



Benefits of risk-adapted and mould-specific antifungal prophylaxis in childhood leukaemia

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Received 23 April 2020; accepted for publication 8 June 2020

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Summary

Fluconazole is one of the most commonly used drugs for antifungal prophylaxis in childhood leukaemia. However, its interaction with vincristine may induce neuropathy and the emergence of antifungal drug resistance contributes to substantial mortality caused by invasive fungal infections (IFIs). In a retrospective single-centre study, we compared tolerability and outcome of different antifungal prophylaxis strategies in 198 children with acute leukaemia (median age 5.3 years). Until 2010, antifungal prophylaxis with fluconazole was offered to most of the patients and thereafter was replaced by liposomal amphotericin-B (L-AMB) and restricted to high-risk patients only. Vincristine-induced neurotoxicity was significantly reduced under L-AMB, as the percentage of patients with severe constipation decreased (15.4% vs. 3.7%, before vs. after 31 December 2010, $P = 0.01$) and stool frequency increased by up to 38% in polyene-treated patients ($P = 0.005$). Before 2011, 10 patients developed confirmed IFIs, most of them were infected with *Aspergillus* species. After risk adaption in 2011, IFIs were completely prevented ($P = 0.007$).

L-AMB prophylaxis is beneficial in childhood leukaemia patients, as it offers effective antifungal activity with improved tolerability as compared to fluconazole. The potential impact of our risk-adapted antifungal treatment should be included in current prophylaxis guidelines for childhood leukaemia.

Keywords: cancer, childhood leukaemia, invasive fungal infection, prophylaxis, liposomal amphotericin-B.

The 5-year survival rates for childhood acute leukaemia have risen from <20% in the 1960s to 60% in 1975 and surpass 90% today.^{1,2} Thus, the focus is currently not only on further improvement of therapy, but also on patient quality of life. When treating childhood acute leukaemia, invasive fungal infections (IFIs) still constitute a major cause of morbidity and mortality,^{3,4} including in patients undergoing haematopoietic stem cell transplantation (HSCT).^{5,6} Although guidelines for the prevention and management of IFIs have been developed,^{7,8} current clinical practice is highly variable, depending on the treating medical team.⁹ Presently, implementation of these recommendations is difficult, as administration of antifungals differs⁹ and local incidence of fungal disease (such as mould infections) varies.¹⁰ The quest for randomised studies in children is futile and does not follow the current concepts of regulatory authorities to make

treatments available to children. Finally, concerns have been raised regarding the toxicity profile of antifungal agents and the rates of drug interaction associated with vincristine and azole exposure.¹¹ In fact, triazole antifungals (namely, fluconazole, itraconazole, voriconazole and posaconazole) are inhibitors of cytochrome P450 enzymes and of the membrane transporter P-glycoprotein and decrease the metabolism of vincristine.⁹ This is clinically illustrated by recent reports of severe neuropathy occurring within a few days after co-administration of vincristine and azoles.^{12–17} Most commonly, severe constipation as part of autonomic neuropathy can occur while patients are in aplasia during induction therapy and consequently put them at risk for compromising gastrointestinal complications, including the development of ileus and sepsis.^{12–14} In view of the conceptual debate about whether to give azoles or alternative

antifungals during induction therapy, we performed a retrospective cohort study of children and adolescents undergoing treatment for acute leukaemia and compared the toxicities of antifungal treatment, the impact on patient quality of life and the occurrence of fungal infections in patients undergoing different antifungal prophylaxis strategies.

Patients and Methods

Ethics

Informed consent for data acquisition and analysis was obtained from all parents. This study was approved by the Ethics Committee of the Medical University of Innsbruck.

