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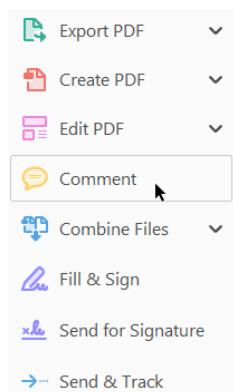
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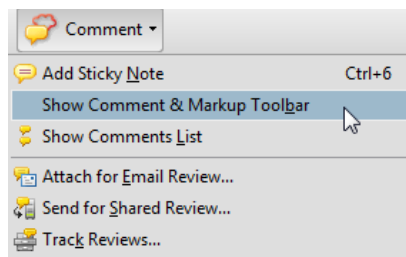


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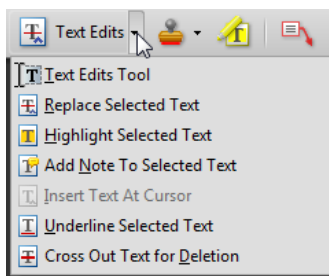


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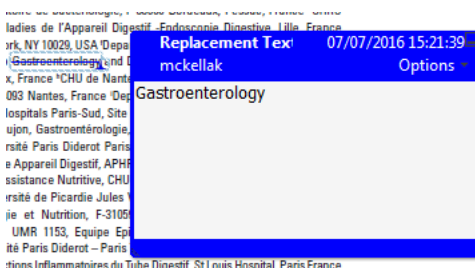
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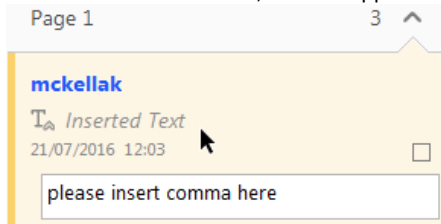


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Efficacy of pre-emptive versus empirical antifungal therapy in children with cancer and high-risk febrile neutropenia: a randomized clinical trial

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Objectives: To compare the efficacy of pre-emptive versus empirical antifungal therapy in children with cancer, fever and neutropenia.

Methods: This was a prospective, multicentre, randomized clinical trial. Children presenting with persistent high-risk febrile neutropenia at five hospitals in Santiago, Chile, were randomized to empirical or pre-emptive antifungal therapy. The pre-emptive group received antifungal therapy only if the persistent high-risk febrile neutropenia was accompanied by clinical, laboratory, imaging or microbiological pre-defined criteria. The primary endpoint was overall mortality at day 30 of follow-up. Secondary endpoints included invasive fungal disease (IFD)-related mortality, number of days of fever, hospitalization and use of antifungal drugs, percentage of children developing IFD, requiring modification of initial treatment strategy and need for ICU.

Results: A total of 149 children were randomized, 73 to empirical therapy and 76 to pre-emptive therapy. Thirty-two out of 76 (42%) children in the pre-emptive group received antifungal therapy. The median duration of antifungal therapy was 11 days in the empirical arm and 6 days in the pre-emptive arm ($P < 0.001$), with similar overall mortality (8% in the empirical arm and 5% in the pre-emptive arm, $P = 0.47$). IFD-related mortality was the same in both groups (3%, $P = 0.97$), as were the percentage of children with IFD (12%, $P = 0.92$) and the number of days of fever (9, $P = 0.76$). The number of days of hospitalization was 19 in the empirical arm and 17 in the pre-emptive arm ($P = 0.15$) and the need for ICU was 25% in the empirical arm and 20% in the pre-emptive arm ($P = 0.47$).

Conclusions: Pre-emptive antifungal therapy was as effective as empirical antifungal therapy in children with cancer, fever and neutropenia, significantly reducing the use of antifungal drugs.

Introduction

Invasive fungal disease (IFD) causes significant morbidity and mortality in paediatric cancer patients with high-risk febrile neutropenia (HRFN), along with high utilization of resources for prevention, diagnosis and treatment.^{1–4} Early diagnosis of IFD and prompt implementation of aggressive antifungal treatment have proven to be critical for patient survival.^{5,6} Nevertheless, early identification of the causal pathogen for an IFD continues to be difficult.

