

Macro-aspartate aminotransferase, a surprising outcome for clinicians

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A 34-year old Polish woman (G2P0) who was 34 weeks pregnant was presented to the gynaecologist with pain in the right upper abdomen. Her medical history revealed severe abdominal and pelvic trauma, nephrolithiasis, benign ovarian cyst, hematocolpos and pregnancy after intracytoplasmic sperm injection procedure. The gynaecologist observed a normal pregnancy without any cause for the abdominal pain. However, initial laboratory examination demonstrated an isolated elevation of ASAT activity (397 IU/L, reference interval ASAT < 30 IU/L). Additional laboratory findings included: white blood cell count $14.9 \times 10^9/L$ ($4-10 \times 10^9/L$), platelets $318 \times 10^9/L$ ($150-400 \times 10^9/L$), LDH 174 IU/L (< 250 IU/L), bilirubin 5 $\mu\text{mol/L}$ (< 17 $\mu\text{mol/L}$), creatinine 44 $\mu\text{mol/L}$ (50-95 $\mu\text{mol/L}$). Consequently, the patient was referred to the department of internal medicine. She denied fever, jaundice, muscle pain or weakness. She occasionally took an antacid and had no history of alcohol use, intravenous drugs use or high-risk sexual behavior. Her physical examination was essentially normal. Imaging studies demonstrated no abnormalities of liver, gallbladder, pancreas and kidneys. Further extensive biochemical investigations once again showed an elevated ASAT 371 IU/L, with ALAT, alkaline phosphatase and gamma-glutamyltransferase within their respective reference ranges. No evidence for other sources of ASAT, such as myocardial disease, skeletal muscle disorders or hemolysis. The finding of normal values for ALAT and γ -GT made hepatic disease very unlikely. Acute viral hepatitis was excluded by serological measurements for hepatitis A, - B, - C, EBV and CMV. During this period of time the patient's abdominal pain spontaneously resolved and the patient was asymptomatic. At this point, the presence of macro-ASAT was suggested by the clinical chemist and ultimately confirmed by means of a polyethylene glycol precipitation assay.

Methods

ASAT activity was determined in human plasma of our patient and two control subjects before and after treatment with polyethylene glycol. To this end, 100 μL plasma was added to either 100 μL of PEG 6000 solution (250 g/L in 9 g/L saline) or 100 μL saline (9 g/L) and held at roomtemperature for 1 min prior to

centrifugation at 15000g for 10 minutes (1). ASAT-activities were measured using the standard IFCC ASAT-assay (Roche Diagnostics Systems, Basel, Switzerland) on both the supernatant and the saline dilution. Next, the polyethylene glycol-precipitable activity (%PPA) for ASAT was calculated as: $\%PPA = 100 \times [(ASAT\text{-activity} - ASAT\text{-activity}_{PEG}) / (ASAT\text{-activity})]$. The results of the polyethylene glycol precipitation procedure are shown in table 1.

Results

Our patient demonstrated that 98% of ASAT-activity was precipitated with polyethylene glycol, whereas two controls showed 24 and 37 %PPA (reference values 18-53 % PPA (1)), confirming the presence of macro-ASAT.

Discussion

Aspartate aminotransferase is a well-known enzyme present in clinically significant amounts in heart, liver, skeletal muscle and to a lesser extent in erythrocytes. Injury or death to any of these cells can result in the release of ASAT into the circulation. Consequently, increased serum activities of ASAT should prompt extensive, but rational assessment for different causes. Follow-up may include laboratory evaluation of hepatocellular, muscular, or cardiac causes and abdominal imaging studies.

In pregnant women, however, differential diagnosis may include HELLP syndrome. HELLP syndrome is an acute condition which most often develops before delivery with a peak frequency in the third trimester (2). The most common clinical symptoms are hypertension, proteinuria, right upper abdominal quadrant or epigastric pain, nausea and vomiting. Although diagnosis of HELLP syndrome requires the presence of three major components, i.e. haemolysis (H), elevated liver enzymes (EL) and low platelets (LP), partial HELLP syndrome consists of just one or two prerequisites of this diagnostic triad (2). On admission,

Table 1. Effect of polyethylene glycol (PEG) precipitation. + PEG = 250 g PEG 6000 per liter 0.9% saline; - PEG = 0.9% saline.