Patients and data collection

Childhood acute leukaemia patients (aged ≤ 18 years at diagnosis) undergoing induction therapy according to acute lymphoblastic leukaemia (ALL)-Berlin-Frankfurt-Münster (BFM) 2000, Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP)-BFM ALL 2009, acute myeloid leukaemia (AML)-BFM 2004 and AML-BFM 2012 protocols [National Cancer Institute (NCI)/European Union Drug Regulating Authorities Clinical Trials (EudraCT) Number: NCT00430118, 2007-004270-43, NCT00111345, 2013-000018-39] at the Department of Paediatrics of the Medical University of Innsbruck were included. Using an institutional cancer registry, 215 patients were retrospectively identified between 1 January 2000 and 31 December 2018. A total of 17 patients were excluded from the study, because they were transferred to an outside hospital within the first month of induction therapy. For efficacy analysis 198 patients were included. The safety and tolerability analysis comprised 195 patients, because in three patients' medical records regarding constipation and stool frequency were missing. Both ALL and AML protocols comprise the same induction protocol for all patients, followed by risk stratification and various consolidation and re-induction elements. According to ALL-BFM 2000 and AML-BFM 2004 protocols, all patients received fluconazole (3–5 mg/kg, once daily), whereas AIEOP-BFM 2009 and AML-BFM 2012 protocols restricted antifungal prophylaxis to patients at high risk of developing IFI. The criteria for 'high risk' included patients with AML, patients after stem cell transplantation, relapsed patients and patients with ALL and cytogenetics with poor prognosis or delayed response to chemotherapy (prednisone poor response, $\geq 10\%$ blasts on day 15 bone marrow or $\geq 5\%$ blasts on day 33 bone marrow), as described previously.^{7,18} Therefore, after 2010 antifungal prophylaxis was considered necessary only for high-risk patients (27 out of 82) and consisted of intravenous liposomal amphotericin-B (L-AMB) at a dose of 3–5 mg/kg given three-times a week. In the minority of patients L-AMB was not tolerated (11% of patients treated after 31 December 2010), voriconazole (4–8 mg/kg, twice

daily) was offered to patients aged < 13 years and posaconazole (300 mg/m², twice daily) was offered to older patients. Dose selection of fluconazole, voriconazole and posaconazole were based on European Medicines Agency (EMA) approval. Blood concentration of voriconazole and posaconazole were monitored and dose was adapted. The dosing regimen of L-AMB followed the recommendation of empirical treatment, but limited to 3 days/week. Low-risk patients did not receive any antifungal prophylaxis. The diagnostic algorithms in patients with persistent fever and/or clinical signs and symptoms have been described previously and did not change in the two decades.¹⁰

Demographic data, including age, sex, race and ALL classification (B- and T-cell phenotype, high risk of fungal infection) were collected. Probable invasive fungal infections were defined by the European Organisation for the Research and Treatment of Cancer (EORTC) criteria and considered proven only by a positive biopsy or the presence of positive microbiology from sterile body sites. Gradual investigation of all possible IFI patients (50 of 198) was performed, receiving a chest computed tomography (CT) within 96 h of fever of unknown origin. In the patients with positive CT findings (12 of 198; which is identical to the probable patients by EORTC criteria), lung biopsy confirmed a proven IFI in 10 patients. Two patients could be excluded from the probable group because of bronchiolitis obliterans pneumonia. During hospitalisation, bowel movement was assessed by the nurses and standardised daily record of stool number, form and consistency into the medical chart. Defecation frequency was calculated by the number of daily stools. Constipation was defined as the lack of stools for > 3 days in a week and was graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0.

Statistical analysis

Descriptive statistics were performed for all variables of interest, giving medians and interquartile ranges for quantitative variables, and absolute and relative frequencies for qualitative variables. Group differences for quantitative variables were assessed using non-parametric tests (Kruskal–Wallis test and Mann–Whitney *U*-test). The chi-square test was used to test for associations between choice of antifungal prophylaxis and grade of constipation. The Cochran–Armitage test was applied to test for an increase in constipation before and after 31 December 2010. Differences across diagnosed leukaemia (B-ALL, T-ALL, and AML) in outcome variables were assessed using the non-parametric two-way Scheirer–Ray–Hare test. The risk of fungal infections before and after 31 December 2010 was assessed via Kaplan–Meier curves, and compared with log-rank tests. All tests of statistical significance were two-sided, and a $P < 0.05$ was considered statistically significant. Data visualisation and analysis was performed using the IBM Statistical Package for the Social Sciences (SPSS®), version 24 (IBM Corp., Armonk, NY, USA).