The classical approach is currently based on clinical, imaging, microbiological (cultures from sterile sites) and histological studies. Major advances for early diagnosis of IFD have been made by the development of non-culture assays such as detection of galactomannan (GM) antigen, mannan antigen and antibody, (1-3)- β -D-glucan antigen and nucleic acid detection, by PCR techniques.^{7,8} Despite these advances, IFD diagnosis continues to be a challenge^{9,10} and current recommendations propose to initiate

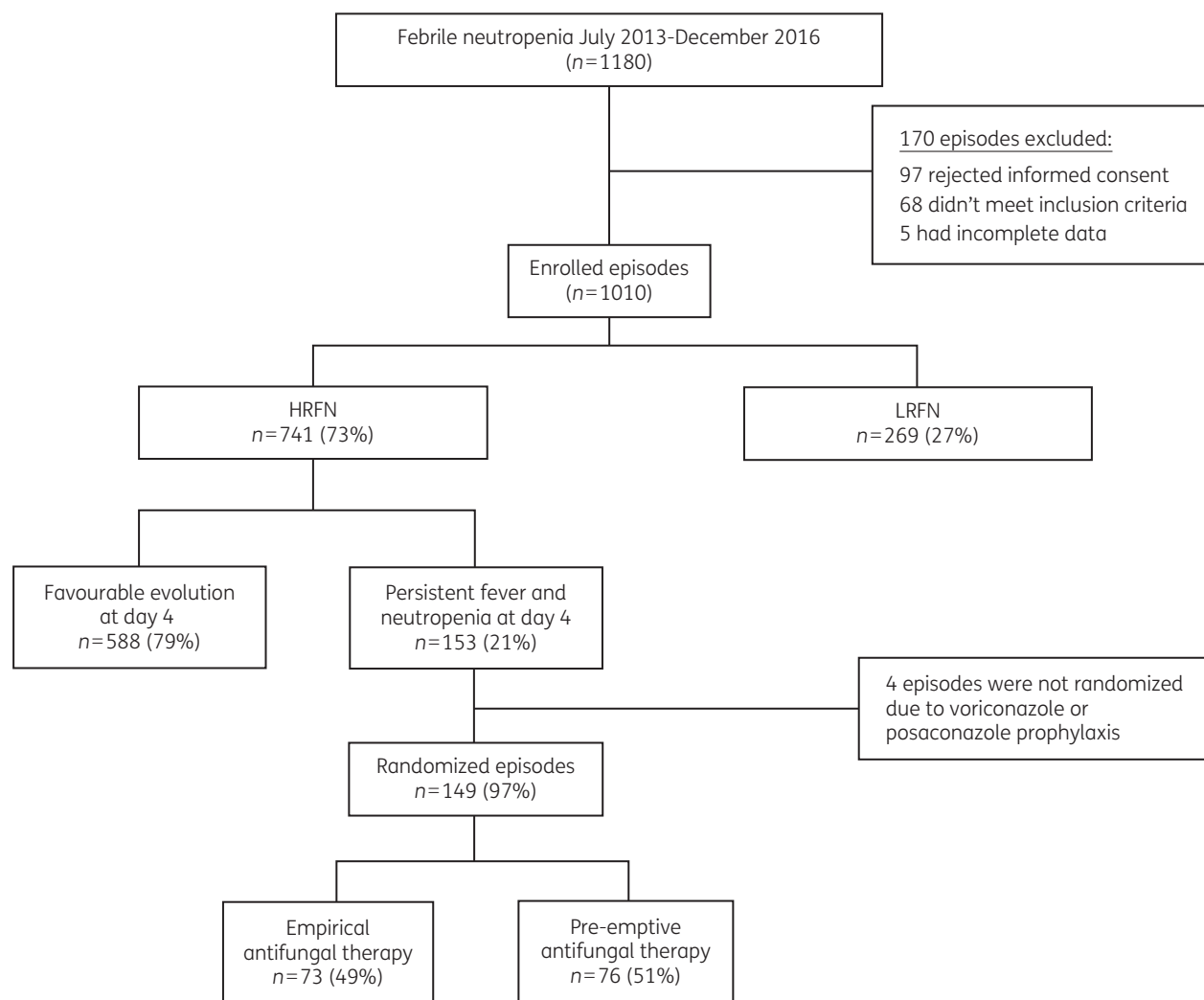


Figure 1. Episodes of fever and neutropenia evaluated, enrolled and randomized during the study period.

empirical antifungal therapy in IFD high-risk paediatric patients with persistent (≥ 96 h) fever and neutropenia unresponsive to broad-spectrum antibacterial agents, as a strong recommendation with high-quality evidence.¹¹ The downside of this approach is the overtreatment of patients meeting the above criteria but who do not have an IFD, leading to an increase in adverse events, prolonged hospitalizations and elevated costs associated with the use of antifungal drugs.¹²

A more reasonable approach in cancer subjects would be to consider early identification of patients at high risk of IFD, application of a complete screening diagnosis strategy followed by a rational approach for antifungal therapy based on results of this early and extensive diagnostic workup, adopting a more selective pre-emptive treatment strategy in patients with persistent fever and neutropenia.¹³

