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Authors

Wollersheim, Susan K

Humphries, Romney M

Cherry, James D

et al.

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Serological Misdiagnosis of Acute Liver Failure Associated with Echovirus 25 Due to Immunological Similarities to Hepatitis A Virus and Prozone Effect

Susan K. Wollersheim,[a]* Romney M. Humphries,[b] James D. Cherry,[a] Paul Krogstad[a]

Department of Pediatrics, Division of Infectious Diseases, David Geffen School of Medicine at UCLA, Los Angeles, California, USA[a]; Department of Pathology and Laboratory Medicine, David Geffen School of Medicine at UCLA, Los Angeles, California, USA[b]

We describe a case of acute liver failure caused by echovirus 25 (E25) in a previously healthy 2-year-old boy. Initial serological studies were consistent with hepatitis A virus (HAV), with prozone phenomenon. The similarity of E25 to HAV may obscure accurate diagnosis in some cases of hepatitis.

Echoviruses are well-known causes of meningitis, skin rash, and respiratory illnesses (1). Echoviruses have also been identified as a cause of hepatitis and liver failure in some cases. Here, we describe a case of echovirus 25 (E25)-associated liver failure in which immunological cross-reaction and a prozone phenomenon initially led to a misdiagnosis of hepatitis A virus (HAV) infection.

A 2-year-old boy presented to an outside hospital with a 10-day history of vomiting. His skin had been jaundiced for 2 days, but he was afebrile and had no changes in mental status or stool habits. Initial lab results on day 10 of illness included an aspartate transaminase (AST) level of 3,202 U/liter, an alanine aminotransferase (ALT) level of 2,898 U/liter, and a total bilirubin level of 10.8 mg/dl. Serologic tests for hepatitis A virus IgM, hepatitis B virus surface antigen, hepatitis C virus IgG, hepatitis E virus IgG/IgM, cytomegalovirus (CMV) IgM, and parvovirus B19 IgG were all negative. He continued to have jaundice and intermittent vomiting and was seen for follow-up with subsequent laboratory findings of an AST level of 5,015 U/liter, an ALT level of 4,422 U/liter, and a total bilirubin level of 20.5 mg/dl. A liver biopsy was performed on the 22nd day of illness, with histological evidence of severe acute hepatitis with centrilobular necrosis. His initial clinical picture was consistent with acute HAV infection, despite a history of receiving one HAV vaccine dose 8 months prior to his illness and a positive total HAV antibody test.

Upon admission to our institution, and following consultation with the laboratory regarding the concern for false-negative HAV IgM, serial dilutions of the patient's serum were prepared in Advia Centaur diluent (Siemens, USA) and tested with the Advia Centaur hepatitis A virus IgM chemiluminescence assay. HAV IgM was nonreactive when neat and at 1:10, 1:50, and 1:100 dilutions, whereas the 1:500 and 1:1,000 dilutions were reactive, suggesting a prozone phenomenon. However, a serum enterovirus PCR test (Focus Diagnostics) was positive. A stool specimen was cultured on MRC-5, A549, and rhesus monkey kidney tissue culture cell lines and showed cytopathic effects consistent with enterovirus replication after 20 days of incubation. The presence of an enterovirus was confirmed using the Light Diagnostics panenterovirus detection reagent (Chemicon, Temecula, CA). Liver failure developed, and the patient underwent orthotopic liver transplantation (OLT) on day 39 of illness. Histological examination of his explanted liver pathology revealed massive hepatic necrosis with loss of normal hepatic architecture. He was discharged home 12 days postoperatively, in good condition.

