Azole-based chemoprophylaxis of invasive fungal infections in paediatric patients with acute leukaemia: an internal audit

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Objectives: Children and adolescents with acute myeloid leukaemia (AML) and recurrent acute leukaemias (RALs) are at high risk of life-threatening invasive fungal infections (IFIs). We analysed implementation, safety and efficacy of a standard operating procedure for oral, azole-based, mould-active antifungal prophylaxis.

Methods: Patients with AML and RALs aged ≥13 years received 200 mg of posaconazole three times daily and patients aged 2–12 years received 200 mg of voriconazole two times daily from the completion of chemotherapy until haematopoietic recovery. Algorithms for fever or focal findings in all patients with haematological malignancies included blood cultures, high-resolution CT and other appropriate imaging, serial serum galactomannan, invasive diagnostics and pre-emptive therapy with change in class if on antifungal medication.

Results: From 2006 to 2010, 40 patients (0.8–17 years; 21 males) with newly diagnosed AML (n = 31) or RAL (n = 9) were admitted, of whom 36 received a total of 149 courses of chemotherapy (reasons for exclusion: contraindications and early death ≤3 days). Azole prophylaxis was given in 87.2% (n = 130/149) of episodes. Pre-emptive anti-fungal therapy for pulmonary infiltrates was initiated in 5/36 (13.9%) patients or 6/130 (4.6%) episodes for a duration of 3–22 days. No proven or probable IFIs occurred. Adverse events (AEs) were common but mostly low grade and reversible. Three courses (2.3%) were discontinued due to AEs. In simultaneously admitted new patients with acute lymphatic leukaemia (ALL; n = 101) and paediatric lymphomas (n = 29) not receiving standard antifungal prophylaxis, proven/probable IFIs occurred in 4 patients with ALL (4.0%) and 7/130 patients (5.4%) received pre-emptive therapy.

Conclusions: Azole-based, mould-active antifungal prophylaxis in high-risk paediatric patients with AML and RALs was satisfactorily implemented, well tolerated and effective. The low rate of IFIs in patients with ALL/lymphoma supports the lack of a general indication for prophylaxis in this population in the presence of a diagnostic and therapeutic algorithm.

Keywords: mycoses, children, cancer, prevention, voriconazole, posaconazole

Introduction

Invasive fungal infections (IFIs) due to Candida, Aspergillus and a variety of more rarely encountered fungal species are important causes of morbidity and mortality in paediatric patients with haematological malignancies. Whereas the risk in patients with acute lymphatic leukaemia (ALL) or lymphoma is <10% in the majority of contemporary series, infection rates in patients with acute myeloid leukaemia (AML) and recurrent acute leukaemia (RAL) of either origin are consistently reported to exceed 10%.1–11 Together with ongoing problems in rapidly establishing a microbiological diagnosis,12,13 the well-established relationship between early treatment and favourable outcome,14–16 and case fatality rates of documented infections in the order of 20%–70%,1–11 primary chemoprophylaxis is justified in the population of paediatric patients with AML and RAL17,18 and recommended by current European guidelines.19

Based on the results of the pivotal Phase III clinical trial of posaconazole in adults with AML/myelodysplastic syndrome (MDS),20 pharmacokinetic data,21–24 dosing recommendations and regulatory approval of second-generation triazoles in paediatric patients,17,18,25 broad-spectrum, oral, mould-active primary antifungal prophylaxis with posaconazole or voriconazole coupled with a diagnostic and therapeutic algorithm was...
instituted at our centre as a standard operating procedure in patients with newly diagnosed AML and those with RAL during the phase of intensive chemotherapy. In patients with ALL and lymphoma, primary antifungal prophylaxis was not mandated but was optional and given on the basis of an individual risk–benefit assessment.

Few structured published data exist on primary antifungal prophylaxis and its implementation in paediatric patients with haematological malignancies. In this report, we present an analysis of the implementation of and adherence to a standard operating procedure for oral, azole-based, mould-active antifungal prophylaxis in paediatric patients with haematological malignancies. We also provide data on its preventative efficacy and safety, in order to audit the overall appropriateness of the selected strategy for the prevention of IFIs in this highly vulnerable patient population.

Patients and methods

Study design

The study was a single-centre, retrospective, non-comparative cohort study of all paediatric patients with haematological malignancies starting intensive chemotherapy between January 2006 and December 2010 at the Department of Paediatric Haematology and Oncology, Children’s University Hospital Münster. Patients eligible for inclusion were ≤18 years of age, had received the diagnosis of de novo or first relapse of acute leukaemia or lymphoma during the 5-year study period and were undergoing induction, consolidation or reinduction chemotherapy. Patients scheduled to receive allogeneic haematopoietic stem cell transplantation (HSCT) were eligible for inclusion and analysis until the start of the preparative regimen. All patients received antineoplastic chemotherapy according to current Berlin–Frankfurt–Münster (BFM) protocols.

