Marrow Transplantation From Hepatitis C Virus Seropositive Donors: Transmission Rate and Clinical Course

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Bone marrow transplant recipients are at risk for acquiring hepatitis C infection from the donated marrow. Twelve patients who were hepatitis C virus (HCV) RNA-negative pretransplant received marrow from anti-HCV seropositive donors. HCV RNA was present in the sera of seven of these recipients after marrow infusion; none cleared virus from the serum. All five recipients of marrow from anti-HCV seropositive donors remained free of HCV RNA in serum up to day 100. Abnormal serum aminotransferases were common in both HCV RNA-negative and HCV RNA-positive marrow recipients. One HCV-infected recipient developed marked elevation in aminotransferases after immunosuppressive drugs were stopped. We conclude that the presence of HCV RNA in the serum of marrow donors is an accurate predictor of HCV infection in marrow recipients. The acute infection was subclinical in all patients. The long-term risk of chronic hepatitis C virus infection in these patients remains to be determined.

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Hepatitis C Virus (HCV) is the predominant agent of transfusion-associated non-A, non-B hepatitis and carries a significant risk of chronic infection and cirrhosis in the normal host. There is evidence that HCV infection poses further risks for immunosuppressed patients, including bone marrow transplant (BMT) recipients. Marrow transplant recipients remain profoundly immunocompromised until BM engraftment occurs (day 15 to 25). Cellular immunity remains abnormal for 3 to 6 months posttransplant, and full reconstitution of humoral immunity may take months to years. Patients with chronic graft-versus-host disease experience further delays in immune reconstitution. Studies have shown that immunocompromised patients infected with HCV have a high incidence of chronic infection and rapid progression to cirrhosis. Furthermore, acute hepatitis B has been described in HCV-infected marrow transplant patients on withdrawal of immunosuppressive therapy.

Before routine screening of blood products for HCV antibodies, the prevalence of hepatitis C virus infection in BMT recipients was reported to be 10% to 20%. Since the institution of routine screening, the prevalence of transfusion-related HCV infection has fallen. However, the problem remains for potential marrow recipients because some were exposed to HCV before the advent of screening. Another potential source of HCV infection is the allogeneic marrow donor. We identified 12 marrow transplant recipients whose related donors tested positive for antibodies to HCV by a sensitive second-generation immunoblot assay. None had alternate HLA-matched family members available to donate marrow. We prospectively studied the recipients of these donor marrows in order to answer three questions: What is the risk of transmitting HCV from a seropositive marrow donor? Is the risk correlated with the presence of HCV RNA in serum as detected by the polymerase chain reaction? Does immunosuppression posttransplant influence the course of illness in HCV-infected marrow recipients?

MATERIALS AND METHODS

Patient selection. All marrow transplant recipients and their donors were screened before transplant for the presence of antibodies to HCV, as described below. We identified 12 anti-HCV seronegative patients who received marrow from anti-HCV seropositive donors between July 1991 and July 1993. In accordance with US Public Health Service guidelines, each marrow recipient gave informed consent to receive marrow from a known anti-HCV seropositive donor. Eight of the donor/recipient pairs were evaluated at the Fred Hutchinson Cancer Research Center (Seattle, WA). They are identified by Unique Patient Number (UPN), assigned at the time of transplantation. The remaining four patients were treated at Memorial Sloan-Kettering Cancer Center (New York, NY), Dana-Farber Cancer Institute (Boston, MA), the Comprehensive Cancer Center of Wake Forest University (Winston-Salem, NC), and City of Hope Medical Center (Duarte, CA), respectively. They are identified by center initials (MSK-I, DF-I, WF-I, and COH-I). Individual conditioning regimens and graft-versus-host disease (GVHD) prophylaxis are listed in Table 1. The day of marrow infusion is day 0.

Serologic testing for hepatitis viruses. Before transplant, all marrow transplant recipients and donors were screened for antibodies to HCV using a second-generation ELISA (Ortho Diagnostic System, Inc, Raritan, NJ). This ELISA uses antigens from the core region (c22-3) and the nonstructural NS-3 and NS-4 regions (c200, c100-3) of the virus. The test was performed and results were calculated according to the manufacturer’s instructions. Serum samples that tested positive by second generation ELISA were tested by second-generation recombinant immunoblot assay (RIBA) (Chiron Corp, Emeryville, CA) for antibodies to four recombinant HCV-encoded antigens (c100-3, 5-1-1, c33-C, c22-3). The test was per-

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Supported by Grants No. CA18029 and CA15704 from the National Institutes of Health, National Cancer Institute.