Results

Clinical characteristics and cohort stratification of childhood leukaemia patients

The study cohort consisted of 198 patients receiving induction chemotherapy for childhood leukaemia; 194 (98%) patients were Caucasian and 110 (55.6%) were male. The majority of patients underwent treatment for either B-ALL (68.2%) or T-ALL (15.7%), while 14.1% were AML patients. Although overall survival reached 94.1% for B-ALL, 83.9% for T-ALL and 75.0% for AML Table I, some patients succumbed to confirmed IFI despite standard antifungal prophylaxis. Patients treated before 31 December 2010 (ALL-BFM 2000 and AML-BFM 2004 protocols, $n = 116$) immediately received antifungal prophylaxis with induction treatment. Azole prophylaxis was administered to 104 patients, the majority of whom (94%, $n = 98$) were treated with fluconazole Table II. In the case of suspected invasive fungal infection, biopsy was performed and antifungal treatment was adjusted. After 31 December 2010 (AEIOP-BFM 2009 and AML-BFM 2012 protocols, $n = 82$) patients were classified in two groups depending on their risk for fungal infection. The high-risk (HR) group ($n = 27$) received intravenous L-AMB, whereas the patients with standard and medium risk (non-HR, $n = 41$) received no prophylaxis Table II. Clinical characteristics stratified for the period before and after 31 December 2010 are listed in Table I. No significant differences in the presence of diagnosis, cytogenetics, central nervous system disease, or therapy response were found between the two groups, although classification as HR *versus* non-HR was slightly increased in the cohort after 2010.

Tolerability and safety of antifungal treatment with fluconazole and L-AMB

The occurrence of neuropathy due to drug interaction between vincristine and triazoles is commonly observed. A total of 20 patients in our study cohort had severe constipation, all of whom were vincristine-treated ALL patients. Severe constipation was observed in patients mostly before 31 December 2010 (15.4% *vs.* 3.7% for before *vs.* after 31 December 2010, Table III; $P = 0.01$). The rates of severe constipation were different across diagnosed leukaemia, with highest rates in T-ALL (29.4% *vs.* 7.1% for before *vs.* after 31 December 2010), and lowest rates in AML (no instances of severe constipation observed) (Table III, $P < 0.01$). However, the rates went uniformly down after 2010 across diagnosis groups ($P_{\text{int}} = 0.48$). Importantly, there was a significant shift in the number of patients with severe constipation allocated to the non-HR group (Table III), indicating that mainly non-HR patients benefited from risk-adapted antifungal strategy after 2010. Of the patients, 90% with severe constipation received antifungal prophylaxis with

fluconazole (Table III, $P = 0.012$). Daily stool frequency was increased by the risk-adapted use of antifungal prophylaxis in patients treated after 2010 (0.52 *vs.* 0.65 for before *vs.* after 31 December 2010, Table IV; $P = 0.014$). Accordingly, patients with fluconazole prophylaxis exhibited the lowest daily stool frequency as compared to L-AMB and no prophylaxis ($P = 0.005$ and $P = 0.01$ respectively, Table IV). Stool frequency was increased by up to 38% in patients treated with L-AMB. While there were differences across diagnosis groups (B-ALL: 0.51 *vs.* 0.65, T-ALL: 0.41 *vs.* 0.52, AML: 0.7 *vs.* 0.89; $P < 0.01$), the increase in daily stool frequency after 2010 was similar across diagnosis groups ($P_{\text{int}} = 0.52$).

As gastrointestinal symptoms are the major adverse events (AEs) of the fluconazole/vincristine interaction, the safety and tolerance analysis was expanded to include nephrotoxic AEs, the major adverse event of chronic L-AMB administration. The clinically relevant and often treatment-limiting, hypokalaemia and increases in serum creatinine were evaluated in L-AMB *versus* no L-AMB patients (Table V). More patients treated with L-AMB (44.4% *vs.* 21.8%, L-AMB *vs.* no L-AMB) were affected by hypokalaemia, but the difference did not reach statistical significance. Hypokalaemia was usually mild and always reversible; no patient was hospitalised because of severe hypokalaemia (Table V). In a minority of patients the level of serum creatinine was elevated but mostly only slightly to CTCAE Grade 1 (Table V).

