Invasive fungal breakthrough infections, fungal colonization and emergence of resistant strains in high-risk patients receiving antifungal prophylaxis with posaconazole: real-life data from a single-centre institutional retrospective observational study

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Objectives: The broad-spectrum triazole posaconazole showed promising results in preventing invasive fungal infections (IFIs) in high-risk patients. Concerns rise over the relevance of breakthrough IFIs (bIFIs) and the emergence of azole-resistant strains. The current retrospective analysis was undertaken to evaluate the incidence of bIFIs and to study fungal colonization and resistance following posaconazole exposure.

Methods: Ninety-five patients who underwent 202 courses of primary antifungal prophylaxis with 200 mg of posaconazole three times daily during neutropenia after chemotherapy/haematopoietic stem cell transplantation between September 2008 and September 2010 were evaluated. An IFI was considered to be a bIFI if its occurrence was detected ≥ 4 days after initiation of preventative posaconazole prophylaxis.

Results: The incidence of bIFIs was 13% (27/202), with 11/27 (41%) proven and 16/27 (59%) probable bIFIs. Proven infections were mainly localized in the lungs (85%). Species diagnosis exclusively revealed non-Aspergillus species, i.e. mucormycetes in 55% and yeasts in 45%. The median overall survival for patients with bIFIs was 5.2 months. Sixteen of 27 patients with bIFIs (proven and probable) succumbed. Regarding only proven cases, 8/11 patients died, whereas only 1/16 deaths was caused by fungal disease. Prospective screening confirmed colonization with yeasts in 42/202 (21%) courses; moulds were not identified. The spectrum of colonizing yeasts changed slightly over time, shifting to more rare yeasts. There were no deaths due to invasive yeast infections.

Conclusions: A significant proportion of bIFIs, compared with historical data, with a shift to non-Aspergillus spp. and in particular to mucormycetes was observed in patients at high risk for IFI during posaconazole prophylaxis.

Keywords: aspergillosis, shift, non-Aspergillus species, mucormycetes, Candida

Introduction

Invasive fungal infections (IFIs) remain a leading cause of morbidity and mortality in patients suffering from haematological malignancies and those undergoing haematopoietic stem cell transplantation (HSCT). The rationale for antifungal prophylaxis was provided by randomized controlled trials showing the superiority of fluconazole in the prevention of Candida infections in stem cell transplant recipients compared with placebo, reducing case fatality rates and overall mortality.1–3

Recently, two large randomized trials evaluating the prophylactic use of posaconazole compared with fluconazole and itraconazole in patients with acute myeloid leukaemia (AML) or high-risk myelodysplastic syndrome (MDS) and prolonged neutropenia, as well as in patients receiving corticosteroids >1 mg/kg for treatment of graft-versus-host disease (GVHD), clearly demonstrated a significantly lower risk for breakthrough IFIs (bIFIs) with posaconazole.4,5 Whereas survival in neutropenic patients was significantly longer among recipients of posaconazole than among recipients of fluconazole or itraconazole,
there was no benefit in overall survival, but the number of deaths from IFI was lower in the posaconazole group of patients with GVHD. Consequently, routine prophylactic care in patients at high risk for fungal infections was changed, whenever possible, to posaconazole at our institution in 2008. The aim of this retrospective single-centre surveillance was to evaluate the real-life impact of the implementation of posaconazole prophylaxis on the incidence of bifIs, as well as on colonization and emergence of resistant strains in a cohort of 95 immunocompromised patients.

Methods

Patients at high risk for the development of IFIs during the treatment of various haematological malignancies between September 2008 and September 2010 were included. As this analysis represents a retrospective evaluation, approval of the local research Ethics Committee was not required. Patients were defined as being at high risk for an IFI in the case of an anticipated neutropenia >7 days, receiving antibiotic treatment, having acute leukaemia, receiving autologous or allogeneic HSCT, suffering from GVHD or receiving steroids (i.e. prednisone) ≥1 mg/kg of body weight/day. Primary antifungal prophylaxis consisted of orally administered 200 mg of posaconazole thrice daily. Whenever possible, posaconazole was administered with fatty food or nutritional supplements, as well as with carbonated beverages, and, in the absence of hyperacid symptoms, proton pump inhibitors were omitted. One course of antifungal prophylaxis was defined as the period of time in which a patient received posaconazole during hospitalization. Historical data from a similar cohort of patients served as a control cohort (see Table 1).6

