

Fluctuating Serum Aspartate Aminotransferase Activity in a Complicated Pregnancy

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CASE DESCRIPTION

A 29-year-old para 0 gravida 2 woman with a history of infertility and spontaneous abortion presented to her local hospital at 9 weeks gestation with severe nausea and vomiting. Symptoms persisted for 10 weeks, leading to the diagnosis of hyperemesis gravidarum and treatment with intravenous fluids (3 times/week) and Zofran. At 8 weeks gestation, laboratory tests were unremarkable with the exception of increased aspartate aminotransferase (AST)⁵ measured at a regional reference laboratory [105 U/L; reference interval (RI), 10–40 U/L]. AST continued to be monitored at the same laboratory, peaking at 132 U/L (9 weeks gestation) and gradually declining to 38 U/L by 19 weeks gestation. By 20 weeks gestation, the symptoms of hyperemesis gravidarum resolved. At 32 weeks gestation the patient returned to the hospital with significant right upper quadrant (RUQ) pain. Serum AST, measured this time at the local hospital laboratory, was markedly increased [336 U/L (RI, 14–36 U/L)], whereas alanine aminotransferase (ALT; EC 2.6.1.2), γ -glutamyltransferase, alkaline phosphatase, and bile acids were within reference intervals. RUQ ultrasound findings were unremarkable. Symptoms persisted 1 week later (33 weeks gestation) and the AST activity measured at the local hospital laboratory remained increased (311 U/L). However, a paired sample evaluated at the regional reference laboratory indicated that AST activity was within the reference interval (17 U/L). All other laboratory values were consistent between the regional reference laboratory and the local hospital laboratory (data not shown). For the next 3 weeks (33–36 weeks gestation) AST was monitored at the regional reference labo-

QUESTION TO CONSIDER

1. What is the differential diagnosis in a patient with increased serum AST activity and normal serum ALT activity?
2. What differences between AST assays could account for the observed differences in AST results?
3. What additional laboratory tests could be performed to help determine the source of the different AST assay results?

ratory and the AST results were within reference intervals (16 U/L, 15 U/L, and 25 U/L). By 36 weeks gestation the RUQ pain was continuous and the patient's liver enzymes were closely monitored at the local hospital laboratory, where AST activity was again increased (312 U/L, 309 U/L, and 294 U/L). A second RUQ ultrasound was performed and was unremarkable. At this time, bile acids were mildly increased [17 μ mol/L (RI, <10 μ mol/L)], but other liver and pancreatic enzymes tested were within reference intervals. All things considered, the physician and the patient agreed on an elective cesarean delivery at 37 weeks gestation. Following an uneventful delivery, serum AST remained increased; hepatitis serology was negative. Additional ultrasound imaging results of the gall bladder, pancreas, and liver were normal.

CASE RESOLUTION

The physician engaged the laboratory directors to identify the cause of the fluctuating AST. On further review it was determined that the local hospital and regional reference laboratories used different instruments/reagents that were differentially supplemented with pyridoxal 5' phosphate (P5P) (Fig. 1). Also revealing, serum samples analyzed at 2 different national reference laboratories, both using Roche Cobas c501/502 chemistry analyzers, reported discordant results (Fig. 1). The national reference laboratory reporting a lower result [8 U/L (RI, 10–31 U/L)] did not use P5P-supplemented reagent, whereas the laboratory reporting an increased AST [243 U/L (RI, 8–43 U/L)] did.

Measurement of AST and ALT activity in serum is used to assess liver injury because damaged hepatic cells

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⁵ Nonstandard abbreviations: AST, aspartate aminotransferase; RI, reference interval; RUQ, right upper quadrant; ALT, alanine aminotransferase; P5P, pyridoxal 5' phosphate; PEG, polyethylene glycol.

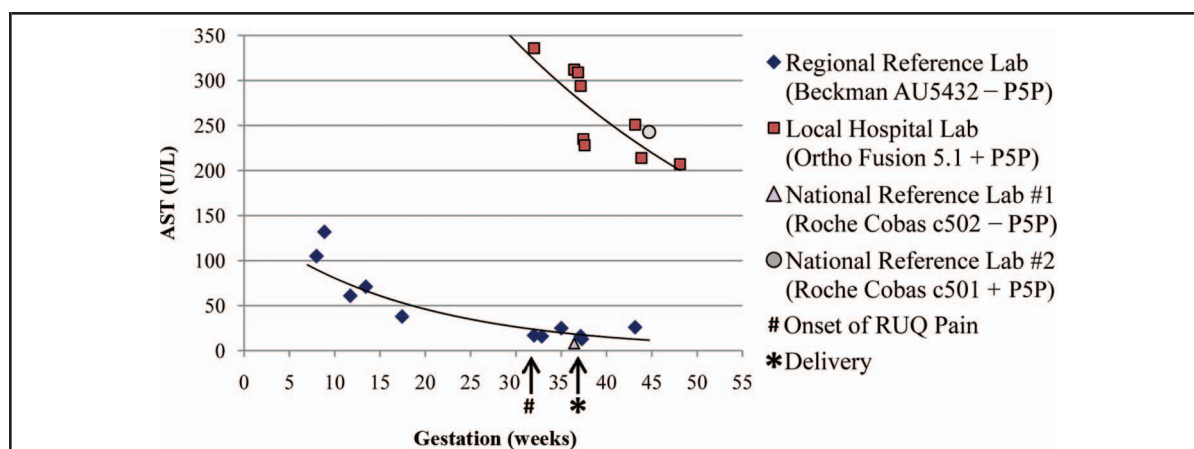


Fig. 1. Monitoring serum AST activity during the course of pregnancy.