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formed according to the manufacturer's instructions. A specimen reacting with two or more antigens was considered positive by RIBA.

Polymerase chain reaction (PCR) for HCV RNA. All anti-HCV seropositive donors and their recipients had pretreatment serum tested for HCV RNA, with the exception of the donor for recipient DF-1 (this seropositive donor was tested for HCV RNA 2 months after his BM harvest). Sera from marrow recipients were serially tested for HCV RNA posttransplant up to day 100, or up to the time of death if death occurred before day 100. Marrow recipients who developed HCV viremia were tested beyond day 100 for persistence of viremia up to the time of last follow-up.

HCV RNA was detected by the nested PCR.16 One hundred microliters of serum was added to 400 µL of a 5 mol/L guanidinium thiocyanate solution and viral RNA was extracted by phenol and chloroform and precipitated with isopropanol using standard procedures.12 Reverse transcription was performed in a volume of 30 µL with 0.25 µmol/L of the right-hand outer primer and AMV reverse transcriptase at 42°C for 15 minutes. The reaction was stopped by treatment at 95°C for 5 minutes. The first PCR was performed in 100 µL (Buffer with 0.15 mol/L dNTPs, 0.2 µmol/L primers) for 40 cycles (94°C for 30 seconds, 55°C for 30 seconds, 72°C for 1 minute, with a final 72°C for 7 minutes). Five microliters of the product was added to 45 µL of new PCR buffer containing the internal concentric primers (0.5 µmol/L primers) and run for 25 cycles. Second-reaction PCR products were analyzed by gel electrophoresis on a 2% agarose gel. Equivocal results were assessed by Southern Blot using an internal viral primer marker specific for HCV and an internal primer in the cuspidine region.

RESULTS

Patient characteristics. Characteristics of marrow recipients and details of their preparative regimens and GVHD prophylaxis are listed in Table 1.

Results of pretransplant tests for hepatitis B and C viruses. Recipient COH-1 and marrow donors for UPN 6684, MSK-1, and DF-1 tested positive for anti-HBc. Hepatitis B virus markers were otherwise negative in all donors and recipients. Results of anti-HCV testing and HCV RNA by PCR are listed in Table 2. All marrow recipients were HCV RNA-negative by PCR before transplant.

Transmission of HCV. All seven recipients of marrow from HCV RNA-positive donors tested positive for HCV RNA in the first available serum sample posttransplant (range day 7 to day 36) (Table 2). No recipient of marrow from a seropositive, HCV RNA-negative donor became positive for HCV RNA posttransplant.

Clinical course in HCV-infected patients. Serumaminotransferase levels over time in HCV RNA-negative and HCV RNA-positive recipients are illustrated in graphic form in Figs 1 and 2, respectively. Of seven recipients who were infected by HCV from their donor marrow, only one (MSK-1) developed overt clinical hepatitis felt secondary to HCV. One patient (DF-1) developed marked AST elevation coincident with septicemia; both serum and liver tissue were positive for hepatitis B virus surface antigen. The five remaining HCV-infected marrow recipients had mild to moderate sepsis that responded to empiric antibiotics and antifungal treatment.

Assessment of liver disease. All marrow graft recipients underwent clinical and laboratory assessment of liver disease during the posttransplant period. Serum tests for aspartate aminotransferase (AST), bilirubin, and alkaline phosphatase were carried out at frequent intervals. Patients underwent liver biopsy if clinically indicated. Viral hepatitis C was diagnosed according to recently published histologic criteria.19 The diagnoses of GVHD and venocclusive disease of the liver were based on clinical and histologic evidence.30,31
Table 2. Pretransplant HCV Results and Liver Disease in Marrow Donors; Posttransplant HCV RNA, Liver Disease and Survival in Marrow Recipients. All Marrow Recipients Were HCV RNA-Negative Pretransplant