Diagnosis of bifIs

Only probable or proven IFIs according to the revised consensus criteria of the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) were considered for analysis.7 An IFI was considered to be a bIFI if its occurrence was detected ≥4 days after initiation of primary antifungal prophylaxis with posaconazole. The day of diagnosis was defined as the day on which the first diagnostic procedure identifying an IFI was performed. In the case of postmortem diagnosis, the day of death was considered to be the day of diagnosis. Whenever possible, CT-guided percutaneous biopsies of the suspected lesions in the lung were obtained. Samples (tissue and blood) were assessed by culture, PCR and serology, and blood samples additionally by galactomannan enzyme immunoassay (GM EIA). Biopsy specimens were transferred to 2 mL of NaCl, minced and homogenized aseptically. Samples were then vortexed, stored at room temperature for 30 min and centrifuged. Supernatants and homogenized tissues were examined for the presence of fungi by application of the Fungi-Fluor solution (CFWS) (Polysciences), by Aspergillus PCR and by GM EIA; a 0.5 cut-off optical density was used. Selected samples that showed unsceptate hyphae by CFWS and that yielded negative results for GM EIA and Aspergillus PCR were evaluated by a PCR specific for mucormycosis.8

Colonization and antifungal susceptibility testing

Within the infection control programme applied during the treatment/transplantation period, surveillance cultures were obtained on a weekly basis by use of swabs from nares, throat, skin and the perianal region. The effect of posaconazole exposure on the prevalence of fungal pathogens was analysed in our patient cohort. Species distribution obtained from start to finish of surveillance was evaluated and compared. For uncommon species, the ITS1-5.8S-ITS2 region was sequenced (where ITS stands for internal transcribed spacer). In vitro susceptibility testing was performed with the Etest methodology according to the manufacturer’s recommendation. The following epidemiological cut-offs were used for defining susceptibility: itraconazole ≤0.5 mg/L, voriconazole ≤2 mg/L and posaconazole ≤0.25 mg/L (based on EUCAST wild-type distributions compiled from several European reference centres).

Statistical analysis

Survival data were analysed as of September 2011 with SPSS 16.0 (SPSS Inc., Chicago, IL, USA). Overall survival was calculated from the date of diagnosis to the date of death from any cause or date of last follow-up. Deaths from any cause were regarded as events.

Results

Patients and diagnosis

The majority of patients (65/95, 68%) suffered from acute leukaemia, receiving remission induction or remission consolidation therapy with an anticipated duration of neutropenia >7 days. Fifty-seven patients (60%) underwent allogeneic HSCT and 16 patients (17%) evaluated had severe (≥grade II) GVHD requiring corticosteroids ≥1 mg/kg/day. Historical data from a similar cohort of patients served as a control cohort, comparable in diagnostic procedures and antifungal treatment strategies; however, patients did not receive posaconazole prophylaxis, but 400 mg of oral or intravenous fluconazole daily or 6 mg/kg/day

<table>
<thead>
<tr>
<th>Variable</th>
<th>Historical cohort</th>
<th>Recent cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>bIFI</td>
<td>12% (62/520)</td>
<td>13% (27/202 courses)</td>
</tr>
<tr>
<td>proven</td>
<td>39% (24/62)</td>
<td>41% (11/27 patients)</td>
</tr>
<tr>
<td>probable</td>
<td>61% (38/62)</td>
<td>59% (16/27 patients)</td>
</tr>
<tr>
<td>Localization of bIFI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lung</td>
<td>98% (61/62)</td>
<td>85% (23/27)</td>
</tr>
<tr>
<td>maxillary sinus blood</td>
<td>2% (1/62)</td>
<td>18% (2/11)</td>
</tr>
<tr>
<td>pulmonary and cutaneous</td>
<td></td>
<td>18% (2/11)</td>
</tr>
<tr>
<td>Organisms (proven bIFI)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucor</td>
<td>13% (3/24)</td>
<td>55% (6/11)</td>
</tr>
<tr>
<td>Aspergillus terreus</td>
<td>38% (9/24)</td>
<td></td>
</tr>
<tr>
<td>Aspergillus fumigatus</td>
<td>21% (5/24)</td>
<td></td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>4% (1/24)</td>
<td></td>
</tr>
<tr>
<td>yeasts (Candida spp.)</td>
<td>25% (6/24)</td>
<td>45% (5/11)</td>
</tr>
<tr>
<td>Antifungal treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AMB mono</td>
<td>52% (32/62)</td>
<td>33% (9/27)</td>
</tr>
<tr>
<td>AMB combination</td>
<td>6% (4/62)</td>
<td>41% (11/27)</td>
</tr>
<tr>
<td>VRC mono</td>
<td>21% (13/62)</td>
<td>11% (3/27)</td>
</tr>
<tr>
<td>VRC combination</td>
<td>5% (3/62)</td>
<td>7% (2/27)</td>
</tr>
<tr>
<td>AMB deoxycholate</td>
<td>11% (7/62)</td>
<td></td>
</tr>
<tr>
<td>others</td>
<td>5% (3/62)</td>
<td>7% (2/27)</td>
</tr>
</tbody>
</table>

