Implication of liver enzymes in viral and alcoholic liver diseases

Seung Ha Park, M.D.

Department of Internal Medicine, Inje University Haeundae Paik-Hospital, Inje University College of Medicine, Busan, Korea

Introduction

Hepatocyte injury is commonly encountered in the practice of medicine, and recognition of chronic hepatic injury has increased. Liver disease is often clinically silent until late in its course. For this reason, liver enzyme tests are usually needed for the recognition and characterization of the type of liver injury present. The most common cause of liver injury worldwide is infection with viruses that primarily infect the liver, often termed hepatitis viruses. Serologic and nucleic acid based tests are required to document exposure to and presence of these viruses, and are also used to monitor treatment of infected individuals. Exposure to ethanol and other drugs can also cause hepatic injury; clinical information is the most reliable means to recognize these potential causes of liver damage. Accordingly, physicians caring for patients with liver disease have long been aware that measurements of liver enzyme activities (serum aminotransferases, including alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) are critical in the diagnosis and assessment of liver disease. This article reviews the physiology of aminotransferases and their role as diagnostic and prognostic tests in viral and alcoholic liver diseases. In particular, a promising immunoassay for ALT measurement determining the extent of liver disease severity is addressed.

Physiology of aminotransferases

AST and ALT, sometimes termed SGOT and ALT respectively, are widely distributed in cells throughout the body. AST is found primarily in heart, liver, skeletal muscle and kidney. ALT is responsible for the reverse transamination between alanine and α-ketoglutarate to form pyruvate and L-glutamate, participating in cellular nitrogen metabolism and in liver gluconeogenesis. It is found primarily in liver and kidney, with less amount in heart and skeletal muscle. AST and ALT activity in liver are about 7,000 and 3,000 times serum activities, respectively. ALT is exclusively cytoplasmic, whereas AST has both mitochondrial and cytoplasmic forms in all cells. The half-life of total AST is 17 ± 5 hours, while that of ALT is 47 ± 10 hours. The half-life of mitochondrial AST averages 87 hours. Accordingly, in acute hepatocellular injury, serum AST levels usually rise immediately, reaching higher level than ALT initially, due to the higher activity of AST in hepatocytes and its release with liver injury. Within 24 to 48 hours, particularly if ongoing damage occurs, ALT will become higher than AST, because of its longer plasma half-life. In adults, AST and ALT activities are significantly higher in males than in females, and reference intervals vary with age. Unexpectedly abnormal results are often normal on repeat testing. Liver disease is the most important
cause of increased ALT activity and a common cause of increased AST activity. However, a number of factors other than liver disease affect AST and ALT activities; body mass index, exercise, specimen storage (stable for years with freezing in AST; marked decrease with freezing/thawing in ALT), hemolysis, and muscle injury.

**Aminotransferases activities in acute hepatic injury**

Acute hepatic injury can be recognized by the presence of jaundice or non-specific symptoms of acute illness accompanied by elevation of AST and/or ALT activities. Serum AST levels usually rise immediately, reaching a higher level than ALT initially, due to the higher activity of AST in hepatocytes and its release with liver injury. Within 24 to 48 hours, particularly if ongoing damage occurs, ALT will become higher than AST, because of its longer plasma half-life. The best discriminant values for recognizing acute hepatic injury appear to be 200 U/L for AST (sensitivity 91%, specificity 95%) and 300 U/L for ALT (sensitivity 96%, specificity 94%). In uncomplicated alcoholic hepatitis, AST and ALT values are almost never over 10× the upper reference limit, while AST/ALT ratio is over 2 in 80%. Other liver diseases are sometimes encountered as the causes of acute increase of AST or ALT activities; in a recent study, 25% of those with AST more than ten times the upper reference limit turned out to have obstruction. Rarely, acute hepatic injury causes severe liver damage and acute liver failure. Aminotransferase activities are more related to the cause of hepatic injury, rather than to its severity. Slight elevations in AST (< 10× the upper reference limit), particularly with AST/ALT ratio > 1, suggest acute alcoholic hepatitis, while marked elevations (> 100× the upper reference limit) with early AST/ALT ratio > 1 strongly suggest ischemic or toxic liver injury. In those with intermediate values with AST/ALT ratio < 1, an acute viral hepatitis is suspected. Peak aminotransferase activities bear no relationship to prognosis, and may fall with worsening of the patient’s condition; in all causes of hepatic injury, aminotransferase activities begin to fall before bilirubin reaches its peak regardless of recovery or deterioration.