Invasive fungal infections

Before 2011, the majority of patients were treated with fluconazole, and in 10 (8.6%) patients proven IFIs were diagnosed with a positive percutaneous CT-guided biopsy followed by microscopic analysis and culture (Table VI). Most of these patients were positive for *Aspergillus* species, which are resistant to fluconazole.¹⁰ Based on these epidemiological data, the antifungal strategy was adapted and IFIs were completely prevented in all patients after 2010 (Fig 1, $P = 0.007$).

Discussion

Currently azoles, in particular fluconazole, are the most commonly recommended/used agents for antifungal prophylaxis in high-risk paediatric patients treated for leukaemia. Although the term 'high risk' is not always properly defined, it is suggested that patients undergoing HSCT, receiving chemotherapy for AML or relapsed ALL are considered at high risk of developing an IFI.⁷ The guidelines issued by the Infectious Diseases Society of America (IDSA), Fever and Neutropenia (FN) in children with cancer and the European Conference on Infections in Leukaemia (ECIL) are not uniform and therefore the use of antifungal prophylaxes are quite diverse. In their recent therapeutic guidelines from 2018, the expert panel of the IDSA does not explicitly differentiate between adults and paediatric patients, and

Table I. Clinical characteristics of the 198 paediatric patients.

Characteristic	Patient cohort		Total	P ^a
	2000–2010	2011–2018		
Total no. of patients	116	82	198	
Age, years, median (IQR)	6.10 (2.92–11.18)	4.69 (2.74–8.64)	5.30 (2.86–10.11)	0.48
Sex, n (%)				0.80
Male	63 (54.4)	46 (56.1)	109 (55.1)	
Female	53 (45.6)	36 (43.9)	89 (44.9)	
Underlying diagnosis, n (%)				0.63
B-ALL	82 (70.7)	53 (64.6)	135 (68.2)	
T-ALL	17 (14.7)	14 (17.1)	31 (15.7)	
AML	14 (12.1)	14 (17.1)	28 (14.1)	
Infant leukaemia	3 (2.6)	1 (1.2)	4 (2.0)	
Cytogenetic, n (%)				0.84
Negative	82 (70.7)	55 (67.1)	137 (69.2)	
TEL/AML	24 (20.7)	19 (23.2)	43 (21.7)	
BCR/ABL	3 (2.6)	1 (1.2)	4 (2.0)	
MLL-AF	4 (3.4)	5 (6.1)	9 (4.5)	
Other	3 (2.6)	2 (2.4)	5 (2.5)	
Therapy response, n (%)				0.17
PGR	91 (78.4)	55 (67.1)	146 (73.7)	
PPR	10 (8.6)	13 (15.9)	23 (11.6)	
Not applicable	15 (12.9)	14 (17.1)	29 (14.6)	
Hyperleucocytosis, n (%)				0.78
No	102 (87.9)	71 (86.6)	173 (87.4)	
Yes	14 (12.1)	11 (13.4)	25 (12.6)	
CNS, n (%)				0.67
No	108 (93.1)	75 (91.5)	183 (92.4)	
Yes	8 (6.9)	7 (8.5)	15 (7.6)	
Risk group, n (%)				0.052
Non-HR	92 (79.3)	55 (67.1)	147 (74.2)	
HR, including relapse	24 (20.7)	27 (37.9)	51 (25.8)	
Allogeneic HSCT	14	14	26	
Survival, n (%)				
B-ALL	75 (91.5)	52 (98.1)	127 (94.1)	0.11
T-ALL	14 (82.4)	12 (85.7)	26 (83.9)	0.80
AML	11 (78.6)	10 (71.4)	21 (75.0)	0.66
Infant leukaemia	2 (66.7)	0 (0)	2 (50)	0.25

ALL, acute lymphoblastic leukaemia; AML, acute myeloblastic leukaemia; CNS, central nervous system; (non-)HR: (non-)high risk; HSCT, haematopoietic stem cell transplantation PGR, prednisone good response; PPR, prednisone poor response.

