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Invasive fungal disease in children with acute myeloid leukaemia: An Australian multicentre 10-year review

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Abstract

Background: Invasive fungal disease (IFD) is a common and important complication in children with acute myeloid leukaemia (AML). We describe the epidemiology of IFD in a large multicentre cohort of children with AML.

Abbreviations: AML, acute myeloid leukaemia; COG, Children's Oncology Group; EORTC, European Organization for Research and Treatment of Cancer; GM, galactomannan; HSCT, haematopoietic stem cell transplant; IFD, invasive fungal disease; IQR, interquartile range; PCR, polymerase chain reaction; TERIFIC, The Epidemiology and Risk factors for Invasive Fungal Infections in immunocompromised Children.

Methods: As part of the retrospective multicentre cohort TERIFIC (The Epidemiology and Risk factors for Invasive Fungal Infections in immunocompromised Children) study, proven/probable/possible IFD episodes occurring in children with primary or relapsed/refractory AML from 2003 to 2014 were analysed. Crude IFD prevalence, clinical characteristics, microbiology and treatment were assessed. Kaplan–Meier survival analysis was used to estimate 6-month survival.

Results: There were 66 IFD episodes diagnosed in 63 children with AML. The majority (75.8%) of episodes occurred in the context of primary AML therapy. During primary AML therapy, the overall prevalence was 20.7% (95% CI 15.7%–26.5%) for proven/probable/possible IFD and 10.3% (95% CI 6.7%–15.0%) for proven/probable IFD. Of primary AML patients, 8.2% had IFD diagnosed during the first cycle of chemotherapy. Amongst pathogens implicated in proven/probable IFD episodes, 74.4% were moulds, over a third (37.9%) of which were non-*Aspergillus* spp. Antifungal prophylaxis preceded 89.4% of IFD episodes, most commonly using fluconazole (50% of IFD episodes). All-cause mortality at 6 months from IFD diagnosis was 16.7% with IFD-related mortality of 7.6% (all in cases of proven IFD).

Conclusions: IFD is a common and serious complication during paediatric AML therapy. Mould infections, including non-*Aspergillus* spp. predominated in this cohort. A systematic approach to the identification of patients at risk, and a targeted prevention strategy for IFD is needed.

KEYWORDS
acute myeloid leukaemia, antifungal, children, invasive fungal disease, prevalence, prophylaxis

1 | INTRODUCTION

The prognosis of children with acute myeloid leukaemia (AML) has improved significantly over recent years with improvements in treatment and supportive care, along with advances in risk stratification according to cytogenetics and molecular markers.[1–3] Despite this progress, treatment-related morbidity and mortality, particularly in relation to infective complications, remain a significant challenge in children with AML.[4,5] Invasive fungal disease (IFD) remains an important complication in children with AML and is associated with significant mortality.[4–7] In a limited number of recent cohort studies, prevalence of IFD remains high in children with AML at 20%–25%.[8–10]

Previous studies describing IFD in Australian children with AML precede modern diagnostic and prophylactic strategies.[11,12] In a 4-year single-centre study from 2002 to 2005, 15% (5/34) of children with primary AML were diagnosed with an IFD.[12] Data from a national paediatric cancer registry between 1997 and 2008 found that 20% of 413 children diagnosed with AML had a microbiologically confirmed fungal infection during treatment; specific details on risk factors, microbiology, treatment and outcome were not reported.[11]

With ongoing advances in chemotherapeutic regimens and supportive care measures, it is likely that the epidemiology of fungal infection in children with AML is evolving. Moreover, the recent shift towards non-albicans *Candida* species,[13–15] along with the emergence of azole-resistant *Aspergillus* spp.[16] and non-aspergillus moulds[17–20] highlight the importance of accurate epidemiological data to inform prophylactic, diagnostic and treatment strategies.

The aim of this study is to further describe the epidemiology, diagnosis, management and prognosis of IFD in a cohort of children with AML, analysing data from a retrospective, multicentre cohort study of IFD in immunocompromised children in Australia from 2003 to 2014[21,22]

2 | METHODS

2.1 | Study design and population

The Epidemiology and Risk factors for Invasive Fungal Infections in immunocompromised Children (TERIFIC) study is a retrospective multicentre cohort study including all children treated for cancer or undergoing haematopoietic stem cell transplant (HSCT) across four Australian tertiary paediatric centres. IFD episodes in immunocompromised children were retrospectively identified in Brisbane between 2004 and 2014, Melbourne between 2004 and 2014, Perth between 2003 and 2014 and Sydney between 2012 and 2013. IFD episodes were ascertained through review of hospital oncology, pharmacy and microbiology databases and diagnostic coding data as previously described.[21,22] Data on baseline demographics, IFD details, IFD

management and treatment outcome to 6 months from IFD diagnosis were collected and entered into a centralised and secure web-based database. Human research ethics approval was granted at each study centre prior to study commencement.

In all contributing hospitals, administration of antifungal prophylaxis and diagnostic investigations were physician dependent. Antifungal prophylaxis was prescribed according to physician preference, largely consistent with previous national guidelines recommending fluconazole prophylaxis, with itraconazole, an echinocandin or liposomal amphotericin B as alternatives.[23] Regarding diagnostic workup for IFD, patients were typically investigated after 3–5 days of persistent fever including radiological (X-ray, computed tomography and magnetic resonance imaging) and microbiological (fungal culture, galactomannan [GM] and *Aspergillus* polymerase chain reaction [PCR]) investigations. The GM assay was available at Brisbane and Sydney for the entire relevant study periods at those sites and was introduced during the study period in Melbourne (2005) and Perth (2013). *Aspergillus* PCR was available at all sites throughout the study period but was infrequently utilised.

The total number of patients diagnosed with AML at each centre during the study period was collected.

2.2 | Inclusion and exclusion criteria

Data on children with a diagnosis of AML were extracted from the study database. IFD episodes occurring in children with AML post-HSCT were included in the analysis only if IFD was diagnosed in the context of relapse of AML, with receipt of cytotoxic chemotherapy within the last 30 days. IFD episodes occurring in the immediate post-HSCT period (<30 days post HSCT) or as a late complication post HSCT in patients not receiving ongoing AML therapy were excluded.

