Invasive fungal infections in children with acute lymphoblastic leukaemia: results from four Australian centres, 2003 – 2013

Stacie S. Wang, MBBS¹, Rishi S. Kotecha, MBChB, PhD²⁻⁴, Anne Bernard, PhD⁵, Christopher C. Blyth, MBBS, PhD⁶⁻⁸, Brendan J. McMullan, MBBS⁹⁻¹¹, Megan P. Cann, MBBS¹², Daniel K. Yeoh, MBBS⁷, Adam W. Bartlett, MBBS^{10,11,13}, Anne L. Ryan, MBBS¹⁴, Andrew S. Moore, MBBS, PhD^{15,16}, Penelope A. Bryant, MBBS, PhD¹⁷⁻¹⁹, Julia Clark, MBBS^{12,20,21} and Gabrielle M Haeusler, MBBS, PhD^{*9,17,22-24}

1. Children's Cancer Centre, Royal Children's Hospital, Melbourne, Victoria
2. Department of Haematology and Oncology, Perth Children's Hospital, Perth, Western Australia
3. Telethon Kids Cancer Centre, Telethon Kids Institute, University of Western Australia, Perth, Australia
4. School of Pharmacy and Biomedical Sciences, Curtin University, Perth, Australia
5. QFAB Bioinformatics, Institute for Molecular Bioscience, The University of Queensland, Australia
6. School of Medicine and Telethon Kids Institute, University of Western Australia, Australia
7. Department of Infectious Diseases, Perth Children's Hospital, Perth, Western Australia
8. Department of Microbiology, PathWest Laboratory Medicine, Western Australia
9. NHMRC National Centre for Infections in Cancer, Sir Peter MacCallum Department of Oncology, University of Melbourne, Victoria, Australia
10. Department of Immunology and Infectious Diseases, Sydney Children's Hospital, Sydney, New South Wales
11. School of Women's and Children's Health, University of New South Wales, Sydney, Australia
12. Infection Management Service, Queensland Children's Hospital, Brisbane, Queensland
13. Biostatistics and Databases Program, Kirby Institute, UNSW Sydney, Australia
14. Department of Haematology and Oncology, Perth Children's Hospital, Perth, Western Australia
15. Oncology Services Group, Queensland Children's Hospital, Brisbane, Queensland
16. Diamantina Institute & Child Health Research Centre, The University of Queensland, Brisbane, Australia
17. Infectious Diseases Unit, Department of General Medicine, Royal Children's Hospital, Melbourne Victoria

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1002/pbc.27915](https://doi.org/10.1002/pbc.27915).

This article is protected by copyright. All rights reserved.

18. Clinical Paediatrics, Murdoch Children's Research Institute, Victoria, Australia
19. Department of Paediatrics, University of Melbourne, Victoria, Australia
20. School of Clinical Medicine, University of Queensland, Brisbane, Queensland.
21. Centre for Children's Health Research, Children's Health Queensland, Brisbane, Queensland.
22. The Paediatric Integrated Cancer Service, Victoria, Australia
23. Department of Infectious Diseases, Peter MacCallum Cancer Centre, Victorian Comprehensive Cancer Centre, Melbourne, Victoria
24. Sir Peter MacCallum Department of Oncology, University of Melbourne, Victoria, Australia

*Correspondence to:

Dr Gabrielle M. Haeusler, Department of Infectious Diseases, Peter MacCallum Cancer Centre, 305 Grattan Street, Melbourne, Australia, 3000, P: +61 3 9656 5853 F: +61 3 9656 1185, E: gabrielle.haeusler@petermac.org

Text word count 3172; abstract 204

Brief running title: Invasive fungal infection in children with leukaemia

Keywords: Invasive fungal infection, Aspergillus, prevalence, children, acute lymphoblastic leukaemia, antifungal

Figures: 2, Tables: 3, Appendices: 2.