AMB, amphotericin B; VRC, voriconazole.
itraconazole orally during neutropenia until haematopoietic recovery or for 75 days after allogeneic HSCT. This group of patients consisted of 94 patients treated for haematological malignancies at our institution in the years 2001–04, also mainly for acute leukaemia receiving remission induction or remission consolidation therapy with an anticipated duration of neutropenia ≥7 days. Forty-nine patients (70%) in this cohort underwent allogeneic hematopoietic stem cell transplantation and 19 patients (20%) had severe (≥grade II) GVHD requiring corticosteroids ≥1 mg/kg/day.

**Incidence, classification and diagnosis of bIFIs**

In total, 95 patients at high risk for the development of IFI, admitted to the Department of Hematology and Oncology of the Medical University Hospital for the treatment of various haematological malignancies between September 2008 and September 2010, were included. The incidence of bIFIs was 13% (27/202 courses of primary antifungal prophylaxis with posaconazole). Patients received posaconazole for a median of 13% (27/202 courses of primary antifungal prophylaxis with posaconazole). Patients received posaconazole for a median of 16 days (range 4–34) before diagnosis of bIFI. According to the revised EORTC/MSG criteria, 16/27 patients (59%) had probable disease, whereas 11/27 (41%) patients were diagnosed with proven bIFI. Proven diagnosis (n=11) was mainly confirmed by biopsy or by detection of fungal pathogens in blood cultures. Probable bIFIs (n=16) were, besides host factors, diagnosed by CT scan.

**Localization, species diagnosis and susceptibility patterns of bIFIs**

For details, see Table 2. The bIFIs (n=27) were primarily localized in the lungs (23/27, 85%), in the lungs and skin (2/27, 7%) and in the bloodstream (2/27, 7%). Species diagnosis and susceptibility patterns of proven bIFIs are depicted in Table 2. For 42/202 courses of antifungal prophylaxis, species diagnosis revealed *C. glabrata* in 18/42 (43%), *C. albicans* in 15/42 (36%), *Saccharomyces cerevisiae* in 4/42 (10%), *C. dubliensis* in 12/42 (29%), *C. krusei* in 1/42 (2%), *C. globosa* in 1/42 (2%), *C. glabrata* in 1/42 (2%) and *Trichosporon asahii* in 1/42 (2%) (Figure 1b). Antifungal susceptibility testing showed 12/42 (29%) pathogens being resistant to fluconazole (*C. glabrata*, n=11; *C. krusei*, n=1), and only 1/42 fungi being non-susceptible to voriconazole or posaconazole, respectively (*C. glabrata*, n=1, *C. krusei*, n=1).

**Table 2.** Localization, species diagnosis and susceptibility patterns of proven bIFIs

<table>
<thead>
<tr>
<th>Localization</th>
<th>Organism</th>
<th>AMB</th>
<th>VRC</th>
<th>FLC</th>
<th>CAS</th>
<th>POS</th>
<th>ITC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lungs</td>
<td><em>L. corymbifera</em></td>
<td>S</td>
<td>R</td>
<td>NE</td>
<td>R</td>
<td>S</td>
<td>NE</td>
</tr>
<tr>
<td>Lungs</td>
<td><em>C. albicans</em></td>
<td>S</td>
<td>R</td>
<td>NE</td>
<td>R</td>
<td>S</td>
<td>NE</td>
</tr>
<tr>
<td>Blood</td>
<td><em>C. glabrata</em></td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Blood</td>
<td><em>C. dubliensis</em></td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Lungs</td>
<td><em>C. krusei</em></td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>NE</td>
</tr>
<tr>
<td>Lungs</td>
<td><em>T. asahii</em></td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>NE</td>
</tr>
<tr>
<td>Lungs and skin</td>
<td><em>Mucor spp.</em></td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td><em>Mucor spp.</em></td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Lungs and skin</td>
<td><em>Rhizomucor spp.</em></td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>Lungs</td>
<td><em>Rhizopus arrhizos</em></td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td><em>S. cerevisiae</em></td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td></td>
</tr>
</tbody>
</table>

AMB, amphotericin B; VRC, voriconazole; FLC, fluconazole; CAS, caspofungin; POS, posaconazole; ITC, itraconazole; NE, not evaluated; S, susceptible; R, resistant.

