Prolonged Enteroviral Infection in a Patient Who Developed Pericarditis and Heart Failure After Bone Marrow Transplantation

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We describe a patient who developed heart failure and pericarditis after bone marrow transplantation for a hematologic malignancy. The patient died of heart failure complicated by pneumonia. Despite extensive surveillance, an infectious cause for the heart failure was not found while he was alive. In addition, cultures of specimens obtained at autopsy did not reveal a cause for the heart failure. Enterovirus was detected by the polymerase chain reaction (PCR) in two samples of pleural fluid that were obtained 21 days apart while he was alive. After the patient died, enteroviral RNA was also detected in his lungs, liver, and spleen, indicating a generalized infection. Analysis of the PCR products revealed sequences sharing close homology with the coxsackie B–like group of enteroviruses. In addition to reporting this case, we review the literature regarding enteroviral infections after transplantation.

Viral infections occur frequently after bone marrow transplantation (BMT) and are an important cause of morbidity and mortality. The main group of viruses that are responsible for infection after BMT are the herpesviruses, which may either be transmitted by the graft or cause a clinically manifest reactivation of infection [1]. Enteroviruses, which comprise the polioviruses, coxsackie viruses, and echo viruses, have seldom been reported to cause severe infections after transplantation. Herein, we describe a patient with heart failure and pericarditis that developed after he underwent BMT. No virus could be cultured, but enteroviral RNA was found in his pleural fluid, lungs, liver, and spleen with use of PCR.

Case Report

A 22-year-old man was admitted to the hospital for autologous BMT. His medical history was remarkable for Hodgkin's disease (stage III B), which was diagnosed in 1990 and partially responded to chemotherapy with MOPP (mechloretamine, vincristine, procarbazine, and prednisone). A complete remission was achieved only after he underwent total nodal irradiation. The disease recurred within 1 year, and the patient was referred to our hospital. Second-line chemotherapy with M-CAV-CIEC (methotrexate, cyclophosphamide, Adriamycin [doxorubicin], vinblastine, carmustine, etoposide, chlorambucil) was started in March 1992 but was interrupted because of a febrile episode that was associated with anemia, thrombocytopenia, and a pleural effusion of unknown origin. Third-line therapy with chlorambucil, bleomycin, vincristine, and prednisone was initiated because progression of the disease was suspected. In December 1992, a major partial remission was achieved.

The patient received a conditioning regimen that consisted of carmustine, cyclophosphamide, and etoposide before undergoing BMT. Autologous bone marrow was reinfused on 21 January 1993. On this day, a prophylactic regimen was started with oral co-trimoxazole (1,920 mg twice weekly), oral ciprofloxacin (2.5 g/d), oral acyclovir (400 mg q.i.d.), and amphotericin B lozenges (10 mg q.i.d.). Six days later a pleural rub was heard, but findings on a repeated radiograph were unchanged in comparison to those on a radiograph obtained 15 days earlier on admission. On day 10, fever (temperature, >39°C) developed, and therapy with iv ceftazidime (2 g t.i.d., the agent routinely administered under such circumstances) was started. Blood cultures yielded Staphylococcus haemolyticus, and therapy with iv teicoplanin (starting dose of 800 mg on the first day, 400 mg/d thereafter) was instituted; later, iv vancomycin (500 mg q.i.d.) was administered because his fever persisted. A pleural effusion developed, from which S. haemolyticus was also isolated. Therefore, iv rifampin (600 mg/d) was added to the antibiotic regimen. At the same time, treatment with iv fluconazole (200 mg/d) was started because Candida albicans was isolated from his mouth.