P value calculated using chi-square for categorical variables and Mann–Whitney U-test for age as a continuous variable.

recommends antifungal prophylaxis with an oral azole or parenteral echinocandins for patients with AML/MDS or undergoing HSCT.¹⁹ Based on three randomised controlled trials on empirical antifungal treatment,^{20–22} the 2017 FN CPG strongly recommended either caspofungin or L-AMB in IFI high-risk paediatric patients. For low-risk neutropenic children with persistent fever a weak recommendation to withhold empirical antifungal therapy is given.²³ The paediatric-specific ECIL recommendations are based on randomised trials in adults, paediatric pharmacokinetics and safety data and regulatory approval of the appropriate doses. The ECIL-4 guidelines, the latest update for treatment of IFI

in high-risk paediatric patients with cancer, recommend fluconazole (A-I), itraconazole or voriconazole (B-I) for allogeneic HSCT, voriconazole, posaconazole and itraconazole (B-I) in the presence of graft-versus-host disease and in patients with *de novo* or recurrent acute leukaemia itraconazole, posaconazole (B-I) or L-AMB (B-II).⁷ The updated ECIL-6 still recommends for adult patients with AML/MDS the use of posaconazole (A-I), itraconazole or fluconazole (B-I), but indicates that there is currently no approved standard care for patients with ALL, although the European Working Group for Adult ALL (EWALL) argues against the use of azoles because of potentially hazardous neurotoxic

Table II. Allocation to treatment groups before and after 31 December 2010.

	Patient cohort	
	2000–2010	2011–2018
Antifungal prophylaxis		
Total no. of patients	116	82
Antifungal prophylaxis, <i>n</i> (%)		
Echinocandins	8 (6.9)	2 (2.4)
L-AMB	4 (3.4)	30* (36.6)
Azole†	104 (89.7)	9‡ (11.0)
No prophylaxis	0 (0)	41 (50)

*Three patients were treated empirically with L-AMB.

†Fluconazole was given in 98 and five patients before and after 31 December 2010, respectively.

‡Four patients were prematurely L-AMB prophylaxis and due to allergic reactions switched to either voriconazole or posaconazole; five patients were treated with oral fluconazole for <5 days because of oral candidiasis.

interactions with vincristine.²⁴ Neurotoxicity is the most common dose-limiting factor of vincristine, resulting in a narrow therapeutic index.²⁵ Specifically, by drug interaction

with azole it could lead to deleted/omitted vincristine doses. Genetically polymorphic enzymes involved in the metabolism of vincristine revealed that the metabolic clearance for vincristine is significantly greater with cytochrome P450 (CYP) isoenzymes (CYP3A5, CYP3A).²⁶ In addition, all azoles inhibit CYP enzymes, resulting in decreased metabolism of vincristine. However, the inhibitory potential varies greatly, itraconazole and posaconazole are more potent inhibitors of CYP3A than are fluconazole or voriconazole.⁹ It needs to be considered that the exact molecular mechanisms of the interaction between triazoles and vincristine are not completely elucidated. These factors strongly impact the use of vincristine and may potentiate the vincristine-induced side-effects in individual patients.

Confronted with the dilemma of the most suitable antifungal treatment approach, we found that our refinement of risk stratification accompanied by reduced and replaced antifungal prophylaxis has reduced the risk of potentially life-threatening complications (e.g. ileus) without the occurrence of fungal infections. In our present study cohort, prophylaxis with fluconazole accounted for 90% of the cases of severe constipation and, moreover, fluconazole-treated patients had

Table III. Constipation in patients treated before and after 31 December 2010.

	Patient cohort			<i>P</i>
	2000–2010	2011–2018	Total	
Stool characteristic				
Total no. of patients	113	82	195	
Degree of constipation, <i>n</i> (%)				
Diarrhoea	1 (0.9)	4 (4.95)	5 (2.6)	
Regular	27 (23.9)	28 (34.1)	55 (28.2)	
Low	40 (35.4)	27 (32.9)	67 (34.4)	
Medium	28 (24.8)	20 (24.45)	48 (24.6)	
Severe	17 (15.4)	3 (3.66)	20 (10.25)	0.01*
Severe constipation, <i>n</i> (% of cohort)	17 (15.4)	3 (3.7)		0.01†
Severe constipation by risk group, <i>n</i> (% of severe cases)				
non-HR	16 (80)	1 (5)	17 (85)	
HR	1 (5)	2 (10)	3 (15)	
Severe constipation by prophylaxis, <i>n</i> (% of severe cases)				0.012‡
Fluconazole	17 (85)	1 (5)	18 (90)	
L-AMB	0	2 (10)	2 (10)	
No prophylaxis	–	0	0	
Severe constipation by diagnosis, <i>n</i> (% of diagnosis group)				
B-ALL	12 (15)	2 (3.8)	14 (10.5)	0.01§
T-ALL	5 (29.4)	1 (7.1)	6 (19.4)	0.09§
AML	0	0	0	1.0§