<table>
<thead>
<tr>
<th>Recipient UPN</th>
<th>ELISA</th>
<th>RNA</th>
<th>HCV RNA</th>
<th>Liver Disease</th>
<th>HCV RNA (d)</th>
<th>Acute/Chronic Hepatitis*</th>
<th>Status (d survived)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5777</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Chronic AST elevation</td>
<td>+</td>
<td>No/n/a</td>
<td>Deceased (154)</td>
</tr>
<tr>
<td>6199</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>CAH with cirrhosis</td>
<td>+</td>
<td>Yes/n/a</td>
<td>Deceased (136)</td>
</tr>
<tr>
<td>MSK-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Chronic AST elevation</td>
<td>+</td>
<td>Yes/yes</td>
<td>Deceased (481)</td>
</tr>
<tr>
<td>6968</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Chronic AST elevation</td>
<td>+</td>
<td>No/n/a</td>
<td>Deceased (154)</td>
</tr>
<tr>
<td>DF-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>History of IVDU; normal AST</td>
<td>+</td>
<td>No/no</td>
<td>Deceased (234)</td>
</tr>
<tr>
<td>COH-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Chronic AST elevation</td>
<td>+</td>
<td>No/yes</td>
<td>Alive (368)</td>
</tr>
<tr>
<td>7533</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Normal AST</td>
<td>+</td>
<td>No/n/a</td>
<td>Deceased (87)</td>
</tr>
<tr>
<td>6684</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Normal AST</td>
<td>(11, 70)</td>
<td>Yes/no</td>
<td>Alive (777)</td>
</tr>
<tr>
<td>6636</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Normal AST</td>
<td>(10, 26, 56, 87)</td>
<td>No/n/a</td>
<td>Deceased (96)</td>
</tr>
<tr>
<td>6986</td>
<td>+</td>
<td>IS</td>
<td>-</td>
<td>Normal AST</td>
<td>(8, 24, 81, 163)</td>
<td>No/no</td>
<td>Alive (640)</td>
</tr>
<tr>
<td>7209</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>Normal AST</td>
<td>(13, 39, 66, 94)</td>
<td>No/no</td>
<td>Deceased (179)</td>
</tr>
<tr>
<td>WF-1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>History of IVDU and hepatitis; normal AST</td>
<td>(20, 53, 122)</td>
<td>No/no</td>
<td>Alive (475)</td>
</tr>
</tbody>
</table>

Abbreviations: IVDU, intravenous drug use; AST, aspartate aminotransferase; CAH, chronic active hepatitis.
* Acute hepatitis, AST > 3 times upper limit of normal 6 to 12 weeks after exposure to HCV; chronic hepatitis, AST > 1.5 times upper limit of normal for greater than 6 months; n/a, unable to determine because of patient’s death.
† HCV RNA by PCR determined 2 months after bone marrow harvest.
§ HCV RNA-negative with biopsy-proven GVHD of the skin and lip.
$ Indeterminate (strongly positive reaction to single antigen, c22-3).

prednisone GVHD of the liver developed and a lip biopsy was later positive for GVHD. However, clinical GVHD remained mild and prednisone was tapered and was discontinued on day 100. Cyclosporine was continued until day 127, when the patient presented with fever and pulmonary infiltrates. She died of respiratory failure of unclear etiology on day 153.

UPN 6199 received methotrexate and cyclosporine for GVHD prophylaxis. She had mild venocclusive disease of the liver early posttransplant. Acute skin GVHD developed on day 28 and was associated with abnormal liver tests also consistent with GVHD (AST 113 U/L, alkaline phosphatase 261 U/L, bilirubin 1.5 mg/dL). Skin disease improved with maximum cyclosporine doses, but liver test abnormalities persisted. Bilirubin peaked at 21 mg/dL on day 69, at which time a liver biopsy showed partial central vein occlusion consistent with venocclusive disease and bile duct changes consistent with GVHD. Skin and lip biopsies also showed GVHD, and prednisone was added on day 77. The patient was maintained on immunosuppressive therapy until she was diagnosed with a pulmonary Aspergillus infection on day 130. She died of disseminated Aspergillosis on day 136.

Recipient MSK-1 received a T-cell-depleted marrow and was given antithymocyte globulin (ATG) and prednisone for graft rejection prophylaxis. Prednisone was tapered and discontinued on day 36. The patient had no evidence for liver disease until day 57, when AST increased to 289, eventually peaking at 1,790 on day 222 (see Fig 3). Serum HBsAg was negative. She remained asymptomatic until day 130, when symptoms of fatigue and intermittent arthralgias developed. A percutaneous liver biopsy sample obtained on day 177 showed chronic active hepatitis consistent with HCV infection. Fatigue and arthralgias eventually resolved, but the patient continued to have fluctuating AST levels in the 100 to 300 U/L range. On day 460 she was hospitalized with pulmonary infiltrates of unclear etiology. She developed progressive respiratory failure and died on day 481. (Of note, this patient had stable mixed chimerism in peripheral blood and BM, first documented on day 75.)

UPN 6968 received prednisone and FK 506 for GVHD prophylaxis. From day 18 through 84 he received α-interferon to prevent relapse. He had no clinical evidence for venocclusive disease or acute GVHD early posttransplant. AST became mildly elevated on day 21 and fluctuated over the next 4 months between 50 and 150 U/L. During this time serum bilirubin remained normal and alkaline phosphatase
was mildly elevated. Given that there was no clinical evidence for GVHD, prednisone was tapered and was discontinued on day 88. The patient remained on FK 506 until his leukemia relapsed on day 135. He sought no further treatment and died on day 154.