Eighteen days after undergoing BMT, the patient developed heart failure due to pericarditis with pleural effusion. Repeated cultures of pleural fluid samples (for bacteria, fungi, and viruses) remained negative. The pericardial fluid was cultured only for bacteria and fungi, and the results were also negative. Treatment consisted of antibiotics (iv vancomycin, rifampin, and ceftazidime and oral fluconazole [150 mg/d]) and gradual dehydration because of persistent exudative pericarditis and pleural effusion. From day 40 until day 53 after BMT, intravenous prednisone (60 mg/d) was added to the regimen for management of the pericarditis; after 1 week, this dose was tapered, and therapy with prednisone was stopped on day 53. The patient
needed frequent thrombocyte transfusions because he developed hemorrhagic cystitis as a complication of the high dose of cyclophosphamide he had received during conditioning. Reconstitution of the bone marrow (neutrophils in blood, >0.5 x 10^9/L) was achieved on day 49 after BMT.

On day 47, therapy with all intravenous antibiotics was discontinued. Because of the patient's impaired immune status, prophylactic therapy with oral ciprofloxacin and acyclovir was continued. His clinical condition temporarily improved, but on day 59 after BMT, a low-grade fever returned, and his condition deteriorated. All bacterial cultures performed during this febrile episode remained negative. On 28 March 1993, 9 1/2 weeks after undergoing BMT, the patient died of respiratory failure.

At autopsy, bilateral pleural effusions and pulmonary edema were observed. Fibrinous, proliferative, hemorrhagic pericarditis was present, and the heart was slightly dilated but of normal weight. Terminal cardiomyopathy was diagnosed by means of an enzymatic reaction that revealed a diffuse loss of lactate dehydrogenase from myocardial tissue. Furthermore, ascites, marked atrophy of the adrenal glands, and severe hemorrhagic ureteritis were present. Permission to autopsy the CNS was denied by the patient’s family.

Microscopic examination of the myocardium showed local fibrosis. A small inflammatory infiltrate consisting of lymphocytes was found in the left ventricle. A partly resolving, partly organizing pneumonia was observed on histopathologic examination of the lungs, and Lactobacillus jensenii was recovered from a culture of lung tissue. Iron deposits were seen in the liver and spleen. The bone marrow was normocellular as well as normocytic, with marked siderosis. There were no signs of recurrence of Hodgkin's disease. Viral cultures were negative. We concluded that respiratory failure was the cause of death.

**Laboratory Methods**

**Serology.** Before undergoing transplantation, the patient was routinely screened for antibodies to hepatitis B virus and hepatitis C virus, HIV, cytomegalovirus (CMV), herpes simplex virus 1 and herpes simplex virus 2, variella-zoster virus, Toxoplasma gondii (by ELISA), and Epstein-Barr virus (by indirect immunofluorescence). After the onset of his pleural symptoms, CF tests were performed to detect antibodies to adenoviruses; respiratory syncytial virus; influenza virus A and influenza virus B; coronaviruses; parainfluenza virus 1, parainfluenza virus 2, and parainfluenza virus 3; enteroviruses; CMV; Mycoplasma pneumoniae; Chlamydia psittaci; and Coxiella burnetii. In addition, ELISAs for IgG, IgM, and IgA antibodies to enterovirus [2], CMV, and *M. pneumoniae* were performed.

**Isolation of viruses.** On days 12, 9, 6, and 2 before BMT and on days 1, 5, 8, and 12 after BMT, throat washings were routinely cultured for viruses; thereafter, viral cultures were performed when an infection was clinically suspected. Virus isolation was performed on human embryonal lung fibroblasts, tertiary cynomolgus monkey kidney cells [3], and Hep-2 (human laryngeal carcinoma) cells. Cultures were maintained for 4 weeks. Isolates from cultures of stool and cultures of autopsy samples were blindly repassaged after 2 weeks. CMV cultures were performed in shell vials and read after 48 hours by means of immunofluorescence with a monoclonal antibody to early antigen. For detection of enterovirus, mice (a single litter of 1-day-old Swiss outbred mice) were inoculated by combined intracerebral and intraperitoneal injection of samples, as described by Melnick et al. [4]. The mice were killed after 14 days; homogenized samples of their brains and torsos were inoculated into a second litter whose brains and torsos were also tested for enterovirus by means of PCR.

