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STANFORD MULTIDISCIPLINARY SEMINARS

Misdiagnosis of Alpha-1 Antitrypsin Phenotype in an Infant with CMV Infection and Liver Failure

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Case Presentation and Evolution

A 4-month-old female with a history of neonatal cholestasis was initially evaluated with jaundice, ascites, coagulopathy, and worsening aminotransferase levels.

At 5 weeks of age, her parents had noticed "yellowing" of her eyes and skin accompanied by a slightly more pale appearance of her regularly yellow-colored stools. Initially, she was noted to have conjugated hyperbilirubinemia, with total bilirubin 6.5 and conjugated 4.5, aspartate aminotransferase (AST) 163, alanine aminotransferase (ALT) 103, and elevated alkaline phosphatase. Liver ultrasound was normal. A percutaneous liver biopsy, obtained due to her continued unexplained cholestasis, was reported as showing possible large duct obstruction and bile duct proliferation, suggestive of extrahepatic biliary atresia. A subsequent endoscopic retrograde cholangiopancreatography (ERCP) and intraoperative cholangiogram showed no anatomic abnormalities in the intrahepatic and extra hepatic biliary system, inconsistent with the diagnosis of biliary atresia (Fig. 1).

During her hospital admission, a serum alpha-1 antitrypsin (A1AT) concentration was 34 mg/dl (normal 100–250 mg/dl), with A1AT MZ phenotype reported. Her conjugated hyperbilirubinemia improved slightly, and her liver numbers stabilized. She was discharged home with a close follow-up outpatient appointment in the pediatric gastroenterology clinic. The remainder of her evaluation for neonatal cholestasis, including an echocardiogram and ophthalmology examination for Alagille's syndrome, was negative. Infectious studies were negative as well. Hepatosplenomegaly or ascites were not detected by physical examination, with reassuring and age-appropriate biometrics noted. Ongoing outpatient medical care revealed elevated cytomegalovirus (CMV) IgM titers (0.97). Due to persistent cholestasis, a repeat A1AT serum concentration was low level at 32 mg/dl, although a repeat ELISA was now reported as the Z phenotype consistent with the ZZ genotype. Due to conflicting tests of mutant serum A1AT proteins, her liver biopsy was re-analyzed with intrahepatic positive periodic acid–schiff stain (PAS) granules now reported, consistent with the diagnosis of A1AT deficiency (Fig. 2). Concomitantly, CMV viremia was confirmed with an elevated CMV polymerase chain reaction (PCR) assay of 12,800.

In the clinic, she exhibited significantly increased abdominal distension and marked ascites. Her bilirubin (total 8.8 mg/dl; conjugated 5.3 mg/dl) and aminotransferases (AST/ALT 500/309 units/l) were elevated. Additionally, a significant coagulopathy was noted with an international normalized ratio (INR) of 2.2 and low fibrinogen of 88. Hyponatremia (119 mg/dl) and metabolic acidosis were also present. She did not have any changes in her mental status, and her venous ammonia concentration was mildly elevated to 68 μmol/l. She was admitted to Lucile Packard Children's Hospital at Stanford for correction of her electrolyte abnormalities and coagulopathy. She was treated with ganciclovir for CMV viremia.

During her first 48 h in the hospital, despite the use of IV phytonadione, her hepatic synthetic liver function deteriorated rapidly, with daily doses of fresh-frozen plasma required to treat her coagulopathy. Due to her

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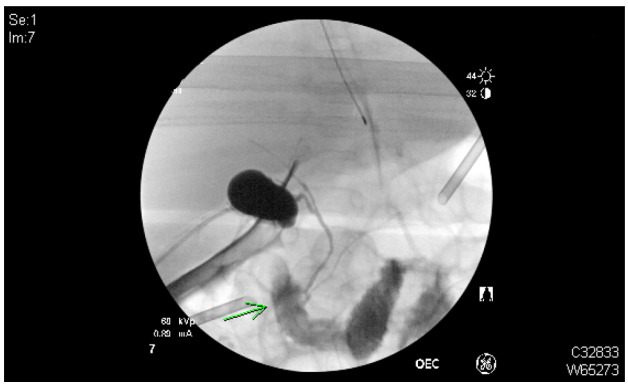

Fig. 1 Intraoperative cholangiogram that shows normal extrahepatic biliary system after injection of contrast (*arrow*)