Differences across diagnosed leukaemias were assessed using the non-parametric two-way Scheirer–Ray–Hare test and were significant for time and diagnosis ($P < 0.01$). Rates went uniformly down after 2010 across diagnosis groups ($P_{\text{int}} = 0.48$).

ALL, acute lymphoblastic leukaemia; AML, acute myeloblastic leukaemia; (non-)HR: (non-) high risk; L-AMB, liposomal amphotericin-B.

*Cochran–Armitage test for trend indicates more severe constipation before 2011 ($P = 0.01$).

†Reduced number of patients with severe constipation after 31 December 2010 (Pearson's chi-square test, $P = 0.01$).

‡Significant correlation between severe constipation and antifungal prophylaxis and fluconazole (calculated with total patients, Pearson's chi-square test, $P = 0.012$).

§Decreased number of patients with ALL with severe constipation after 31 December 2010. Comparison within each diagnosis group before and after 31 December 2010 ($P = 0.01$, $P = 0.09$, $P = 1.0$, linear by linear chi-square test).

Table IV. Daily stool frequency in patients treated before and after 31 December 2010.

Stool characteristic	Patient cohort		Total	P
	2000–2010	2011–2018		
Total no. of patients	113	82	195	
Daily stool frequency by risk group, median (range)	0.52 (0.38–0.73)	0.65 (0.46–0.89)		0.014*
non-HR	0.5 (0.35–0.71)	0.67 (0.46–0.87)		0.012†
HR	0.63 (0.47–0.78)	0.61 (0.5–0.97)		
Daily stool frequency by prophylaxis, median (range)				0.009‡
Fluconazole			0.50 (0.35–0.73)	
L-AMB			0.69 (0.5–1.00)	
No prophylaxis			0.64 (0.48–0.76)	
Daily stool frequency by diagnosis, median (range)				
B-ALL	0.51 (0.36–0.70)	0.65 (0.47–0.84)	0.57 (0.4–0.76)	0.015§
T-ALL	0.41 (0.22–0.59)	0.52 (0.33–0.75)	0.46 (0.31–0.67)	0.15§
AML	0.7 (0.54–1.03)	0.89 (0.48–1.03)	0.87 (0.52–1.0)	0.91§

ALL, acute lymphoblastic leukaemia; AML, acute myeloblastic leukaemia; (non-)HR: (non-) high risk; L-AMB, liposomal amphotericin-B.

*Increased stool frequency in patients after 2010 ($P = 0.014$, Mann–Whitney U -test).

†Higher stool frequency in non-HR patients after 2010 ($P = 0.012$, Mann–Whitney U -test).

‡Significant differences in daily stool frequency and antifungal prophylaxis for all patients (Kruskal–Wallis test, $P = 0.009$). Comparison of fluconazole with L-AMB ($P = 0.005$, Mann–Whitney U -test) and fluconazole with no prophylaxis ($P = 0.03$, Mann–Whitney U -test).

§Increased daily stool frequency after 31 December 2010. Comparison within each diagnosis group before and after 31 December 2010 ($P = 0.015$, $P = 0.15$, $P = 0.91$, Mann–Whitney U -test). Differences across diagnosed leukaemias were assessed using the non-parametric two-way Scheirer–Ray–Hare test and were significant for diagnosis ($P < 0.01$) and time ($P = 0.013$). The increase in daily stool frequency after 2010 was similar across diagnosis groups ($P_{\text{int}} = 0.52$).

Table V. Renal adverse events in the cohort after 2010.