**PCR.** Primers were selected in the 5' nontranslated region, which is highly conserved among enteroviruses. The primers, the extraction of RNA, reverse transcription, and the PCRs have been described before [5]. Samples were run in duplicate. RNA and cDNA from coxsackievirus B3 (Nancy strain) were used as positive controls. Distilled water was tested in duplicate as a negative control. A PCR with β-actin primers was used as an internal control for the quality of the RNA extracted from the specimens. PCR testing was also performed for detection of encephalomyocarditis virus and *M. pneumoniae* [6, 7]. A positive finding was confirmed by repeating the PCR, starting with the original clinical sample. Standard precautions to avoid contamination were followed [8]. The PCR product was directly sequenced using the AmpliCycle sequencing kit according to the instructions of the manufacturer (Perkin & Elmer, Roche Molecular Systems, Branchburg, NJ). DNA sequence alignment studies were performed, with use of the European Molecular Biology Laboratory database (Heidelberg, Germany) and the sequence analysis software package of the Genetics Computer Group of the University of Wisconsin implemented on a VAX computer.

**Results**

Routine screening tests for hepatitis viruses and HIV were negative. Our patient had antibodies to the different herpesviruses, but there was no evidence of an active infection at the time he underwent BMT (serologies for IgM and IgA antibodies were negative). He had no antibodies to *T. gondii*. Serologies for respiratory pathogens, enteroviruses, and CMV were performed on days 13, 19, 33, 48, and 63 after BMT. High titers or significant rises in titers were not observed. CF titers of antibodies to enteroviruses ranged between 1:8 and 1:16 for all samples. ELISAs were negative for IgA and IgM antibodies and showed no increases in IgG antibody levels. Repeated attempts to isolate viruses from the patient's throat, stool, and pleural fluid yielded negative results, and repeated buffy-coat cultures for CMV were also negative. In addition, viral cultures of autopsy specimens from the heart, spleen, liver, and lungs remained negative (table 1).

PCRs with enterovirus-specific primers performed on pleural-fluid samples obtained on days 12 and 33 after BMT were positive, with consistent results for all duplicate reactions. The
Table 1. Detection of enterovirus in a patient who developed peri- carditis and heart failure after bone marrow transplantation.

<table>
<thead>
<tr>
<th>No. of days after transplantation</th>
<th>Specimen tested</th>
<th>Mouse inoculation</th>
<th>PCR of specimen from patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Throat washings</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>5</td>
<td>Throat washings</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>Throat washings</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>Throat washings</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>12</td>
<td>Pleural fluid</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>Feces</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>19</td>
<td>Buffy coat</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>33</td>
<td>Pleural fluid</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>38</td>
<td>Feces</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>64</td>
<td>Buffy coat</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>67</td>
<td>Lung</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Liver</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Spleen</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Myocardium</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

NOTE: ND = not done, + = enterovirus (or enterovirus RNA) detected; – = no enterovirus detected.

* Tissues cultures of all samples were all negative (theuffy coat was cultured only for cytomegalovirus).

Discussion

Our patient developed heart failure after undergoing BMT. Enterovirus was detected in several organs by PCR but not by culture. However, enterovirus was not found in his heart; this may be due to a sampling error [9]. The heart failure might have been secondary to persistent pericarditis with effusion or to the extensive irradiation and chemotherapy given for inducing remission of Hodgkin’s disease. Nevertheless, detection of results of repeated testing were the same. Enterovirus-specific RNA was detected by PCR in autopsy specimens of the lungs, liver, and spleen but not in the heart muscle (table 1). PCR failed to detect M. pneumoniae or encephalomyocarditis virus. PCR performed with β-actin primers was positive for all samples. Because of the discrepancy between PCR results and the results of viral cultures, samples were inoculated in newborn mice in an attempt to isolate an enterovirus (table 1). None of the mice became ill or died, and PCR results for samples from killed mice (brains and torsos) were negative.

The enterovirus-specific PCR product in the 5' nontranslated region was sequenced, and the sequence was aligned to all enteroviral sequences available (figure 1). The PCR fragment had a sequence that showed the closest homology with coxsackievirus B3 (table 2), indicating that the virus belongs to the coxsackie B-like group of enteroviruses.

