Persistent Increase in Aspartate Aminotransferase in an Asymptomatic Patient

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CASE

A 66-year-old woman without a preexisting history of liver disease presented with chest discomfort and dyspnea. Laboratory studies revealed an isolated increase in aspartate aminotransferase (AST)3 that prompted consultation with a hepatologist. The patient was a retired schoolteacher and took no medications. She affirmed alcohol use estimated at <2 oz of spirits daily.

A physical examination revealed a healthy-appearing woman with no obvious abnormalities. The sclerae were anicteric. The abdomen was soft, flat, and without palpable organomegaly. There was no edema.

Laboratory studies revealed the following: AST, 544 U/L (reference interval, 11–47 U/L); albumin, 38 g/L (36–50 g/L); alkaline phosphatase (ALP), 95 U/L (38–126 U/L); total bilirubin, 4.0 mg/L (3.0–11 mg/L); direct bilirubin, 2.0 mg/L (0.0–3.0 mg/L); γ-glutamyltransferase (GGT), 25 U/L (11–50 U/L); lactate dehydrogenase (LDH), 373 U/L (100–250 U/L); hemoglobin, 139 g/L (121–151 g/L); reticulocytes, 0.008 (0.005–0.015); haptoglobin, 0.97 g/L (0.27–2.20 g/L); thyroid-stimulating hormone, 3.60 mIU/L (0.35–4.60 mIU/L); antinuclear antibodies, reactive at 1:80 (negative); antimitochondrial antibodies, negative; α1-antitrypsin, 1.67 g/L (0.7–2.1 g/L); ferritin, 202 mg/L (10–291 mg/L); ceruloplasmin, 380 mg/L (180–460 mg/L); hepatitis B surface antigen, nonreactive; anti–hepatitis C virus, nonreactive; aldolase, 4.5 U/L (<8.0 U/L); and creatine kinase (CK), 116 U/L (38–234 U/L). The results of radiographic studies, including abdominal and chest computed tomography scans were unremarkable. Given the isolated increase in AST without signs or symptoms of liver disease, the patient was advised to discontinue alcohol consumption, and the clinical laboratory was contacted for additional studies.

DISCUSSION

INCREASES IN THE RESULTS OF LIVER FUNCTION TESTS

Tests for liver “function” consist of those for aminotransferases (ALT and AST), bilirubin, ALP, LDH, GGT, albumin, and prothrombin time (1). Of these tests, only albumin, bilirubin, and prothrombin time truly assess hepatic function. For other functions of the liver, such as drug metabolism, nutrient storage, intermediary metabolism, and bile production, there are few tests available. Proper tests of hepatic function usually entail administration of a drug, dye, or a carbohydrate such as galactose and measurement of metabolites or clearance rates. The other traditional liver “function” tests are in reality markers of liver cell damage or death and not of functional capacity. Because of the large reserve capacity of the liver, the results of true tests of liver function such as albumin and prothrombin time can be nonpathologic when markers of hepatic cell damage are increased.

ALT, GGT, and 5′-nucleotidase are most useful as markers of cholestatic liver injury. All 3 of these enzymes are glycosylphosphatidylinositol-anchored membrane proteins. The aminotransferases and LDH show the greatest magnitude of increase in hepatitis because these enzymes are released into the circulation when hepatic cells become damaged or die. ALT and AST are present in high concentrations in the cytoplasm of the liver, kidney, and myocardial and skeletal muscle and occur in other organs at lower activities. There is also a mitochondrial form of AST. The biochemical function of aminotransferases is to transfer an amino group from an α-amino acid to an α-ketoacid with pyridoxal phosphate (vitamin B6) as a cofactor. This reaction is an important step in intermediary metabolism. Laboratory methods for aminotransferases should be supplemented with pyridoxal phosphate to avoid falsely decreased activities in samples obtained from malnourished individuals with low endogenous vitamin B6 concentrations. The activities of AST in the liver, kidney, heart, and skeletal muscle are 7000-, 4500-, 8000-, and 5000-fold higher, respec-
isolated AST increase. AST (macro-AST) was considered as the cause of the increased AST. A macroenzyme form of the aminotransferase was not malnourished. A macroenzyme form of the AST method did not increase. This was not the case in our laboratory, and, furthermore, the patient was not malnourished. A macroenzyme form of AST (macro-AST) was considered as the cause of the isolated AST increase.

Nonliver causes for increases in AST include damage to cardiac or skeletal muscle cells and hemolysis. Indeed, before the advent of assays for CK isoenzyme MB, increases in AST and CK were sensitive but nonspecific markers for myocardial infarction. Muscle diseases that cause myocyte damage (such as muscular dystrophies, polymyositis, and rhabdomyolysis) and muscle trauma all cause much greater increases in AST than in ALT because of the higher relative activity of AST in skeletal muscle. CK, aldolase, and myoglobin are more sensitive markers of skeletal muscle damage. The fact that the patient did not have an increased CK or aldolase activity excluded skeletal muscle as the source of the increased AST. Although this patient had a modest increase in LDH, her nonpathologic values for hemoglobin, reticulocytes, haptoglobin, and total and indirect bilirubin are inconsistent with hemolysis as the cause of the increased AST.