Studies aiming to reduce empirical antifungal overtreatment based on pre-emptive strategies have been published in adult patients with cancer and persistent fever and neutropenia.^{14–19} A meta-analysis published in 2015 reviewed nine studies, including randomized controlled trials, cohort studies and feasibility studies,

and demonstrated that in adult populations a pre-emptive strategy was associated with significantly lower antifungal exposure (relative risk 0.48; 95% CI 0.27–0.85) without an increase in IFD-related mortality (relative risk 0.82; 95% CI 0.36–1.87) or overall mortality (relative risk 0.95; 95% CI 0.46–1.99).²⁰ Similar studies in paediatric populations, to our knowledge, have not been performed.

In this study we aim to determine the efficacy of pre-emptive treatment compared with current standard empirical antifungal treatment in children with cancer and HRFN.

Methods

Population

From July 2013 to December 2016, a prospective, randomized, multicentre, government-sponsored study was conducted in five hospitals in Santiago, Chile, that belong to the National Child Programme of Antineoplastic Drugs network. Children and adolescents with cancer, ≤ 18 years of age, admitted because of a febrile neutropenic episode were invited to participate and enrolled after parental and child informed consent or assent (when older

Pre-emptive versus empirical antifungal therapy in children with cancer

than 8 years of age). Children with HSCT or under prophylaxis with voriconazole or posaconazole were excluded. The study was approved by the Ethics Committee of each participating institution.

Overall study design

- 5 Each child with an episode of febrile neutropenia was classified at admission as having low-risk febrile neutropenia (LRFN) or HRFN based on the following parameters: type of cancer, type and date of last chemotherapy,

Table 1. Admission characteristics of 149 children with persistent fever and neutropenia, randomized into empirical versus pre-emptive antifungal therapy

Characteristic	Intervention		P
	empirical, N = 73	pre-emptive, N = 76	
Age (years), median (IQR)	6 (4–12)	7 (3–11)	0.84
Male, n (%)	39 (53)	43 (57)	0.69
Type of cancer, n (%)			
leukaemia/lymphoma	46 (63)	49 (64)	0.85
leukaemia relapse	16 (22)	18 (24)	0.79
solid tumours	11 (15)	9 (12)	0.56
Use of granulocyte colony stimulating factor, n (%)	17 (23)	15 (20)	0.59
Use of central venous catheter, n (%)	62 (85)	64 (84)	0.90
Hours of fever prior to admission, median (IQR)	1 (1–3)	2 (1–4)	0.10
Admission clinical diagnosis, n (%)			
fever without focus	35 (48)	37 (49)	0.92
respiratory infection	17 (23)	16 (21)	0.74
intestinal focus	18 (25)	19 (25)	0.96
others ^a	3 (4)	4 (5)	0.73

Categorical variables were compared using the χ^2 test. Continuous variables were compared using the Mann–Whitney test.

^aOthers: skin/soft-tissue infection.

blood pressure, haematological status [absolute neutrophil count (ANC) and platelet count] and quantitative C-reactive protein (CRP).^{21,22} The microbiological diagnosis protocol included central and peripheral automated blood cultures and other cultures if clinically indicated. After this initial evaluation, all children were treated according to the guidelines for the management of febrile neutropenia in children with cancer.^{23,24} Briefly, all children were hospitalized and those at LRFN were treated with a third-generation cephalosporin (ceftriaxone) while children at HRFN were treated with an anti-pseudomonal third-generation cephalosporin (ceftazidime) plus amikacin, with or without an anti-Gram-positive β -lactam or glycopeptide antimicrobial. Children with HRFN episodes who continued with fever and neutropenia at day 4 of antimicrobial treatment were subject to a 1:1

Table 3. Demographic characteristics and clinical outcome of 18 children with final diagnosis of proven or probable IFD, randomized into empirical versus pre-emptive antifungal therapy

Characteristic	Intervention		
	empirical, N = 9	pre-emptive, N = 9	P
age (years), mean (SD)	9 (5)	8 (6)	0.81
male, n (%)	7 (78)	7 (78)	1.00
type of cancer, n (%)			
leukaemia/lymphoma/leukaemia relapse	8 (89)	9 (100)	1.00
solid tumours	1 (9)		
Clinical outcome			
mortality, n (%)	2 (22)	2 (22)	1.00
days of fever, mean (SD)	13 (5)	17 (6)	0.18
days of hospitalization, median (IQR)	21 (20–38)	26 (23–32)	0.50
days of antifungal, median (IQR)	21 (18–33)	17 (14–26)	0.35
antifungal modification required, n (%)	3 (33)	3 (33)	1.00
need for ICU, n (%)	5 (56)	4 (44)	1.00

Categorical variables were compared using Fisher's exact test. Continuous variables were compared using Student's *t*-test or the Mann–Whitney test according to data distribution.