Recipient DF-1 had no clinical evidence for venoocclusive disease or acute GVHD posttransplant. His early posttransplant course was complicated by diffuse alveolar hemorrhage. He was treated with high-dose prednisone, which was tapered and discontinued on day 40. He also developed respiratory syncytial virus infection and was treated with ribavirin and respiratory syncytial virus Ig. On day 118 the patient was admitted with hypoxia and pulmonary infiltrates and was treated with high-dose steroids for probable bronchiolitis (he also received antibiotics for organisms isolated from sputum and bronchoalveolar lavage). Prednisone was rapidly tapered and was discontinued on day 137, but was resumed on day 167 for inflammatory lung disease possibly related to GVHD. AST was first elevated on day 18, fluctuated between 50 and 125 U/L through day 200, then began to increase further, peaking at 1,322 U/L on day 231. Liver biopsy on day 231 showed acute hepatitis with hepatitis B surface antigen detected on immunoperoxidase staining. Serum was also positive for HBsAg. After the liver biopsy he developed abdominal pain and was found to have toxic megacolon. He developed septicemia and expired on day 234.

Recipient COH-1 received methotrexate, cyclosporine, and prednisone for graft-versus-host disease prophylaxis. He had no evidence for liver disease early posttransplant. His posttransplant course was complicated only by prednisone-induced insulin-requiring hyperglycemia. His AST began to increase on day 34, and continues to fluctuate between 100 and 200 U/L. Bilirubin remains normal and alkaline phosphatase has been mildly elevated since day 44, peaking at 165 U/L on day 175. He has no signs or symptoms of hepatitis or GVHD. He continues on a slow taper of prednisone and cyclosporine for GVHD prophylaxis.
HEPATITIS C VIRUS AND MARROW TRANSPLANTATION

Fig 3. Serum AST over time in recipient MSK-1. Immunosuppressive regimen and HCV RNA results are indicated in the top of the figure.

UPN 7633 received cyclosporine and prednisone for GVHD prophylaxis. She had no clinical evidence for venoocclusive disease or GVHD early posttransplant. While tapering the prednisone dose, she developed gastrointestinal symptoms of nausea, vomiting, and diarrhea improved on high-dose prednisone (2 mg/kg/d) and cyclosporine. On day 73 she was hospitalized with fever. At this time AST was mildly elevated at 121 U/L (AST later peaked at 290 U/L on day 81, at which time alkaline phosphatase and bilirubin were 193 U/L and 2.8 mg/dL, respectively). Blood cultures grew Enterococcus, Staphylococcus, and Xanthomonas, and Aspergillus was cultured from her oropharynx and from stool. Despite antibiotic and antifungal therapy, her condition deteriorated and she expired on day 87. Postmortem examination showed fungal endocarditis with dissemination to the lungs, liver surface and parenchyma, ileum, and colon. In addition to scattered fungal lesions, liver histology showed a mild lymphocytic portal infiltrate with occasional biliary duct cell dropout (Fig 4). These findings were not sufficiently marked to make a diagnosis of HCV hepatitis or GVHD.

DISCUSSION

All donors whose serum was HCV RNA-positive transmitted virus to the recipients of their marrow, whereas no donor who was HCV RNA-negative transmitted virus. Our findings are consistent with studies that demonstrate that the presence of viremia detected by PCR is predictive of infectivity.25 Because marrow infusions generally contain a large volume of plasma, it is not surprising that all donors with documented viremia transmitted virus to their recipients. However, it is less certain whether HCV infection can be transmitted from a donor with evidence for past infection in the absence of viremia. Mononuclear cells may serve as a non-hepatic reservoir for replicating virus,3-25 raising the possibility that infusion of marrow lymphoid cells or peripheral mononuclear cells can transmit HCV. Although our numbers are small, we saw no evidence for cell-associated transmission of virus from HCV seropositive donors.

