

Brief Report

HEPATITIS C VIRUS-ASSOCIATED
FULMINANT HEPATIC FAILURE

PATRIZIA FARCI, M.D., HARVEY J. ALTER, M.D.,
ATSUSHI SHIMODA, M.D.,
SUGANTHA GOVINDARAJAN, M.D., LING C. CHEUNG, M.D.,
JACQUELINE C. MELPOLDER, B.Sc.,
RONALD A. SACHER, M.D., JAMES W. SHIH, PH.D.,
AND ROBERT H. PURCELL, M.D.

FULMINANT hepatic failure is a dramatic clinical syndrome characterized by massive necrosis of liver cells.¹ It is most often caused by hepatitis A virus and hepatitis B virus (HBV)²; whether hepatitis C virus (HCV) can cause it is still controversial.^{3,4} Among patients with non-A, non-B fulminant hepatitis, antibodies against HCV (anti-HCV) or serum HCV RNA were found in 40 to 60 percent in Japan^{5,6} and Taiwan,⁷ but in only 2 percent (range, 0 to 12 percent) in Western countries,⁸⁻¹³ with one exception: a recent study conducted in California reported a prevalence of 60 percent associated with low socioeconomic status and Hispanic ethnicity.¹⁴ Whether these discrepancies reflect geographic differences in the epidemiology of HCV infection or the pathogenicity of the prevalent viral strains is not known. Furthermore, because of the dramatic course of fulminant hepatic failure, in most patients only a single serum sample, often obtained late in the course of the disease, was studied.

In this report, we describe a patient with HCV-associated fulminant hepatitis in whom serial studies were done that provided a unique opportunity to establish a temporal association between the acquisition of HCV infection and the development of fulminant hepatitis and to define the clinical, virologic, and histologic profile of fulminant hepatitis C.

From the Hepatitis Viruses Section, Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases (P.F., A.S., R.H.P.), and the Department of Transfusion Medicine, Warren G. Magnuson Clinical Center (H.J.A., L.C.C., J.C.M., J.W.S.), National Institutes of Health, Bethesda, Md.; the Department of Pathology, Rancho Los Amigos Medical Center, Downey, Calif. (S.G.); and the Division of Clinical and Laboratory Service, Georgetown Medical Center, Washington, D.C. (R.A.S.). Address reprint requests to Dr. Farci at the Istituto di Medicina Interna, University of Cagliari, Via San Giorgio 12, 09124 Cagliari, Italy.
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CASE REPORT

A 68-year-old white man, who has been described previously,¹⁵ was admitted to Georgetown University Hospital in Washington, D.C., to undergo coronary-artery bypass grafting and aortic-valve replacement on March 21, 1990, about two months before the introduction of anti-HCV screening for blood donors. He was enrolled in a prospective study of post-transfusion non-A, non-B hepatitis being conducted at the National Institutes of Health at that time.¹⁶ He had no evidence of liver disease and had not received any transfusions in the previous six months (these were exclusion criteria for entry into the study).¹⁶ During surgery, the patient received a total of 39 units of red-cell concentrate, 15 units of platelets, 21 units of fresh-frozen plasma, and 2 units of plasma cryoprecipitate; no halothane was administered. He recovered quickly and was discharged seven days after surgery. He was readmitted four weeks later because of increasing malaise and nausea, at which time his serum alanine aminotransferase concentration was 4493 U per liter (it was 27 U per liter at the time of discharge). After readmission, icterus and progressive encephalopathy and coagulopathy developed, and the patient died in hepatic coma on the 11th hospital day, almost 7 weeks after surgery. His peak serum bilirubin concentration was 15 mg per deciliter (256 μ mol per liter), and the longest prothrombin time was 70 seconds.

The diagnosis of fulminant hepatitis C was based on the assessment of clinical,¹⁷ virologic, and histologic measures. None of the medications that were administered to the patient were known to be hepatotoxic. Serial serum samples were tested for the presence and amount of HCV RNA, the HCV genotype, the degree of genetic heterogeneity within individual isolates, and the immune response to HCV. None of the serum samples had detectable levels of hepatitis B surface antigen, IgM antibodies against hepatitis A or hepatitis B core antigens, or antibodies against cytomegalovirus, Epstein-Barr virus, or the human immunodeficiency virus. To exclude the possibility of coinfection with HBV and hepatitis G virus (HGV), all serum samples were tested for the presence of HBV DNA and HGV RNA by the polymerase chain reaction (PCR), and all were negative.