Causes of hepatitis are many but include viruses (e.g., hepatitis A, B, and C), toxins (e.g., acetaminophen), alcohol, ischemia, Reye syndrome, and autoimmune diseases. The aminotransferases can often be increased by as much as 50 times the upper reference limit in acute viral, ischemic, and toxic hepatitis, whereas in alcoholic hepatitis the increases are generally <10-fold. The usually higher value for ALT than for AST is most likely due to the exclusively cytoplasmic distribution of ALT and its longer half-life in the blood (approximately 50 h) than for AST (approximately 16 h). The exception is alcoholic liver disease, in which the AST/ALT ratio is often >2. Regardless of cause, chronic hepatitis is characterized by milder—and fluctuating—increases in the aminotransferases.

Other hepatic causes for increases in aminotransferases include hemochromatosis, nonalcoholic fatty liver disease, and Wilson disease. Regardless of the pathology causing the damage to hepatic cells, both aminotransferases are usually increased, which was not the case in this patient. If liver disease was the cause of her AST increase, the only possible explanation could be that the AST method included supplemented pyridoxal phosphate and the ALT method did not. This was not the case in our laboratory, and, furthermore, the patient was not malnourished. A macroenzyme form of AST (macro-AST) was considered as the cause of the isolated AST increase.

MACROENZYMES

Macroenzymes are usually due to the formation of an autoantibody–enzyme complex, which has a higher molecular mass and a delayed clearance that leads to an increase in the amount of circulating enzyme (3). Binding of enzymes to substances such as hydroxyethyl starch from intravenous fluids, lipid aggregates, and α2-macroglobulins has also been described (3). Macroenzymes have been reported for amylase, CK, ALP, AST, GGT, LDH, and lipase (4, 5). The frequency of macroenzymes remains uncertain. Previous publications have calculated an incidence of macroamylasemia of 0.98% in patients with typical amylase concentrations and 2.56% in those with hyperamylasemia. Macro-LDH has been estimated to occur in <1 in 10 000 people (4). Macroenzymes are reported less frequently in children and adolescents, with 13 cases of macro-AST having previously been reported in this age group (3).

Although macroenzymes have been reported to be associated with autoimmune disorders, including rheumatoid arthritis, systemic lupus erythematosus, ankylosing spondylitis, cryoglobulinemia, and inflammatory bowel disease, there is no convincing evidence of antienzyme antibodies causing disease (5–8), although some antienzyme antibodies (such as antithyroid peroxidase and anti-LKM1) are considered markers of autoimmune diseases. Interestingly, our patient did have a reactive antinuclear antibody. AST–IgA complexes in adult patients have been reported to be associated with hepatologic malignancies or chronic liver disease (7). In a study of 128 patients with liver disease, AST–IgA complexes were found in 41.8% of patients with chronic hepatitis, 62.2% of liver cirrhosis patients, 90% of hepatocellular carcinoma patients, and 66.7% of patients with alcoholic liver disease (9). Although macroenzymes are generally not considered pathologic, the persistently increased enzyme values can lead to multiple invasive and/or expensive diagnostic tests.

Several methods for detecting macroenzymes have been described, including electrophoresis, differential precipitation with polyethylene glycol or ammonium sulfate, measurements of heat stability, and gel filtration chromatography (10). To investigate this patient, we used a simple and fast way to establish the presence of macroenzymes: removing immunoglobulin from the serum with protein A or protein G.

LABORATORY TESTING FOR MACRO-AST

Eight hundred microliters of 50% slurries of protein A–Sepharose and protein G–Sepharose beads (Sigma–Aldrich) were added to separate tubes and washed 4 times with normal saline. The supernatant was removed, and 600 mL of either patient or control plasma
from a patient with liver disease was added to 400 mL of the washed protein A or protein G beads. The beads were resuspended and incubated at 32 °C with gentle rocking for 3 h. The beads were removed by centrifugation, and the supernatants were analyzed for AST and ALT on a Roche Modular system.

Table 1 demonstrates that there was a >95% reduction in AST when this patient’s plasma was absorbed with protein A or protein G. The ALT values and the control patient’s AST and ALT values were decreased about 40%, as expected from simple sample dilution after mixing with the beads. Taking the dilution into account indicated full recovery of these enzymes (Table 1).

We concluded that the patient had an immunoglobulin–AST complex macroenzyme and that no further testing or treatment was needed.

CONCLUSION

Macroenzymes are an important but rare consideration in an asymptomatic patient with isolated increases in enzymes. Macroenzymes often persist for long periods and can lead to expensive follow-up testing. Documentation of a macroenzyme should be established in suspected patients to avoid future follow-up testing or treatment.