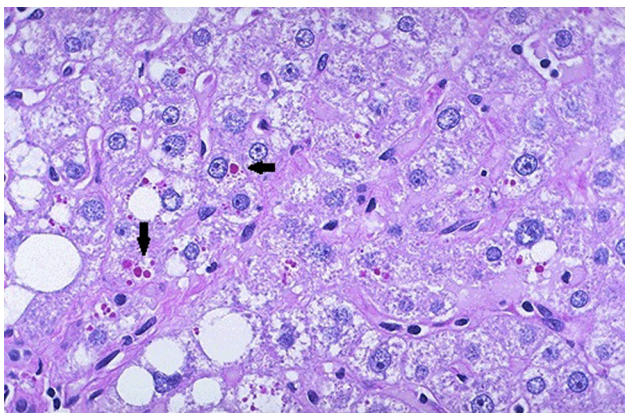

Fig. 2 Abnormal alpha-1 antitrypsin protein accumulates as PAS-positive diastase-resistant globules within hepatocytes

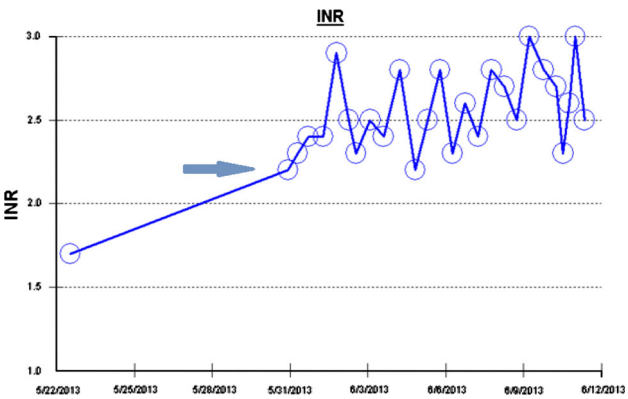

Fig. 3 Since the first day of her second admission, progressive rise in the patient's INR was noted despite daily infusions of fresh-frozen plasma (*arrow* corresponds to the first day of second admission)

worsening conjugated hyperbilirubinemia, coagulopathy, and rising ammonia levels, she was evaluated for a liver transplant for hepatic disease due to A1AT deficiency compounded by CMV viremia.

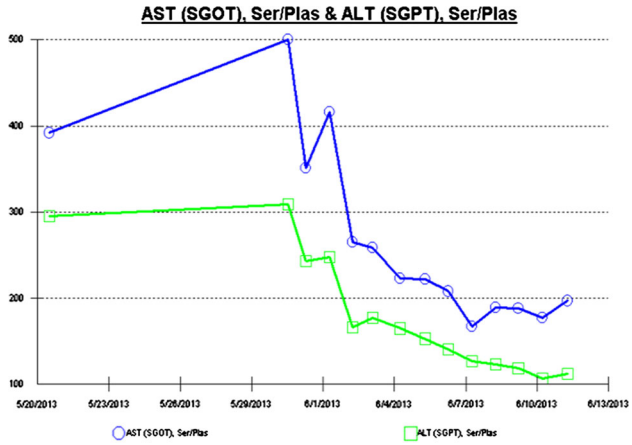

Fig. 4 Trends in AST and ALT with the patient's progression of her liver failure. Normalization of AST/ALT in the context of coagulopathy, hyperammonemia, and worsening clinical status represent hepatocyte "burnout" secondary to irreversible liver injury

The patient was transferred to the intensive care unit (ICU) due to worsening ascites, severe coagulopathy (INR 2.9), and rising ammonia levels with a change in her mental status (Figs. 3 and 4). Rifaximin and lactulose were used in alternate dosing to treat hepatic encephalopathy, with ammonia concentrations now exceeding >100 μmol/l.

Her CMV PCR count responded very well to the use of ganciclovir, falling to 806; however, her synthetic liver function remained abnormal with a continuous infusion of fresh-frozen plasma required to treat her worsening coagulopathy. Based on her persistent liver synthetic dysfunction despite supportive measures and treatment of her CMV viremia, ganciclovir was discontinued due to worsening leucopenia. After several days in the ICU, CMV PCR showed an increased 10,800 copies, for which she received two doses of CytoGam followed by re-starting ganciclovir. At this point, her liver injury was considered irreversible, prompting listing her for urgent liver transplantation. In the ICU, her ammonia concentrations remained elevated despite oral medications. She was intubated and started on continuous venous–venous hemodialysis (CVVH). She received a successful left lateral segment hepatic transplant at 5 months of age.

The explant obtained at the time of the liver transplantation showed a liver that was grossly diffusely nodular. Variable trichrome staining in the septa between the nodules suggested early bridging fibrosis. PAS stain highlighted fine granules within hepatocytes at the periphery of the regenerative nodules, consistent with her diagnosis of A1AT deficiency. Her immediate post-transplant course was unremarkable; ganciclovir was continued for CMV viremia with basiliximab, prednisone, and tacrolimus given for immunosuppression. After 49 days in the hospital, she was discharged home to the care of her parents on a regular diet and oral tacrolimus/prednisone for immunosuppression.