Two cohorts were analysed: patients with AML and RALs considered to be at high risk of IFIs, for whom a standard operating procedure of primary antifungal prophylaxis had been implemented in January 2006 (Cohort 1); and patients with ALL and lymphoma, for whom no primary antifungal prophylaxis was provided as per the standard operating procedure (Cohort 2). In both cohorts, the observation period was restricted to the phase of intensive chemotherapy, the phase of maintenance chemotherapy was not included in the analysis.

The primary endpoint of the study was the implementation of and adherence to the standard operating procedure for primary prophylaxis; secondary endpoints included the incidence of breakthrough fungal infections (Cohorts 1 and 2) and the safety and tolerance of azole-based antifungal chemoprophylaxis (Cohort 1), with the ultimate goal to audit the appropriateness of the institution’s strategy for the prevention of IFIs.

Relevant data were collected by authorized study personnel with the aid of a standardized case report and entered in an anonymized form into an electronic database. Written informed consent for antifungal medication as part of the medically indicated measures of supportive care and for data collection was obtained within the consent procedure for cancer treatment and specialized medical care approved by the local ethics committee.

Institutional standard operating procedure

Starting in January 2006, oral, azole-based, mould-active primary antifungal prophylaxis was instituted as the standard operating procedure in patients with newly diagnosed AML and those with RAL during the phase of intensive induction, consolidation and reinduction chemotherapy. In patients with ALL and lymphoma, primary antifungal prophylaxis was not mandated but was optional and provided on an individual basis.

Based on the results of the pivotal Phase III clinical trial in adults with AML/MDS, pharmacokinetic data and regulatory approval of second-generation triazoles and the intention to provide oral medication, patients aged ≥13 years were to receive 200 mg of posaconazole three times daily by mouth and those aged 2–12 years were to receive 200 mg of voriconazole twice daily by mouth during phases of granulocytopenia with careful consideration of contraindications and drug–drug interactions. Prophylaxis was to be started on the first day after completion of a course of chemotherapy and continued until granulocyte recovery or the start of the next course of chemotherapy if there was no granulocyte recovery. Alternatives in patients unable to tolerate oral medication or having contraindications forazole treatment included intravenous voriconazole, 1 mg/kg liposomal amphotericin B once daily or every other day, and 1 mg/kg micafungin once daily. In children ≤2 years of age in whom no dosage exists for posaconazole and voriconazole, alternatives include liposomal amphotericin B and micafungin at similar dosages.

Algorithms for fever during granulocytopenia and focal findings included monitoring with blood cultures, high-resolution computed tomography (HR-CT) of the chest in patients with fever persisting for ≥48–72 h or with respiratory symptoms and other imaging as appropriate; optional switching to empirical antifungal therapy in the case of completely negative results; switching to targeted antifungal therapy in the case of positive blood cultures; and switching to pre-emptive antifungal therapy, serial serum galactomannan antigen assays and optional invasive diagnostics in the case of pulmonary infiltrates or other suggestive imaging findings, in all cases with change in class upon change in antifungal therapy.

Assessment of implementation and adherence

Implementation of azole-based primary antifungal prophylaxis per the standard operating procedure in Cohort 1 was assessed by (i) the proportion of patients with AML and RALs who received azole-based prophylaxis; (ii) correct allocation according to age to either voriconazole or posaconazole; and (iii) the percentage target attainment calculated by the ratio of days with prophylaxis received to days with indication for prophylaxis and the ratio of episodes with prophylaxis received to episodes with indication for prophylaxis, respectively. Assessment of the use of antifungal prophylaxis in patients of Cohort 2 included (i) the proportion of patients who had received primary antifungal prophylaxis at any time during the phase of intensive chemotherapy; (ii) the number of distinct episodes with primary antifungal prophylaxis; and (iii) the antifungal agents used for primary antifungal prophylaxis.

