

Undetectable Serum Alkaline Phosphatase Activity in a Patient with Fulminant Hepatic Failure and Hemolytic Anemia

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CASE

A 21-year-old woman presented with a 5-day history of loss of appetite, malaise, nausea, and vomiting. On examination, she appeared acutely ill, with jaundice and right-sided hypochondrial tenderness. The stigmata of chronic liver disease were absent, and the results of her neurologic examination were typical. She had previously been well except for bouts of somnolence with occasional emesis during the previous year. There was no history of recent travel, alcohol or illicit drug use, or liver disease in her family. Viral hepatitis was considered the most likely diagnosis, but the patient's clinical deterioration necessitated hospital admission a day later. The results of serology tests for acute infection with hepatitis A virus, hepatitis B virus, or Epstein–Barr virus were negative, and the complete blood count revealed a macrocytic anemia. Liver function tests revealed the following: total bilirubin, 136 $\mu\text{mol/L}$ [upper reference limit (URL),² 26 $\mu\text{mol/L}$]; direct bilirubin, 62 $\mu\text{mol/L}$ (URL, 7 $\mu\text{mol/L}$); albumin, 31 g/L [reference interval (RI), 35–50 g/L]; γ -glutamyltransferase, 165 U/L (URL, 44 U/L); aspartate aminotransferase (AST), 166 U/L (URL, 35 U/L); alanine aminotransferase (ALT), 24 U/L (URL, 35 U/L). Serum alkaline phosphatase (ALP) was noteworthy for being undetectable (<5 U/L; RI, 51–117 U/L) on 3 consecutive days. Methodologic interference in the ALP assay by anticoagulants, such as EDTA and fluoride, was excluded on the basis of sodium, potassium, and calcium concentrations that were within reference intervals. Evidence of hemolysis was provided by the results for serum lactate dehydrogenase (786 U/L;

QUESTIONS TO CONSIDER
1. What are the causes and characteristics of fulminant hepatic failure?
2. Which causes of fulminant hepatic failure are associated with hemolysis?
3. What are causes of low ALP activity, and how are they related to fulminant hepatic failure?

RI, 120–230 U/L) and haptoglobin (<60 mg/L; RI, 300–2000 mg/L). The hemoglobin concentration decreased from 83 g/L to 35 g/L (RI, 124–167 g/L), requiring transfusion of 9 units of packed red blood cells. The patient also received 18 units of fresh frozen plasma during 4 plasma-exchange procedures. Although citrate chelation after transfusion of blood products may cause low ALP activity, undetectable ALP activity had been documented before the transfusions. The results for the direct Coombs test were negative, and the international normalized ratio was 2.0 (RI, 0.9–1.2). An initial serum urea concentration of 5.3 mmol/L (RI, 2.1–7.1 mmol/L) and a creatinine value of 78 $\mu\text{mol/L}$ (RI, 53–97 $\mu\text{mol/L}$) indicated that the patient's renal function was well preserved (Table 1).

DISCUSSION

Fulminant hepatic failure (FHF) is a sudden deterioration of liver function in an otherwise healthy individual that is associated with jaundice, coagulopathy, any degree of encephalopathy, and multiple organ dysfunction (including renal insufficiency in 50% of cases) (1). Causes may be classified as: (a) viral (hepatitis A, B, and/or D; Epstein–Barr virus; and others); (b) drug- and toxin-induced (notably acetaminophen); (c) vascular (“shock liver,” Budd–Chiari syndrome, and veno-occlusive disease); (d) metabolic [acute fatty liver of pregnancy, HELLP (hemolysis, increased liver enzymes, low platelets) syndrome, Reye syndrome, Wilson disease (WD), and other inherited metabolic disorders]; and (e) di-

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Received June 24, 2010; accepted September 1, 2010.

DOI: 10.1373/clinchem.2010.152447

² Nonstandard abbreviations: URL, upper reference limit; RI, reference interval; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; FHF, fulminant hepatic failure; HELLP, hemolysis, elevated liver enzymes and low platelets; WD, Wilson disease; AIH, autoimmune hepatitis; WD-FHF, FHF associated with WD; dw, dry weight; OLT, orthotopic liver transplantation.

Table 1. Sequential results for this patient.