Adverse event	Patient cohort, n (%)		Total, n (%)	P
	No L-AMB	L-AMB		
Total no. of patients	55	27	82	
Grade of hypokalaemia (CTCAE)				0.105*
Any grade	12 (21.8)	12 (44.4)	24 (29.3)	
<LLN (3.0 mmol/l)	4 (7.3)	1 (3.7)	5 (6.1)	
<LLN (3.0 mmol/l); symptomatic, intervention indicated medium	2 (3.6)	4 (14.8)	6 (7.3)	
<3.0–2.5 mmol/l	5 (9.1)	5 (18.5)	10 (12.2)	
<2.5 mmol/l	1 (1.8)	2 (7.4)	3 (3.7)	
Grade of creatinine increase (CTCAE)				0.322*
Any grade	7 (12.7)	6 (22.2)	13 (15.9)	
>ULN – 1.5 × ULN	6 (10.9)	6 (22.2)	12 (14.6)	
>1.5–3.0 × baseline; >1.5–3.0 × ULN	1 (1.8)	0 (0)	1 (1.2)	

L-AMB, liposomal amphotericin-B; LLN, lower limit of normal; ULN, upper limit of normal.

*No difference in number of patients with hypokalaemia and creatinine increase adverse events (Pearson's chi-square test).

the lowest daily stool frequency (Table IV). Before 2011, most of the patients with severe constipation were stratified as non-HR, and particularly this group of patients benefited the most from withdrawal of antifungal prophylaxis after 2010. As a result of our risk stratification after 2010, 50% of the patients did not receive antifungal prophylaxis, and consequently possible drug interactions and side-effects were decreased. After 2010 only high-risk patients received antifungal prophylaxis with L-AMB. Although L-AMB is currently not approved as antifungal prophylaxis, it is approved as first-line indication for empirical therapy of persistently

neutropenic patients and for treatment of invasive aspergillosis and candidiasis.^{27–29} In a recent randomised study, caspofungin and L-AMB had comparable tolerability, safety and efficacy as a single agent for empirical antifungal therapy in neutropenic febrile children.²¹ In addition, a prospective cohort analysis concluded that L-AMB was well tolerated and an effective preventive antifungal approach for paediatric cancer patients at high risk of IFIs compared to azole treated historical controls.³⁰ Hypokalaemia, as an AE of L-AMB administration, occurred in 13.5% of the prophylactic episodes, but was usually mild and always reversible. Also one

Table VI. Characteristics of 10 patients with suspected IFI.

Patient number	Disease	Age, years	EORTC criteria	Underlying pathology
#014	AML	15.0	++	<i>Aspergillus fumigatus</i>
#028	HR-ALL	14.7	+	<i>Saccharomyces cerevisiae</i>
#034	ALL-R	9.3	+	<i>Aspergillus</i> species
#039	ALL-R	16.0	+	<i>Aspergillus terreus</i>
#048	ALL	15.0	+	<i>Aspergillus</i> species
#050	ALL	15.0	+	<i>Aspergillus</i> species + <i>Rhizopus</i>
#084	HR-ALL	15.5	+	<i>Aspergillus</i> species
#106	AML	1.45	+	<i>Aspergillus terreus</i>
#129	HR-ALL	16.7	+	<i>Aspergillus flavus</i>
#218	AML-R	14.1	++	<i>Aspergillus</i> species + <i>Candida</i>

ALL(-R), acute lymphoblastic leukaemia (-relapse); AML(-R), acute myeloblastic leukaemia (-relapse); HR-ALL, high-risk ALL; IFI, invasive fungal infection.