There was likely some overlap between the cohort included in this study and the previous report from the Australia Paediatric Cancer Registry (1997–2008) by Foresto et al.[11] There was no overlap with the single-centre study reported by Hale et al.[12] as this centre was not a site in the TERIFIC study.

2.3 | Definitions

IFD episodes were classified as proven, probable or possible according to the European Organization for Research and Treatment of Cancer (EORTC)/Invasive Fungal Infections Cooperative Group criteria.[24] Disseminated IFD was defined as either microbiological or radiological findings consistent with an IFD in two or more noncontiguous sites. Consistent with previous analysis,[25] patients in whom antifungal prophylaxis had been administered for at least 7 consecutive days in the month prior to IFI diagnosis were classified as having received antifungal prophylaxis. Mortality attributed to an IFD was determined according to the documented opinion of the treating physician and review by the principal investigator at the respective study centre.

2.4 | Statistical analysis

Descriptive analyses were used to report demographic, clinical and mycological characteristics using median and interquartile range (IQR) for continuous data and frequency and percentage for categorical data. Chi-square test or Fisher's exact test were used to assess the relationship between categorical variables, and Mann–Whitney *U* test or Kruskal–Wallis test for continuous variables (including age and treatment duration). Crude IFD prevalence with 95% confidence intervals (CI) was calculated for patients at study centres with available denominator data (Brisbane, Melbourne and Perth) by dividing the number of children with an IFD during primary AML therapy by the total number of patients newly diagnosed with AML during the study period. Denominator data for relapsed AML were not available. Overall survival probability was estimated for those who met EORTC criteria for proven/probable/possible IFD using the Kaplan–Meier method at 6 months from IFD diagnosis. Statistical analyses were performed using R statistical software.

3 | RESULTS

Amongst 348 IFD episodes in 331 children, there were a total 66 separate IFD episodes diagnosed in 63 individual patients with AML (Table 1). A total of 23 episodes of proven IFD, 13 episodes of probable IFD and 30 episodes of possible IFD were diagnosed. There were 50 IFD (75.8%) episodes diagnosed during primary AML therapy in 49 patients. The most common study protocols for AML therapy in children diagnosed with IFD were Children's Oncology Group (COG) AAML0531 (53.1%) and COG AAML1031 (16.3%).[26,27] Of the IFD episodes during primary AML therapy, 38.0% (n = 19/50) were diagnosed during the first cycle of chemotherapy at a median of 21 days following AML diagnosis (IQR 17.5–24.5). Of patients with IFD in the setting of treatment for relapsed/refractory AML, 37.5% (n = 6/16) had previously undergone HSCT; IFD was diagnosed at a median 354 days post-HSCT (IQR 198–652).

3.1 | Prevalence

IFD prevalence was calculated for episodes occurring in from Melbourne, Brisbane and Perth. There were a total of 232 children diagnosed with primary AML during the study period at these centres (Melbourne n = 108, Brisbane n = 62 and Perth n = 62). There were 65 IFD episodes in 62 patients at these sites (Table 2). IFD prevalence during primary AML therapy was 20.7% (95% CI 15.7%–26.5%) overall, and was similar between sites (Table 2). IFD prevalence during the first cycle of primary AML therapy was 8.2% (n = 19/232). For proven and probable IFD, prevalence during primary AML therapy was 10.3% (95% CI 6.7%–15.0%) overall, 9.3% (95% CI 4.5%–16.4%) in Melbourne, 9.7% (95% CI 3.6%–19.9%) in Brisbane and 12.9% (95% CI 5.7%–23.9%) in Perth.

TABLE 1 Patient characteristics per IFD episode

Total number of IFD episodes	***n* = 66**
Median age, years (IQR)	11.7 (5.8–14.6)
Gender (F)	38 (57.6%)
Disease stage, *n* (%)	
- Primary	50 (75.8%)
- Nonprimary (relapse/refractory)	16 (24.2%)
Cycle of therapy at diagnosis: primary disease (*n* = 50)	
- 1	19 (38.0%)[a]
- 2	5 (10.0%)
- 3	8 (16.0%)
- 4 or greater	18 (36.0%)
previous HSCT (*n* = 6)	
Median days post HSCT (IQR)	354 (198–652)
Donor type: matched sibling	3 (50.0%)
Donor type: matched unrelated donor	3 (50.0%)
- Umbilical cord	2 (33.3%)
Graft-versus-host disease within last month	2 (33.3%)
Fungal prophylaxis, *n* (%)	
Any prophylaxis	59 (89.4%)
Fluconazole	33 (50.0%)
Mould active	26 (39.4%)
- Posaconazole	5 (7.6%)
- Voriconazole	3 (4.5%)
- Itraconazole	15 (22.7%)
- Liposomal amphotericin	3 (4.5%)
Other IFD risk factors	
Central venous access device in situ	63 (95.5%)
Steroids in last 3 months	11 (16.7%)
- Median duration, days (IQR)	7 (5–8)
Neutropenia (<0.5 μmol/L) within 30 days, *n* (%)	65 (98.5%)
- Median duration, days (IQR)	21 (13–24)

Abbreviation: IFD, invasive fungal disease.
[a]Median day of diagnosis: day 18 (range 7–25).

3.2 | Microbiology and site of infection

There were 39 fungal pathogens identified in 36 proven and probable IFD episodes on microbiological or histopathological examination (Table 3) (two episodes had two mould pathogens and one episode had two yeast pathogens identified). Invasive mould infection (IMI) predominated in both primary AML (80.8% (*n* = 21/26) of IFD isolates) and relapsed/refractory AML (61.5% [*n* = 8/13] of IFD isolates). Non-aspergillus moulds comprised 37.9% (*n* = 11/29) of IMI isolates overall; *Lomentospora prolificans* (formerly *Scedosporium prolificans*) was the most common species, comprising 63.6% [*n* = 7/11] of non-aspergillus mould isolates. All yeast infections were caused by *Candida* spp. (as classified at time of diagnosis) including one *Candida albicans* and nine non-albicans *Candida* infections (including four due to *Pichia kudriavzevii* [formerly referred to as *Candida krusei*]).[28]

There was no significant difference in age between children with yeast and mould infections IFD (median age 7.5 years vs. 11.3 years; $p = .289$) (Table 4). There was no significant change in the proportion of mould infections comparing the first and second halves of the study period (65.2% vs. 92.3%; $p = .114$). Similarly, the number of mould infections as a proportion of proven/probable IFD episodes was similar comparing IFD episodes diagnosed during primary AML therapy and episodes occurring during relapsed/refractory AML therapy ($p = .409$). Non-aspergillus moulds were implicated in 20% (*n* = 5/25) of proven/probable IFD episodes during primary AML therapy and 36.3% (*n* = 4/11) of episodes during relapsed/refractory AML therapy.