Abbreviations	
IFI	Invasive fungal infections
ALL	Acute lymphoblastic leukaemia
TERIFIC	The Epidemiology and Risk Factors for Invasive Fungal Infections in Immunocompromised Children study
HSCT	Haematopoietic stem cell transplant
EORTC	European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group
PCR	Polymerase chain reaction
MSG	National Institute of Allergy and Infectious Diseases Mycoses Study Group
COG	Children's Oncology Group
CCG	Children's Cancer Group
SR	Standard Risk
HR	High Risk
GM	Galactomannan
BAL	Bronchoalveolar Lavage

Acknowledgments: All authors contributed to research design, analysis, critical review of the manuscript and have approved the final version. GMH and SW wrote the paper, and GMH, SW and AB performed statistical analyses. GMH was supported by a Victorian Cancer Agency early career fellowship. This investigator-initiated study was supported from a grant from Gilead Sciences.

Conflict of Interest Statement: The authors have no conflict of interest to declare.

Data Availability Statement: Data that support the findings of this study are available from the corresponding author upon reasonable request.

ABSTRACT

Background: Invasive fungal infections (IFI) are an important complication of acute lymphoblastic leukaemia (ALL) treatment. Our study describes the prevalence and outcomes of IFI in children with ALL.

Methods: IFI episodes in children with primary or relapsed ALL, identified for The Epidemiology and Risk Factors for Invasive Fungal Infections in Immunocompromised Children (TERIFIC) study, were analysed. IFI were classified according to EORTC criteria with a 'modified possible' category included.

Results: A total of 123 IFI episodes in 119 patients with ALL were included. A proven, probable, possible and modified possible IFI was diagnosed in 56 (45.5%), 22 (17.9%), 39 (31.7%), 6 (4.9%) episodes, respectively. The prevalence was 9.7% (95% CI 8-11.4%) overall and 23.5% (95% CI 14.5-32.5%) for relapsed/refractory ALL. For non-relapsed ALL the IFI prevalence was significantly higher for children with high-risk compared to standard-risk ALL (14.5% vs 7.3%, $p=0.009$), and IFI were more common during induction, consolidation and delayed intensification phases. Mould infections occurred more frequently than non-mould infections. Thirteen children (10.9%) died within 6 months of IFI diagnosis with 5 deaths (4.2%) attributable to an IFI.

Conclusions: IFI is more common in children with high-risk ALL and in relapsed disease. Overall survival was encouraging, with IFI contributing to very few deaths.

INTRODUCTION

Acute lymphoblastic leukaemia (ALL) is the most common cancer in children and adolescents.¹ Over the last five decades, refinements in risk-directed therapy and use of allogeneic haematopoietic stem cell transplant (HSCT) have led to dramatic improvements in treatment outcomes, with five year survival as high as 90% for certain risk groups.² However, despite improvements in supportive care, treatment-related deaths occur in one of every 25 children treated for ALL.³ An important cause of morbidity and mortality in children with ALL is infection, of which invasive fungal infection (IFI) remains one of the most challenging to prevent, diagnose and treat.⁴

Predictors for IFI in children with cancer include neutropenia, HSCT, graft versus host disease and prolonged corticosteroid exposure.^{5,6} The degree of IFI risk has also been ascribed to underlying cancer diagnosis, with overall risk often considered low for ALL.⁵ However, children with ALL are a heterogeneous group and this low risk categorisation may not be applicable to patients with underlying high-risk leukaemia and during intensive chemotherapy phases.⁷ To date, few paediatric studies have explored the changing pattern of IFI risk during ALL treatment phases and between risk categories with many combining data for all types of acute leukaemia and HSCT.⁸⁻¹³

The prevalence of IFI in children with ALL is reported to range between 4 and 35%, depending on era, chemotherapy protocol, risk category and prophylaxis regimen.⁸⁻¹³ In an early Australian study, the IFI prevalence in children with high-, standard- and low-risk ALL of was 35%, 30% and 6%, respectively.¹³ However this included patients treated on an intensive chemotherapy protocol that is now no longer used and may, in part, explain the high IFI rates observed.¹⁴ An evaluation in the US of infection-related complications in children with ALL identified an increased incidence of IFI during the more intensive induction and re-induction treatment phases.⁷ Similarly, a recent European study

of children with ALL found the majority of proven and probable IFI occurred during the induction phase, with an overall attributable mortality of 13%.⁸ However, the proportion of patients with relapsed ALL or post HSCT were not reported in several of these studies and may have impacted the overall rates.