Table 2. Clinical outcome of 149 children with persistent fever and neutropenia, randomized into empirical versus pre-emptive antifungal therapy

	Intervention		P	Relative risk (95% CI)
	empirical, N = 73	pre-emptive, N = 76		
Primary endpoint				
overall mortality at day 30, n (%)	6 (8)	4 (5)	0.47	0.64 (0.19–2.18)
Secondary endpoints				
IFD-related mortality at day 30, n (%)	2 (3)	2 (3)	0.97	0.96 (0.13–6.6)
days of fever, median (IQR)	9 (7–13)	9 (6–14)	0.76	
days of hospitalization, median (IQR)	19 (14–23)	17 (13–22)	0.15	
days of antifungal, median (IQR)	11 (7–16)	6 (3–13)	<0.001	
developing IFD, n (%)	9 (12)	9 (12)	0.92	0.96 (0.40–2.28)
antifungal start required, n (%)		32 (42)		
antifungal modification required, n (%)	15 (21)	12 (16)	0.45	0.77 (0.39–1.53)
need for ICU, n (%)	18 (25)	15 (20)	0.47	0.80 (0.43–1.46)

Categorical variables were compared using the χ^2 test. Continuous variables were compared using the Mann–Whitney test.

Table 4. Demographic, clinical, imaging and microbiological characteristics of 18 children with proven or probable IFD

Age [years (y) or months (m)]/sex	Type of cancer	Clinical focus and imaging	Maximum GM	Microbiological findings	Outcome	Randomization group
Proven IFD						
14 y/male	ALL relapse	sepsis/enterocolitis	0.2	BC(+) <i>Candida albicans</i> , BC(+) <i>Klebsiella pneumoniae</i>	died	empirical
17 y/male	ALL relapse	enterocolitis	0.4	BC(+) <i>Candida glabrata</i>	favourable	empirical
4 y/male	ALL	none	0.1	BC(+) <i>C. albicans</i>	favourable	empirical
4 y/male	ALL relapse	central venous catheter infection	0.3	BC(+) <i>Candida parapsilosis</i> , BC(+) <i>Escherichia coli</i>	favourable	pre-emptive
1 y/female	AML	enterocolitis	0.1	BC(+) <i>Candida tropicalis</i> , BC(+) <i>Pseudomonas</i> sp.	favourable	pre-emptive
17 y/male	ALL relapse	central venous catheter infection	0.2	BC(+) <i>Candida lusitanae</i> , BC(+) CoNS	favourable	pre-emptive
4 m/male	AML	enterocolitis	0.3	BC(+) <i>C. albicans</i>	favourable	pre-emptive
12 y/female	ALL relapse	pneumonia	0.7	<i>Aspergillus fumigatus</i> in lung biopsy	favourable	pre-emptive
3 y/male	AML	pneumonia	0.7	BC(+) <i>F. solani</i>	favourable	empirical
3 y/female	ALL	brain abscess	0.4	<i>S. apiospermum</i> in brain biopsy	favourable	empirical
13 y/male	lymphoma	none	0.2	BC(+) <i>S. kiliense</i>	favourable	empirical
11 y/male	AML	sinusitis/pneumonia	0.5	<i>Rhizopus</i> sp. in sinus biopsy	favourable	empirical
Probable IFD						
14 y/female	ALL	pneumonia	4.6	BC(+) <i>E. coli</i>	favourable	empirical
3 y/male	AML	pneumonia	6.4 (BAL)	BC(+) CoNS	died	empirical
9 y/male	solid tumour	pneumonia	0.8 (BAL)	none	favourable	empirical
11 y/male	ALL relapse	enterocolitis/pneumonia	4.6	BC(+) <i>E. coli</i>	died	pre-emptive
5 y/male	ALL	pneumonia	1.5	none	favourable	pre-emptive
10 y/male	AML relapse	pneumonia	7.7 (BAL)	BC(+) CoNS	died	pre-emptive

BC, blood culture.

simple randomization by the blinded study coordinator using statistical software (GraphPad Prism, version 6.01; GraphPad, San Diego, CA, USA) into two groups: one group followed current recommendations receiving empirical antifungal treatment starting on day 4 and the second group followed a novel pre-emptive treatment protocol receiving antifungal therapy, at any time of the evolution, only if persistent fever and $\text{ANC} \leq 500/\text{mm}^3$ were accompanied by any of the following findings suggesting IFD:²⁰ (i) clinical/imaging documented pneumonia or sinusitis (characteristic chest or sinus CT scan); (ii) skin lesions suggesting IFD; (iii) clinical/imaging enterocolitis; (iv) unexplained CNS symptoms; (v) splenic or hepatic characteristic imaging; (vi) single positive GM; or (vii) positive mycological finding.

