

# Isolated elevated aspartate aminotransferase: a surprising outcome for clinicians

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## ABSTRACT

In this report a case of macro-aspartate aminotransferase in a 34-year-old pregnant woman is presented. Awareness of the existence of a macroenzyme is important because of their ability to cause diagnostic confusion, which leads to unnecessary investigations. Confirmation with a polyethylene glycol precipitation test is simple to perform and not expensive.

## KEYWORDS

Macro-enzyme, macro-ASAT, polyethylene glycol precipitation

## INTRODUCTION

Although increased enzyme activity in serum is usually associated with disease, benign conditions have been described in which serum enzymes are abnormal. Indeed, several enzymes can form high-molecular-mass complexes either by self-polymerisation or by association with other plasma components, i.e. immunoglobulins.<sup>1,2</sup> This phenomenon was first recognised in 1964.<sup>3</sup> Although serum activity may be unaffected, in some cases, immunoglobulin binding to circulating enzymes may lead to an increased activity by mechanisms involving reduced inactivation, clearance or excretion.<sup>1</sup> Unfortunately, routine laboratory analysis is inapt to differentiate between enzyme activity and a corresponding macroenzyme species. As a result, the presence of a macroenzyme may cause diagnostic confusion if remained undetected. Here, a case of a persistent and isolated elevation of aspartate aminotransferase (ASAT) activity in serum due to a macroenzyme of ASAT is presented. This report illustrates the importance of recognising macroenzyme species and

clear documentation of this biochemical abnormality to prevent misinterpretation and to avoid excessive investigations.

## CASE REPORT

A 34-year old Polish woman (G2P0), who was 34 weeks pregnant, presented with pain in the right upper abdomen. Her medical history revealed severe abdominal and pelvic trauma, nephrolithiasis, benign ovarian cyst, haematocolpos and pregnancy after an intracytoplasmic sperm injection procedure. She denied fever, jaundice, muscle pain or weakness. She occasionally took an antacid. Full physical examination was normal, including a normal pregnancy. However, laboratory examination demonstrated a persistent, isolated elevation of ASAT activity (397 IU/l; 371 IU/l, reference interval ASAT <30 IU/l). Additional laboratory findings included: haemoglobin 7.6 mmol/l (7.5 to 10 mmol/l), erythrocytes  $3.9 \times 10^{12}/l$  ( $4.0$  to  $5.0 \times 10^{12}/l$ ), white blood cell count  $14.9 \times 10^9/l$  ( $4$  to  $10 \times 10^9/l$ ), platelets  $318 \times 10^9/l$  ( $150$  to  $400 \times 10^9/l$ ), creatinine 44  $\mu\text{mol}/l$  ( $50$  to  $95 \mu\text{mol}/l$ ), lactate dehydrogenase 174 IU/l (<250 IU/l), creatine kinase 58 IU/l (<45 IU/l), bilirubin 5  $\mu\text{mol}/l$  (<17  $\mu\text{mol}/l$ ), and haptoglobin 1.15 g/l (0.40 to 2.45). Imaging studies demonstrated no abnormalities of the liver, pancreas, gallbladder and kidneys. Normal values for alanine aminotransferase and gamma glutamyltransferase made hepatic disease very unlikely. Acute viral hepatitis was excluded by serological measurements for hepatitis B and C, Epstein-Barr virus and cytomegalovirus. No evidence for other sources of ASAT, such as myocardial disease, skeletal muscle disorders or haemolysis, was found. The patient's abdominal pain spontaneously resolved and the patient became asymptomatic. At this point, the presence of macro-ASAT was suggested by the

clinical chemist. ASAT activity was determined in the human plasma of our patient and two control subjects before and after treatment with polyethylene glycol as described previously.<sup>4</sup> The results are shown in *table 1*. Our patient demonstrated that 98% ASAT activity was precipitated with polyethylene glycol, whereas two controls showed 24 and 37% PPA (polyethylene glycol-precipitable activity, reference values 18 to 53% PPA),<sup>4</sup> confirming the presence of macro-ASAT.