	Reference interval	Before admission	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	At discharge
Sodium (S) ^a , mmol/L	136–145		135		134	138	134		136	136
Potassium (S), mmol/L	3.5–5.1		3.9		4.5	3.7	3.8		3.7	3.9
Urea (S), mmol/L	2.1–7.1				5.3	6.3	7.0		6.1	3.7
Creatinine (S), μmol/L	53–97				78	90	31	<27	<27	48
Calcium (S, corrected), mmol/L	2.20–2.55		2.26			2.28	2.21	2.16	2.19	
Albumin (S), g/L	35–50	29	31	27		26	25	27	24	19
Total bilirubin (S), mmol/L	2–26	66	136	89			143	195	271	80
Direct bilirubin (S), μmol/L	<7	35	62	57						44
ALP (S), U/L	51–117	13	<5	<5	<5	16				58
γ-Glutamyltransferase (S), U/L	<44	175	165	100	74	56				51
AST (S), U/L	13–35	136	166	115	93	72				68
ALT (S), U/L	<35	25	24	14	7	12				25
Lactate dehydrogenase (S), U/L	120–230		786							
Haptoglobin (S), mg/L	300–2000			<60						
Hemoglobin, g/L	124–167	85			35	75	66	85	71	95
Total copper (S), μmol/L	12.6–24.3			43.6						
Copper (U), μmol/day	<0.6			29.8			63.5			
Transfusion of packed RBCs, units					4		3		2	
PEX (fresh frozen plasma), units					5	3		5	5	

^a S, serum; U, urine; RBC, red blood cell; PEX, plasma exchange.

verse [sepsis, autoimmune hepatitis (AIH), and hepatic infiltrations] (1). Ingestion of hepatotoxins was not apparent, and the results of an autoantibody screen and serologic tests for common hepatitis viruses were negative. A computed tomography evaluation demonstrated hepatomegaly but no infiltrative or vascular lesions.

Given the prominence of hemolysis in this case, a consideration of FHF etiologies associated with hemolysis is indicated. Hemolysis in FHF may be immune mediated and/or non-immune mediated. Immune-mediated mechanisms are implicated in sepsis, fulminant viral hepatitis, and autoimmune hemolysis associated with AIH (2). Non-immune mediated mechanisms may be categorized as (a) microangiopathic (HELLP syndrome, disseminated intravascular coagulation in sepsis, disseminated malignancy), (b) oxidative (fulminant WD, viral hepatitis in glucose-6-phosphate dehydrogenase deficiency), (c) extravascular destruction (spur cell anemia, hypersplenism), and (d) hemolysin production (*Clostridium perfringens* sepsis) (2–4). Many of these conditions could be excluded in our patient on the basis of her clinical features and platelet and white cell counts that were within reference intervals.

Methodologic interference in the ALP assay by anticoagulants has been discussed. Other causes of low ALP (EC 3.1.3.1) activity are hereditary hypophosphatasemia, pernicious anemia, hypothyroidism, hypomagnesemia, and zinc deficiency (5). Pernicious anemia and hypothyroidism can be associated with FHF due to AIH in the context of polyglandular autoimmune syndrome, but FHF is unlikely in the other causes. Low serum ALP activity is frequently observed in FHF associated with WD (WD-FHF), which has been called pathognomonic of fulminant WD because it is uncommon in chronic presentations of WD (5, 6). Other findings in FHF that are suggestive of WD are higher bilirubin and lower hemoglobin concentrations, and lower aminotransferase activities (4, 7). Incorporating these features into indices, such as an ALP/total bilirubin ratio <4 and an AST/ALT ratio >2.2, has yielded sensitivities and specificities of 86%–100% for WD-FHF (7). Applying these ratios to our patient's initial results would have led to a WD diagnosis (ALP/total bilirubin ratio indeterminate and an AST/ALT ratio of 6.9). AST/ALT ratios in WD-FHF are high, owing to AST release from necrotic hepatocytes (mitochondrial isoenzyme) and lysing erythrocytes.

CASE RESOLUTION

The most likely diagnosis in this case was WD-FHF, which was subsequently confirmed by finding a 50-fold increase in urinary copper excretion (29.8 $\mu\text{mol/day}$; URL, 0.6 $\mu\text{mol/day}$). The concentration of total copper in the serum was also increased (43.6 $\mu\text{mol/L}$; RI, 12.6–24.3 $\mu\text{mol/L}$), but because ceruloplasmin was not measured, the concentration of free copper could not be estimated. A histologic analysis of a liver biopsy sample demonstrated cirrhosis and copper deposition in hepatocytes. WD is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in the *ATP7B* gene (ATPase, Cu^{++} transporting, beta polypeptide) on chromosome 13q14.3. The gene encodes a copper-transporting adenosine triphosphatase required for biliary copper excretion and incorporation of copper into ceruloplasmin. Copper accumulation in tissues manifests as hepatic, neurologic, hemolytic, or psychiatric disease, generally between the first and fourth decades (8). The diagnosis is based on finding decreased serum ceruloplasmin (<200 mg/L), urinary copper excretion >1.57 $\mu\text{mol/day}$ (>100 $\mu\text{g/day}$), and a hepatic copper content >3.9 $\mu\text{mol/g}$ dry weight (dw) or >250 $\mu\text{g/g}$ dw (URL, 0.8 $\mu\text{mol/g}$ dw or 50 $\mu\text{g/g}$ dw) (8). Heterozygotes are asymptomatic but may exhibit hypoceruloplasminemia, increased urinary copper excretion after penicillamine treatment, and a hepatic copper content of 1.6–3.9 $\mu\text{mol/g}$ dw (100–250 $\mu\text{g/g}$ dw) (9). The heterozygote status should be confirmed to prevent unwarranted lifelong chelation therapy (9).