	- PEG	ASAT-activity (IU/L) + PEG	%PPA
Patient	397	10	98
control 1	210	160	24
control 2	264	166	37

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our patient was 34 weeks pregnant, presented with pain in her right upper abdominal quadrant and her laboratory findings included strong elevation of ASAT, suggesting the possibility of partial HELLP syndrome. Women with partial HELLP syndrome have fewer symptoms and develop less complications than those with the complete form, but a partial HELLP syndrome may well develop to a complete form with considerable complications, both maternal and neonatal. HELLP syndrome was ruled out by the gynecologist. In the absence of disease, analytical interferences should be part of the differential diagnosis. Indeed, measurement of ASAT is greatly affected by hemolysed samples as erythrocytes contain ASAT activities 15 times greater than normal serum. In our patient, however, lactate dehydrogenase activity which is also highly concentrated in red blood cells was well within its respective reference range excluding *in vitro* hemolysis as analytical interference.

More importantly, our experiments with polyethylene glycol clearly demonstrated the presence of a macro-enzyme species for aspartate aminotransferase. To date, approximately one hundred cases of macro-ASAT have been reported in literature, including apparently healthy individuals with ASAT activities as high as 40 times the upper limit of the reference range (3-5). While the majority of reported cases were asymptomatic, patients have been described with various conditions, e.g. acute and chronic hepatitis, autoimmune hepatitis, primary or metastatic liver malignancies, inflammatory bowel disease, epigastric distress, colitis, diverticulosis and after specific allergen injection immunotherapy. Nevertheless, the absence of pathology over a long period of time in healthy individuals with macro-ASAT argues for the benign nature of this phenomenon (3, 6). It is noteworthy that the presence of macroenzyme is not a transient phenomenon. Indeed, while our patient already demonstrated a single increase of ASAT in 2004 (in Poland), several other cases have been documented in which macro-ASAT was still present after more than 10 years (3, 6). Hence, it is imperative to document this information prominently in the patient's medical records to avoid diagnostic confusion, perhaps years in the future.

The presence of macro-enzyme species can be determined by several laboratory techniques including size exclusion chromatography, protein electrophoresis, and polyethylene glycol precipitation (1, 7). Protein electrophoresis and chromatography require expertise, special equipment and time, whereas the polyethylene glycol precipitation assay is (or should be) a clear-cut, readily available method in all laboratories. This precipitation technique is considerably less expensive and can be used for the screening of all macro-enzymes (e.g. aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, amylase, creatine kinase,

gamma-glutamyltransferase, lactate dehydrogenase). We recommend the polyethylene glycol technique as a rapid initial screening method for the detection of macro-enzyme species provided carefully defined protocols and reference ranges are used (1, 8). However, we would like to point out that if results are inconclusive other techniques should be considered. Nevertheless, if the presence of macro-enzyme species is suspected, evaluation should always start with a review of the patient's clinical background and medical records for routine laboratory findings. Individuals with macro-enzyme species often have a persistently elevated activities for an enzyme in serum that either is not associated with any clinical manifestations or is uncharacteristic for the clinical course and routine laboratory tests.

Conclusion

In conclusion, our case illustrates the need to consider a macro-enzyme species as a cause of persistent elevated ASAT to avoid unwarranted, invasive and expensive investigations. The use of polyethylene glycol is a simple and effective test for the detection of macroenzyme species as exemplified by our case. Given that the presence of macro-ASAT is not a transient phenomenon, it is imperative to record this information prominently in the patient's notes to avert diagnostic confusion in the future. More importantly, it is vital to reassure patients presenting with this phenomenon that macro-enzyme species, i.e. macro-ASAT, has a benign evolution and consequently does not require any specific treatment.

References

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