The site of infection for proven and probable IFD episodes varied according to causative organism (Figure 1). For mould IFD, the lung was the primary site in 81.5% (*n* = 22/27) of episodes. For yeast IFD, blood was primary site in 88.9% (*n* = 8/9) of episodes. Disseminated IFD occurred in 37.0% (*n* = 10/27) of patients with mould infection, and 66.7% (*n* = 6/9) of those with yeast infections. In 96.7% (*n* = 29/30) of possible IFD episodes, the primary site was the lung, based on radiological findings meeting the EORTC definition.

3.3 | Impact of prophylaxis

Overall, 89.4% (*n* = 59/66) of IFD episodes occurred in patients who had received antifungal prophylaxis for at least 7 consecutive days in the month prior to diagnosis of IFD, including 97.2% (*n* = 35/36) of proven/probable IFD episodes. There was variation between centres in antifungal prophylactic agent preceding breakthrough-proven/probable IFD episodes with most occurring in patients on fluconazole in Brisbane (70.0%, *n* = 7/10) and Perth (64.3%, *n* = 9/14), whilst itraconazole was the most common agent used prior to breakthrough IFD in Melbourne (66.7%, *n* = 8/12).

A mould was implicated as the causative organism in 77.8% of proven/probable IFD episodes in patients receiving fluconazole prophylaxis compared to 76.5% in those receiving a mould-active prophylactic agent ($p = 1.0$) (Table 4). Amongst patients with breakthrough-proven/probable IFD on mould active prophylaxis (*n* = 17), the

TABLE 2 IFD episodes by disease status and study site

Study site		Melbourne	Brisbane	Perth	Total
Total primary AML diagnoses		**108**	**62**	**62**	**232**
IFD episodes					
Proven IFD	Primary AML (prevalence)	6 (5.6%)	4 (6.5%)	6 (8.1%[a])	16 (6.5%[a])
	Nonprimary AML	0	2	5	7
	total	6	6	11	23
Probable IFD	Primary AML (prevalence)	4 (3.7%)	2 (3.2%)	3 (4.8%)	9 (3.9%)
	Nonprimary AML	2	2	0	4
	total	6	4	3	13
Possible IFD	Primary AML (prevalence)	10 (9.3%)	7 (11.3%)	7 (11.3%)	24 (10.3%)
	Nonprimary AML	4	1	0	5
	total	14	8	7	29[b]
IFD total	Primary AML (prevalence) [95% CI]	20 (18.5%) [11.7%–27.1%]	13 (21.0%) [11.7%–33.2%]	16 (24.2%[a]) [14.2%–36.7%]	49 (20.7%a[a]) [15.7%–26.5%]
	Nonprimary AML	6	5	5	16
	Total episodes	26	18	21	65[b]

Abbreviation: IFD, invasive fungal disease.
[a]One patient had two proven IFD during primary AML therapy.
[b]Excluding Sydney (one case of possible IFD).

proportion of mould infections was similar between agents (Table 4). The majority of the proven/probable breakthrough IFD in the mould-active triazole group occurred in patients on itraconazole prophylaxis (61.5%, $n = 8/13$) only one of whom had had a level measured, which was sub-therapeutic (<0.5 mg/L).

3.4 | Treatment and survival

The median duration of antifungal treatment was 107 days (IQR 37–179 days) for IFD episodes: 103 days (IQR 28–180.5) for proven IFD, 151 days (IQR 59–186) for probable IFD and 107 days (IQR 47–154.8) for possible IFD ($p = .68$). Of the 14 patients with invasive aspergillosis, five (35.7%) received initial treatment with voriconazole monotherapy, four (28.6%) with voriconazole in combination with liposomal amphotericin B (>7 days), four (28.6%) with liposomal amphotericin B followed by voriconazole and one (7.1%) with caspofungin monotherapy. Of the nine patients with invasive candidiasis, eight (88.9%) received initial treatment with liposomal amphotericin B (followed by caspofungin in two and fluconazole in one) and one (11.1%) received caspofungin initially. Of the seven patients with *Lomentospora prolificans*, three received combination therapy with voriconazole and terbinafine, one was treated with posaconazole, one with voriconazole and two passed away before initiation of directed therapy.

Eleven deaths occurred within 6 months of IFD diagnosis, with an overall all-cause mortality of 16.7% (Figure 2A). Five deaths (7.6% IFD-related mortality) were attributed to IFD, all in patients with proven IFD: four with *Lomentospora prolificans* infection and one with candidaemia (*C. glabrata*). There were six deaths not attributed to IFD including in three patients with possible IFD, two with probable IFD (*Aspergillus* spp.) and one with proven IFD (*C. guilliermondii*).

Overall 6-month mortality was not significantly different between proven/probable IFD episodes (22.2% [95% CI 8.6%–35.8%]) and possible IFD episodes (10.0% [95% CI 0.0%–20.7%]) (Figure 2B). Pathogen-specific mortality was highest for *Lomentospora prolificans* IFD at 57.1% ($n = 4/7$), followed by *Candida* spp. 20.0% ($n = 2/10$) and *Aspergillus* spp. 14.3% ($n = 2/14$).