METHODS

Anti-HCV Testing

First- and second-generation enzyme immunoassays (Ortho Diagnostic Systems, Raritan, N.J.) were used to test for anti-HCV.

Detection, Titration, Genotyping,
and Sequencing of HCV RNA

Total RNA extracted from 100 μ l of serum by the guanidinium isothiocyanate-phenol-chloroform method¹⁸ was amplified by PCR with two sets of nested primers. The first set, derived from the 5' noncoding region,¹⁹ was used to investigate the course of HCV viremia, and the second set, from the *E1* and *E2* genes,¹⁹ including the hypervariable region 1,²⁰ was used to determine the HCV genotype²¹ and the degree of variation within individual viral isolates. The sensitivity and specificity of this nested-PCR assay have been reported previously.¹⁸ The concentration of HCV RNA in serum was measured by the branched-chain DNA test with the Amplex Chiron assay (Chiron, Emeryville, Calif.).²²

The PCR products derived from the *E1* and *E2* regions were analyzed both by direct sequencing, as previously described,¹⁹ and by sequencing of molecular clones. The amplified PCR products were also cloned into pGEM-T vector systems (Promega Biotech, Madison, Wis.), and 8 to 10 clones from each sample were sequenced with an automated DNA sequencer (model 373, Applied Biosystems, Foster City, Calif.) by a modified Sanger method.

Detection of Serum HGV RNA

Total RNA extracted from 100 μ l of serum was amplified by PCR with primers derived from the putative fifth nonstructural region of the HGV genome, as previously reported.²³ The PCR

products were analyzed by dot blot hybridization with a ^{32}P -labeled oligonucleotide probe.²³

Detection of Serum HBV DNA

Serum HBV DNA was extracted,²⁴ and PCR was performed with nested primers derived from the *preS1* gene and the major *S* gene of the HBV genome. The outer primer pair consisted of F1 (5'GGGTGAGCCCTCAGGCTCAGGGCA3'), starting at map position 1679, and F2 (5'GAAGATGAGGCATAGCAGCAGGAT3'), starting at map position 2254. The inner primer pair consisted of F3 (5'CCTCCTGCCTCCACCAAT3'), starting at map position 1730, and F4 (5'GAGGTTGGTGGTGGTGGTGGT3'), starting at map position 2166.

Liver-Biopsy Studies and Immunohistochemical Staining for the Detection of HCV Antigen in the Liver

Liver tissue obtained at autopsy was stained for an HCV antigen encoded by the fourth nonstructural (*ns4*) gene; the percentage of cells that were antigen-positive was determined. Deparaffinized tissue sections were pretreated with protease XXIV solution and rinsed in TRIS-hydrochloric acid buffer. The sections were incubated for 30 minutes with a mouse monoclonal antibody against protein expressed from the *NS4* region of the HCV genome (MA 292, Biogenex, San Ramon, Calif.). The sections were washed and then incubated with biotinylated antimouse antibody (HK 335-5K, Biogenex) for 30 minutes. The samples were rinsed, and a streptavidin-phosphatase label (HK 331-5K, Biogenex) was applied, followed by a rinse and application of 6-bromo-2-hydroxy-3-naphtholic blue substrate solution. Fifteen minutes later, the sections were rinsed again and coverslips were applied with mounting medium.

RESULTS

Serial serum samples were tested for the presence and concentration of HCV RNA and for anti-HCV.

HCV viremia was not detected before or 1 week after transfusion, but it was detected in the next available sample, obtained 5 weeks after transfusion, when the patient was readmitted to the hospital, and remained detectable until the patient's death, 11 days later (Fig. 1). Before surgery, anti-HCV antibodies were not detected in serum, but these antibodies were detected by a second-generation enzyme immunoassay one day before the patient's death. The level of HCV viremia, as measured by the branched-chain DNA test, increased in parallel with serum alanine aminotransferase concentrations to a peak value of more than 10^8 genome equivalents per milliliter, and then rapidly decreased below the level of sensitivity of this test, despite the fact that serum HCV RNA was continuously detected by PCR until the patient's death (Fig. 1). No HBV or HGV sequences were detected in any of the serial samples tested. Postmortem examination revealed areas of submassive and massive necrosis of the liver (Fig. 2A), and immunohistochemical staining for intrahepatic HCV NS4 antigen was positive in 20 percent of the residual liver cells (Fig. 2B).

