Breakthrough Fungal Infections in Stem Cell Transplant Recipients Receiving Voriconazole

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Infection with voriconazole-resistant fungi may become problematic, because organisms with decreased susceptibility have been noted. Breakthrough fungal infections occurred in 13 of 139 patients who received voriconazole at our center during the period of September 1998 through September 2003. Zygomycetes were found in 6 patients, and Candida glabrata bloodstream infection occurred in 4 patients. Minimal inhibitory concentrations were ≥1 μg/mL for all available isolates. Yeasts and molds with decreased susceptibility to voriconazole may cause invasive infection in patients treated successfully for aspergillosis.

Invasive fungal infections (IFIs) are an important cause of morbidity and mortality in patients receiving cytotoxic chemotherapy for leukemia or undergoing stem cell transplantation (SCT) [1]. Treatment with voriconazole, a broad-spectrum triazole antifungal, has been shown to result in favorable outcomes in patients with multiple opportunistic fungal infections, including those caused by Aspergillus species [2–4] and less common invasive fungi, such as Fusarium species [5]. Despite advantages associated with voriconazole therapy, organisms with decreased susceptibility have been noted. Voriconazole shows limited activity against pathogenic Zygomycetes [6, 7]. We hypothesized that an increased incidence of infections due to Zygomycetes might be encountered in cases of extensive exposure to the drug, particularly in patients who were treated successfully for invasive aspergillosis due to prolonged immunosuppression. We evaluated the clinical records of all patients treated with voriconazole at the Fred Hutchinson Cancer Research Center (FHCRC; Seattle) to assess the significance of breakthrough infections and antimicrobial resistance.

Patients and methods. We retrospectively analyzed the clinical records of 139 patients at FHCRC who were treated with voriconazole during the period of 1 September 1998 through 30 September 2003. During this period, patients received voriconazole for primary therapy for proven invasive aspergillosis or probable invasive aspergillosis (as defined by consensus criteria [8]) and, infrequently, as preventative therapy in the presence of high risks (at the discretion of the patients’ clinicians). Age, sex, underlying disease, type of SCT, presence of neutropenia (neutrophil count, <500 neutrophils/mm3), severity of graft-versus-host disease (GVHD) [9, 10], administration of corticosteroids, and use of other antifungals were recorded. This retrospective study was approved by the FHCRC institutional review board.

An invasive fungal infection was considered to be a breakthrough infection if infection with a different organism was detected (compared with the infection that led to initiation of voriconazole therapy) or if infection was shown to develop >3 days after initiation of preventative voriconazole therapy [11]. Twelve isolates were available for susceptibility testing for voriconazole. The isolates had been stored frozen at −70°C until recovery by subculture. Voriconazole susceptibility was determined by microbroth dilution assay, as described by the NCCLS standards for Candida glabrata [12, 13].

Results. During a 5-year period, 13 patients receiving voriconazole developed a breakthrough invasive fungal infection, according to our definitions. All patients had undergone allogeneic SCT. Underlying diagnoses, patient demographic characteristics, and types of infections are summarized in table 1.

Most patients (10 of 13) developed breakthrough infection late after transplantation; the median day of diagnosis was 180 days after SCT (range, 11–949 days). All but 3 patients who developed infection had severe acute or chronic GVHD. All patients had received antifungal prophylaxis withazole drugs before the development of breakthrough infection. At the time that breakthrough infection was diagnosed, patients were receiving voriconazole therapy for proven (n = 6) or probable (n = 2) aspergillosis or proven fusariosis (n = 1). The remaining patients were receiving voriconazole as prophylaxis in the presence of perceived high risks (receipt of corticosteroids; n = 2) or as empirical therapy for refractory fever (n = 2). Caspofungin was used in combination with voriconazole for 2 patients (patients 12 and 13), whereas voriconazole alone was