Assessment of antifungal efficacy

Successful azole-based primary antifungal prophylaxis in Cohort 1 was defined by the absence of a switch to empirical, pre-emptive and targeted antifungal therapy for breakthrough infections, the absence of drug discontinuation due to adverse events (AEs) and survival of the patient; it was assessed per patients enrolled and per episodes of antifungal prophylaxis received. Coding of antifungal treatment strategies was performed by the investigators responsible for data analysis (S. Y. and A. H. G.). The criterion for coding of empirical antifungal therapy was persisting fever during granulocytopenia and that for pre-emptive antifungal therapy was the detection of new pulmonary infiltrates by HR-CT or new suspicious infiltrates in other relevant imaging studies. Coding of a proven or probable IFI was made on the basis of the revised criteria of the EORTC/MSG Consensus Group. The assessment of antifungal efficacy in Cohort 2 was limited to the use of prophylactic, empirical and pre-emptive antifungal therapy and the incidence of proven or probable IFIs.

Assessment of safety and tolerance

Laboratory parameters of hepatic function at baseline, during treatment (maximum pathological value) and at the end of treatment and clinical AEs during azole exposure were recorded in Cohort 1 and graded according
to current Common Terminology Criteria for Adverse Events guidelines of the US National Cancer Institute. A clinical AE attributable to azole treatment was defined as an event that was not present at baseline but developed during the treatment and resolved completely after cessation of therapy.

Measurement of plasma concentrations
In order to assess drug exposure in the cohort receiving primary antifungal prophylaxis, plasma samples were obtained under conditions of steady-state at trough prior to the next dose. Blood samples were collected in heparinized vials, promptly centrifuged and concentrations of voriconazole and posaconazole in plasma were determined by validated high-performance liquid chromatography methods.

Statistical considerations
Clinical and laboratory data were tabulated and analysed using descriptive statistics. Comparisons of continuous data were performed by the Mann–Whitney U-test. A two-sided P value of ≤0.05 was considered statistically significant.

Results

Patients
During the 5 year observation period, a total of 40 patients with newly diagnosed AML or RALs and indication for azole-based primary antifungal prophylaxis were admitted to our department and constitute the study population.

Twenty-one of the 40 patients were male and 19 were female. All were of Caucasian origin and the median age at the time of diagnosis was 9 years (range: 10 months to 17 years). The majority of patients (77.5%) had AML as the underlying condition, followed by recurrent ALL (17.5%) and recurrent AML (5%). The total number of episodes with indication for azole-based primary antifungal prophylaxis before the start of the phase of maintenance chemotherapy or admission for allogeneic HSCT was 160.

Primary allocation to antifungal prophylaxis
Thirty-six of the 40 patients ultimately received primary azole prophylaxis. Reasons for not being allocated to primary azole prophylaxis included concomitant use of contraindicated drugs (n = 1), pre-existing grade III elevations of hepatic transaminases (n = 1), early death due to haematological complications (n = 1) and unknown reasons (n = 1). In patients aged ≤12 years, 22/23 patients received voriconazole and 1 received posaconazole; in patients aged ≥13 years, 6/13 received posaconazole and 7 were placed on voriconazole.

Target attainment following allocation
Azole-based prophylaxis was administered in 130 of 149 total episodes with indication for prophylaxis, accounting for a target attainment rate of 87.2%. In 13 episodes, no prophylaxis was given and in 6 episodes, azole-based prophylaxis was replaced by prophylaxis with liposomal amphotericin B. Related to the calculated number of days with indication for prophylaxis, the target attainment rate was 77% (mean number of days per course with indication: 25.8; mean number of days per course with prophylaxis: 19.3).

Preventative efficacy
Azole-based primary antifungal prophylaxis was successful in 111/130 episodes (85.4%); related to patients and the entire period of intensive chemotherapy until the start of maintenance therapy or admission for allogeneic HSCT, the success rate was 58.3% (21/36) (Table 1). There were no deaths and no probable or proven IFIs were diagnosed.

In 10 episodes or seven patients, empirical antifungal therapy was given for a mean of 10.7 days (range: 6–20) for persistent fever during granulocytopenia. In six episodes or five patients, pre-emptive antifungal therapy was administered for a mean of 12 days (range: 3–22) on the basis of new pulmonary infiltrates detected by HR-CT without microbiological evidence for a fungal aetiology. Antifungal prophylaxis was discontinued due to exanthema in two patients allocated to voriconazole (subsequent switch to posaconazole (n = 1)/liposomal amphotericin B (n = 1)) and in one patient allocated to posaconazole (subsequent switch to voriconazole).

Safety and tolerance
Independent of causal relationship and allocated azole prophylaxis, increased hepatic transaminases (47/130 episodes; 36%) and serum bilirubin (16/130 episodes; 12.3%) while on treatment were frequent but mostly (41/47 and 14/16) of grade I or II, not exceeding grade III, and generally reversible. At the end of treatment, elevated hepatic transaminase and bilirubin levels were noted in 24 and 4 episodes (18.4% and 3%, respectively), with grade III changes limited to transaminase elevations of <300 U/L in two episodes. Moreover, mean serum bilirubin levels at the end of treatment were significantly lower (P < 0.001) and mean aspartate aminotransferase (AST) and alanine aminotransferase (ALT) values were not different from their respective baseline values (Figure 1).