Hepatic involvement manifests as self-limited acute hepatitis, fatty liver, “autoimmune-like” chronic hepatitis, and cirrhosis (9). Rarely (as seen in this case), the initial presentation is with FHF and Coombs-negative hemolysis (10). Without prompt intervention to remove copper from the circulation, the outcome is fatal unless orthotopic liver transplantation (OLT) is performed (10). Given that chelating agents require weeks to appreciably reduce the plasma copper concentration, modalities such as plasmapheresis have been added (10). By attenuating hemolysis and stabilizing the clinical condition with a combination of chelation and plasma exchange, OLT may be averted or postponed (10). Early and definitive diagnosis of WD-FHF is therefore essential, but it is not accomplished without difficulty. Kayser–Fleischer rings, although present in >90% of individuals with neuropsychiatric WD, occur in only 50% of those with liver disease (7, 9). There were no obvious Kayser–Fleischer rings in our patient, but slit-lamp examination was not performed.

The biochemical assessment of copper status in FHF is unreliable, and the tests are subject to long turn-

around times. Ceruloplasmin assays based on oxidase activity are preferred for WD diagnosis, because immunonephelometric methods measure both holo- and apoceruloplasmin (8, 9). Nonpathologic concentrations may be seen in WD-FHF, because ceruloplasmin is an acute-phase reactant and hypoceruloplasminemia is not uncommon in non-WD FHF, owing to impaired hepatic synthesis (4, 7). Total copper concentrations in serum, which are reduced in chronic WD, are frequently increased in WD-FHF because of copper release from necrotic hepatocytes (4, 7). Hypercupremia occurs in non-WD FHF for the same reason, but concentrations >31.4 $\mu\text{mol/L}$ (>200 $\mu\text{g/dL}$) are highly suggestive of WD (7). Urinary copper excretion is often dramatically increased in WD-FHF and is less so in non-WD FHF [mean (SD), 56.6 (9.8) $\mu\text{mol/day}$ or 3602 (622) $\mu\text{g/day}$, vs 9.2 (3.6) $\mu\text{mol/day}$ or 584 (231) $\mu\text{g/day}$, in one study], but assessment may be hampered by oliguria (4). Estimating the tissue copper concentration is of limited value because hepatic copper is increased in several chronic liver diseases. Copper concentrations in WD are not consistently high and can vary between biopsies because of the patchy distribution of copper in cirrhosis (8, 9). In patients with FHF, the presence of coagulopathy may preclude liver biopsy.

Liver disease in WD is hypothesized to be due to oxidative damage caused by reactive oxygen species produced by the copper excess. This hypothesis is supported by studies of hepatic tissues from affected patients, which show decreased ratios of reduced to oxidized glutathione, increased lipid peroxidation products, and lower activities of antioxidant enzymes (11). FHF in WD is believed to be initiated by extensive apoptosis of hepatocytes triggered by extremely high loads of oxidant stress (11). The sudden massive release of hepatic copper precipitates hemolysis via direct damage to erythrocyte membranes, by inhibiting erythrocyte enzymes, and oxidative stress (4, 5). Low ALP activity in WD-FHF has similarly been ascribed to oxidative damage by hydroxyl free radicals generated during Cu^{2+} -catalyzed ascorbate oxidation (6). Another proposed mechanism is that transient marked hypercupremia leads to incorporation of Cu^{2+} instead of Zn^{2+} at the ALP active site, yielding an enzyme with reduced activity; however, attempts to simulate this phenomenon in vitro have been unsuccessful (5, 6).