## DISCUSSION

Aspartate aminotransferase is present in significant amounts in the liver, heart, skeletal muscle and erythrocytes. Injury to any of these organs or cells can result in the release of the enzyme into the circulation. Consequently, increased serum activities of ASAT should prompt clinical evaluation, which may include abdominal imaging studies and laboratory assessment of hepatocellular, muscular, or cardiac causes. Furthermore, several types of medication (i.e. erythromycin) can cause solitarily elevated serum ASAT.<sup>5</sup> As mentioned earlier, our patient was not on any medication and no evidence of hepatic disease, skeletal muscle disorders or myocardial disease was found in our patient. Hence, in the absence of disease, analytical interferences should be an intrinsic part of the differential diagnosis.

Measurement of ASAT activity is clearly affected by haemolysed samples as erythrocytes contain ASAT activity up to 20 times greater than normal serum. In our case, however, lactate dehydrogenase activity, which is also abundantly present in erythrocytes, was within its reference range. In addition, measurement of the serum haptoglobin level was not decreased, excluding haemolysis as analytical interference.

Our experiments with polyethylene glycol, however, clearly demonstrated the presence of a macroenzyme species for ASAT. Until now, several cases of macro-ASAT have been reported in the literature, including apparently healthy individuals with ASAT activities as high as 50 times the

upper limit of the reference range.<sup>6-8</sup> Although some patients have been described with various conditions, including malignancies and autoimmune disorders, the majority of reported cases are asymptomatic. Indeed, the absence of pathology over a long period of time in healthy individuals with macro-ASAT argues for the benign nature of this phenomenon.<sup>6,9</sup> Remarkably, the presence of macro-ASAT is not a transient phenomenon. In addition to our patient, who already demonstrated an isolated increase in ASAT in 2004, numerous cases have been reported in which macro-ASAT was persistently present for more than ten years.<sup>6,9</sup> Hence, it is imperative to document this essential information in the patient's medical records to avoid diagnostic confusion, perhaps years in the future.

Laboratory test results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings. However, an important clue to the presence of a macroenzyme species is the persistent elevation of a single enzyme activity in serum. Indeed, our patient demonstrated persistently increased ASAT activity, whereas all other laboratory results were within their respective reference ranges. Moreover, several studies have shown that macro-aspartate aminotransferase is the culprit in 40 to 100% of healthy cases with an isolated increase in ASAT activity.<sup>4,6,10</sup>

The presence of macroenzyme species can be determined by laboratory techniques including polyethylene glycol precipitation, size exclusion chromatography and protein electrophoresis. The polyethylene glycol precipitation technique is a low-cost method and can be used for the screening of all macroenzyme species (e.g. amylase, creatine kinase, prolactin). We recommend this method as a rapid initial screening method for the detection of macroenzyme species provided carefully defined protocols and reference ranges are used.<sup>4,11</sup>

Nowadays, several laboratory expert systems that permit real-time validation of biochemical data are readily available.<sup>12,13</sup> These automated systems use artificial intelligence techniques to aid decision making by the clinical chemist or physician. Clearly, a simple algorithm could trigger a reflex test strategy, including the polyethylene glycol precipitation technique and consequently prevent unwarranted investigations. Still, even in the absence of such expert systems, one should strongly consider the presence of a macroenzyme species if only a single enzyme activity is elevated.

## CONCLUSION

Our case demonstrates the need to consider a macroenzyme species as a cause of persistent isolated elevation of ASAT to prevent unwarranted, invasive and expensive investigations. We recommend the use of the

**Table 1.** Effect of polyethylene glycol (PEG) precipitation

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

The polyethylene glycol-precipitable activity (% PPA) for ASAT was calculated as: % PPA = 100 x [(ASAT activity - ASAT activity PEG) / (ASAT activity)]; + PEG = 250 g PEG 6000 per litre 0.9% saline; - PEG = 0.9% saline.

polyethylene glycol precipitation technique as a simple and effective screening test for the detection of macroenzyme species.

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