Reported AST results are shown from 7 weeks gestation through 12 weeks postpartum ($n = 22$). Reference intervals for AST at different laboratories were as follows: national reference laboratory no. 1 (10–31 U/L), national reference laboratory no. 2 (8–43 U/L), local hospital (10–36 U/L), and regional reference laboratory (10–40 U/L). The black lines represent quadratic fitting of the data.

release the enzymes into circulation. The extent to which AST or ALT activities are increased in serum has diagnostic utility. In most instances of liver damage the AST/ALT ratio is ≤ 1 due to the longer half-life of ALT. However, in cases of hemolytic anemia, myocardial infarction, skeletal muscle damage, cirrhosis, macro-AST, and chronic alcoholism, AST may be $>3\times$ the upper reference limit of the reference interval, while ALT remains within the reference interval. This can occur due to much higher concentrations of AST released by some cells (i.e., red blood cells) or release of enzymatically inactive ALT (chronic alcoholism or cirrhosis) (1).

AST and ALT catalyze the interconversion of amino acids and α -keto acids by transfer of amino groups. Both AST and ALT require vitamin B₆ (pyridoxine) in its physiologically active form, P5P, as a catalytic cofactor (2). Studies looking at in vitro P5P supplementation have demonstrated that serum aminotransferases are not fully saturated with P5P and this varies widely between AST sources (cardiac vs liver; cytoplasmic vs mitochondrial) and by nutritional states (3). In an attempt to standardize aminotransferase assays, the IFCC recommends that laboratories measure enzyme activity in the presence of excess P5P to eliminate confounding variables (4). Yet, in the 2013 College of American Pathologists proficiency survey approximately 50% of laboratories surveyed did not supplement these assays with P5P, and this varied among manufacturers (only approximately 5% of Roche, but the majority of Siemens users supplemented).

Two approaches were used to support that the patient's discrepant results were due to differences in P5P supplementation. First, AST and ALT were measured on

the Beckman AU5432 (which is not supplemented with P5P) with and without exogenous P5P (0.1 mmol/L). Exogenous P5P increased AST activity from 11 to 186 U/L (1690% increase; Table 1). ALT modestly increased from 25 to 30 U/L (20% increase). Second, the patient's vitamin B₆ status was assessed using LC-MS/MS to quantify 2 B6 vitamers, P5P (4 $\mu\text{g/L}$; RI, 5–50 $\mu\text{g/L}$) and pyridoxic acid (<2 $\mu\text{g/L}$; RI, 3–30 $\mu\text{g/L}$). These results corroborate a scenario in which the patient's AST results were abnormally low in unsupplemented assays due to vitamin B₆ deficiency.

However, the observed increase in AST activity upon P5P supplementation far exceeded reported observations in B6-deficient patients and failed to explain the cause of the increased AST activity (5). Beyond nonspecific RUQ pain associated with pregnancy there was little clinical explanation for the increased AST. Macro-AST was not initially part of the differential diagnosis because the elevation of AST activity was not persistent. However, after delivery and once symptoms had resolved, AST remained increased. The reference laboratory director suggested evaluating the sample for the presence of macro-AST. Macro-AST is suspected in asymptomatic patients with sustained and unexplained increased AST. Macroenzyme complexes are formed when either immunoglobulins bind to circulating enzymes or enzymes self-associate to form large complexes which can delay clearance from circulation leading to benign elevations in enzyme activity. The presence of macro-AST can be detected by measuring the amount of AST activity precipitated by polyethylene glycol (PEG). For this patient, AST before PEG precipitation was 243 U/L (RI, 8–43 U/L). Post-PEG precipitation AST activity was 46 U/L,

Table 1. Laboratory results demonstrating P5P-dependent AST activity, vitamin B6 deficiency, and the presence of macro-AST.^a

	ALT, U/L	AST, U/L	AST/ALT
Without P5P	25	11	0.4
With P5P	30	186	6.2
Percent increase with P5P	20%	1690%	
	Pyridoxic acid, µg/L	P5P, µg/L	
Reference interval	3–30	5–50	
Patient result	<2	4	
	Total AST, U/L	Residual AST after PEG precipitation, U/L	Precipitated AST activity (% of total activity)
AST activity (with P5P)	243	46	81%

^a Paired serum and plasma samples collected 11 weeks postpartum were used to assess vitamin B6 status in the patient: AST and ALT were measured on a Beckman AU5432 chemistry analyzer with and without P5P supplementation; vitamin B6 vitamers (pyridoxic acid and P5P) were quantified using LC-MS/MS. Macro-AST was identified using a serum sample collected 8 weeks postpartum by measuring residual AST activity after removal of PEG-precipitated AST. Macro-AST is confirmed when greater than 80% of AST activity precipitates upon PEG treatment of the sample.