*a*Additional severe mucositis and multisite colonization with *C. krusei*.

**Figure 1.** (a) Early-stage fungal colonization obtained by the weekly surveillance programme in 27 patients. (b) Late-stage fungal colonization obtained by the weekly surveillance programme (n=42).

was classified with proven fungal disease (*Mucor* spp.). Probable bIFIs were exclusively localized in the lungs and entirely diagnosed by CT scan.

**Colonization and susceptibility patterns of colonizing fungal pathogens**

Colonization was defined as early stage when cultures obtained at the beginning of surveillance revealed colonization. Early- and late-stage colonization was unilocular as well as multilocular. Early-stage colonization identified *Candida albicans* in 14 (52%) cases, *Candida glabrata* in 11 (41%) cases and *Lichtheimia corymbifera* and *Candida dubliensis* in 1 (4%) case each (see Figure 1a). Colonization was multilocular in seven cases. Post-posaconazole prophylaxis colonization was noted in 42/202 (21%) courses of antifungal prophylaxis. Species diagnosis revealed *C. glabrata* in 18/42 (43%), *C. albicans* in 15/42 (36%), *Saccharomyces cerevisiae* in 4/42 (10%), *C. dubliensis* in 1/42 (2%), *C. globosa* in 1/42 (2%), *C. krusei* in 1/42 (2%) and *Trichosporon asahii* in 1/42 (2%) (Figure 1b). Antifungal susceptibility testing showed 12/42 (29%) pathogens being resistant to fluconazole (*C. glabrata*, n=11; *C. krusei*, n=1), and only 1/42 fungi being non-susceptible to voriconazole or posaconazole, respectively (*C. glabrata*, n=1, *C. krusei*, n=1).
Invasive breakthrough infections under posaconazole prophylaxis

Antifungal treatment and overall survival

Antifungal treatment mainly consisted of liposomal amphotericin B (LAMB) in combination in 11/27 cases (41%), mainly with an echinocandin (8/11) or an azole (3/11), whereas 9/27 cases of bIFIs were treated with liposomal amphotericin as a single agent. In the case of lacking evidence for mucormycosis or in proven yeast infections, 3/27 cases were treated with voriconazole monotherapy and posaconazole in combination (not LAMB) in 2/27 (7%), and caspofungin and anidulafungin in 1/27 (4%) each.

The median overall survival for patients with bIFI was 5.2 months from the diagnosis of fungal disease. Sixteen of 27 patients (59%) with bIFI died during the observation period. In patients with proven disease (n = 11), 8/11 patients died (73%), whereas only 1 death was attributed, at least in part, to fungal disease (6%). Causes of death were bacterial septic multi-organ failure in 7/16 patients (44%), transplant-related mortality in 5/16 (31%), progressive disease in 3/16 (19%) and breakthrough invasive fungal disease in 1/16 (6%). The latter patient additionally suffered from severe GVHD of the skin and gut, and bIFI remained classified as being a probable disease (clinical signs and CT scan) even after post mortem examination.

Discussion

The current analysis revealed an incidence of 13% bIFIs among high-risk patients receiving extended-spectrum antifungal prophylaxis with posaconazole. Remarkably, all bIFIs were caused exclusively by non-Aspergillus species, and in particular by filamentous fungi belonging to the class of mucormycetes.9–11 This is of peculiar interest, as posaconazole is distinguished as being effective in the treatment of this species of fungal pathogen.12 Furthermore, the recently described fungal spectrum does not correspond to that noticed in the two large posaconazole prophylaxis studies, where breakthrough mucormycosis was not a serious issue.12,13 Breakthrough mucormycosis has been increasingly observed in patients with leukaemia and recipients of H SCT receiving Aspergillus-active drugs, such as voriconazole or echinocandins.13–16 However, a recent study comparing voriconazole prophylaxis with other azoles in H SCT patients did not show a significant increase in the incidence of mucormycosis.17 Interestingly, the incidence of bIFIs in the current analysis remained stable compared with a historical cohort treated at our institution in the years 2001–04 with another antifungal prophylaxis strategy (13% versus 12%, respectively). In contrast to the posaconazole prophylaxis studies by Ullmann et al.18 and Cornely et al.,19 this is a remarkably high incidence, even though the current analysis included patients with haematological diseases other than MDS or AML. Despite the stable incidence over time, the fungal spectrum clearly changed to non-Aspergillus spp. (Table 1).5