**Aminotransferases activities in alcoholic liver diseases**

In most types of liver disease, ALT activity is higher than that of AST. However, in alcoholic liver injury, AST activity is characteristically elevated in comparison to ALT activity, although mild elevation of ALT level is common. The reasons for the higher AST activity in alcoholic hepatitis appear to be multiple: 1) Alcohol increases mitochondrial AST activity in plasma, while other forms of hepatitis do not; 2) Pyridoxine deficiency common observed in alcoholics, which is a cofactor for the enzymatic activity of ALT, decreases hepatic ALT activity; 3) Alcohol induces the release of mitochondrial AST, which has longer half-life, from cells without visible cell damage.

**Aminotransferases activities in advanced fibrosis**

In chronic hepatocellular injury including viral liver disease, ALT is more commonly elevated than AST; however, as fibrosis progresses, ALT activities typically decline, and the ratio of AST to ALT gradually increases, so that by the time cirrhosis is present, AST is often higher than ALT. Although the reason for this finding is still not clear, there are some plausible explanations for increased AST/ALT ratio in process of fibrosis. This appears to be due to the reduction of ALT
production in damaged liver. The half-life of mitochondrial AST released into circulation by progressive damage to mitochondria is the longest. Because intrahepatic shunting is a common phenomenon in cirrhosis and clearance of AST is primarily performed within the liver by sinusoidal cells, shunting of functional liver blood flow may be responsible for the increase in the AST/ALT ratio as liver disease progresses.

**ALT activities for prediction of histological severity in viral liver disease**

The major significance of viral liver disease is its possible progression to liver-related complications such as cirrhosis or liver cancer. Chronic hepatitis has two major components: inflammatory damage and fibrosis. While the extent of inflammation reflects the degree of damage at that point in time, the extent of fibrosis more closely relates to the likelihood of developing liver-related morbidity and mortality including cirrhosis and liver cancer. Plasma activities of aminotransferases are not related to the degree of fibrosis, and there is at best a weak correlation between plasma ALT activity and histological activity. Thus, it is noteworthy that ALT activity is not suitable for determining the severity of liver disease, despite of the elevated ALT activity as an indicator of liver disease. Although the reasons for the lack of correlation between liver histopathology and ALT activities remain unclear, one explanation may be that the latter is shaped by dynamic events such as immunologic activity, frequency and intensity of exacerbations, and the total duration of disease. Hence, ALT activity, which is often performed on a limited number of samples, is likely to give misleading information about overall disease intensity in a chronic and dynamically changing condition like chronic viral liver disease.

**Fibrosis prediction models including aminotransferases activities**

It is well known that liver biopsy, as the gold standard for assessing histological severity in liver disease, has substantial limitations (complications, cost, and sampling error). ALT activity alone is unsatisfactory to provide comparable information with respect to the level of liver disease activity. Because of this limitation of ALT activity and as a part of ongoing efforts to improve predictive accuracy toward histological disease severity, ALT or AST activities as well as routine laboratory data such as age, other liver enzyme, and platelet count have been incorporated into algorithms for fibrosis assessment in viral liver disease, AST/ALT ratio, cirrhosis discriminant score, AST-to-platelet ratio index. Although all of these models were reported to predict the presence of significant fibrosis or cirrhosis with considerable accuracy, substantial improvements in the understanding of the molecular mechanisms in fibrosis have accelerated the enthusiasm to evaluate direct serum assays reflecting dynamic turnover, thereby the clinical and translational implications of these advances hold some promise as simpler alternatives to liver biopsy. These newer biomarker panels have included markers of inflammatory response modifiers such as α2-macroglobulin and haptoglobin, synthetic ability of the liver such as apolipoprotein A1, and/or the actual process of fibrosis such as hyaluronate, procollagen peptides, and matrix metalloproteinases and their inhibitors.

However, new biomarkers do not always mean that they are more accurate than routine liver enzyme test. Recently a few studies explicitly addressed the added value of new biomarkers over a model consisting of liver enzyme. In a study of patients with chronic hepatitis B, the simultaneous addition of several new biomarkers adds only modestly to the overall prediction for fibrosis based on simple Age-AST model, by about 2% in the ROC area. The lack of added value of new biomarkers over
Age-AST model is also true to predict advanced fibrosis in chronic hepatitis B virus-infected patients with high viral load and normal or minimally raised ALT,\textsuperscript{21} in whom antiviral therapy is not indicated on current guidelines. In addition, none of the new biomarkers studied had value in addition to Cirrhosis Score formulated using platelet count and $\gamma$-glutamyl transpeptidase for differentiating cirrhosis from chronic hepatitis B.\textsuperscript{22} Likewise in chronic hepatitis B, the use of contemporary biomarkers adds only moderately to the AST-to-platelet ratio index for risk assessment of significant fibrosis in patients with chronic hepatitis C (Park SH, unpublished observations).