CID 2004:39 (1 September) 743

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<table>
<thead>
<tr>
<th>Patient</th>
<th>Age, years</th>
<th>Sex</th>
<th>Type of malignancy</th>
<th>Transplant donor type</th>
<th>Indication for voriconazole</th>
<th>Organism</th>
<th>Site of infection</th>
<th>Day of breakthrough infection, days</th>
<th>Duration of VOR exposure, days</th>
<th>MIC of VOR, μg/mL</th>
<th>GVHD classification (grade)</th>
<th>Steroid dose, mg/kg</th>
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<td>F</td>
<td>CML</td>
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<td>IA</td>
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<td>7</td>
<td>2</td>
<td>Chronic</td>
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<td>Death</td>
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<td>F</td>
<td>AML</td>
<td>MM-UR</td>
<td>Prevention</td>
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<td>Lung</td>
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<td>IA</td>
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<td>Blood</td>
<td>75</td>
<td>37</td>
<td>2</td>
<td>Acute (g3)</td>
<td>28</td>
<td>Death</td>
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<td>M</td>
<td>CML</td>
<td>MR</td>
<td>IA</td>
<td>Aspergillus terreus</td>
<td>Lung</td>
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<td>38</td>
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<td>30</td>
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<td>Lung</td>
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<td>MM-UR</td>
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</tbody>
</table>

**NOTE.** ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; GI, gastrointestinal; MDS, myelodysplastic syndrome; MM, multiple myeloma; MM-UR, HLA-mismatched or unrelated donor; MR, HLA-matched, related donor; NA, information not available; renal cell, renal cell carcinoma; Zygomycetes NOS, histopathologic evidence of organisms consistent with Zygomycetes.

* Identified cause of breakthrough infection.
* Site of documented involvement. "Disseminated" was defined as involvement of >1 organ, as determined by clinical factors and autopsy results.
* Day of breakthrough infection diagnosis relative to day of hematopoietic stem cell transplantation (day 0, receipt of stem cells).
* Duration of VOR exposure immediately before infection.
* "Chronic" is defined as clinically extensive.
* Cumulative dose of corticosteroids received in 2 months immediately before breakthrough infection, relative to body weight.
* Death due to relapsed malignancy is indicated, where appropriate.
* These patients received oral beclamethasone for gastrointestinal graft-versus-host disease.
being administered to the other 11 patients at the time of breakthrough infection. Voriconazole had been prescribed for a median of 47 days (range, 4–118 days) before diagnosis of breakthrough infection.

Zygomycetes were the most common cause of breakthrough infection (in 6 [46%] of 13 patients), followed by C. glabrata (in 4 [31%] of 13 patients). In 2 patients, >1 fungal species was isolated from sterile tissue. Twelve isolates were available for susceptibility testing. Results revealed MICs of voriconazole of ≥1 μg/mL (range, 1–32 μg/mL; table 1) for all isolates examined. Zygomycetes and Scedosporium species typically had the highest MICs. The breakthrough cases of candidemia that occurred during voriconazole therapy were caused by C. glabrata; the 2 isolates that were available for susceptibility testing had MICs of 2 μg/mL.

All patients but one were treated for their breakthrough infection with a lipid formulation of amphotericin B. Six patients initially responded to the change in therapy, but 9 patients ultimately died of progressive fungal infection or alternative infections (e.g., cytomegalovirus infection and sepsis due to gram-negative bacteria) in the presence of severe GVHD or relapsed malignancy. In 1 patient (patient 13), the diagnosis of Zygomycetes infection was not recognized until after death, at autopsy.

**Discussion.** Multiple antifungal drugs are now available to treat invasive fungal infections. Voriconazole, a broad-spectrum triazole antifungal, is an appropriate agent for therapy for invasive aspergillosis; superior outcomes were obtained for patients with aspergillosis who were treated with voriconazole, compared with conventional amphotericin B, in a large randomized trial [4]. It is now licensed for treatment of documented aspergillosis and other less common mold infections. Although it has not been licensed for preventative use, it is currently being evaluated as a prophylactic drug in patients undergoing SCT. Clinicians should be aware of the potential for breakthrough infection caused by Zygomycetes and C. glabrata.