4 | DISCUSSION

Our multicentre study is one of the largest cohort studies examining IFD in children with AML. We found a high prevalence of IFD in children with AML, despite the majority of patients receiving antifungal prophylaxis prior to IFD diagnosis. The overall IFD prevalence of 20.7% and prevalence of 10.3% for proven/probable IFD during primary AML therapy in our study are comparable to recent data from Asia,[8,10] North America[29] and Europe.[9,30,31] The proportion of patients on antifungal prophylaxis varied in these previous studies, with only 5% receiving prophylaxis in one study,[8] whilst in other studies, the majority of patients received prophylaxis.[9,10,30] Similarly the choice of antifungal agent for prophylaxis was heterogenous amongst studies, with fluconazole[29,31] and mould active triazoles[10,30] the most common agents used. The overall prevalence of proven/probable/possible IFD in our study was also similar to the groups receiving caspofungin (18.9%) and fluconazole (19.9%) prophylaxis in the recently published trial in children and young adults with AML by Fisher et al.[32] In contrast to several previous reports,[8,29,30] we found a predominance of moulds amongst causative pathogens, including a significant proportion of

TABLE 3 Causative pathogens—proven and probable IFD episodes

	Primary AML		Non-primary AML		
Pathogen	**Proven**	**Probable**	**Proven**	**Probable**	**Total**
Moulds	12	9	3	5	29[e]
Aspergillus spp.	3	8	0	3	14
- *A. fumigatus*	1	2		2	5
- *A. flavus*	1				1
- *A. niger*		1			1
- *Aspergillus* spp.	1[a]	5[b]	0	1[c]	7
Non-*Aspergillus* moulds	**6**	**–**	**3**	**2**	**11**
- Mucormycete	2				2
- *Lomentospora prolificans*	3		3	1	7
- *Penicillium* spp.	1			1	2
Other mould (not specified)[d]	**3**	**1**			**4**
Yeasts	**5**	**–**	**5**	**–**	**10**[e]
Candida spp.					
- *C. albicans*	1				**1**
- *C. parapsilosis*	1		1		**2**
- *Pichia kudriavzevii*	3		1		**4**
- *C. guillermondii*			1		**1**
- *C. glabrata*			1		**1**
- Other *Candida* spp.			1		**1**

Abbreviation: IFD, invasive fungal disease.
[a]Lung biopsy +ve on histo, culture –ve, serum GM +ve.
[b]GM +ve 5/6, aspergillus DNA +ve BAL 1/6.
[c]Serum GM +ve only.
[d]Histopathology demonstrating fungal hyphae, microbiology negative.
[e]36 Episodes of proven/probable IFI—39 causative pathogens.

non-*Aspergillus* spp. Together with the paucity of *C. albicans* infections, this data may represent an extension of previously observed trends towards increases in non-aspergillus moulds and non-albicans *Candida* infections in children with cancer[6] and more broadly.[13,17]

A notable finding in our study was the high proportion of IFD occurring within a month of AML diagnosis; 8.2% of all patients with primary AML had IFD diagnosed during the first cycle of chemotherapy. The early onset of IFD observed in our study is in keeping with recent adult data demonstrating high prevalence of IFD early in AML therapy, consistent with the inherent immunodeficiency associated with the AML disease process.[33] A role for baseline computerised tomography (CT) imaging for early detection of IFD in adult patients with de novo AML has been proposed[33]; however, early imaging is not considered standard practice at present at adult centres.[34] Our findings of high prevalence of IFD in the period immediately following AML diagnosis suggest a broad systems based approach is required to identify patients at highest risk of IFD, implement prophylaxis from the start of treatment and facilitate timely diagnostic testing to minimise time to initiation of appropriate antifungal therapy.

Almost 90% of patients in our study received antifungal prophylaxis prior to diagnosis of IFD, highlighting the potential limitations of fluconazole and itraconazole prophylaxis. Fluconazole, which was the prophylactic agent used in half of IFD cases in our study, may have insufficient spectrum for the purpose of IFD prophylaxis in this setting, considering the predominance of moulds and non-albicans *Candida* spp.[13] It is also possible that use of fluconazole prophylaxis may have led to selection of more resistant organisms[35] although fluconazole prophylaxis in children with AML has been associated with a survival benefit, suggesting an overall beneficial impact.[36] Itraconazole prophylaxis preceded almost one quarter of IFD episodes in our study. Although itraconazole has a broader spectrum than fluconazole, including anti-mould activity, efficacy may have been

TABLE 4 Proven and probable IFD episodes—yeasts versus moulds by age and prophylactic agent

Pathogen	Yeasts	Moulds			Proven/probable IFD
		Aspergillus	Non-Aspergillus	Total moulds	
Median age, years (IQR)	7.5 (3.5–12.5)	11.3 (8.8–16.1)	9.7 (3.8–12.8)	11.4 (7.3–14.3)	11.1 (5.6–13.6)
Primary AML, *n* (%)	5 (20%)	11	5	20[a] (80%)	25
Non-primary AML, *n* (%)	4 (36.4%)	3	4	7 (63.6%)	11
Timing of diagnosis					
2003–2008, *n* (%)	8 (34.8%)	8	4	15[a] (65.2%)	23
2009–2014, *n* (%)	1 (7.7%)	6	5	12[a] (92.3%)	13
Fungal prophylaxis, *n* (%)					
No prophylaxis	1 (100%)	0	0	0	1
Fluconazole	4 (22.2%)	7	4	14[a] (77.8%)	18
Mould active	4 (23.5%)	7	5	13[a] (76.5%)	17
- Posaconazole	–	1	2	3 (100%)	3
- Voriconazole	1 (33.3%)	–	2	2 (66.7%)	3
- Itraconazole	1 (12.5%)	6	1	7 (87.5%)	8
- Liposomal amphotericin	2 (66.7%)	–	–	1[a] (33.3%)	3
Total	**9 (25%)**	**14**	**9**	**27[a] (75%)**	**36**

[a]Including patients with histopathology demonstrating hyphae and negative microbiology (total $n = 4$).

limited in these patients due to a lack of appropriate therapeutic drug monitoring and the use of older formulations with poor oral bioavailability.[37]