To sum up, these observations highlight the importance of evaluating putative biomarkers with the use of explicit quantitative assessments to measure the ability to classify risk. The future success of biomarker strategies may depend on the discovery of new biomarkers to complement the readily available, existing ones such as AST-to-platelet ratio index, perhaps with the help of new, unbiased approaches.

**ALT activities for prediction of long-term outcome in viral liver disease**

The predictive value of ALT activity for long-term outcome including the development of cirrhosis or liver cancer and liver-related mortality is major importance to clinicians to predict morbidity and to judge the criteria for treatment. Since the test for hepatitis C virus was developed in 1989, it is not ethically justified to select treatment-naive patients and include them in a prospective study without providing therapy (which is currently very effective; in fact, antiviral therapy affects the progression of hepatic damage, and liver-related mortality is reduced in patients achieving eradication of hepatitis C virus infection even if the disease is advanced, but compensated.

The first landmark population-based study was from the REVEAL-HBV Study Group in Taiwan which prospectively followed 3,653 HBsAg positive subjects from 1991-1992. They found a relative risk of cirrhosis of 1.6 in those who had ALT activity $\geq$ 45 IU/L compared with those who had ALT activity $<$ 45 IU/L\textsuperscript{23} and a 2.3 times higher liver disease-related mortality in individuals with ALT activity $\geq$ 45 IU/L compared with those with ALT activity $<$ 45 IU/L during a mean follow-up period of 12.5 years.\textsuperscript{24} There were biological gradients of overall cumulative lifetime incidences of liver cancer (67.2% in ALT $\geq$ 45 IU/L, 24.6% in 15 IU/L $\leq$ ALT $<$ 45 IU/L, 10.9% in ALT $<$ 15 IU/L, respectively) and cirrhosis (70% in ALT $\geq$ 45 IU/L, 41.9% in 15 IU/L $\leq$ ALT $<$ 45 IU/L, 28% in ALT $<$ 15 IU/L, respectively) across ALT levels.\textsuperscript{25} Although the REVEAL study is an important study, it has certain limitations. As a community study the patients have less severe disease than a hospital clinical population in whom decisions about treatment are made. For example, of the 3,653 HBsAg-positive subjects in the REVEAL study, only 6% had an elevated ALT and only 2% had cirrhosis. They also represented an older population (30-65 years) and 85% were HBeAg negative. 80% formed a sub-cohort negative for HBeAg, had a normal ALT, and no cirrhosis. But it is unlikely that a study larger than the REVEAL study will ever be carried out.

In a further hospital-based study of 4,376 HBeAg-negative hepatitis B surface antigen carrier from Taiwan, the cumulative liver-related mortality was significantly higher in patients with maximal ALT of 2 times upper limit of normal, compared with patients with maximal ALT less than 1 time or 1 to 2 times upper limit of normal during a mean follow-up period of 13.4 years.\textsuperscript{26} Nearly half the patients with normal baseline ALT became abnormal during the study and patients with persistently normal ALT had an excellent long-term prognosis, regardless of their virus replication level, highlighting that serial ALT measurements over a long period may provide more prognostic information than a single time measurement at entry or during...
Taken together, it can be speculated that ALT activity is a good prognostic tool for chronic hepatitis B, especially in HBeAg negative Asian patients.

**Immunooassay for ALT**

There is little correlation between serum ALT activity and hepatic injury assessed through biopsy, which might be explained by ALT activity measured using a spectrophotometric assay, in which the transamination reaction is coupled to a second reaction that reduces pyruvate to lactate via lactate dehydrogenase and NADH. Although this assay quantifies the ALT activity, ALT activity may be altered by various modification processes such as proteolysis, protein modification, or antibody against ALT within the circulation following release from liver tissue, which are common for serum and tissue proteins, and several factors involved in protein modifications are well known to reduce enzymatic activity. The altered activity of ALT does not necessarily reflect the amount of ALT released from liver. Thus, spectrophotometric assay cannot determine the exact amount of serum ALT, usually ending up in underestimation of liver function.

However, an immunologic approach could surmount this problem. Recently, Korean pioneering researchers developed a sandwich immunoassay that uses murine monoclonal antibodies generated against human recombinant ALT1 protein to quantify serum ALT mass concentration. They found that patients with cirrhosis displayed a pattern of high mass/low activity, suggesting that a considerable amount of immunologically active but catalytically inactive ALT enzyme exists in the sera of cirrhosis patients. Furthermore, ALT mass concentration had a tendency to increase as liver disease progressed, whereas ALT activity decreases. These results show that ALT immunoassay deserves a further validation study to elucidate its usefulness in clinical practice. Another potential of immunoassay comes from the stable measurement of ALT mass compared to unstandardized ALT activity measurement across-and within-laboratories.

**References**