demonstrating that a majority of activity (81%) was precipitated with PEG, consistent with macro-AST (Table 1). We cannot conclude if the residual AST activity present was due to incomplete precipitation of macro-AST complexes or if the patient had increased AST activity independent of macro-AST, but given the lack of any other liver enzyme or bilirubin abnormalities or clinical signs we hypothesize the former. AST activity due to the presence of macro-AST has been reported to be exceptionally responsive to P5P supplementation, but can be increased without exogenous P5P (6). Supporting this, in 8 confirmed cases of macro-AST identified at the Mayo Clinic, 5 of the 8 cases had increased AST activity regardless of assay supplementation with P5P (unpublished data). Therefore, it should be cautioned that the lack of P5P supplementation is unlikely to prevent the detection of all macro-AST cases. Plausible explanations for the variable requirement of exogenous P5P in macro-AST cases include enzyme half-life in circulation, the amount of macroenzyme complex, and/or the origin of the AST found in the complex.

This case highlights an instance in which both awareness and harmonization with P5P-supplemented reagents would have prevented unnecessary testing and improved patient care. Literature supports identifying macro-AST by the presence of persistently increased AST in the absence of other increased liver enzymes and the absence of clinical findings. In this case, there was a failure to recognize persistent elevation of AST due to nonstandardized use of P5P reagent across laboratories. Furthermore, there was a general lack of awareness that AST activity due to macro-AST could be reagent specific. Certain populations are likely to have discordant AST results when testing

methods alternate between P5P-supplemented and -unsupplemented assays. Populations most at risk are those with B6 deficiency (susceptible patients include chronic alcoholics, women using oral contraceptives, and women who are pregnant) and those with macro-AST, as reported here. This case aims to provide an example of why patients and the medical community would benefit from adoption of universal reagents for measuring AST/ALT activity. The IFCC endorses P5P supplementation in an attempt to ensure that maximum potential cata-

POINTS TO REMEMBER

- Despite maternal supplementation, severe hyperemesis gravidarum during pregnancy can accelerate the onset of vitamin B₆ deficiency.
- Commercial vendors differ in whether they offer P5P-supplemented and/or -unsupplemented reagents for AST and ALT measurements.
- The IFCC recommends that laboratories supplement AST and ALT assays with P5P to ensure that the maximum potential catalytic activity is measured in patient samples.
- Patients with vitamin B₆ deficiency are likely to have discordant results between P5P-supplemented and -unsupplemented AST assays.
- Clinicians may encounter large fluctuations in serum AST activity if testing alternates between P5P-supplemented and -unsupplemented assays in patients with macroenzyme AST.

lytic activity is measured (4). Despite these recommendations, in vitro diagnostic companies have not standardized the use of P5P reagents—not even across chemistry analyzers sold by the same company. For instance, Beckman Coulter does not offer P5P-supplemented reagent for the AU series but offers reagents with and without P5P for the UNICEL DxC. Similarly, Siemens offers P5P-supplemented reagent only for the Dimension and Vista but offers both types of reagents for the Advia. Roche offers reagents with and without P5P for the Cobas c500/700 series. Laboratorians and clinicians must be familiar with their laboratory assays; as described here, understanding and

communicating how P5P affects AST/ALT results can improve patient care.

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Commentary

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The case reported by Mills and colleagues represents several challenges for the hepatologist. Elevation that is present before pregnancy (an often unknown fact) may indicate an underlying hepatic disease that requires further evaluation and/or treatment. Elevation of liver-associated enzymes that develops during pregnancy is usually considered to be an important finding and traditionally has been felt to be due to one of several severe conditions that can affect the mother and/or her baby, including those related to preeclampsia [including acute fatty liver of pregnancy and HELLP (hemolysis, elevated liver enzymes, and low platelet count) syndrome], hyperemesis gravidarum, and intrahepatic cholestasis of pregnancy (1). In one study, this occurred in 3% of all pregnancies (2). A recent study found a high frequency of mild, clinically insignificant elevation in women who had become pregnant using assisted reproductive techniques (3). Most likely, the high AST in this case was initially attributed to hyperemesis gravidarum, although (given the his-

tory of infertility) one would wonder about use of assisted reproductive techniques to achieve pregnancy.

From a clinical standpoint, several features should have led to earlier suspicion of another reason for the increased AST. First, most liver disorders are associated with greater elevation of ALT than AST (exceptions would include alcoholic hepatitis, cirrhosis, Wilson disease, and very early liver damage). Even in muscle injury, such high AST activities should be associated with at least mild ALT elevation. AST has a half-life of about 16–18 h, and discordance of AST between different laboratories using samples collected at the same time (as seen in this case) or more rapid than expected fall in AST should indicate that this is not really liver (or muscle) disease, but a laboratory measurement issue. Clinicians should be alert to unlikely laboratory patterns and call the laboratory to request further evaluation.

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