CONCLUSIONS

The patient recovered sufficiently after treatment with penicillamine and plasmapheresis to permit discharge after 12 days. She continues to do well on chelation therapy, and listing for OLT is not currently being con-

POINTS TO REMEMBER

- The combination of Coombs-negative hemolysis and low serum ALP activity in a patient with FHF should arouse suspicion of WD. Most of the other causes of low ALP activity are easily excluded and unlikely to be associated with FHF.
- Pronounced hyperbilirubinemia, low ALP activity, and relatively mild increases in aminotransferases are suggestive of WD as the cause of FHF. ALP/total bilirubin and AST/ALT ratios have high sensitivity and specificity for diagnosing WD-FHF, particularly in combination.
- Of the biochemical indicators of copper status, serum total copper is the best predictor of WD in the setting of FHF. Dramatic increases in urinary copper excretion are likewise suggestive, but assessment may be hampered by renal insufficiency. Ceruloplasmin measurement and tissue copper estimation remain useful in the workup of chronic WD.
- Rapid reduction of plasma copper levels by plasmapheresis may be lifesaving in WD-FHF, allowing OLT to be averted or postponed.
- Confirmation of the heterozygote status is essential in asymptomatic individuals to prevent inappropriate life-long chelation therapy.

sidered. Genetic testing will be offered to the proband and her first-degree relatives.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Commentary

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Low or undetectable alkaline phosphatase activity is a relatively uncommon finding. Artifactual decreases in

Authors' Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

Acknowledgments: I am grateful to Dr. Lani Frantzen for introducing me to the case and thank Lancet Laboratories and Vermaak and Partners' Pathologists for making laboratory data available to me. I thank Dr. Robert Bond, Dr. Theunis Botha, and Prof. Ernie Song for sharing clinical case details.

References

1. Sass DA, Shakil AO. Fulminant hepatic failure. *Liver Transplant* 2005;11:594–605.
2. Beutler E. Hemolytic anemia resulting from infections with microorganisms. In: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT, eds. *Williams hematology*. 7th ed. New York: McGraw-Hill; 2006. p 723–7.
3. Baker KR, Moake J. Hemolytic anemia resulting from physical injury to red cells. In: Lichtman MA, Beutler E, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT, eds. *Williams hematology*. 7th ed. New York: McGraw-Hill; 2006. p 709–16.
4. McCullough AJ, Fleming CR, Thistle JL, Baldus WP, Ludwig J, McCall JT, Dickson ER. Diagnosis of Wilson's disease presenting as fulminant hepatic failure. *Gastroenterology* 1983;84:161–7.
5. Shaver WA, Bhatt H, Combes B. Low serum alkaline phosphatase activity in Wilson's disease. *Hepatology* 1986;6:859–63.
6. Hoshino T, Kumasaka K, Kawano K, Yamagishi F, Koyama I, Fujimori-Arai Y, et al. Low serum alkaline phosphatase activity associated with severe Wilson's disease. Is the breakdown of alkaline phosphatase molecules caused by reactive oxygen species? *Clin Chim Acta* 1995;238:91–100.
7. Korman JD, Volenberg I, Balko J, Webster J, Schiodt FV, Squires RH, et al. Screening for Wilson disease in acute liver failure: a comparison of currently available diagnostic tests. *Hepatology* 2008;48:1167–74.
8. Medici V, Rossaro L, Sturniolo GC. Wilson disease—a practical approach to diagnosis, treatment and follow-up. *Dig Liver Dis* 2007;39:601–9.
9. Cox DW, Roberts E. Wilson disease: hepatolenticular degeneration. *GeneReviews*. NCB Bookshelf. <http://www.ncbi.nlm.nih.gov/books/NBK1512/> (Accessed May 2010).
10. Asfaha S, Almansori M, Qarni U, Gutfreund KS. Plasmapheresis for hemolytic crisis and impending acute liver failure in Wilson disease. *J Clin Apher* 2007;22:295–8.
11. Nagasaka H, Inoue I, Inui A, Komatsu H, Sogo T, Murayama K, et al. Relationship between oxidative stress and antioxidant systems in the liver of patients with Wilson disease: hepatic manifestation in Wilson disease as a consequence of augmented oxidative stress. *Pediatr Res* 2006;60:472–7.

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Received October 21, 2010; accepted October 28, 2010.