The genotype of the HCV strain recovered from this patient was 1b. Direct sequencing of 525 nucleotides derived from the *E1* and *E2* genes showed that the two viral isolates recovered during the course of the disease, at week 5 and just before death 11 days later, were identical. Sequencing of molecular clones confirmed that the viral strain was the same but re-

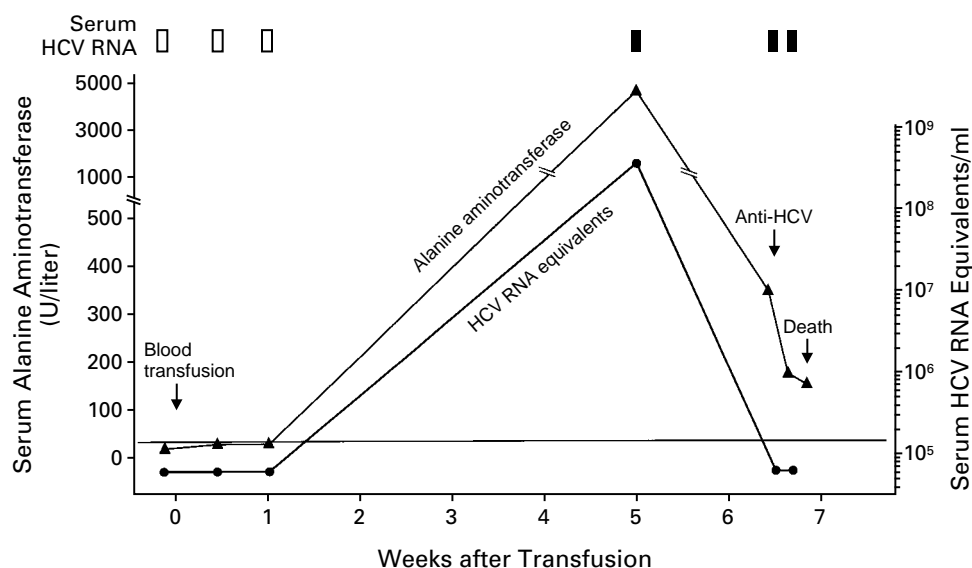
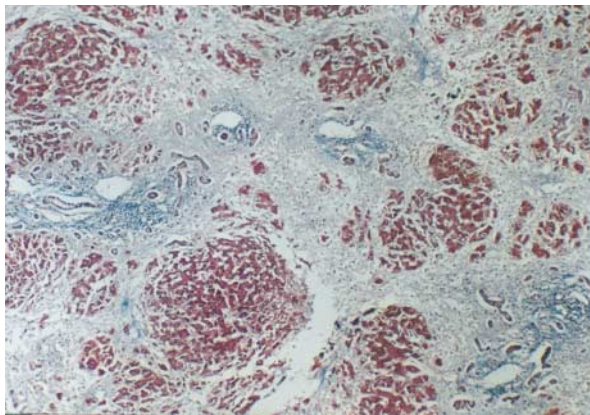
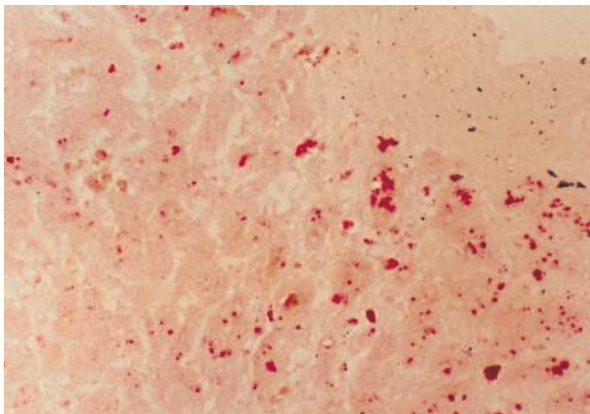


Figure 1. Biochemical, Serologic, and Molecular Profiles of Fulminant Hepatitis C in a Patient Who Acquired HCV Infection after Blood Transfusion for Heart Surgery.

Open bars indicate negative assays for serum HCV RNA by PCR, and solid bars positive assays. A logarithmic scale is used to show the titer of serum HCV RNA; the horizontal line indicates the limit of sensitivity of the assay. Anti-HCV was detected by a second-generation enzyme immunoassay one day before the patient's death.



