Risk Factors for Anaerobic Bloodstream Infections in Bone Marrow Transplant Recipients

Rebecca L. Lark,1 Shelly A. McNeil,1 Kristi VanderHyde,2 Zehra Noorani,4 Joseph Uberti,2 and Carol Chenoweth1,3

Divisions of 1Infectious Diseases and 2Hematology-Oncology, Department of Internal Medicine, and 3Department of Infection Control and Epidemiology, University of Michigan Health System, Ann Arbor, and 4Department of Internal Medicine, St. Joseph Mercy Oakland, Pontiac, Michigan

The incidence of anaerobic bloodstream infections (BSI) in patients who underwent bone marrow transplantation (BMT) recently increased at our institution. A retrospective case-control study of patients undergoing BMT from January 1995 through December 1998 was performed to determine the microbiological characteristics, epidemiology, and outcome of anaerobic BSI and to identify independent risk factors for infection. Anaerobic BSI occurred in 23 patients, for a rate of 4 BSIs per 100 BMT procedures, and it accounted for 17% of all BSIs that occurred during the study period. Infection occurred at a mean (± standard deviation) of 7 ± 4 days after BMT and 7 ± 5 days after the onset of neutropenia. Fusobacterium nucleatum was the most frequently isolated pathogen (in 17 patients), followed by Leptotrichia buccalis (in 4), Clostridium septicum (in 1), and Clostridium tertium (in 1). Two case patients (9%) died. Severity of mucositis was an independent predictor of anaerobic BSI (odds ratio, 4.4; P = .01). Controlling mucositis is critical for the prevention of anaerobic BSI in this patient population.

Bone marrow transplantation (BMT) has become a successful therapy for many terminal diseases. However, infectious complications remain a major cause of morbidity and mortality in patients who undergo BMT [1, 2]. These patients are at particularly high risk for bacterial infection during the early posttransplantation period as a consequence of intensive conditioning regimens that result in profound granulocytopenia and the loss of normal physical barriers (e.g., mucous membranes) [2, 3]. Bloodstream infections (BSI) are among the most common bacterial infections in this population. We recently identified an increase in the rate of anaerobic BSI among patients who had undergone BMT at our institution. Therefore, we performed an investigation to determine the microbiological characteristics, epidemiology, and outcome of such infections and to identify risk factors for such infections. We were particularly interested in the potential impact of mucositis severity on the development of anaerobic BSI.

PATIENTS AND METHODS

Setting. The University of Michigan Medical Center is an 800 adult-bed referral center with a 20-bed medical intensive care unit. The hospital supports an active BMT program, and 611 bone marrow transplants were performed at our institution from January 1995 through December 1998.

Case definition and ascertainment. A “case patient” was defined as any patient who underwent BMT from 1 January 1995 through 31 December 1998 and who developed a primary BSI as a result of an obligate anaerobe during the time from initiation of induction chemotherapy until 30 days after transplantation. The Centers for Disease Control and Prevention’s definition for primary BSI was used [4]. Case patients were identified by review of infection control records, medical...
records, microbiological data, and the BMT database. An incidence rate, defined as the number of anaerobic BSIs per 100 BMT procedures performed, was calculated for each year of study and for the preceding 5-year period (1990–1994).

**Case-control study.** A matched, retrospective, case-control study was performed to identify risk factors for infection. For each case patient, 2 control patients were selected and matched according to the type of BMT (i.e., allogeneic or autologous). Matched control patients were chosen from among the cohort of patients who underwent BMT during the 4-year study period and who had no cultures of blood samples that tested positive for anaerobic bacteria. Data were abstracted from medical records, and case patients and control patients were characterized with regard to demographic characteristics and potential risk factors. Risk factors were evaluated at the time of admission until the occurrence of anaerobic BSI for case patients and during a matched period of observation for control patients.