Three of our ELISA-positive marrow donors tested negative for anti-HCV by immunoblot confirming test (RIBA) and were negative for HCV RNA. These likely represent false-positive ELISA tests, as, in addition to testing negative by RIBA, none of these donors had risk factors for HCV or evidence of liver disease.26,27 One of the HCV RNA-negative donors tested positive for anti-HCV by confirmatory RIBA; a second HCV RNA-negative donor was RIBA-indeterminate. Although some studies suggest that a positive RIBA result accurately predicts HCV viremia and liver disease,26 others have shown that RIBA-confirmed seropositive patients are not universally viremic.27 The pattern in these two donors is consistent with resolution of HCV infection despite persistence of antibody,22,28 but may also represent intermittent viremia.28,29

Abnormal serum aminotransferases were common after transplant in both HCV-infected and noninfected marrow recipients (Figs 1 and 2). Abnormal serum liver tests post-transplant are frequently caused by venoocclusive disease, GVHD, drug toxicity, systemic infection, and hepatic fungal and viral infections.21 In four of the seven HCV-infected marrow recipients, another cause of liver test abnormalities could be implicated. Two patients (UPNs 5777 and 6199) had clinical GVHD, and in one patient this was confirmed on liver biopsy. (Although bile duct abnormalities can be seen in HCV-induced liver disease,20 this patient had biopsy-proven GVHD in two other sites and had cholestasis by serum liver tests consistent with GVHD of the liver.) One patient (UPN 7633) had fungal liver disease confirmed at autopsy; one patient (recipient DF-1) had biopsy-proven acute hepatitis felt secondary to HBV. Three patients had liver disease that could not be explained by other causes; two had mild AST elevations consistent with hepatitis C (UPN 6968 and recipient COH-1) and the third had an exacerbation of liver disease with biopsy-proven chronic active hepatitis C (recipient MSK-1).

Immunosuppression may have influenced HCV disease activity in our patients. Although HCV may cause liver disease via a direct cytopathic effect of the virus, there is also both clinical and immunological evidence for immune-mediated injury.20,21,30-34 Serum levels of markers of T-cell activation are significantly higher in patients with chronic active hepatitis C infection than in normal controls, and are higher during disease exacerbation than during remission.31 Furthermore, HCV-specific HLA class I restricted CD8+ cytotoxic lymphocytes have been demonstrated in both serum and liver of patients with chronic HCV infection.22-24 The onset of
liver disease in recipient MSK-I coincided with the discontinuation of prednisone, suggesting that an enhanced immune system contributed to the exacerbation of disease. This patient’s host marrow had been incompletely ablated by conditioning therapy, as she was found later to have mixed chimerism. It is possible that the continued presence of host marrow cells led to more rapid immune reconstitution. Acute hepatitis after immune reconstitution has been described in HCV-infected marrow transplant recipients,10,11 and has been reproduced in chimpanzee studies.12 Immunosuppression is associated with enhanced viral replication.13 We speculate that once the immune system is reconstituted, the resulting immune response to a large viral load can lead to hepatic injury.

The mild AST abnormalities in patients who remained on immunosuppressive therapy and the bland liver histology in two of these patients (UPNs 6199 and 7633) lend some support to the hypothesis that liver injury in HCV infection is immune-mediated. Immunosuppressive therapy may have subdued the hepatic inflammatory response to HCV. Mild hepatic inflammation is seen in similarly immunosuppressed liver transplant recipients with HCV viremia.15,16

The short-term morbidity of HCV infection in marrow recipients did not appear to be severe; clinical hepatitis developed in only one patient. However, overall mortality was higher in the HCV-infected recipients than in the noninfected group. Although we have no evidence that HCV infection contributed to these deaths, we can make no definitive statement regarding the effect of HCV infection on survival in these patients. The long-term risks of chronic hepatitis and cirrhosis remain uncertain.

In conclusion, we found that the risk of transmission of HCV by seropositive marrow donors was limited to those donors who were viremic as assessed by PCR. PCR was thus a more effective test of HCV transmissibility than ELISA with RIBA confirmation. Therefore, we recommend testing anti-HCV seropositive, RIBA-confirmed marrow donors for HCV RNA in serum. If a donor tests positive for HCV RNA, another HLA-matched sibling or related donor should be used. If no alternate HLA-matched, related donor is available, the marrow recipient must be counseled that he or she is very likely to become infected with HCV. Although the acute infection is expected to be mild or subclinical, chronic HCV infection is likely to develop and may lead to cirrhosis over several years to decades. Alternatively, the transplant could be performed using autologous marrow or peripheral blood stem cells, HLA-mismatched family donor marrow, or unrelated donor marrow. However, the short-term morbidity of tumor relapse or acute GVHD appears greater than the morbidity associated with transplantation of marrow from a hepatitis C-infected HLA-matched sibling donor. Finally, if BMT can safely be delayed for several months, one could treat the donor with α-interferon and harvest the marrow if and when serum HCV RNA becomes negative. However, no data are available regarding this approach.

REFERENCES


Marrow transplantation from hepatitis C virus seropositive donors: transmission rate and clinical course

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