The Epidemiology and Risk Factors for Invasive Fungal Infections in Immunocompromised Children (TERIFIC) study is a retrospective, multi-centre, cohort study of IFI in immunocompromised children in Australia. The epidemiological data from this large cohort has been previously described.¹⁵ The aim of this study is to describe the prevalence and outcomes of IFI in children with ALL.

METHODS

Episodes of IFI in immunocompromised children at four Australian tertiary paediatric centres in Brisbane (Queensland Children's Hospital), Melbourne (Royal Children's Hospital), Perth (Perth Children's Hospital) and Sydney (Sydney Children's Hospital) were retrospectively identified (Perth enrolled between 2003 to 2014, Brisbane and Melbourne enrolled between 2004 to 2014, and Sydney between 2012 to 2013).

Participants. Detailed methodology, including participant identification and inclusion and exclusion criteria have been described.¹⁵ Briefly, children with IFI were identified from hospital pharmacy, microbiology and oncology databases and hospital diagnostic coding. Data collected included patient demographics, IFI details, clinical management and treatment outcome over a 6-month period from date of IFI diagnosis. Data were entered into a centralised and secure web-based application. Ethics approval was granted at each individual site.

Invasive fungal infections were classified as proven, probable or possible as per the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC) criteria.¹⁶ A positive *Aspergillus* polymerase chain reaction (PCR) was also included as mycological evidence within the diagnostic criteria for probable IFI. Although not included in the current EORTC criteria, the EORTC/MSG (National Institute of Allergy and Infectious Diseases Mycoses Study Group) have proposed future inclusion within the diagnostic criteria for probable invasive aspergillosis.¹⁷ Furthermore, given the real-world difficulties of applying the EORTC criteria to children, a further category of 'modified possible' was also included in this analysis. 'Modified possible' was defined as (i) presence of host factors and clinical criteria suggestive of IFI not listed by EORTC criteria (i.e. lesions suggestive of hepatosplenic candidiasis with negative blood cultures) or ii) host and mycology criteria but no EORTC clinical criteria. Adapted. After primary data collection, children with a diagnosis of ALL were extracted from the database. IFI episodes occurring in children with ALL post HSCT were excluded from the analysis.

Across all sites, prophylaxis was physician dependant and not routinely prescribed to patients with ALL. Data on the total number of patients with ALL receiving prophylaxis was unavailable. Regarding diagnostic workup for IFI, patients were typically investigated after three to five days of persistent fever. Investigations available at all sites included radiological (x-ray, CT and MRI) and microbiological (fungal culture, galactomannan and *Aspergillus* PCR). Investigations performed were physician dependent and individualised to the patient and presenting symptoms.

Denominator data. The total number of patients diagnosed with ALL at each centre during the study period was collected from Perth (n=224), Brisbane (n=375) and Melbourne (n=617). Denominator

data were unavailable for Sydney. Compared to our previous publication, denominator data from Perth were also included for the year 2003.¹⁵

To allow for risk group comparison, data were collected on the number of children treated with specific ALL protocols. The treatment protocols were classified by two paediatric haematologists (RSK, AM) into standard risk (Children's Oncology Group (COG) trials AALL0331, AALL0932 and the Children's Cancer Group (CCG) 1991 trial) and high risk (COG trials AALL0631, AALL1122, AALL0232, AALL1131, AALL0031, AALL0434, AALL08P1, P9407 and the CCG1961 trial). The standard-risk protocols incorporated all children that were classified as standard, average or low risk, whereas the high-risk protocols incorporated all children that were classified as high or very high risk according to COG criteria. For children treated on alternative protocols, the ALL risk status documented in the medical record was used.

Data were obtained from individual centres regarding the number of patients treated on high-risk protocols (Perth, 88; Brisbane, 153), standard-risk protocols (Perth, 113; Brisbane, 200), and relapse protocols (Perth, 23; Melbourne 63). Data on number of patients treated on high- and standard-risk protocols were unavailable for Melbourne.

Statistical methods. Descriptive statistics were presented 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 two categorical variables.