The following baseline information was collected for each patient: age, sex, underlying disease, and type of BMT (i.e., allogeneic or autologous). Allogeneic transplants were further characterized as either related or matched-unrelated donor transplants. In addition, the following information was obtained for each patient: the date of BMT, chemotherapy regimen, central venous catheter use (type of catheter and duration), catheter-related complications (e.g., thrombosis), severity of mucositis, severity and duration of diarrhea, duration of neutropenia, development of graft versus host disease (GVHD), invasive procedures performed (e.g., surgery or endoscopy), presence of *Clostridium difficile* colitis, presence of other infectious complications, duration of hospital stay, and outcome (i.e., discharge or death). Stop and start dates were recorded for antibacterial, antiviral, and antifungal agents that had been given either as prophylaxis or as treatment. Dosage and duration of therapy with any immunosuppressive medications (e.g., corticosteroids, tacrolimus) were noted. For case patients, pertinent clinical parameters and laboratory data from the day of onset of infection were recorded. These included the patient’s blood pressure, highest temperature (or lowest temperature, if the patient was hypothermic [temperature, <36.0°C]), WBC count, and serum creatinine level.

Neutropenia was defined as an absolute neutrophil count <500 cells/mm³. Mucositis scores for each patient were obtained from the physicians’ daily progress notes. The Southwest Oncology Group grading system was used to evaluate the severity of mucositis, and these scores ranged from 1 (mild) to 4 (life-threatening). Nursing flow sheets were reviewed to assess diarrhea, which was defined as ≥300 mL of loose stool in a 24-h period. The severity of diarrhea was graded from 0 (none) to 4 (hemorrhagic dehydration), according to the classification system of the World Health Organization [5]. The diagnosis of *C. difficile* colitis was made if a patient had diarrhea and a stool toxin assay result that was positive for *C. difficile*. Patients were considered to have GVHD if it had been documented by histopathologic examination or if empirical treatment with high-dose steroids had been given on the basis of clinical criteria consistent with GVHD.

As part of standardized protocols, all patients received antimicrobial prophylaxis that began at the time of the conditioning regimen. This prophylaxis included acyclovir (if herpes simplex virus antibodies were present in serum), fluconazole, and a quinolone (i.e., norfloxacin or ciprofloxacin). In addition, patients were prescribed daily antiseptic oral rinses during the period of neutropenia. Any patient who developed a fever while neutropenic had 2 or more sets of blood samples drawn for culture and was immediately started on intravenously administered antimicrobial therapy. Most patients received cefazidime and vancomycin as empirical treatment.

**Microbiological analysis.** Blood samples were drawn for culture during the study period at the discretion of the clinical teams; most samples were drawn because of the presence of fever and neutropenia. All blood samples that were drawn for culture were inoculated onto media for processing on the Bac T/Alert System (Organon Teknika). A set of blood cultures consisted of both aerobic and anaerobic FAN bottles, which were each inoculated with 5–10 mL of blood. Cultures were incubated at 36°C until flagged as positive or for 5 days, and no blind subcultures were performed. After growth in the anaerobic culture bottle, isolates were subcultured onto laked blood agar, phenylethyl alcohol agar, and kanamycin-vancomycin agar for incubation in an anaerobic chamber. Aerobic sheep blood agar plates were inoculated and incubated at 35°C in a CO₂ chamber. Identification of anaerobes was performed by means of standardized techniques [6].

**Statistical analysis.** The primary outcome was the development of an anaerobic BSI. Univariate analysis was performed by means of conditional logistic regression with single variables. Multivariable conditional logistic regression analysis was then performed by significant variables from the univariate analysis and potential confounding variables. Estimates of relative risk, expressed as OR, and 95% CIs were calculated. Tests of significance were 2-tailed, and *P* values of <.05 were considered significant. Statistical analysis was performed by use of SPSS for Windows, version 9.0 (SPSS), and Stata, version 6.0 (Stata Corporation).

**RESULTS**

**Description of cases.** During the 4-year study period, 23 episodes of anaerobic BSI were identified in 23 patients. This yielded a rate of 4 anaerobic BSIs per 100 BMT procedures performed. The incidence of anaerobic BSI increased from 0.64 BSIs per 100 BMT procedures during the period of 1990–1994.
Figure 1. Incidence of bloodstream infections (BSI) among patients who underwent bone marrow transplantation (BMT) at the University of Michigan, January 1990–December 1998.

to 6.0 BSIs per 100 BMT procedures in 1996 (figure 1). The rate of anaerobic BSI subsequently decreased to 4.7 BSIs per 100 BMT procedures in 1997 and 2.2 BSIs per 100 BMT procedures in 1998.