**Table 1. Recovery of enzyme activity following protein A and protein G absorption, corrected for sample dilution.*

<table>
<thead>
<tr>
<th>Sample</th>
<th>Test</th>
<th>Unabsorbed, U/L</th>
<th>Protein A, U/L</th>
<th>Protein G, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>AST</td>
<td>568</td>
<td>11 (3%)</td>
<td>13 (4%)</td>
</tr>
<tr>
<td>Control</td>
<td>AST</td>
<td>564</td>
<td>395 (117%)</td>
<td>358 (106%)</td>
</tr>
<tr>
<td>Patient</td>
<td>ALT</td>
<td>25</td>
<td>17 (113%)</td>
<td>16 (107%)</td>
</tr>
<tr>
<td>Control</td>
<td>ALT</td>
<td>863</td>
<td>599 (116%)</td>
<td>545 (105%)</td>
</tr>
</tbody>
</table>

* Recoveries are indicated in parentheses: Percent recovery = [(Absorbed value)/(0.6 × Unabsorbed value)] × 100.

**POINTS TO REMEMBER**

- Of the common liver “function” tests, only albumin, bilirubin, and prothrombin time truly assess liver function.
- Other common liver “function” tests are markers of hepatic cell damage or death, but not liver function.
- Isolated increases in AST can occur with skeletal or cardiac damage, and with macroenzymes.
- Macroenzymes are not known to cause disease but have been associated with autoimmune diseases.
- The presence of a macroenzyme can be confirmed with electrophoresis, polyethylene glycol precipitation, gel filtration chromatography, or immunoglobulin binding with protein A– or protein G–Sepharose beads.
- Identifying and documenting macroenzymes help prevent additional expensive testing and treatment.

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**References**

Commentary

D. Robert Dufour

A recurring theme in this series is interferences in laboratory testing. In many cases, as in the case reported by Krishnamurthy et al., such interference has led to extensive additional investigation, producing unnecessary costs and, at the least, patient inconvenience. Although most of these cases have had falsely abnormal results, falsely normal results can also occur, leading to delays in disease recognition.

Healthcare providers rely on laboratory test results for much of the objective information in patient care, but providers usually have little training in laboratory tests and seldom are taught about interferences. To prevent administration of unnecessary or inappropriate care, laboratories must minimize reporting of misleading results.

Potential approaches to reduce misleading results fall into 3 main areas. The first involves selection of methods that show minimal interferences. This approach would not likely have prevented reporting of high AST in this case. A second approach involves use of computerized techniques; common tools include use of delta checks, which can detect unlikely changes from previous results, and autoverification rules to detect medically unlikely results. If selected appropriately, these rules call attention to sample results with a high likelihood of being misleading. In this case, such rules may have detected the very high ratio of AST to ALT, which is not typically found in disease, and could have led to testing for macro-AST.

Unfortunately, many misleading results are not obviously erroneous to laboratory personnel. Good communication between the laboratory and providers, and education of providers on the possibility of interferences, are critical to preventing adverse effects on patient care. Although compendia of nondisease causes for abnormal results are available (1, 2), most healthcare providers are unaware of their existence, and only rarely is such information included in clinical textbooks. Laboratorians should continue efforts to make providers more aware of the limitations of laboratory tests and should make themselves readily available to consult and assist in the process of resolving misleading results, as exemplified by the current case.

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References


Commentary

Henry C. Bodenheimer

The case presented by Krishnamurthy and coworkers is an excellent example of the multiple clinical and analytical variables that should be considered when interpreting serum aminotransferase activities. Alcoholic liver disease deserves inclusion in the initial differential diagnosis in this patient’s case because of the increased susceptibility of women to alcohol hepatotoxicity. This increased susceptibility may be attributable to weight difference of women compared to men that leads to higher circulating concentrations of ethanol per kilogram of body weight. Additionally, gastric alcohol dehydrogenase activity is reduced in women. This sex difference may contribute to higher portal vein ethanol...
concentrations for similar amounts of ethanol ingestion in women compared to men. The amount of alcohol ingested by the case patient was reportedly low; however, patient recall and reporting are often inaccurate. Because consumption of large amounts of alcohol increases GGT activity, the fact that the measured values in this case were within the reference interval argues against a significant alcohol effect. The authors are correct to note that an AST of 544 U/L is excessive for alcohol hepatotoxicity, during which AST commonly remains below 200 U/L.

In blood donors, multivariate analysis has revealed that sex, body mass index, and serum triglycerides, all of which are commonly measured variables in the US population, correlate with ALT. Indeed, serum ALT activity may also be predictive for risk of cardiovascular disease, possibly through association with lipid levels, hypertension, and diabetes (1).

An additional point to consider in the evaluation of the clinical significance of aminotransferase activity is intraindividual variability within the laboratory-reported reference interval. Individuals with measured activity at the upper region of the reference interval may be at risk for serious liver injury, even cirrhosis. In patients with chronic hepatitis C who undergo successful treatment, for example, ALT may fall from 38 U/L to 20 U/L, within the reference interval. This variation suggests not only that aminotransferase activities are not specific indicators of liver disease, but also that values within reference intervals do not assure a lack of liver injury.

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