Viral RNA was extracted from the stool viral culture and serum specimens, and seminested PCR amplification of and partial sequencing of the VP1 coding sequence were performed as previously described (2), and sequences were submitted to GenBank (see below). The VP1 stool and serum viral RNA sequences were most similar to the VP1 region of known E25 isolates. The serotype of this virus isolate was confirmed by neutralization assay in rhesus monkey kidney cells, using echovirus serotype 20- to 26-specific antisera, by a standard protocol (3). Neutralization of cytopathic effect (CPE) was evident only in the anti-E25 tubes. Acute- and convalescent-phase serum samples collected from the patient during weeks 3 and 12 of illness neutralized E25 at 1:2 and 1:256 (Fig. 1), respectively, providing additional confirmation of acute E25 infection. Stool viral cultures and plasma enterovirus PCR were negative on days 56 and 119 following the onset of illness.

E25 was associated with, and likely the cause of, fulminant hepatitis in this child. The patient was viremic with the same virus that he was shedding in his stool during his time of acute liver failure and demonstrated a rise in neutralizing antibody titers. In current *Picornaviridae* family taxonomy, E25 is classified within the *Enterovirus* genus and the *Enterovirus B* species, which also includes most pathogenic enteroviruses that circulate in the United States (1, 4). The National Enterovirus Surveillance System (NESS) ranked E25 as the 20th most common enterovirus in circulation from 1970 to 2005, accounting for 1.1% of all reports with a known serotype; its highest ranking was fifth for 1 year (5).

E25 is known to cause mild respiratory disease and skin rash in

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Address correspondence to Paul Krogstad, pkrogstad@mednet.ucla.edu.

* Present address: Susan K. Wollersheim, Department of Pediatrics, Georgetown University Medical Center, Washington, DC, USA.

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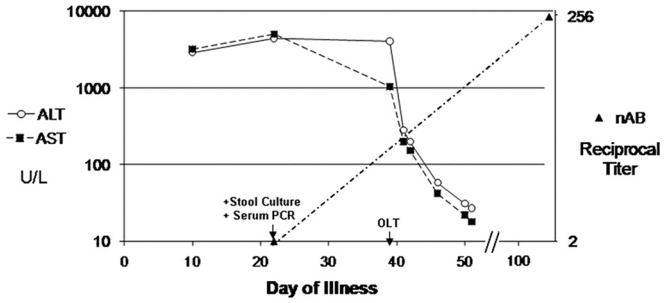

FIG 1 Time course of illness. Arrows indicate detection of echovirus 25 detection in stool culture and plasma PCR and the day of orthotopic liver transplantation (OLT). Left axis, transaminase values; right axis, echovirus 25 reciprocal neutralizing antibody titer (nAB).

infants, as well as meningitis in all ages (1); rare cases of E25 hepatitis have been described. Among these is one previous report of E25 being identified in the stool culture of a patient with acute hepatitis (6); this echovirus isolate was serologically indistinguishable from HAV by immune electron microscopy.

The majority of clinical immunoserology testing is done by automated methods, such as chemiluminescence, and is performed using a single serum dilution. In contrast, historically, serological studies were done by complement fixation (CF), immunofluorescence assays (IFA), or neutralization assays and involved serial dilutions of serum (7). As noted in our patient, the initial HAV IgM test was falsely negative due to a prozone effect. The frequency of false-negative results due to such a prozone effect is currently unknown. This potential problem can be overcome by doing all tests with two dilutions; however, this would be cost-prohibitive and is associated with regulatory challenges for the laboratory. In cases such as the one presented here, where the clinical index of suspicion for a viral etiology is high, physicians may consider requesting the laboratory to test several dilutions of serum, if possible, to aid with diagnosis. The antigenic similarity between E25 and HAV warrants further evaluation, as it is possible that the occurrence of hepatitis caused by E25 has been underestimated in the past. In addition, false-positive serologies may have confounded studies of the impact of HAV infection on the outcome of pregnancies and exacerbation of preexisting chronic liver diseases. Moreover, it is possible that routine immunization against HAV will lead to a reduction in circulation of E25, an unexpected beneficial effect of vaccination.

Nucleotide sequence accession numbers. Viral RNA sequences were submitted to GenBank under accession numbers JF297596.1 and JF297597.1.

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