Notably, whilst previous regional guidelines from North America[38] and Australia[23] recommended fluconazole prophylaxis for children with primary AML, recent European[39] and international[40] consensus guidelines recommend the use of a mould-active agent in this population. Newer mould-active antifungal agents and formulations are now available, presenting a number of options for prophylaxis in children with AML; the body of data demonstrating safety and efficacy of these agents in children is growing. Posaconazole has been shown to be superior to itraconazole or fluconazole in preventing proven/probable IFD in patients ≥13 years of age with AML or myelodysplasia[41] yet is not licensed for use in children less than 13 years of age. Furthermore, attainment of target serum levels is poor with the available liquid posaconazole formulation.[42] There are promising emerging paediatric data including in children less than 13 years of age suggesting posaconazole delayed-release tablets may be a suitable option for prophylaxis in children with AML with superior attainment of target trough levels.[43] Similarly, a superior bioavailability formulation of itraconazole (SUBA-itraconazole) has shown promise in adult IFD prophylaxis,[44,45] with emerging safety and dosing data in children.[46] Echinocandins are another potential option for prophylaxis in paediatric AML, as demonstrated in the recent randomised trial by Fisher et al. showing significant reduction in proven/probable IFD with caspofungin compared with fluconazole prophylaxis.[32] Intermittent liposomal amphotericin B is another approach used in clinical practice that circumvents the drug interactions associated with triazoles and the daily intravenous infusion required for echinocandin prophylaxis, although efficacy data in children are relatively limited.[47] Longer extended dosing intervals for both amphotericin B and echinocandin prophylaxis have been explored, yet efficacy data to support widespread paediatric use remain limited.[48] Additional comparative data, ideally from prospective paediatric-specific studies are required to inform an optimal IFD prophylaxis strategy in paediatric AML. Based on data from the TERIFIC study demonstrating a high proportion of mould IFD in children with AML, and in line with recent international guidelines,[39,40] the updated national Australian guidelines for antifungal prophylaxis recommend prophylaxis with a mould-active antifungal agent for children with AML.[49] Ensuring implementation of these updated recommendations across paediatric centres is a priority together with efforts to optimise antifungal prescribing, including improving consistency of therapeutic drug monitoring for mould-active triazoles. Furthermore, with ongoing advances in chemotherapeutic regimens and supportive care measures, together with the changing epidemiology of fungal pathogens, it is likely that the epidemiology of IFD in children with leukaemia will continue to evolve. Real-time surveillance of IFD epidemiology in oncology patients will be important to assess impact of changes in antifungal prophylaxis prescribing and to inform future prophylactic strategies. Promisingly, recent technological advances including natural language processing of radiology reports have shown potential in facilitating prospective surveillance in adult patients,[50] yet there are limited paediatric data examining this approach to date.

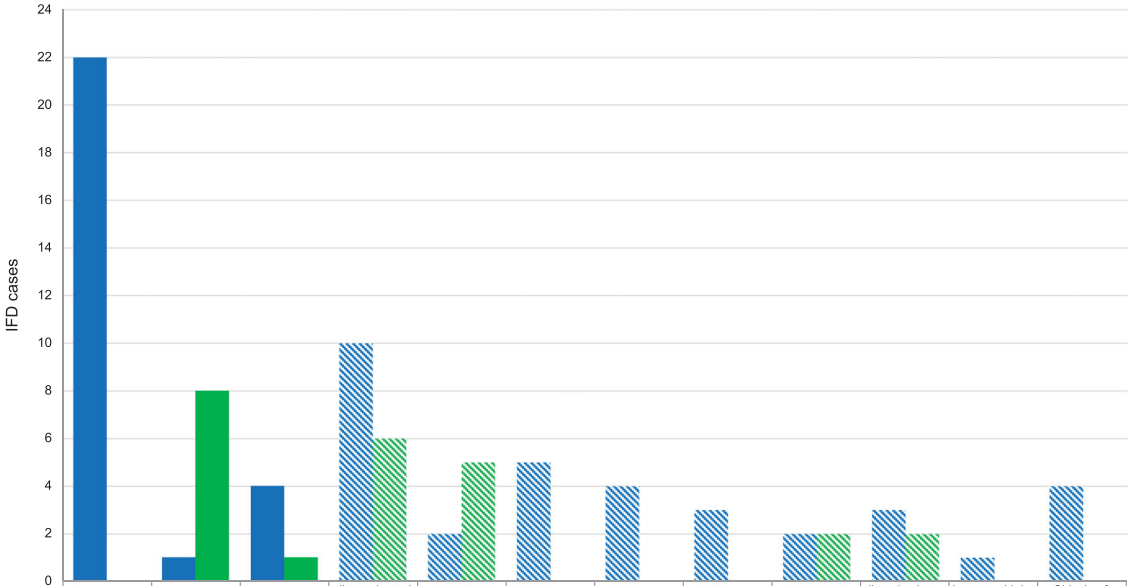

FIGURE 1 Site of infection: proven and probable invasive fungal disease

The overall mortality of 16.7% in our study is lower compared to some previous reports,[8,10,31] possibly due to differences in period of follow-up, availability of antifungal agents and underlying disease-related risk factors. In contrast, mortality attributable to proven/probable IFD of 7.6% in our study is higher than reported in other series.[9,29] *Lomentospora prolificans*, a pathogen with intrinsic antifungal resistance associated with a high mortality, was the causative agent in 80% of proven/probable IFD-attributable deaths in our study, highlighting the challenges in managing infections caused by this pathogen.[51,52] The relatively high number of *Lomentaspora prolificans* infections in this cohort compared with international studies in children with AML may be due to the epidemiological niche of this organism, which has been predominantly isolated in regions with arid climates including Australia.[53]