Further attributable clinical AEs included phototoxic erythema (n = 6) and photophobia (n = 2) following voriconazole and skin rash (n = 2) following posaconazole treatment. Three courses (2.3%) were discontinued due to skin eruptions that were considered related to azole prophylaxis (Table 1).

Plasma concentrations at trough
A total of 23 plasma samples were obtained from 14 patients receiving voriconazole. Trough plasma concentrations of voriconazole were determined by validated high-performance liquid chromatography methods.31,32

Table 1. Preventative efficacy in 36 patients (130 episodes) with AML or RALs allocated to azole-based primary antifungal prophylaxis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Episodes [n (%)]</th>
<th>Patients [n (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empirical therapy</td>
<td>10 (7.7)</td>
<td>7 (19.4)</td>
</tr>
<tr>
<td>Pre-emptive therapy</td>
<td>6 (4.6)</td>
<td>5 (13.9)</td>
</tr>
<tr>
<td>Proven/probable IFI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Discontinuation</td>
<td>3 (2.3)a</td>
<td>3 (8.3)</td>
</tr>
<tr>
<td>Death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Success</td>
<td>111 (85.4)</td>
<td>21 (58.3)</td>
</tr>
</tbody>
</table>

IFI, invasive fungal infection.

Two patients receiving voriconazole developed exanthema and were switched to posaconazole (n = 1) and liposomal amphotericin B (n = 1); one patient receiving posaconazole developed exanthema and was switched to voriconazole.
voriconazole ranged from <0.2 to 6.4 mg/L (mean ± SD: 1.43 ± 1.90; median: 0.60 mg/L); in 26%, 39% and 56% of the samples, voriconazole concentrations were <0.2, ≤0.5 and <1.0 mg/L, respectively. Similarly, a total of seven samples were obtained from five patients receiving posaconazole. Trough plasma concentrations of posaconazole ranged from non-detectable to 0.424 mg/L (mean ± SD: 0.229 ± 0.158; median: 0.292 mg/L); in two and three of the samples, posaconazole concentrations were <0.100 and <0.250 mg/L, respectively.

**Antifungal prophylaxis and outcomes in patients with ALL or lymphoma**

During the 5 year observation period, there were 136 patients with newly diagnosed ALL or newly diagnosed paediatric lymphoma and no indication for azole-based primary antifungal prophylaxis, of whom 130 were available for analysis (6 patients had insufficient data). The majority of these patients had ALL (78%), followed by mature B-cell lymphoma/leukaemia (11%), lymphoblastic lymphoma (8%) and anaplastic large cell lymphoma (3%) (Table 2). The median age of these patients was 6 years (range: 0.75–18); 83 were male and 47 were female.

Thirty-four patients (26.2%) had received a total of 80 distinct episodes of primary antifungal prophylaxis at any time during the phase of intensive chemotherapy. The antifungal agents used for primary antifungal prophylaxis included fluconazole (n=13 patients), voriconazole (n=11), different sequences of agents (n=8) and liposomal amphotericin B (n=2). Empirical antifungal therapy for persisting fever during granulocytopenia was administered to 26 patients (20%) and pre-emptive antifungal therapy to 7 patients (5.4%) based on the detection of new pulmonary infiltrates by HR-CT without microbiological evidence for a fungal aetiology. Two patients developed probable and two patients proven invasive *Aspergillus fumigatus* aspergillosis. Invasive aspergillosis occurred during induction or reinduction chemotherapy for ALL, accounting for an incidence rate of 4% in ALL patients. One of the four patients had invasive pulmonary and cerebral aspergillosis, while three had invasive pulmonary aspergillosis, which was fatal in one case.