All children, randomized to empirical or pre-emptive therapy, were evaluated with a standardized clinical, laboratory, imaging and microbiological panel for IFD. The evaluation included ANC, absolute monocyte count (AMC), CRP, repeat blood cultures, serum GM, chest and sinus CT scan, abdominal ultrasound and other imaging studies and diagnostic evaluations according to clinical presentation [fundoscopy, echocardiography, MRI, bronchoalveolar lavage (BAL), skin or other tissue biopsy].

Antifungal agents, in the empirical and pre-emptive groups, included liposomal amphotericin B, echinocandins or voriconazole, according to clinical and laboratory findings. Both groups were monitored until day 30 after enrolment for clinical, laboratory, imaging and microbiological resolution. Two investigators evaluated all cases at day 30, according to the primary and secondary endpoints, blind to information on randomization. Antifungal treatment was stopped or prolonged according to clinical, laboratory, imaging and microbiological findings in each individual case. Children who died were evaluated by a panel of experts including

oncologists and infectious diseases specialists from all participating hospitals to determine if the death was or was not related to IFD.

According to Ethics Committee requirements, it was possible to randomize each child in only one episode of febrile neutropenia that met study criteria; for this reason, one randomized episode of HRFN was equivalent to one randomized patient.

Study endpoints

The primary endpoint was overall mortality at day 30 of follow-up. Secondary endpoints included IFD-related mortality, number of days of fever, number of days of hospitalization, number of days of antifungal use, percentage of episodes that developed an IFD (proven, probable or possible), percentage of episodes requiring modification of initial treatment strategy (introduction of antifungal and antifungal modification) and percentage of episodes that needed ICU admission.

Definitions

- (i) Neutropenia: $\text{ANC} \leq 500/\text{mm}^3$.
- (ii) Fever: single axillary temperature $\geq 38.5^\circ\text{C}$ or $\geq 38^\circ\text{C}$ in two measurements separated by ≥ 1 h.
- (iii) HRFN: a febrile neutropenic episode with one or more of the following risk factors at the time of admission: (a) relapse of leukaemia as cancer type, (b) hypotension or (c) quantitative CRP ≥ 90 mg/L, or a febrile neutropenic episode with the following two factors at the time of admission: (d) ≤ 7 days between the end of the last

chemotherapy and the beginning of the fever and (e) platelet count $\leq 50\,000/\text{mm}^3$.

(iv) LRFN: a febrile neutropenic episode without the above mentioned factors at the time of admission.

(v) Persistent HRFN: fever and neutropenia ≥ 96 h in a child with HRFN.

(vi) IFD status was defined according to the European Organization for Research and Treatment of Cancer (EORTC) as follows: proven if the child had microbiological or histological evidence of fungal tissue invasion or a positive fungal culture obtained from a sterile site, in addition to clinical or radiological findings consistent with fungal infection; probable IFD required the presence of one or more host factors, a clinical criterion and one or more mycological criterion by a direct test (cytology, direct microscopy or culture) or an indirect test (detection of antigen); and possible IFD was defined as a case meeting host factor and clinical criteria but lacking any mycological documentation.²⁵

(vii) Overall mortality at day 30 of follow-up: mortality for any cause during the study period.

(viii) IFD-related mortality: mortality attributable to IFD, according to a panel of experts, occurring during the study period, in a child with proven or probable IFD.

Sample size

The primary endpoint for this non-inferiority study was overall mortality at day 30 of follow-up. Sample size was calculated considering that overall mortality at day 30 of follow-up in children with persistent HRFN is 6%, as previously reported by our group.^{26,27} Considering the same frequency of overall mortality in both groups, a type I error of 0.05 and a potency of 90%, we calculated that 140 episodes of persistent HRFN were required. Based on our previous experience, we have determined our capacity to enrol 300 febrile neutropenic episodes per year for a total of 1000 episodes in 3.5 years.

Statistical analysis

Categorical variables were compared using the χ^2 test or Fisher's exact test depending on the number of episodes per comparison group. Continuous variables were compared according to distribution using Student's *t*-test or the Mann-Whitney test. Overall mortality risk and the risk for secondary endpoints were evaluated for the exposed compared with the non-exposed groups, calculating the relative risk with the respective 95% CI. Statistical analyses were performed using GraphPad Prism version 6.01 and Stata IC version 14.0.