Crude prevalence of IFI in ALL was calculated by dividing the number of patients with IFI by the total number of new patients diagnosed with ALL during the study period and was reported with 95% confidence interval (CI). Due to availability of specific denominator data, IFI prevalence for relapsed disease was calculated for Melbourne and Perth and for standard- and high-risk disease for Brisbane and Perth. Overall survival was calculated from the earliest date that the IFI was suspected or confirmed to date of death from all causes or date of last follow-up with patients censored at six months. Mean overall survival was calculated using Kaplan-Meier estimates and reported with standard error (SE) and Kaplan-Meier curves. Median time from diagnosis of ALL to the first documented diagnosis or treatment for IFI was calculated and reported with IQR. All tests were 2-tailed and a p-value of <0.05 was considered statistically significant. Analyses were performed using R statistical software.¹⁸

RESULTS

There were 348 episodes of IFI in 331 children. Of these, 149 episodes occurred in 142 children with ALL. Twenty-six IFI episodes occurred in children who had undergone HSCT for ALL a median of 48.5 days (IQR 9.5-274 days) prior to IFI diagnosis. After excluding these, there were 123 episodes in 119 patients included in this analysis (table 1). Ninety-four IFI episodes occurred in 94 patients with primary, non-relapsed disease and 29 IFI episodes occurred in 25 patients with relapsed or refractory disease (4 patients had two IFI's).

Of the children with B-cell ALL, the most common standard risk study protocol was COG AALL0331 (NCT00103285) (51.4% of all episodes with standard risk B-cell ALL) and the most common high-risk study protocol was COG AALL0232¹⁹ (50.0% of all episodes with high risk B-cell ALL). For children with T-cell ALL, the most common protocol was AALL0434²⁰ (85.7%). For children with relapsed

disease the most common treatment protocol was UKALLR3²¹ (n=16, 55.2%). Prevalence of IFI according to each treatment protocol is available in Supplementary Appendix 1.

Overall, a proven, probable and possible IFI was diagnosed in 56 (45.5%), 22 (17.9%) and 39 (31.7%) episodes, respectively. A modified-possible IFI was diagnosed in 6 episodes (clinical details in Supplementary Appendix 2). In the 94 patients with non-relapsed ALL a proven, probable, possible and modified-possible IFI was diagnosed in 42 (44.7%), 18 (19.1%), 29 (30.9%) and 5 (5.3%), respectively. For these 94 patients, 28 (29.8%) occurred during induction, 26 (27.7%) during consolidation and 25 (26.6%) during delayed intensification (Figure 1). The median time from diagnosis of ALL to IFI diagnosis was 3.3 months (IQR 1-7.5 months).

The median age was 6.5 years (IQR 3-11 years), 8 years (IQR 6-14 years) and 9 years (6.75-9.75 years) in the proven/probable group, possible and modified-possible group, respectively. For the non-relapsed group, there was no difference between the proven/probable, possible and modified-possible groups with respect to disease risk status, with 34 patients (57%) in the proven/probable group, 21 (72%) in the possible group and 4 (80%) in the modified-possible group being high-risk (p=0.29). Similarly, no difference was found between the three groups with respect to treatment phase (Intensive vs. Maintenance, p=0.27). A total of 50 (86%) proven/probable IFIs (with a known treatment phase), 28 (97%) possible IFIs and 3 (100%) modified-possible IFIs were identified in intensive chemotherapy phases.

Prevalence

The IFI prevalence was calculated for episodes occurring in Melbourne, Brisbane and Perth. During the study period, there were 1216 patients with ALL treated at these three sites (Table 2). Overall the prevalence was 9.7% (95% CI 8-11.4) for proven/probable/possible/modified-possible IFI and 6.2% (95% CI 4.8-7.5) for proven/probable IFI. There was no significant difference in the overall IFI prevalence between centres ($p>0.05$, data not shown).