Of note, anaerobic infections accounted for 17% of all BSIs that occurred in this population during the study period. Anaerobic BSI developed at a mean (± SD) of 7 ± 4 days after BMT; 17 infections occurred in patients who had undergone allogeneic BMT (8 patients with related donors and 9 with matched-unrelated donors) and 6 infections occurred in patients who had undergone autologous BMT. These infections occurred after a mean duration (± SD) of 7 ± 5 days of neutropenia and 4 ± 3 days of mucositis. All patients had neutropenia at the time of infection, with a mean WBC count of 214 cells/mm³ (range, 100–500 cells/mm³). The absolute neutrophil count could not be calculated because a WBC differential was not performed when the total WBC count was <500 cells/mm³. Five patients (22%) had hypotension while they had bacteremia; this included 3 patients who underwent allogeneic BMT and 2 patients who underwent autologous BMT. All case patients received ceftazidime and vancomycin for the empirical treatment of neutropenic fever.

Microbial etiology. The organisms that were isolated included *Fusobacterium nucleatum* in 17 patients (74%), *Leptotrichia buccalis* in 4 patients (17%), *Clostridium tertium* in 1 patient (4%), and *Clostridium septicum* in 1 patient (4%). The overwhelming majority of infections (87%) were caused by a single organism. *Fusobacterium nucleatum* was isolated from each of the 3 patients who had polymicrobial infections, which involved viridans streptococci in 2 patients and *Haemophilus parainfluenzae* in 1 patient.

All anaerobic organisms that were identified grew only in the anaerobic blood culture bottles, except for the cultures of blood samples obtained from 2 patients, in which *Clostridium* species grew in both the aerobic and anaerobic bottles. Blood culture bottles turned positive for anaerobes after a mean duration of 42.7 h (range, 11.2–93.6 h) of incubation. Fourteen of the 23 case patients had only a single set of positive results of blood cultures. The remaining 9 patients had 2 or 3 sets of positive results of cultures of blood samples. Repeated positive blood culture results were not identified on subsequent days for any patient, with the exception of 2 patients whose cultures tested positive for *Clostridium* species on the day after their first positive culture result.

Case-control study. The demographic data for case patients and control patients are presented in table 1. The mean age of both case patients and control patients was 43 years, and the majority of the patients were men. The most common underlying diseases in both groups were leukemia and lymphoma. In addition, 1 case patient and 4 control patients had myelodysplastic syndrome. Seven of the control patients had an indication for BMT that had not been present in any of the case patients. These diagnoses included breast cancer in 5 patients, multiple myeloma in 1 patient, and aplastic anemia in 1 patient. However, this finding was not statistically significant.

Univariate analysis revealed that severe mucositis (Southwest Oncology Group grade of 3–4) was significantly associated with the development of anaerobic BSI (OR, 4.3; 95% CI, 1.3–14.0; \( P = .01 \); table 2). Nearly 80% of all case patients and control patients developed diarrhea, and no significant differences in the duration or severity of diarrhea were found. In addition, no significant differences were found with regard to duration of neutropenia, chemotherapy regimen, year of BMT (1995–1996 vs. 1997–1998), other infectious complications, or invasive

<table>
<thead>
<tr>
<th>Table 1. Characteristics of case patients and control patients who underwent bone marrow transplantation.</th>
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<tr>
<td><strong>Characteristic</strong></td>
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<tr>
<td>--------------------</td>
</tr>
<tr>
<td>Age, mean years ± SD</td>
</tr>
<tr>
<td>Male sex</td>
</tr>
<tr>
<td>Underlying disease</td>
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<tr>
<td>Leukemia</td>
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<td>Lymphoma</td>
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<tr>
<td>Myelodysplastic syndrome</td>
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<tr>
<td>Multiple myeloma</td>
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<td>Aplastic anemia</td>
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<td>Breast cancer</td>
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**NOTE.** Data are no. (%) of patients, unless otherwise indicated. Analysis was performed by means of conditional logistic regression with single variables. NS, not significant (\( P > .05 \)).
procedures. Very few patients (4 control patients and no case patients) developed *C. difficile* colitis.

The initial antibiotic regimen given for the empirical treatment of fever and neutropenia did not differ significantly between case patients and control patients. Although 2 control patients received piperacillin, gentamicin, and vancomycin, the remainder of the control patients and all of the case patients received vancomycin and ceftazidime. In addition, 10 control patients (22%), compared with no case patients, had received antibiotics with anaerobic activity at some time before the onset of infection.