![Homology between sequences of enterovirus detected in a patient (PAT) and known sequences of coxsackieviruses A and B (CA and CB), echoviruses (EV), swine vesicular disease virus (SVDV), and polioviruses (PV). A total of 153 nucleotides were compared. The patient developed pericarditis and heart failure after bone marrow transplantation.](image-url)
Enteroviral Infection After BMT

viral RNA in several organs (the spleen, liver, and lungs) implies that the infection was generalized. A serological response to enterovirus infection was not observed; this lack of response might be due to delayed recovery of lymphocyte function [1]. On the other hand, these enterovirus-specific tests are based on cross-reactive serological responses and inherently lack sensitivity. By using both a CF test and ELISA, we can detect 70%—80% of acute enteroviral infections caused by a variety of serotypes [2].

The other pathogens that were isolated during the course of our patient’s illness or at autopsy could not have caused his heart failure. After the sepsis due to S. haemolyticus was treated, bacterial cultures of pleural fluid, pericardial fluid, and blood samples obtained while he was alive were consistently negative. L. jenseni was the only organism recovered after he died. Lactobacillus species sometimes cause endocarditis [10]; however, there were no signs of endocarditis in our patient, and L. jenseni was isolated only from his lungs.

Enteroviruses, particularly the coxsackie B viruses, are important causes of pericarditis and myocarditis. In healthy individuals, such complications usually have a benign course [11]. In neonates and in patients with agammaglobulinemia, however, enteroviruses can cause chronic, fatal infections such as myocarditis, hepatitis, and meningoencephalitis [12, 13]. Severe enteroviral infections do not occur in patients with impaired cellular immunity. The results of a search with use of MEDLINE suggest that indeed only a few cases of severe enteroviral infection have been reported in transplant recipients [14—18]. An outbreak of coxsackie A1 gastroenteritis in a BMT unit was found to be associated with significant mortality [14, 15].

More recently, a fatal case of disseminated infection due to echovirus 11 and CMV after BMT was reported [16]. This patient also had pericarditis and myocarditis. Severe combined infections with CMV and a coxsackie B virus [17] or with CMV and hepatitis A virus [18] have also been observed after transplantation of solid organs. It might be that coinfection with CMV, which has immune-modulating properties [19], played a role in the severity of these infections. In our patient, no evidence of active CMV infection was found.

Isolation of enteroviruses can be difficult in some cases. Enteroviral RNA has been detected in culture-negative samples of CSF during both acute episodes of meningoencephalitis as well as chronic episodes of the infection [20, 21]. Clinical observations with regard to agammaglobulinemic patients support the fact that this is not an uncommon problem [13]. It may be that in such patients, the virus is at least partially neutralized by antibody present in the gamma globulins that are administered. Our patient did not receive gamma globulin preparations. However, he did receive frequent transfusions of RBCs and platelets. Between days 36 and 50 after BMT, 13 units of plasma were also given. These blood products may have contained antibodies that hampered isolation of the virus. Another explanation may be that some enteroviruses fail to grow in the cell lines and mice that are routinely used for isolation.

Finally, it has been reported that enteroviral RNA can be detected in patients with chronic diseases such as dilated cardiomyopathy, postpoliomyelitis syndrome, polymyositis, and chronic fatigue syndrome in the absence of positive viral cultures. It has been suggested that enteroviral RNA can persist in patients with these chronic medical conditions, but the persistence of the virus is still a matter of debate [22, 23]. We believe that our patient had an active infection from which he was unable to recover because of impaired immunity.

The case described in this paper confirms the fact that enteroviruses may cause prolonged infections in patients who undergo BMT. Although the repeated finding of enteroviral RNA as the sole positive result suggests that there was a causal relationship between our patient’s pericarditis and heart failure and the virus, this was not formally proven. However, this finding confirms that enteroviral infections may remain undiagnosed unless molecular techniques are applied.

References

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