The IFI prevalence in standard-risk ALL was 7.3% (95% CI 4.5-10.2) for proven/probable/possible/modified-possible IFI and 5.8% (95% CI 3.2-8.3) for proven/probable IFI. The IFI prevalence in high-risk ALL was 14.5% (95% CI 10.1-19.0) for proven/probable/possible/modified-possible IFI and 8.7% (95% CI 5.1-12.3) for proven/probable IFI (mould $n=17$; yeast $n=4$). IFI prevalence was significantly higher for children with high-risk ALL compared to those with standard-risk ALL (14.5% vs 7.3%; $p=0.009$). Considering the individual centres separately, the IFI prevalence in high-risk ALL was 12.5% (95% CI 9.9-21.4) in Perth and 15.7% (95% CI 9.9-21.4) in Brisbane ($p=0.62$). The IFI prevalence for standard risk ALL was 3.5% (95% CI 0.1-6.9) for Perth and 9.5% (5.4-13.6) in Brisbane ($p=0.08$).

The prevalence in relapsed/refractory ALL was 23.5% (95% CI 14.5-32.5) for proven/probable/possible/modified-possible IFI and 12.9% (95% CI 5.8-20.1) for proven/probable IFI (mould $n=7$; yeast $n=4$). For non-relapsed disease, the prevalence was 6.2% (95% CI 4.4-7.9) for proven/probable/possible/modified-possible IFI and 3.8% (95% CI 2.4-5.1) for proven/probable IFI (mould $n=18$; yeast $n=10$). The IFI prevalence was significantly higher in patients with relapsed/refractory ALL than in non-relapsed ALL (23.5% vs 6.2%; $p<0.001$) for

proven/probable/possible/modified-possible IFI and for proven/probable IFI (12.9% vs 3.8%; $p=0.001$).

Microbiology

There were 82 fungal pathogens (54 mould and 28 non-mould) identified by culture, molecular techniques or histopathology in the 78 episodes with a proven or probable IFI (Table 3) (3 episodes had 2 mould pathogens and 1 episode had 2 non-mould pathogens). *Aspergillus* species was the most frequently identified mould, followed by *Lomentospora* (formerly *Scedosporium*) *prolificans* and *Exserohilum* species. Among the non-mould infections, *Candida* species accounted for the majority of infections, of which *Candida albicans* and *Candida parapsilosis* were most frequently detected. There was no significant relationship between disease status (relapse versus non-relapse) and type of fungus ($p=0.58$). Mould infection accounted for 38/60 (63.3%) episodes in patients with non-relapsed disease compared to 13/18 (72.2%) episodes in relapsed/refractory disease.

Among the proven/probable mould IFI, the most frequent primary site of infection was lung ($n=31$), followed by skin and soft tissue ($n=10$), sinonasal ($n=3$), central nervous system ($n=3$), blood ($n=2$) and bone ($n=2$). For non-mould infections, the most frequent site of infection was blood ($n=27$).

Impact of prophylaxis

Overall, there were 34 (27.6%) IFI episodes in which anti-fungal prophylaxis had been administered for at least seven consecutive days in the month prior to diagnosis of IFI. The median duration of prophylaxis prior to IFI diagnosis was 32 days (IQR 18-92 days, range 8-215 days).

A total of 89 (72.4%) IFI episodes occurred in patients on no prophylaxis, of which 31 (34.8%) were mould and 28 (31.4%) were non-mould (no pathogen identified in 30). Twenty-six (21.1%) IFI episodes occurred in patients on fluconazole prophylaxis, of which 17 (65.4%) were mould and two (7.7%) were non-mould (*Pichia kudriavzevii* and *Rhodotorula* species) (no pathogen identified in 7). *Aspergillus* species was cultured in 10 of the 17 (58.8%) mould infections, with a further four patients having indirect evidence of *Aspergillus* species with a positive PCR. Finally, eight (6.5%) IFI episodes occurred in patients on mould-active prophylaxis (intermittent liposomal amphotericin n=3, itraconazole n=5). Of the episodes occurring on mould-active prophylaxis, two had pulmonary aspergillosis (*Aspergillus niger* and *Aspergillus flavus*) diagnosed by core biopsy, one had *P. kudriavzevii* (formerly *Candida krusei*) cultured from the blood and two had probable pulmonary aspergillosis with positive galactomannan on bronchoalveolar lavage specimen (no pathogen identified in 3). Therapeutic drug levels for itraconazole prophylaxis were only performed in two patients and both were subtherapeutic.