We have recognized 13 patients receiving voriconazole therapy who developed breakthrough fungal infections, according to our predefined criteria. Although these infections were clinically significant, resulting in poor outcomes in almost all patients, it is important to note that the overall incidence of breakthrough infection is likely to be low when considering exposure to the drug, as measured in patient-days. Unfortunately, we do not have the denominator data to enable calculation of risks in all patients exposed to voriconazole or data to enable the evaluation of the potential impact of serum concentrations. Also, the definition of breakthrough infection used in this study may have yielded an overestimation of its true importance, because the 2 patients who had breakthrough infection diagnosed while receiving empirical therapy may have developed infection earlier than was documented by diagnostic evaluation.

Patients who developed breakthrough infections while receiving voriconazole therapy were typically quite immunosuppressed; the majority had received voriconazole for prolonged periods of time and had severe GVHD. In one sense, the development of voriconazole-resistant infections caused by organisms other than A. fumigatus may be considered a problem caused by therapeutic advances, because the risks for resistant organisms may increase as more immunosuppressed patients survive during therapy for aspergillosis. Nevertheless, the observation of documented voriconazole-resistant fungal infections developing during receipt of therapy is important, as it emphasizes the persistent need for diagnostic vigilance for patients who have prolonged immunosuppression.

Several in vitro studies have reported that Zygomycetes typically exhibit high MICs of voriconazole [6, 7, 14–16]; however, few studies have noted the clinical significance of this observation. Marty et al. [17] reported that multiple patients in their transplantation center who were treated with voriconazole as a preventative agent developed breakthrough infections with Zygomycetes, and that rates of infection caused by these organisms appear to be increasing. Our observations are supportive of these findings in that many breakthrough infections that occurred during voriconazole therapy were caused by Zygomycetes. In both studies, these infections typically occurred late after transplantation while the patient had GVHD; this observation is consistent with prior reports noting that zygomycosis is typically a GVHD-associated—rather than a neutropenia-associated—infection in persons undergoing allogeneic SCT [18]. Although voriconazole use may be one factor that provides selective advantage to Zygomycetes, it is likely that the increased recognition of these infections is multifactorial, potentially influenced by increased survival of patients with GVHD.

The emergence of voriconazole-resistant Candida species has not been documented. In large epidemiologic surveys, Candida species have typically exhibited low MICs of voriconazole [19–21], and successful therapy in cases of esophageal candidiasis [22] and invasive candidiasis have been documented [23]. Our observations suggest that there is the potential for voriconazole resistance among C. glabrata isolates. Of note, response rates for voriconazole salvage therapy for C. glabrata infections tended to be lower than for infections with other Candida species in 1 study [23]. The significance of our observation when applied to a broader population of patients exposed to the drug, as well as the specific mechanisms by which C. glabrata isolates may adapt to voriconazole exposure, may be subjects worthy of future study.

We have observed breakthrough infections caused by other molds, including non-*fumigatus* species of Aspergillus (e.g. As-
pergillus terreus and Aspergillus ustus), Scedosporium prolificans, and Acremonium species. Results of treatment of S. prolificans infection have been poor [5], and this organism has tended to demonstrate relatively high MICs of all antifungals studied to date [24]. A. ustus is an unusual cause of infection that has been noted in the past to exhibit high MICs of multiple antifungals [25]. It is not possible to determine whether these infections result from clinically meaningful antifungal drug resistance or are more a function of the severity of the immunocompromised state of the patients at risk.

In conclusion, we describe multiple patients who developed infections while receiving voriconazole therapy for other indications. These findings demonstrate the potential for emergence of resistance among multiple different types of fungal pathogens, and they emphasize the continued requirement of diagnostic vigilance for patients who remain at risk for fungal infections because of prolonged immunosuppression.

Acknowledgments

Financial support. National Institutes of Health (grants K08 AI01571 and R21 AI55928 [to K.A.M.] and RO1 AI-061628 [to D.N.F.]) and the Swiss National Science Foundation (Nr. PBBEA-102316 [to A.I.]).

Conflict of interest. K.A.M. has been a consultant for Pfizer. All other authors: No conflict.

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