Results

Population characteristics

A total of 1010/1180 febrile neutropenic episodes were evaluated between July 2013 and December 2016 in the five participating hospitals; 170 episodes were excluded: 97 rejected informed consent, 68 did not meet inclusion criteria and 5 had incomplete data. Seven hundred and forty-one out of 1010 (73%) episodes were classified as HRFN episodes. At day 4 of evolution, 153/741 (21%) had persistent HRFN and met criteria for randomization, of which 149/153 (97%) were randomized, 73 into the empirical group and 76 into the pre-emptive group. Four episodes were not randomized due to prophylactic use of voriconazole or posaconazole (Figure 1).

Table 1 describes the main clinical characteristics of children with episodes of persistent HRFN randomized to empirical or pre-emptive antifungal therapy. No significant differences were observed in term of age, sex and type of cancer, with mainly haematological malignancy in both groups (85% and 88%, $P = 0.56$). Nearly 50% of the children had episodes of HRFN without

clinical foci at admission; the other 50% of children had respiratory or intestinal foci.

Outcome of children with persistent HRFN by study group

Table 2 shows the clinical outcome of 149 randomized children, according to the intervention. Overall mortality at day 30 of follow-up was 8% (6/73) in the empirical arm and 5% (4/76) in the pre-emptive group, with no difference in risks between groups ($P = 0.47$). In four of the ten children who died the expert panel concluded that mortality was related to an IFD, 2/73 (3%) in the group with empirical therapy and 2/76 (3%) in the group with pre-emptive therapy, with no difference in risk between groups ($P = 0.97$). The median number of days of antifungal therapy was 11 in the empirical arm and 6 in the pre-emptive arm ($P < 0.001$), the median number of days of fever was 9 in both groups ($P = 0.76$), the median number of days of hospitalization was 19 and 17, in the empirical and pre-emptive arms, respectively ($P = 0.15$), 12% of children developed IFD in each group ($P = 0.92$) and the need for ICU was 25% in the empirical arm and 20% in the pre-emptive arm ($P = 0.47$).

After randomization, 32/76 (42%) children in the pre-emptive group received antifungal therapy within the 30 day follow-up period. The median number of days for the initiation of antifungal therapy in this group was 9 (IQR 7–11). The median number of days of neutropenia showed no difference between groups, with 9 days in the empirical arm (IQR 7–13) and 10 in the pre-emptive arm (IQR 7–15), $P = 0.31$.

Diagnosis and outcome of children with proven or probable IFD by study group

Eighteen children developed a proven or probable IFD during the study, nine in the empirical group and nine in the pre-emptive group. Possible cases were not considered for the analysis. The median age of children with IFD was similar in the two groups (9 and 8 years in the empirical and pre-emptive groups, respectively, $P = 0.81$). Most of the children were male (78% in each group, $P = 1.00$) and had a haematological malignancy (89% and 100% in the empirical and pre-emptive groups, respectively, $P = 1.00$). The outcome of children with proven and probable IFD was similar, irrespective of whether they were randomized into the empirical or pre-emptive group. The percentage of children with proven or probable IFD who died was 22% in each group ($P = 1.00$), the median number of days of fever was 13 and 17 in the empirical and pre-emptive groups, respectively, ($P = 0.18$), the median number of days of hospitalization was 21 and 26 in the empirical and pre-emptive groups, respectively, ($P = 0.50$), the median number of days of antifungal use was 21 and 17 in the empirical and pre-emptive groups, respectively, ($P = 0.35$), 33% in each group required antifungal modification ($P = 1.00$) and 56% and 44% in the empirical and pre-emptive groups, respectively, needed ICU admission ($P = 1.00$) (Table 3).

The demographic, clinical, imaging and microbiological characteristics of the eighteen children with proven and probable IFD are described in Table 4. The most common species was *Candida* spp., causing seven cases of candidaemia. We also had seven cases of invasive aspergillosis, one proven and six probable, all with

pneumonia (by clinical and imaging findings) and positive GM detection in serum and/or in BAL. Other diagnoses included *Fusarium solani* infection, *Scedosporium apiospermum* brain abscess, *Sarocladium kiliense* fungaemia and mucormycosis.