DOI: 10.1373/clinchem.2010.158089

alkaline phosphatase activity can occur in the absence of divalent cations needed as cofactors. In a review of almost 70 000 alkaline phosphatase results for adult, mainly male, patients, Lum found low activity in only 0.19% (1). In half of the cases reviewed, there was no explainable cause for the low results. The most common explainable cause was cardiac surgery, and malnutrition and magnesium deficiency were the next most common causes. All of these causes can be associated with either low levels of cations such as magne-

sium and zinc or the presence of chelators, such as citrate, in transfusions that lower alkaline phosphatase activity. Contamination of “serum” by EDTA or citrate during phlebotomy can cause undetectable alkaline phosphatase activity. Clinically apparent causes of low alkaline phosphatase include the rare congenital disorder hypophosphatasia, use of estrogens (including estrogen-containing oral contraceptives), and severe hypothyroidism. As seen in the current case, low alkaline phosphatase activity is also seen in fulminant hepatitis due to Wilson disease. Because none of the other causes of low alkaline phosphatase activity would be associated with jaundice or hemolytic anemia, the combination of these findings should lead the astute laboratorian to contact the clinicians to make sure that they consider Wilson disease as a diagnostic possibility. As described well here, tests more commonly used for Wilson disease diagnosis are often unreliable in the setting of acute liver failure. In a review of cases of acute liver failure in children, 54% of those with “unexplained” causes had never been evaluated for Wilson disease (2). Laboratory professionals should be alert to this uncommon cause of a rare laboratory finding, which can be lifesaving if the correct diagnosis of Wilson disease is made.

Commentary

Eve A. Roberts^{1,2,3,4*}

Strictly speaking, fulminant hepatic failure associated with Wilson disease (WD-FHF) is not “acute liver failure,” because there is an underlying (although unrecognized) chronic liver disease. When WD-FHF was first reported in the late 1970s, it seemed that it might be extremely rare. In fact, it accounts for approximately 5% of patients with acute liver failure. Besides severe coagulopathy and encephalopathy, WD-FHF patients have a typical clinical profile: Coombs-negative hemolytic anemia with features of acute intravascular hemolysis, only modest increases in serum aminotrans-

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:

Employment or Leadership: D.R. Dufour, *Clinical Chemistry, AACC.*

Consultant or Advisory Role: D.R. Dufour, *Ortho Clinical Diagnostics.*

Stock Ownership: None declared.

Honoraria: D.R. Dufour, *Osler Institute and College of American Pathologists.*

Research Funding: None declared.

Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

1. Lum G. Significance of low serum alkaline phosphatase activity in a predominantly adult male population. *Clin Chem* 1995;41:515–8.
2. Narkevicz MR, Dell Olio D, Karpen SJ, Murray KF, Schwarz K, Yazigi N, et al. Pattern of diagnostic evaluation for the causes of pediatric acute liver failure: an opportunity for quality improvement. *J Pediatr* 2009;155:801–6.

ferases, normal or markedly subnormal serum alkaline phosphatase, and rapid progression to renal failure. Females are more likely than males to develop WD-FHF. Thus, the presented case appears classic. The intravascular hemolysis reflects copper toxicity to erythrocyte plasma membranes. The serum aminotransferase activities may be lower than expected because the liver is undergoing extensive hepatocellular apoptosis or because it is already cirrhotic. Serum alkaline phosphatase is typically subnormal, although not always as low as in this case; the mechanism for this feature remains obscure. Serum and urinary copper concentrations are greatly increased in WD-FHF, but these laboratory results may not be available in time for critical clinical decision-making. Whereas serum ceruloplasmin is not informative for making the diagnosis of WD-FHF, formulas that use available laboratory data [a ratio of alkaline phosphatase (in U/L) to total bilirubin (in milligrams per deciliter) <4, concomitantly with an aspartate aminotransferase–alanine aminotransferase ratio >2.2] are useful.

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Received November 15, 2010; accepted December 3, 2010.

DOI: 10.1373/clinchem.2010.158097

The standard teaching is that WD-FHF is uniformly fatal, and thus liver transplantation is required. This concept should be the mindset. It mandates rapid diagnosis, immediate/timely transfer to a liver transplantation center, and extensive communication with the patient and his/her family. The use of standard chelators alone has little efficacy in treating WD-FHF; the role of antioxidants as adjunctive therapy is being investigated. Recent experience has shown that plasmapheresis and hemofiltration, exchange transfusion, or albumin dialysis may stabilize patients with WD-FHF until transplantation. Very occasionally (as in this case), such interventions may obviate transplantation. Arrangements for liver transplantation should be completed anyway. If a brother or sister is a potential donor, he/she needs to be assessed for WD, preferably by genetic diagnosis. WD carriers (heterozygotes, e.g., parents) can be donors.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:*

Employment or Leadership: None declared.

Consultant or Advisory Role: E.A. Roberts, Bristol-Myers Squibb.

Stock Ownership: None declared.

Honoraria: None declared.

Research Funding: None declared.

Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.