Among the patients who had undergone allogeneic BMT, no differences in GVHD prophylaxis (e.g., cyclosporine, tacrolimus, or methotrexate) or the incidence of GVHD were observed. As expected, however, the patients who underwent allogeneic BMT and who received high-dose methotrexate (15 mg/m² on day 1 after BMT and 10 mg/m² on days 3, 6, and 11) were more likely to develop severe oropharyngeal mucositis than were those patients who received low-dose (5 mg/m² on days 1, 3, 6, and 11 after BMT) or no methotrexate (43% of patients who received high-dose methotrexate vs. 19% of patients who received low-dose or no methotrexate; *P* = .09). Multivariable conditional logistic regression analysis revealed that severe mucositis was an independent predictor of anaerobic BSI (OR, 4.4; 95% CI, 1.3–14.7; *P* = .01).

Among the 46 control patients, 7 patients who underwent allogeneic BMT developed BSI due to pathogens other than anaerobes. The organisms that caused these infections included coagulase-negative staphylococci (in 2 patients), methicillin-resistant *Staphylococcus aureus* (in 1 patient), viridans streptococci (in 1 patient), *Candida* species (in 2 patients), and *Enterobacter cloacae* (1 patient). No BSIs due to pathogens other than anaerobes occurred among the patients who underwent autologous BMT.

Outcomes of infection were not significantly different between case patients and control patients. Two (9%) of 23 case patients and 3 (7%) of 46 control patients died during hospitalization (*P* = .75). The mean duration of hospitalization (±SD) for patients who survived was 29 ± 15 days for case patients and 29 ± 9 days for control patients (*P* = .94).

### DISCUSSION

During the past 2 decades, a major shift in the types of microorganisms that cause BSI in patients with neutropenia who have cancer has been documented. Data from large multicenter trials have demonstrated a marked decrease in the incidence of gram-negative infections, with a concurrent increase in the incidence of gram-positive infections [7–9]. Of note, among febrile patients with granulocytopenia who have cancer, the proportion of cases of bacteremia due to gram-positive organisms increased from 37% in the 1970s to nearly 70% in the early 1990s [7, 9]. Bone marrow transplant recipients are at particularly high risk for infection because of the intensive conditioning regimens that are given to these patients.

BSIs due to viridans streptococci have become a common occurrence in patients with neutropenia who have cancer [10, 11]. In fact, these organisms were the leading cause of bacte remia in the most recent European Organization for Research in Cancer Therapy trial [7]. Many potential risk factors for such infections have been cited, including the prophylactic use of quinolones or cotrimoxazole, the use of certain chemotherapeutic agents (e.g., cytarabine), and severe neutropenia [12–15]. In addition, oropharyngeal mucositis has been identified as an independent risk factor for the development of BSI due to viridans streptococci [12].

Mucositis has been identified as a risk factor for BSI due to a variety of microorganisms in patients with neutropenia.

### Table 2. Risk factors for anaerobic bloodstream infection in case patients and control patients who underwent bone marrow transplantation (BMT).

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Case patients (<em>n</em> = 23)</th>
<th>Control patients (<em>n</em> = 46)</th>
<th><em>P</em></th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucositis</td>
<td>20 (87)</td>
<td>31 (67)</td>
<td>.09</td>
<td>3.2 (0.8–12.3)</td>
</tr>
<tr>
<td>Severe mucositis*</td>
<td>10 (43)</td>
<td>6 (13)</td>
<td>.01</td>
<td>4.3 (1.3–14.0)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>18 (78)</td>
<td>36 (78)</td>
<td>1.00</td>
<td>1.0 (0.3–3.3)</td>
</tr>
<tr>
<td>Severe diarrhea*</td>
<td>15 (65)</td>
<td>22 (48)</td>
<td>.18</td>
<td>2.1 (0.7–6.4)</td>
</tr>
<tr>
<td>Duration of diarrhea, mean days ± SD</td>
<td>3.8 ± 2.7</td>
<td>4.6 ± 3.4</td>
<td>.91</td>
<td>—</td>
</tr>
<tr>
<td>Duration of neutropenia, mean days ± SD</td>
<td>6.7 ± 5.0</td>
<td>6.8 ± 4.7</td>
<td>.96</td>
<td>—</td>
</tr>
<tr>
<td>Underwent BMT in 1995–1996</td>
<td>11 (48)</td>
<td>22 (48)</td>
<td>1.00</td>
<td>1.0 (0.4–2.7)</td>
</tr>
<tr>
<td>Anaerobic therapy*</td>
<td>0 (0)</td>
<td>10 (22)</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

*NOTE.* Data are no. (%) of patients, unless otherwise indicated. Analysis was performed by conditional logistic regression with single variables.