There were several limitations of this study. The retrospective study design may have led to incomplete ascertainment of IFD episodes. The impact of this is expected to be small given use of multiple data sources including hospital coding, pharmacy and microbiology databases to identify IFD episodes, a robust approach that has been demonstrated to be superior to use of administrative coding data alone, which misses 40% of IFD episodes in paediatric cancer patients.[54] Data on antifungal prophylaxis in the denominator population was not available, limiting the ability to draw conclusions about efficacy of different prophylactic strategies. Although it is likely that the majority of children with AML who did not develop IFD received antifungal prophylaxis in keeping with local and international guidelines,[23,40] recommendations regarding optimal choice of agent differs between guidelines,[55] which is reflected by variations in prescribing in clinical practice.[56,57] A broader understanding of antifungal prophylaxis prescribing is therefore required to determine effectiveness of prophylactic strategies. Similarly, we were unable to determine denominator data for relapsed/refractory AML, hence prevalence was only calculated for primary AML. The number of IFD episodes occurring outside of primary AML therapy was not insignificant, highlighting the ongoing risk of IFD in patients who are receiving therapy for relapsed/refractory AML and the need for continuing prophylaxis in these patients. Finally, we chose to include possible IFD in determining prevalence, a group where there is, by definition, diagnostic uncertainty. The rationale for inclusion of possible IFD was to allow comparison with previous cohort studies and also to account for expected heterogeneity in diagnostic approach between centres. To account for the limitations of including possible IFD, we also analysed prevalence, impact of prophylaxis, site of infection and survival separately for proven/probable IFD. The high proportion of possible IFD in this cohort highlights the challenges in diagnosing IFD in children, for whom CT imaging findings lack specificity[58,59] and microbiological sampling is often contraindicated, impractical or inconclusive[60]; improving consistency of diagnostic approach using existing tools and further exploration of novel diagnostic strategies should be prioritised.

In conclusion, this study demonstrates the high burden of IFD in a cohort of Australian children with AML, including early in primary chemotherapy, despite widespread use of antifungal prophylaxis, mostly with fluconazole or itraconazole. The predominance of mould infections, including a significant proportion of non-*Aspergillus* species, is reflective of the changing epidemiology of IFD in immunosuppressed patients more broadly, highlighting the importance of prospective IFD surveillance to inform future preventative strategies. Our findings also support recent consensus recommendations for mould-active prophylaxis in children with primary AML.[39,40] A systematic approach incorporating epidemiological data together with comparative efficacy data

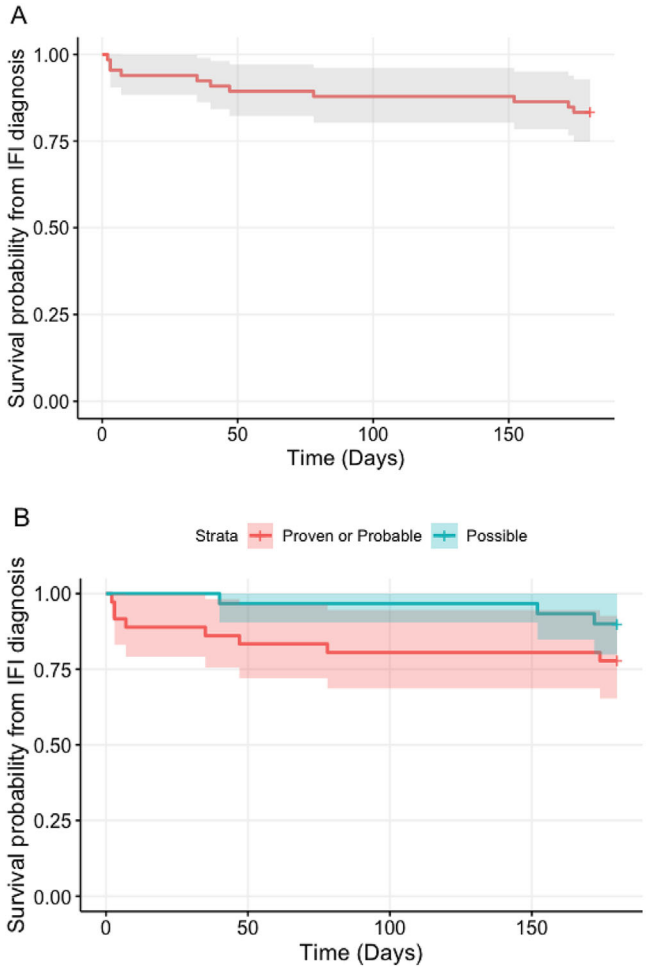

FIGURE 2 Kaplan–Meier estimates for overall survival for patients with acute myeloblastic leukaemia and invasive fungal disease (A) overall (B) according to European Organization for Research and Treatment of Cancer (EORTC)-MSG classification comparing proven/probable and possible cases

on antifungal prophylactic approaches is required to identify patients at highest risk and inform targeted IFD prophylactic and diagnostic strategies for children with AML.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Creutzig U, van den Heuvel-Eibrink MM, Gibson B, et al. Diagnosis and management of acute myeloid leukemia in children and adolescents: recommendations from an international expert panel. *Blood*. 2012;120(16):3187-3205.
2. Reedijk AMJ, Klein K, Coebergh JWW, et al. Improved survival for children and young adolescents with acute myeloid leukemia: a Dutch study on incidence, survival and mortality. *Leukemia*. 2019;33(6):1349-1359.
3. Rasche M, Zimmermann M, Borschel L, et al. Successes and challenges in the treatment of pediatric acute myeloid leukemia: a retrospective analysis of the AML-BFM trials from 1987 to 2012. *Leukemia*. 2018;32(10):2167-2177.
4. Sung L, Lange BJ, Gerbing RB, Alonzo TA, Feusner J. Microbiologically documented infections and infection-related mortality in children with acute myeloid leukemia. *Blood*. 2007;110(10):3532-3539.
5. Sung L, Buxton A, Gamis A, Woods WG, Alonzo TA. Life-threatening and fatal infections in children with acute myeloid leukemia: a report from the Children's Oncology Group. *J Pediatr Hematol Oncol*. 2012;34(1):e30-e35.
6. Pana ZD, Roilides E, Warris A, Groll AH, Zaoutis T. Epidemiology of invasive fungal disease in children. *J Pediatric Infect Dis Soc*. 2017;6(suppl_1):S3-S11.
7. 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;7(3):191-198.
8. Lin G-L, Chang H-H, Lu C-Y, et al. Clinical characteristics and outcome of invasive fungal infections in pediatric acute myeloid leukemia patients in a medical center in Taiwan. *J Microbiol Immunol Infect*. 2018;51(2):251-259.
9. Lehrnbecher T, Schöning S, Poyer F, et al. Incidence and outcome of invasive fungal diseases in children with hematological malignancies and/or allogeneic hematopoietic stem cell transplantation: results of a prospective multicenter study. *Front Microbiol*. 2019;10:681.
10. Kobayashi R, Hori D, Sano H, Suzuki D, Kishimoto K, Kobayashi K. Risk factors for invasive fungal infection in children and adolescents with hematologic and malignant diseases: a 10-year analysis in a single institute in Japan. *Pediatr Infect Dis J*. 2018;37(12):1282-1285.
11. Foresto SA, Youlden DR, Baade PD, Hallahan AR, Aitken JF, Moore AS. The outcomes and treatment burden of childhood acute myeloid leukaemia in Australia, 1997–2008: a report from the Australian Paediatric Cancer Registry. *Pediatr Blood Cancer*. 2015;62(9):1664-1666.
12. Hale KA, Shaw PJ, Dalla-Pozza L, MacIntyre CR, Isaacs D, Sorrell TC. Epidemiology of paediatric invasive fungal infections and a case-control study of risk factors in acute leukaemia or post stem cell transplant. *Br J Haematol*. 2010;149(2):263-272.
13. Chapman B, Slavin M, Marriott D, et al. Changing epidemiology of candidaemia in Australia. *J Antimicrob Chemother*. 2017;72(4):1103-1108.
14. Zeng Z, Tian G, Ding Y, Yang K, Deng J, Liu J. Epidemiology, antifungal susceptibility, risk factors and mortality of invasive candidiasis in neonates and children in a tertiary teaching hospital in Southwest China. *Mycoses*. 2020;63(11):1164-1174.
15. Benedict K, Roy M, Kabbani S, et al. Neonatal and pediatric candidemia: results from population-based active laboratory surveillance in four US locations, 2009–2015. *J Pediatric Infect Dis Soc*. 2018;7(3):e78-e85.