5 Discussion

In our study, pre-emptive antifungal therapy was as effective as empirical antifungal therapy in children with cancer and HRFN, with a significant reduction in antifungal use. A reduction of antifungal use, based on stringent diagnostic criteria, could favour the adoption of evidence-based management strategies in this population. This approach requires optimal laboratory support with rapid turnaround response. Introduction of non-culture-based diagnostic techniques into clinical practice could contribute to better management of these patients, favouring the possibility of patient-based individualized therapy. Active monitoring and early diagnostic workup is a necessary step prior to proposing an evidence-based management strategy.^{28–30}

Studies comparing empirical versus pre-emptive antifungal therapy in adult populations have evaluated different endpoints: overall mortality, IFD-related mortality, percentage of patients with final diagnosis of IFD, percentage of patients receiving antifungal therapy and number of days of antifungal treatment.²⁰ Overall mortality was our primary endpoint in accordance with studies in adult populations and because it represents the most relevant outcome in children.

To our knowledge, this is the first prospective, multicentre, randomized study that compares standard versus pre-emptive antifungal therapy in HRFN children with prolonged fever and neutropenia. The comparative analysis between our study and studies in adult populations must be performed cautiously as populations and outcomes are different.²⁸ The pending question of which is the best approach for antifungal therapy in children with cancer cannot be definitely answered until other groups replicate these findings.

This study had limitations, including the lack of double-blinding, which was decided upon because the majority of these children received antifungal therapy through their central venous catheter. We decided not to use the central venous catheter for a possible placebo in order to avoid potentially deleterious manipulation. Another limitation is that we did not evaluate the toxicity of antifungal therapy. Results in adult populations concluded that pre-emptive antifungal therapy has been related to reduction of antifungal drug use and associated toxicity, without increasing mortality.²⁰

In our experience, 58% of patients of the pre-emptive group did not receive antifungal therapy, with similar clinical outcome to the empirical group, with the aim of reserving antifungal therapy for the subset of patients who have early evidence of IFD by careful clinical, laboratory, imaging and microbiological assessment.

The main findings of our study lead us to propose a step forward in the rational approach of children with cancer focusing on one yet unresolved issue in the management of the patients: adoption of a more selective pre-emptive antifungal treatment strategy in children with prolonged fever and neutropenia.

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Transparency declarations

None to declare.

References

- 1 Mor M, Gilad G, Kornreich L et al. Invasive fungal infections in pediatric oncology. *Pediatr Blood Cancer* 2011; **56**: 1092.
- 2 Drgona L, Khachatryan A, Stephens J et al. Clinical and economic burden of invasive fungal diseases in Europe: focus on pre-emptive and empirical treatment of *Aspergillus* and *Candida* species. *Eur J Clin Microbiol Infect Dis* 2014; **33**: 7–21.
- 3 Villarreal M, Avilés CL, Silva P et al. Risk factors associated with invasive fungal disease in children with cancer and febrile neutropenia: a prospective, multicenter evaluation. *Pediatr Infect Dis J* 2010; **29**: 816–21.
- 4 Fisher BT, Robinson PD, Lehrnbecher T et al. Risk factors for invasive fungal disease in pediatric cancer and hematopoietic stem cell transplantation: a systematic review. *J Pediatric Infect Dis Soc* 2017; doi:10.1093/jpids/pix030.
- 5 Hahn-Ast C, Glasmacher A, Mückter S et al. Overall survival and fungal infection-related mortality in patients with invasive fungal infection and neutropenia after myelosuppressive chemotherapy in a tertiary care centre from 1995 to 2006. *J Antimicrob Chemother* 2010; **65**: 761–8.
- 6 Maschmeyer G. Invasive fungal disease: better survival through early diagnosis and therapeutic intervention. *Expert Review Anti Infect Ther* 2011; **9**: 279–81.
- 7 Colombo AL, Cortes JA, Zurita J et al. Recommendations for the diagnosis of candidemia in Latin America. Latin America invasive mycosis network. *Rev Iberoam Micol* 2013; **30**: 150–7.
- 8 Lehrnbecher T, Robinson P, Fisher B et al. Galactomannan, β -D-glucan, and polymerase chain reaction-based assays for the diagnosis of invasive fungal disease in pediatric cancer and hematopoietic stem cell transplantation: a systematic review and meta-analysis. *Clin Infect Dis* 2016; **63**: 1340–8.
- 9 Groll AH, Castagnola E, Cesaro S et al. Fourth European Conference on Infections in Leukaemia (ECIL-4): guidelines for diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or allogeneic haemopoietic stem-cell transplantation. *Lancet Oncol* 2014; **15**: 327–40.
- 10 Santolaya ME, Alvarado T, Queiroz-Telles F et al. Active surveillance of candidemia in children from Latin America: a key requirement for improving disease outcome. Latin American invasive mycosis network. *Pediatr Infect Dis J* 2014; **33**: 40–4.
- 11 Lehrnbecher T, Robinson P, Fisher B et al. Guideline for the management of fever and neutropenia in children with cancer and hematopoietic stem-cell transplantation recipients: 2017 update. *J Clin Oncol* 2017; **35**: 2082–94.
- 12 Tissot F, Agrawal S, Pagano L et al. ECIL-6 guidelines for the treatment of invasive candidiasis, aspergillosis and mucormycosis in leukemia and hematopoietic stem cell transplant patients. *Haematologica* 2017; **102**: 433–44.
- 13 Morgan J, Hassan H, Cockle J et al. Critical review of current clinical practice guidelines for antifungal therapy in paediatric haematology and oncology. *Support Care Cancer* 2017; **25**: 221–8.