* Grade 3 or 4, according to the grading criteria of the Southwest Oncology Group.

* Grade 3 or 4, according to the classification system of the World Health Organization.

* We were unable to calculate the OR because this factor was not present in any of the case patients.
It is hypothesized that substantial damage to normal mucosal barriers provides a portal of entry for these bacteria. Although this may result in transient bacteremia without consequence in a normal host, the absence of neutrophils in patients who have undergone BMT predisposes them to septicaemia. Of interest, a recent study has also demonstrated that severe mucositis is an independent predictor of vancomycin-resistant enterococcal bacteremia in patients with cancer [20]. This suggests that mucositis may be a marker for mucosal injury elsewhere in the gastrointestinal tract. Anaerobic organisms, such as enterococci and viridans streptococci, are also important constituents of both oropharyngeal and gut flora. Therefore, it is not surprising that severe mucositis also correlates strongly with the development of anaerobic BSI in patients who have undergone BMT.

The reason for the marked increase in the rate of anaerobic BSI in 1996 is likely multifactorial. To begin with, there was a substantial change in the BMT patient population after 1995. Before 1995, virtually all of the patients had undergone autologous BMT; consequently, they had reduced risk and severity of mucositis. After 1995, however, more allogeneic and unrelated transplants were performed. Patients who underwent these latter procedures experienced more-severe mucositis due to the use of methotrexate as well as the more-intensive preparative regimen that they usually received. We found a correlation with the use of high-dose methotrexate among patients who had undergone allogeneic BMT during this period, which likely predisposed them to severe mucositis and resultant bacteremia. Of interest, such high doses of methotrexate were no longer being administered in 1997 and 1998, and the rate of infection decreased during this period. Nonetheless, the incidence of anaerobic BSI at the end of the study period was still substantially higher than it had been from 1990 through 1994.

Gut decontamination with norfloxacin also was not used before 1995. In addition, the use of piperacillin, which has anaerobic activity, was part of standard antimicrobial therapy for patients with febrile neutropenia during the early 1990s. After 1995, neither the prophylactic antimicrobial treatment nor the empirical antimicrobial therapy for febrile neutropenia had activity against anaerobic bacteria. We found that none of the 23 case patients and 10 of the 46 control patients had received antibiotics with activity against anaerobes. Therefore, for those patients with neutropenia and severe mucositis who undergo BMT and who do not have a clinical response to the initial empirical antibiotic regimen, the addition of anaerobic antimicrobial agent should be strongly considered.

We hypothesized that diarrhea may also be a risk factor for anaerobic BSI. However, no differences in the duration or severity of diarrhea were observed between case and control patients, the majority of whom developed severe diarrhea during their hospital stay. Two infections were caused by Clostridium species, which are organisms that are known to inhabit the large intestine. These bacteria have been strongly associated with necrotizing enterocolitis, which was documented in 1 patient at autopsy. The major risk factor for necrotizing enterocolitis is neutropenia, but antecedent mucosal injury may also play an important role. Given that mucositis has been associated with enterococcal bacteremia [20], it follows that patients with severe oral mucositis may also be at increased risk for infections caused by anaerobic bowel flora. We did not find any association between C. difficile colitis and the occurrence of anaerobic BSI. Very few patients (4 control patients and no case patients) developed C. difficile colitis. In addition, all patients with C. difficile infections were treated promptly with metronidazole, an antibiotic that would likely have prevented the development of anaerobic BSI. It is noteworthy that the only 2 deaths among patients with anaerobic BSI were due to clostridial sepsis, and that the in-hospital mortality rate did not differ significantly between case patients and control patients.

In summary, we have demonstrated that severe mucositis significantly increases the risk of anaerobic BSI in patients who undergo BMT. Therefore, controlling mucositis in patients who undergo BMT is critical to prevent these infections. It is important for clinicians to use specific media for the detection of anaerobes when performing blood cultures in this patient population. The use of empirical antimicrobial therapy with activity against anaerobes should be considered for patients who undergo BMT, who have neutropenia and severe mucositis, and who do not respond to initial antimicrobial therapy.

References