Discussion

A1AT deficiency, the most frequent genetic disorder causing liver failure [1], is caused by mutations in the *SERPINA1* gene, which encodes the A1AT enzyme, responsible for degrading neutrophil elastase, a serine protease that is activated in inflammation [1, 2]. Patients with A1AT deficiency have abnormal A1AT protein structure, impairing its release from the hepatocyte endoplasmic reticulum (ER). Abnormal accumulation of A1AT in the liver activates mitochondrial and ER caspases, potentiating hepatocyte destruction [1–3].

A1AT deficiency was first described in 1962 at the University of Lund, Sweden, by Sten Eriksson and Carl-Bertil Laurell [1, 4]. They noticed the absence of the α_1 band in the protein electrophoresis in 5/1,500 serum samples, although it was not until 1969 that Dr. Harvey Sharp from the University of Michigan described liver involvement in these patients [4]. At least 100 alleles of A1AT have been assigned a letter code based upon electrophoretic mobility [1, 5]. Normal alleles, termed "M", are associated with normal serum concentrations of A1AT and normal phenotype, with the normal genotype termed "MM". Mutant alleles are associated with serum A1AT concentrations <100. The most common mutant alleles are termed "Z" and "S" [1, 5, 6], with the homozygous ZZ genotype detected in 1/1,800–1/2,000 live births [1, 6]. A Swedish national prospective screening study conducted during the early 1970s identified more than 180 infants with the ZZ or SZ genotypes. Over a 30-year observation period, only 8–10 % of ZZ homozygotes and SZ heterozygotes developed clinically significant liver disease [4, 7]. Patients with undetectable serum A1AT activity bearing the ZZ genotype had a statistically significant increase in the incidence of cirrhosis and hepatocellular carcinoma [7].

Diagnosis of A1AT deficiency is a two-step process. Serum concentrations of A1AT are measured to detect a quantitative deficiency, and A1AT is characterized by phenotype or genotype to identify the cause of the low A1AT concentration [1, 3]. Identification of the specific A1AT alleles confirms the genetic deficiency and provides information as to whether the patient is at risk of liver damage in addition to lung manifestations [3, 7]. A PCR-based assay is used to detect the Z and S alleles within the alpha-1 antitrypsin SERPINA1 gene [1, 8]. Serum levels are measured by immunonephelometry and reported with the genotyping result [1, 8]. Protein replacement therapy, the presence of other rare variants or the presence of DNA polymorphisms, can yield false-negative and false-positive results [2, 6, 9].

In patients with A1AT-deficiency-related liver disease, environmental and/or other genetic factors inform disease severity and progression. The most common initial clinical manifestation of infants with A1AT deficiency and liver disease is poor feeding, irritability, lethargy, and jaundice [1, 4, 9].

There is no specific therapy for A1AT-deficiency-associated liver disease [1, 9, 10]. Supportive management of symptoms resulting from liver dysfunction and the prevention of complications that are generic to all chronic and acute liver diseases represents the initial treatment strategy. If the patient's clinical course leads to liver failure, orthotopic liver transplantation remains the only alternative, with survival rates well over 90 % at 1 year and 80 % at 5 years [9, 10].

Our patient was initially misdiagnosed with an incorrect A1AT genotype and developed acute liver failure after CMV infection injured her liver. In this age group, neonatal hepatitis secondary to viral etiologies (CMV) is one of the most common reasons for increased serum aminotransferases [8]. Clinically significant liver disease develops in only 10–15 % of A1AT-deficient children with a homozygous ZZ genotype [1]. In our patient, CMV viremia led to rapid clinical deterioration and irreversible liver injury. This case is also unique due to the misdiagnosis of the A1AT genotype MZ, which was actually the homozygous ZZ genotype. Although the liver biopsy was initially interpreted as not being consistent with A1AT deficiency, the second review, interpreted with knowledge of the correct genotype, yielded the correct diagnosis with PAS-positive granules identified in her hepatocytes. This pathologic finding combined with the persistently low A1AT serum concentration of 38 and ZZ phenotype conclusively confirmed the diagnosis of A1AT deficiency, manifesting as progressive failure, which was appropriately treated medically and subsequently with hepatic transplantation.

In summary, the repeat laboratory testing for A1AT genotype was the pivotal diagnostic intervention that facilitated her being diagnosed correctly and treated appropriately.

Key Points

- Infantile cholestasis with aminotransferase elevation requires a careful systematic clinical evaluation, including considerations of obstructive, infectious, and metabolic etiologies.
- A missed A1AT diagnosis based on incorrect A1AT serum protein identification is a rare but clinically important consideration. In certain cases, repeating A1AT level, genotype, and re-interpreting histopathologic analysis is warranted.
- In the neonatal age group, infections leading to neonatal hepatitis can be common and, in susceptible patients, can develop into irreversible acute liver failure.

- When a patient with A1AT deficiency initially manifests with acute liver failure, a second etiology should be investigated, such as infections, medications, or metabolic disease.

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