- 14 Cordonnier C, Pautas C, Maury S *et al*. Empirical versus preemptive antifungal therapy for high-risk, febrile, neutropenic patients: a randomized, controlled trial. *Clin Infect Dis* 2009; **48**: 1042–51.
- 5 15 Girmenia C, Micozzi A, Gentile G *et al*. Clinically driven diagnostic antifungal approach in neutropenic patients: a prospective feasibility study. *J Clin Oncol* 2010; **28**: 667–74.
- 16 Pagano L, Caira M, Nosari A *et al*. The use and efficacy of empirical versus pre-emptive therapy in the management of fungal infections: the HEMA e-Chart project. *Haematologica* 2011; **96**: 1366–70.
- 10 17 Freemantle N, Tharmanathan P, Herbrecht R. Systematic review and mixed treatment comparison of randomized evidence for empirical, pre-emptive and directed treatment strategies for invasive mould disease. *J Antimicrob Chemother* 2011; **66**: i25–35.
- 15 18 Martín-Peña A, Gil-Navarro MV, Aguilar-Guisado M *et al*. Cost effectiveness analysis comparing two approaches for empirical antifungal therapy in hematological patients with persistent febrile neutropenia. *Antimicrob Agents Chemother* 2013; **57**: 4664–72.
- 20 19 Barnes R, Earnshaw S, Herbrecht R *et al*. Economic comparison of an empirical versus diagnostic-driven strategy for treating invasive fungal disease in immunocompromised patients. *Clin Ther* 2015; **37**: 1317–28.
- 20 20 Fung M, Kim J, Marty F *et al*. Meta-analysis and cost comparison of empirical versus pre-emptive antifungal strategies in hematologic malignancy patients with high-risk febrile neutropenia. *PLoS One* 2015; **10**: e0140930.
- 25 21 Santolaya ME, Alvarez AM, Becker A *et al*. Prospective, multicenter evaluation of risk factors associated with invasive bacterial infection in children with cancer, neutropenia and fever. *J Clin Oncol* 2001; **19**: 3415–21.
- 22 Santolaya ME, Alvarez A, Avilés CL *et al*. Prospective evaluation of a model of prediction of invasive bacterial infection risk among children with cancer, fever and neutropenia. *Clin Infect Dis* 2002; **35**: 678–83. 30
- 23 Paganini H, Santolaya ME, Álvarez M *et al*. Diagnóstico y tratamiento de la neutropenia febril en niños con cáncer. Consenso de la Sociedad Latinoamericana de infectología pediátrica. *Rev Chil Infect* 2011; **28**: 10–38.
- 24 Lehrnbecher T, Phillips R, Alexander S *et al*. Guideline for the management of fever and neutropenia in children with cancer and/or undergoing hematopoietic stem-cell transplantation. *J Clin Oncol* 2012; **30**: 4427–38. 35
- 25 De Pauw B, Walsh T, Donnelly J *et al*. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infection Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis* 2008; **46**: 1813–21. 40
- 26 Santolaya ME, Alvarez AM, Avilés CL *et al*. Admission clinical and laboratory factors associated with death in children with cancer during a febrile neutropenic episode. *Pediatr Infect Dis J* 2007; **26**: 794–8.
- 27 Santolaya ME, Alvarez AM, Avilés CL *et al*. Prospective validation of a risk prediction model for severe sepsis in children with cancer and high-risk febrile neutropenia. *Pediatr Infect Dis J* 2013; **32**: 1318–23. 45
- 28 Cordonnier C, Robin C, Alanio A *et al*. Antifungal pre-emptive strategy for high-risk neutropenic patients: why the story is still ongoing. *Clin Microbiol Infect* 2014; **20**: 27–35. 50
- 29 Donnelly JP, Maertens J. The end of the road for empirical antifungal treatment? *Lancet Infect Dis* 2013; **13**: 470–2.
- 30 Klastersky J. Antifungal therapy in patients with fever and neutropenia—more rational and less empirical? *N Engl J Med* 2004; **351**: 1445–7.

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