**Discussion**

Implementation of and adherence to a standard of oral, azole-based, mould-active antifungal prophylaxis in high-risk patients with AML and RALs coupled with a diagnostic and therapeutic algorithm at our centre were satisfactory. Ninety percent of eligible patients were allocated to primary prophylaxis as provided by the standard operating procedure, with reasons for not being allocated including contraindications in three of the four cases. The preferential use of voriconazole in the patients aged ≥13 years may have been related to reluctance to use a newly approved agent and/or the preference for tablets in this age group. Of note, the overall target attainment rate in allocated patients was 87.2% related to episodes and 77% related to the calculated number of days with indication for prophylaxis. Compared with previously published compliance rates for anti-infective preventive measures in

**Table 2. Antifungal prophylaxis, empirical and pre-emptive therapy and occurrence of invasive fungal infections in 130 patients with ALL or non-Hodgkin’s lymphoma without indication for primary antifungal prophylaxis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>ALL (n=101)</th>
<th>B-NHL (n=15)</th>
<th>LBL (n=10)</th>
<th>ALCL (n=4)</th>
<th>Total (n=130)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prophylaxis</td>
<td>23 (22.8)</td>
<td>7 (46.7)</td>
<td>3 (30)</td>
<td>1 (25)</td>
<td>34 (26.2)</td>
</tr>
<tr>
<td>Empirical therapy</td>
<td>20 (19.8)</td>
<td>5 (66.7)</td>
<td>—</td>
<td>1 (25)</td>
<td>26 (20.0)</td>
</tr>
<tr>
<td>Pre-emptive therapy</td>
<td>6 (5.9)</td>
<td>—</td>
<td>1 (10)</td>
<td>—</td>
<td>7 (5.4)</td>
</tr>
<tr>
<td>Proven/probable IFI</td>
<td>4 (4.0)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>4 (3.1)</td>
</tr>
<tr>
<td>Death</td>
<td>2 (2.0)c</td>
<td>1 (6.7)d</td>
<td>—</td>
<td>—</td>
<td>3 (2.3)</td>
</tr>
</tbody>
</table>

ALL, acute lymphoid leukaemia; B-NHL, mature B-cell lymphoma/leukaemia; LBL, lymphoblastic lymphoma; ALCL, anaplastic large cell lymphoma; IFI, invasive fungal infection.

cIncluding one patient with recurrent disease.

dIncluding two patients with recurrent disease.

Death due to invasive pulmonary aspergillosis (n=1) and influenza B (n=1) in haematological remission.

dDeath due to refractory recurrent disease.
Azole-based chemoprophylaxis in paediatric patients

paediatric cancer patients, these data attest to the feasibility of azole-based chemoprophylaxis under real-life circumstances.

Antifungal chemoprophylaxis with either posaconazole or voriconazole was effective, with low rates of empirical (7.7%) and pre-emptive (4.6%) treatment, no breakthrough fungal infections and 100% survival throughout intensive chemotherapy. Even when considering the cases with pre-emptive treatment, these rates are similar to or lower than those reported for prophylaxis with posaconazole or either posaconazole or voriconazole in adult AML/MDS patients, and comparable to the published paediatric cohort studies of voriconazole in patients with ALL and AML or haematological malignancies.

In severely immunocompromised children receiving intensive chemotherapy, azole-based chemoprophylaxis had acceptable safety without occurrence of unexpected toxicities: the rate of discontinuations due to AEs was 2%, with skin eruptions being the cause in all cases. Although increases in parameters of hepatic function during treatment were observed, these increases were mostly mild to moderate and mostly reversible. Indeed, mean AST and ALT values at the end of treatment were not different from their respective baseline values and mean serum bilirubin levels were lower, which suggests anticancer chemotherapy and its sequelae are important drivers of these changes. While the assessment of mild-to-moderate clinical AEs is compromised by the retrospective nature of the audit, these safety data are overall consistent with those reported in the literature for paediatric patients treated with voriconazole and posaconazole.

Consistent with previous data, pharmacokinetic variability was high after oral administration of both voriconazole and posaconazole, and in individual measurements, no or very low concentrations were observed. However, while there is suggestive evidence for therapeutic drug monitoring (TDM) of voriconazole in treatment of IFIs, the dosing target in the setting of prophylaxis is unknown. Similarly, while TDM has been advocated for posaconazole for both treatment and prophylaxis, its value in the prophylactic setting beyond the documentation of exposure is unclear.

An important endpoint of this audit was to assess the outcome of patients with ALL and lymphoma, for whom no primary antifungal prophylaxis was provided per the standard operating procedure. While 25% of the patients received antifungal chemoprophylaxis and 20% empirical antifungal therapy at some time during the phase of intensive chemotherapy, the rates of pre-emptive therapy for pulmonary infiltrates without microbiological evidence and of proven/probable IFIs (5.4% and 3.1%, respectively) were low and do not signal a need for a general indication for primary antifungal prophylaxis in this population. In a broader sense, this study shows the usefulness of institutional audits to validate antifungal strategies in high-risk patients and the notion that different strategies may not be mutually exclusive in complicated patient settings.

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Transparency declarations

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