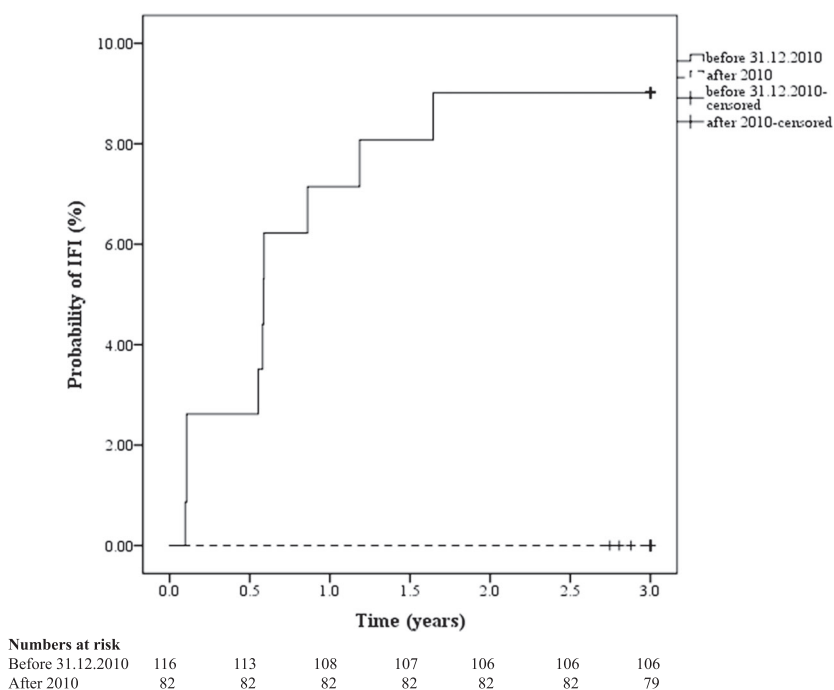


Fig 1. Estimated probability of invasive fungal infections (IFIs, hazard function). Before 2011, the majority of patients were treated with fluconazole and confirmed IFIs were diagnosed in 10 patients (8.6% of the cohort before 2011, $n = 116$). In the year 2011 the antifungal strategy was adapted to the use of liposomal amphotericin-B (L-AMB) and restricted to patients with a high risk of IFIs. IFIs were completely prevented in all patients after 2010 ($n = 82$). The log-rank test was used to compare the risk for IFI before and after 31 December 2010 ($P = 0.007$). [Colour figure can be viewed at wileyonlinelibrary.com]

out of three randomised placebo-controlled studies in adults with haematological malignancies observed significantly more patients with hypokalaemia and increased creatinine in the L-AMB-treated group.^{31–33} In our present cohort, we noticed that more patients treated with L-AMB were affected by hypokalaemia and increased creatinine, but the difference did not reach statistical significance (Table V). Hypokalaemia was usually mild and always reversible. Most importantly our approach, namely to withhold antifungal prophylaxis for non-HR patients and to switch to L-AMB

for HR patients, successfully prevented the occurrence of IFIs. Before 2011, IFIs were diagnosed in 8.6% of our patients, most of them infected with naturally fluconazole-resistant *Aspergillus* species. The newer generation of azoles (itraconazole, voriconazole, and posaconazole) show potent and broad-spectrum activity not only against *Candida* species, but also against most clinically important *Aspergillus* species.³⁴ However, as outlined above, also these agents show drug interaction with vincristine and frequently signs of neuropathy.

The present study has some limitations. The medical record of clinical signs of stool frequency and constipation were routinely recorded, but the hypothesis of the study and analysis of data was retrospectively performed. The criteria for inclusion in the study were diagnosis of leukaemia, including both patients treated with first-line chemotherapy but also with intensive therapies such as HSCT.

In conclusion, precise risk assessment in individual patients is essential to ensure that intensive treatment is limited primarily to high-risk patients, thus sparing low-risk patients undue exposure to toxicities. Our risk-adapted therapy works efficiently to prevent antifungal prophylaxis and reduces neurotoxicity. Finally, to validate our clinical observations, a prospective/randomised study comparing our risk-adapted strategy and azole prophylaxis would be important to consider its efficacy in preventing IFIs and drug interaction with vincristine.

Funding information

This work was supported by grants from 'Kinderkrebshilfe Tirol und Vorarlberg' and 'Kinderkrebshilfe Südtirol-Regenbogen'.

Author contributions

Roman Crazzolaro, Gabriele Kropshofer and Cornelia Lass-Flörl designed the study. Julia Hutter and Christina Salvador collected the data. Andreas Meryk, Josef Fritz and Roman Crazzolaro analysed the data. Andreas Meryk wrote the manuscript. All authors reviewed, revised, and approved the final version of the manuscript.

Conflict of Interest

The authors declare no conflict of interests.

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