16. van der Linden JW, Camps SM, Kampinga GA, et al. Aspergillosis due to voriconazole highly resistant *Aspergillus fumigatus* and recovery of genetically related resistant isolates from domiciles. *Clin Infect Dis*. 2013;57(4):513-520.
17. Wattier RL, Dvorak CC, Hoffman JA, et al. A prospective, international cohort study of invasive mold infections in children. *J Pediatric Infect Dis Soc*. 2015;4(4):313-322.
18. Lionakis MS, Lewis RE, Kontoyiannis DP. Breakthrough invasive mold infections in the hematology patient: current concepts and future directions. *Clin Infect Dis*. 2018;67(10):1621-1630.
19. Douglas AP, Chen SC, Slavin MA. Emerging infections caused by non-Aspergillus filamentous fungi. *Clin Microbiol Infect*. 2016;22(8):670-680.
20. Lamoth F, Chung SJ, Damonti L, Alexander BD. Changing epidemiology of invasive mold infections in patients receiving azole prophylaxis. *Clin Infect Dis*. 2017;64(11):1619-1621.
21. Bartlett AW, Cann MP, Yeoh DK, et al. Epidemiology of invasive fungal infections in immunocompromised children; an Australian national 10-year review. *Pediatr Blood Cancer*. 2019;66(4):e27564.
22. Wang SS, Kotecha RS, Bernard A, et al. Invasive fungal infections in children with acute lymphoblastic leukaemia: results from four Australian centres, 2003–2013. *Pediatr Blood Cancer*. 2019;66(10):e27915.
23. Fleming S, Yannakou CK, Haeusler GM, et al. Consensus guidelines for antifungal prophylaxis in haematological malignancy and haemopoietic stem cell transplantation, 2014. *Intern Med J*. 2014;44(12b):1283-1297.
24. Donnelly JP, Chen SC, Kauffman CA, et al. Revision and update of the consensus definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis*. 2020;71(6):1367-1376.
25. Wang SS, Kotecha RS, Bernard A, et al. Invasive fungal infections in children with acute lymphoblastic leukaemia: results from four Australian centres, 2003–2013. *Pediatr Blood Cancer*. 2019;66(10):e27915.
26. Gamis AS, Alonzo TA, Meshinchi S, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III Children's Oncology Group trial AAML0531. *J Clin Oncol*. 2014;32(27):3021-3032.
27. Richard A, Soheil M, Lillian S, et al. Bortezomib with standard chemotherapy for children with acute myeloid leukemia does not improve treatment outcomes: a report from the Children's Oncology Group. *Haematologica*. 2020;105(7):1879-1886.
28. Kidd SE, Halliday CL, McMullan B, Chen SC-A, Elvy J. New names for fungi of medical importance: can we have our cake and eat it too? *J Clin Microbiol*. 2021;59(3):e02730-20.
29. Johnston DL, Lewis V, Yanofsky R, et al. Invasive fungal infections in paediatric acute myeloid leukaemia. *Mycoses*. 2013;56(4):482-487.
30. Czyzewski K, Galazka P, Fraczkiewicz J, et al. Epidemiology and outcome of invasive fungal disease in children after hematopoietic cell transplantation or treated for malignancy: impact of national programme of antifungal prophylaxis. *Mycoses*. 2019;62(11):990-998.
31. Cesaro S, Tridello G, Castagnola E, et al. Retrospective study on the incidence and outcome of proven and probable invasive fungal infections in high-risk pediatric onco-hematological patients. *Eur J Haematol*. 2017;99(3):240-248.
32. Fisher BT, Zaoutis T, Dvorak CC, et al. Effect of caspofungin vs fluconazole prophylaxis on invasive fungal disease among children and young adults with acute myeloid leukemia: a randomized clinical trial. *JAMA*. 2019;322(17):1673-1681.
33. Bitterman R, Hardak E, Raines M, et al. Baseline chest computed tomography for early diagnosis of invasive pulmonary aspergillosis in hemato-oncological patients: a prospective cohort study. *Clin Infect Dis*. 2019;69(10):1805-1808.
34. Stemler J, Bruns C, Mellinghoff SC, et al. Baseline chest computed tomography as standard of care in high-risk hematology patients. *J Fungi (Basel)*. 2020;6(1):36.
35. Singh N. Trends in the epidemiology of opportunistic fungal infections: predisposing factors and the impact of antimicrobial use practices. *Clin Infect Dis*. 2001;33(10):1692-1696.
36. Fisher BT, Kavcic M, Li Y, et al. Antifungal prophylaxis associated with decreased induction mortality rates and resources utilized in children with new-onset acute myeloid leukemia. *Clin Infect Dis*. 2014;58(4):502-508.
37. Leong YH, Boast A, Cranswick N, Curtis N, Gwee A. Itraconazole dosing and drug monitoring at a tertiary children's hospital. *Pediatr Infect Dis J*. 2019;38(1):60-64.
38. Science M, Robinson PD, MacDonald T, Rassekh SR, Dupuis LL, Sung L. Guideline for primary antifungal prophylaxis for pediatric patients with cancer or hematopoietic stem cell transplant recipients. *Pediatr Blood Cancer*. 2014;61(3):393-400.
39. Groll AH, Pana D, Lanternier F, et al. 8th European Conference on Infections in Leukaemia: 2020 guidelines for the diagnosis, prevention, and treatment of invasive fungal diseases in paediatric patients with cancer or post-haematopoietic cell transplantation. *Lancet Oncol*. 2021;22(6):e254-e269.
40. Lehrnbecher T, Fisher BT, Phillips B, et al. Clinical practice guideline for systemic antifungal prophylaxis in pediatric patients with cancer and hematopoietic stem-cell transplantation recipients. *J Clin Oncol*. 2020;38(27):3205-3216.
41. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *N Engl J Med*. 2007;356(4):348-359.
42. Lai T, Alffenaar J-W, Kesson A, Bandodkar S, Roberts JA. Evaluation of target attainment of oral posaconazole suspension in immunocompromised children. *J Antimicrob Chemother*. 2019;75(3):726-729.
43. Tragiannidis A, Herbruggen H, Ahlmann M, et al. Plasma exposures following posaconazole delayed-release tablets in immunocompromised children and adolescents. *J Antimicrob Chemother*. 2019;74(12):3573-3578.
44. Nield B, Larsen SR, van Hal SJ. Clinical experience with new formulation SUBA-itraconazole for prophylaxis in patients undergoing stem cell transplantation or treatment for haematological malignancies. *J Antimicrob Chemother*. 2019;74(10):3049-3055.
45. Lindsay J, Sandaradura I, Wong K, et al. Serum levels, safety and tolerability of new formulation SUBA-itraconazole prophylaxis in patients with haematological malignancy or undergoing allogeneic stem cell transplantation. *J Antimicrob Chemother*. 2017;72(12):3414-3419.
46. Abbotsford J, Foley DA, Goff Z, Bowen AC, Blyth CC, Yeoh DK. Clinical experience with SUBA-itraconazole at a tertiary paediatric hospital. *J Antimicrob Chemother*. 2021;76(1):249-252.
47. Meryk A, Kropshofer G, Hutter J, et al. Benefits of risk-adapted and mould-specific antifungal prophylaxis in childhood leukaemia. *Br J Haematol*. 2020;191(5):816-824.
48. Lehrnbecher T, Bochennek K, Klingebiel T, Gastine S, Hempel G, Groll AH. Extended dosing regimens for fungal prophylaxis. *Clin Microbiol Rev*. 2019;32(3):e00010-19.
49. Teh BW, Yeoh DK, Haeusler GM, et al. Consensus guidelines for antifungal prophylaxis in haematological malignancy and haematopoietic stem cell transplant. *Intern Med J*. 2021.
50. Ananda-Rajah MR, Bergmeir C, Petitjean F, Slavin MA, Thursky KA, Webb GI. Toward electronic surveillance of invasive mold diseases in hematology-oncology patients: an expert system combining natural language processing of chest computed tomography reports, microbiology, and antifungal drug data. *JCO Clin Cancer Inform*. 2017;1:1-10.