Treatment and outcome

The median duration of antifungal treatment was 131 days (IQR 37.5-236.5) overall and 157 days (IQR 24.7-264.0) for proven IFI, 108 (IQR 58.5-197.0) for probable, 108 (IQR 41.5-188.0) for possible and 275 (IQR 185.8-317.8) for modified possible IFI.

Thirteen (10.9%) children died within 6 months of IFI diagnosis, 6 of whom had relapsed disease and 2 had refractory ALL (Figure 2). Five deaths (4.2%) were attributed to IFI. Causative organisms in these five patients included *Scedosporium prolificans* (n=2), *Aspergillus flavus* (n=1), *Aspergillus fumigatus* complex (n=1), *Trichosporon asahii* and *Candida parapsilosis* (both infections in one patient). The mortality according to pathogen for patients with proven *L. prolificans* and *Aspergillus* spp was 50.0% (n=2/4) and 17.6% (n=3/17), respectively.

DISCUSSION

In this large, multicentre study of IFI in children with ALL, the overall IFI prevalence was 9.7%. The prevalence was significantly higher in children with relapsed or refractory ALL compared to non-relapsed disease (23.5% versus 6.2%). It was also higher for children treated on high-risk chemotherapy protocols compared to standard-risk (14.5% versus 7.3%) and most infections occurred during the dose-intensive chemotherapy phases. Across all groups, mould infections occurred more frequently than non-mould infections.

Together with our previously published paediatric study describing the epidemiology of IFI in immunocompromised Australian children, this is one of the few attempts at extending EORTC criteria to accommodate the nuances of IFI diagnosis in children.¹⁵ This particularly relates to the difficulties in obtaining computed tomography or magnetic resonance imaging in those requiring a general anaesthetic,²² as well as the absence of typical nodular and ground glass radiological changes in the younger population.^{23,24} Furthermore, the inclusion of a 'modified-possible' group reflects the realities of clinical practice where patients are treated for IFI without fulfilling criteria originally intended for research purposes.¹⁶ While highly specific criteria are important for clinical trials assessing new diagnostic markers or drugs, they may be less relevant to understanding the

epidemiology of IFI in children with cancer, the overall clinical impact of these infections and to informing prospective, real-time surveillance and prophylaxis strategies.

To date, few paediatric studies have explored the contribution of chemotherapy intensity and ALL risk status on the prevalence of IFI. This may, in part, explain the broad range of prevalence previously described.⁸⁻¹³, In keep with our results, a recent publication of children with ALL treated on the UKALL2001 protocol found the IFI rate was significantly higher in children treated with a more intensive chemotherapy regimen (11% versus 4%).²⁵ Similarly, the study also showed the majority of IFI occurred in the dose-intense treatment phases of induction, consolidation and delayed intensification that are typically associated with prolonged periods of neutropenia and high-dose steroids.

Limited conclusions can be drawn from this study regarding the impact of antifungal prophylaxis on development of IFI as the total number of patients treated for ALL that received antifungal prophylaxis is unknown. The high prevalence of IFI in children with relapsed/refractory ALL in our study is in keeping with guidelines recommending routine antifungal prophylaxis in this group.²⁶ In contrast, few paediatric guidelines recommend prophylaxis for children with non-relapsed ALL on high-risk treatment protocols. Our study suggests that that this group may also benefit from antifungal prophylaxis, particularly during the more intensive chemotherapy phases. While choice of antifungal agent is frequently limited by drug-chemotherapy interactions, mould-active cover should be considered due to the proportionally higher rates of mould infections in this group. Given the complexities of antifungal use in this population, particularly around adverse effects, drug interactions, optimal dosing and therapeutic drug monitoring, further research in this area is urgently required.^{26,27}

Overall survival in this ALL cohort is encouraging, with a 6-month IFI attributable mortality rate of 4.2%. At an individual pathogen level, 6-month mortality associated with proven invasive aspergillosis (17.6%) was also better than previously reported, with mortality as high as 50%.^{28,29} Unfortunately our study did not capture the challenges associated with treating these infections, including interruptions to chemotherapy, requirements for dose adjustments and drug interactions. As treatment durations extended beyond 130 days for half of these episodes, these are likely to be significant.