A



B

Figure 2. Photomicrographs of a Liver-Biopsy Specimen from a Patient with Fulminant Hepatitis C.

Panel A shows areas of submassive and massive hepatic necrosis (Masson's trichrome stain, $\times 59$). The three portal tracts are close to collapsed reticulin, and there are no viable intervening hepatocytes. Islands of viable hepatocytes are seen in other lobules. Panel B shows positive (red) staining for HCV NS4 antigen within the cytoplasm of the residual liver cells on immunohistochemical analysis with a monoclonal antibody against HCV NS4 peptide ($\times 297$).

revealed the presence of genetic heterogeneity within each of the two isolates. Comparative sequence analysis of 18 clones showed the presence of 11 closely related viral variants, each differing from the consensus sequence by only one or two amino acids (data not shown).

DISCUSSION

The availability of serial serum samples from a patient in whom fulminant hepatic failure developed provided us with the opportunity to demonstrate a temporal association between HCV infection and the development of fulminant hepatitis. The appear-

ance of viremia, followed by seroconversion, unequivocally indicated the acquisition of primary HCV infection. The longitudinal analysis and molecular studies allowed us to rule out the etiologic role of other hepatitis viruses, including HGV, a recently described hepatitis agent.²³ In this patient, fulminant hepatitis C was associated with continuous replication of HCV. As previously documented in acute hepatitis C,¹⁸ the detection of serum HCV RNA was the earliest marker for the diagnosis of fulminant hepatitis C. In contrast, anti-HCV was detected only one day before the patient's death. This suggests that in fulminant hepatitis, because of the extremely rapid course of the disease, there may not always be sufficient time for the development of antibodies. The patient's persistent HCV viremia was a critical marker in the differential diagnosis of non-A, non-B fulminant hepatitis. On the basis of this finding, a negative test for HCV RNA in a patient with fulminant hepatitis makes it extremely unlikely that the patient has HCV infection, even in cases in which only a single serum sample is available for testing. By extension, we infer that the previously described cases of fulminant non-A, non-B hepatitis that tested negative for HCV by PCR were probably unrelated to HCV infection. Whether these cases were due to unidentified infectious agents or, as recently proposed,⁴ to noninfectious hepatotoxic agents is not known.

In our patient, fulminant hepatitis C was characterized by high levels of viremia. Although the pathogenetic mechanism of virally induced fulminant hepatic failure is not known, the extent of liver damage correlated with the magnitude of viral replication in the absence of detectable antibodies. In contrast, in patients with fulminant hepatitis B, HBV replication is barely detectable or is undetectable, and antibody titers are high.²⁵ This suggests that the mechanisms of fulminant liver injury differ in the two types of hepatitis. In viral isolates from our patient, we found a remarkably low degree of diversity, whereas in chronic hepatitis C there is considerable diversity.²⁶ This finding may reflect a lack of selective pressure on the viral population because there is insufficient time for the development of a specific immune response in this rapidly evolving syndrome.

Although the temporal association between the acquisition of HCV infection and the development of fulminant hepatitis in this patient suggests that HCV was the causative agent, we cannot exclude the role of nonviral factors. Nevertheless, the incidence of post-transfusion fulminant hepatitis associated with major surgical operations is exceedingly low.²⁷ Among more than 100 prospectively followed transfusion recipients in whom non-A, non-B hepatitis developed after major surgery, this patient was the only one in whom fulminant hepatitis developed.²⁷ Thus, it is very unlikely that intraoperative factors were responsible for the fulminant hepatic failure.

In summary, HCV can cause fulminant hepatic failure. The disease is characterized by continuous viral replication. The detection of serum HCV RNA by PCR is the earliest and most valuable marker for the diagnosis of fulminant hepatitis C.

Presented in part at the Annual Meeting of the American Association for the Study of Liver Diseases, Chicago, November 11-15, 1994.