51. Jenks JD, Seidel D, Cornely OA, et al. Clinical characteristics and outcomes of invasive *Lomentospora prolificans* infections: analysis of patients in the FungiScope registry. *Mycoses*. 2020;63(5):437-442.
52. Seidel D, Hassler A, Salmanton-García J, et al. Invasive *Scedosporium* spp. and *Lomentospora prolificans* infections in pediatric patients: analysis of 55 cases from FungiScope and the literature. *Int J Infect Dis*. 2020;92:114-122.
53. Ramirez-Garcia A, Pellon A, Rementeria A, et al. Scedosporium and Lomentospora: an updated overview of underrated opportunists. *Med Mycol*. 2018;56(suppl_1):102-125.
54. Valentine JC, Worth LJ, Verspoor KM, et al. Classification performance of administrative coding data for detection of invasive fungal infection in paediatric cancer patients. *PLoS One*. 2020;15(9):e0238889.
55. Morgan JE, Hassan H, Cockle JV, Lethaby C, James B, Phillips RS. Critical review of current clinical practice guidelines for antifungal therapy in paediatric haematology and oncology. *Support Care Cancer*. 2017;25(1):221-228.
56. Osowicki J, Blyth CC, Britton PN, et al. Ahead of consensus: a paediatric antifungal prophylaxis census. *Intern Med J*. 2015;45(3):364-365.
57. Klein K, Hasle H, Abrahamsson J, De Moerloose B, Kaspers GJL. Differences in infection prophylaxis measures between paediatric acute myeloid leukaemia study groups within the international Berlin-Frankfurt-Munster (I-BFM) study group. *Br J Haematol*. 2018;183(1):87-95.
58. Burgos A, Zaoutis TE, Dvorak CC, et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics*. 2008;121(5):e1286-e1294.
59. Thomas KE, Owens CM, Veys PA, Novelli V, Costoli V. The radiological spectrum of invasive aspergillosis in children: a 10-year review. *Pediatr Radiol*. 2003;33(7):453-460.
60. Chellapandian D, Lehrnbecher T, Phillips B, et al. Bronchoalveolar lavage and lung biopsy in patients with cancer and hematopoietic stem-cell transplantation recipients: a systematic review and meta-analysis. *J Clin Oncol*. 2015;33(5):501-509.

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