During a routine examination 10 years earlier, an episode of hematuria and proteinuria associated with mildly impaired renal function had been observed. Since then, his renal insufficiency gradually worsened, especially during the two years preceding this admission.

The patient was pale and thin. His blood pressure was 160/130 mmHg, urea 1.65 g/L, creatinine 76 mg/L, potassium 5.0 mmol/L, calcium 89 mg/L, and phosphorus 69 mg/L. Echographic examination of the pelvic regions and intravenous urography showed bilaterally atrophied kidneys. In the following months his chronic renal insufficiency grew considerably worse and he was treated by hemodialysis. Visual inspection revealed the absence of a distinct $\alpha_1$-globulin peak, which had gone unnoticed despite several previous instances of electrophoresis of his serum proteins, performed in other laboratories with results for $\alpha_1$-globulin ranging between 2 and 3% of total protein. The AAT concentration was 0.5 g/L, and he was identified as having SZ phenotype.

Discussion

Because AAT normally accounts for at least 90% of the $\alpha_1$-globulin fraction in serum, a pronounced decrease in circulating AAT should be revealed by a greatly reduced $\alpha_1$-globulin fraction. Indeed, the first description of AAT deficiency was based on the visual observation of an "empty" $\alpha_1$-globulin zone (12). Because of their much greater sensitivity and specificity, immunological techniques have since emerged as the methods of choice for detecting AAT deficiency, and visual inspection of the electrophoretic strip has largely been replaced by automated densitometric scanning (13). However, as illustrated above, the latter procedure tends to overestimate $\alpha_1$-globulin concentrations. This relative insensitivity seems method-related rather than instrument-related, for we observed it with several densitometric systems. The fact that the AAT deficiency of patient 5 had gone unnoticed despite repeated electrophoretic analyses in another laboratory further supports this view. Consequently, many cases of profound AAT deficiency might escape early diagnosis in the absence of a general population screening of the disease. This report therefore represents a plea for the re-emphasis of the systematic visual inspection of every electrophoreogram of serum proteins. Even with the limitation that low concentrations of AAT might be obscured by increases in other $\alpha_1$-globulin fractions, such a strategy should still improve detection of affected individuals. A more efficient detection of monoclonal gammopathies might also result from such visual inspection.

The above case reports illustrate the remarkable variability in the clinical manifestations of the disease. A result of this variability is that the clinical diagnosis may easily be overlooked. However, given that much of the population of developed countries sooner or later undergoes blood tests at the hospital or the physician's office, often with requests for protein fractionation, systematic visual inspection of all serum protein profiles may contribute to an earlier diagnosis of AAT deficiency—as exemplified by the cases of patients 1, 3, 4, and 5, who consulted for symptoms apparently unrelated to that disease. The near absence of $\alpha_1$-globulins in serum of patient 2 oriented the diagnosis toward AAT deficiency and helped to rule out biliary atresia as a possible cause for the prolonged jaundice. In view of the patient's age, such rapid differentiation was mandatory, surgical management of biliary atresia being of little value after the age of three months (14). Moreover, affected relatives of patient 2 were identified and consequently may benefit from preventive measures and genetic counseling.

Early diagnosis of severe AAT deficiency not only initiates active avoidance of lung tissue damage, thereby improving the patient's life expectancy, but also facilitates prospective studies aiming at identifying factors that protect against or induce tissue damage in populations at high risk for destructive lesions. For example, it is interesting that patient 3 showed no evidence of emphysema, in spite of her advanced age, a very low concentration of AAT, and the presence of a PZZ phenotype; hence, even severe AAT deficiency does not necessarily provoke lung damage (15). Why certain individuals with severe AAT deficiency develop lung lesions exclusively (see patient 1), whereas others display specific hepatic involvement (see patient 3), is another unsolved problem. Although lung and liver lesions are not mutually exclusive, as was initially believed, it is nevertheless intriguing why they are rarely associated (16).

The fact that moderately deficient heterozygous MZ (17) and SZ phenotypes, such as patient 2, can display hepatitis or cirrhosis, whereas the very rare so-called Pi-null phenotype (18, 19)—characterized by complete absence of AAT—are not at risk supports the suggestion that sequestration of Z-type AAT within hepatocytes is involved.

In conclusion, careful examination of electrophoretic profiles of serum profiles provides us with a simple and cost-free
tool to improve the detection of AAT deficiency, thereby contributing to a more effective treatment and a better knowledge of the disease.

References