REFERENCES

1. Bernuau J, Rueff B, Benhamou JP. Fulminant and subfulminant liver failure: definitions and causes. *Semin Liver Dis* 1986;6:97-106.
2. Sherlock S. Fulminant hepatic failure. *Adv Intern Med* 1993;38:245-67.
3. Lee WM. Acute liver failure. *N Engl J Med* 1993;329:1862-72. [Erratum, *N Engl J Med* 1994;330:584.]
4. Wright TL. Etiology of fulminant hepatic failure: is another virus involved? *Gastroenterology* 1993;104:640-3.
5. Muto Y, Sugihara J, Ohnishi H, Moriwaki H, Nishioka K. Anti-hepatitis C virus antibody prevails in fulminant hepatic failure. *Gastroenterol Jpn* 1990;25:32-5.
6. Yanagi M, Kaneko S, Unoura M, et al. Hepatitis C virus in fulminant hepatic failure. *N Engl J Med* 1991;324:1895-6.
7. Chu CM, Sheen IS, Liaw YF. The role of hepatitis C virus in fulminant viral hepatitis in an area with endemic hepatitis A and B. *Gastroenterology* 1994;107:189-95.
8. Wright TL, Hsu H, Donegan E, et al. Hepatitis C virus not found in fulminant non-A, non-B hepatitis. *Ann Intern Med* 1991;115:111-2.
9. Theilmann L, Solbach C, Toex U, et al. Role of hepatitis C virus infection in German patients with fulminant and subacute hepatic failure. *Eur J Clin Invest* 1992;22:569-71.
10. Feray C, Gigou M, Samuel D, et al. Hepatitis C virus RNA and hepatitis B virus DNA in serum and liver of patients with fulminant hepatitis. *Gastroenterology* 1993;104:549-55.
11. Liang TJ, Jeffers L, Reddy RK, et al. Fulminant or subfulminant non-A, non-B viral hepatitis: the role of hepatitis C and E viruses. *Gastroenterology* 1993;104:556-62.
12. Kuwada SK, Patel VM, Hollinger FB, et al. Non-A, non-B fulminant hepatitis is also non-E and non-C. *Am J Gastroenterol* 1994;89:57-61.
13. Sallie R, Silva AE, Purdy M, et al. Hepatitis C and E in non-A non-B fulminant hepatic failure: a polymerase chain reaction and serological study. *J Hepatol* 1994;20:580-8.
14. Villamil FG, Hu KQ, Yu CH, et al. Detection of hepatitis C virus with RNA polymerase chain reaction in fulminant hepatic failure. *Hepatology* 1995;22:1379-86.
15. Sacher RA, Melpolder JJ. Hepatitis C virus and fulminant hepatitis. *Ann Intern Med* 1991;115:984-5.
16. Koziol DE, Holland PV, Alling DW, et al. Antibody to hepatitis B core antigen as a paradoxical marker for non-A, non-B hepatitis agents in donated blood. *Ann Intern Med* 1986;104:488-95.
17. Trey C, Davidson CS. The management of fulminant hepatic failure. *Prog Liver Dis* 1970;3:282-98.
18. Farci P, Alter HJ, Wong D, et al. A long-term study of hepatitis C virus replication in non-A, non-B hepatitis. *N Engl J Med* 1991;325:98-104.
19. Farci P, Alter HJ, Govindarajan S, et al. Lack of protective immunity against reinfection with hepatitis C virus. *Science* 1992;258:135-40.
20. Weiner AJ, Brauer MJ, Rosenblatt J, et al. Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins. *Virology* 1991;180:842-8.
21. Bukh J, Purcell RH, Miller RH. At least 12 genotypes of hepatitis C virus predicted by sequence analysis of the putative E1 gene of isolates collected worldwide. *Proc Natl Acad Sci U S A* 1993;90:8234-8.
22. Alter HJ, Sanchez-Pescador R, Urdea MS, et al. Evaluation of branched DNA signal amplification for the detection of hepatitis C virus RNA. *J Viral Hepatitis* 1995;2:121-32.
23. Linnen J, Wages J Jr, Zhang-Keck Z-Y, et al. Molecular cloning and disease association of hepatitis G virus: a transfusion-transmissible agent. *Science* 1996;271:505-8.
24. Shih JW, Cheung LC, Alter HJ, Lee LM, Gu JR. Strain analysis of hepatitis B virus on the basis of restriction endonuclease analysis of polymerase chain reaction products. *J Clin Microbiol* 1991;29:1640-4.
25. Brechot C, Bernuau J, Thiers V, et al. Multiplication of hepatitis B virus in fulminant hepatitis B. *BMJ* 1984;288:270-1.
26. Martell M, Esteban JI, Quer J, et al. Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. *J Virol* 1992;66:3225-9.
27. Alter HJ. To C or not to C: these are the questions. *Blood* 1995;85:1681-95.

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