The results of our study suggest a certain selection pressure caused by an extended-spectrum antifungal prophylaxis with posaconazole. However, posaconazole reliably prevented invasive aspergillosis and fungal pathogens remained susceptible to LAMB, which proved to have good activity in the first-line as well as in the salvage setting.18–21 Consequently there was only one patient death, being at least in part attributed to fungal infection due to Lichtheimia spp. showing in vitro resistance to posaconazole. Hence, from the clinicians view, survival from IFI appears to have improved over time, probably due to improved diagnostic and therapeutic approaches.6,21–23

Another fact to keep in mind is that the efficacy of posaconazole might be limited by poor absorption.5,5,24,25 A broad line-up of co-medications, inadequate dietary intake and abnormal gastric pH levels are common in critically ill haematological patients, i.e. suffering from mucositis following chemotherapy or from G VHD following HSCT, which may result in unpredictable bioavailability and subtherapeutic plasma concentrations.26,27 Additionally, most patients receive proton pump inhibitors, which are known to cause erratic absorption of posaconazole.28

Current strategies to improve posaconazole serum concentrations are mainly focused on improving drug dissolution and absorption (i.e. administer with high-fat food or acidic beverage) or discontinuing acid suppression therapy (especially proton pump inhibitors).10,28 Therapeutic drug monitoring (TDM), however, might be a helpful tool to ensure compliance and absorption of the antifungal, therefore avoiding breakthrough infections.26,27,29–32 TDM was not undertaken at our hospital throughout the study period, which marks a limitation of this analysis, but probably reflects ‘real life’ in these years in many institutions and underlines the potential importance of TDM. The relationship between drug exposure and response to therapy, however, is discussed controversially.31,34

As is known, the probability of developing IFI is highest within the first 100 days following newly diagnosed acute leukaemia and in patients who did not achieve remission after a first course of induction chemotherapy.35 This seems to be reflected in the current analysis recording 9/27 cases of bIFIs during first remission induction chemotherapy or salvage chemotherapy for refractory of progressive disease (4/27) and bIFIs occurring in a median of 14 days (range 4–34) after initiation of posaconazole prophylaxis (n = 27). On the one hand, patients who developed bIFI while receiving posaconazole for a few days clearly were not able to reach therapeutic drug levels of posaconazole. Hence the appearance of a bIFI in those patients, by definition, has to be called into question. On the other hand, it might be speculated that patients may have already experienced exposure to aerosolized fungal pathogens before admission to the hospital, thereby leading to invasive infection at a time of maximum immunosuppression and thus, strictly speaking, not meeting the definition of a classic breakthrough infection.36 However, clinicians are called for outstanding attention to this particular patient group throughout this treatment period, and diagnostic procedures such as CT scans should be initiated in a timely fashion in the case of suspected fungal infections.

Colonization with Candida spp. is a known risk factor for poor survival in haematological patients.37,38 The current study revealed heavy colonization mainly with non-C. albicans, a fungal spectrum that has already been noticed in the large study of posaconazole prophylaxis in GVHD patients.3 Resistance to posaconazole, voriconazole and echinocandins was uncommon in our study, which is also in line with a recent study by Pfaller et al.39 The spectrum of yeasts before and during posaconazole prophylaxis did change slightly, shifting to rare yeasts. Hence we were
unable to detect a shift to more resistant strains, as was recently shown by a multicentre study implemented in France.\textsuperscript{40}

Summarizing the current study, bIFIs due to non-\textit{Aspergillus} spp. and especially due to mucormycetes were noticed in a considerable proportion of patients at high risk for IFI receiving posaconazole prophylaxis. As a consequence, antifungal treatment must be chosen wisely, as probable breakthrough infections (EORTC/MSG criteria\textsuperscript{7}) in fact might not be caused by \textit{Aspergillus} spp., but by mucormycetes, which lack susceptibility to voriconazole, the recommended first-line therapy for invasive aspergillosis.\textsuperscript{23} LAMB, however, was confirmed as being an effective and potent antifungal therapy even for mucormycosis in our study.

Regarding fungal colonization, long-term and repetitive antifungal prophylaxis with posaconazole resulted in a shift toward rare species.

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This study was carried out as part of our routine work.

Transparency declarations
None to declare.

References
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