Whilst our study is one of the larger reports of IFI in children undergoing treatment for ALL, it is limited by its retrospective nature. Prevalence assessments for ALL risk groups were grouped according to site data availability, although when considered separately there was no significant difference between sites. There was, however a trend toward a higher IFI prevalence at Brisbane, which is unlikely to be explained by differences in approaches to prophylaxis or diagnostic workup as these were similar between sites. Furthermore, our overall prevalence of 9.7% was slightly different to the 10.6% reported in our previous publication, due to our inclusion of 2003 data from the Perth site.¹⁵ However, data produced from the TERIFIC study cohort is unique as it is the first to define a separate diagnostic group (modified possible IFI) that accommodates IFI diagnostic challenges in children as well as the realities of treating clinical findings suspicious of IFI in immunosuppressed children.¹⁵ Moving forward, such criteria may be useful in informing epidemiological studies as well as surveillance criteria in this population.

This study provides further insights into the burden of IFI in children with ALL and highlights the importance of considering standard- and high-risk subgroups, as well as dose-intense treatment

phases, separately. Our study may also inform prophylaxis strategies for children with relapsed ALL and those on high-risk chemotherapy protocols. With the rapid evolution of ALL treatment a collaborative approach is required to ensure we remain abreast of changing patterns of IFI in this population and consideration should be given to inclusion of additional criteria, such as 'modified possible,' into definitions used to monitor prevalence rates.

REFERENCES

1. Smith MA, Altekruse SF, Adamson PC, Reaman GH, Seibel NL. Declining childhood and adolescent cancer mortality. *Cancer*. 2014;120(16):2497-2506.
2. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med*. 2006;354(2):166-178.
3. Lund B, Asberg A, Heyman M, et al. Risk factors for treatment related mortality in childhood acute lymphoblastic leukaemia. *Pediatr Blood Cancer*. 2011;56(4):551-559.
4. Christensen MS, Heyman M, Mottonen M, et al. Treatment-related death in childhood acute lymphoblastic leukaemia in the Nordic countries: 1992-2001. *Br J Haematol*. 2005;131(1):50-58.
5. 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.

6. 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.* 2018;7(3):191-198.
7. Inaba H, Pei D, Wolf J, et al. Infection-related complications during treatment for childhood acute lymphoblastic leukemia. *Ann Oncol.* 2017;28(2):386-392.
8. Sahbudak Bal Z, Yilmaz Karapinar D, Karadas N, et al. Proven and probable invasive fungal infections in children with acute lymphoblastic leukaemia: results from an university hospital, 2005-2013. *Mycoses.* 2015;58(4):225-232.
9. Kobayashi R, Kaneda M, Sato T, Ichikawa M, Suzuki D, Ariga T. The clinical feature of invasive fungal infection in pediatric patients with hematologic and malignant diseases: a 10-year analysis at a single institution at Japan. *J Pediatr Hematol Oncol.* 2008;30(12):886-890.
10. Castagnola E, Cesaro S, Giacchino M, et al. Fungal infections in children with cancer: a prospective, multicenter surveillance study. *Pediatr Infect Dis J.* 2006;25(7):634-639.
11. Rosen GP, Nielsen K, Glenn S, Abelson J, Deville J, Moore TB. Invasive fungal infections in pediatric oncology patients: 11-year experience at a single institution. *J Pediatr Hematol Oncol.* 2005;27(3):135-140.
12. Mor M, Gilad G, Kornreich L, Fisher S, Yaniv I, Levy I. Invasive fungal infections in pediatric oncology. *Pediatr Blood Cancer.* 2011;56(7):1092-1097.
13. 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.
14. Marshall GM, Dalla Pozza L, Sutton R, et al. High-risk childhood acute lymphoblastic leukemia in first remission treated with novel intensive chemotherapy and allogeneic transplantation. *Leukemia*. 2013;27(7):1497-1503.
15. 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.
16. De Pauw B, Walsh TJ, Donnelly JP, et al. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008;46(12):1813-1821.
17. White PL, Wingard JR, Bretagne S, et al. Aspergillus Polymerase Chain Reaction: Systematic Review of Evidence for Clinical Use in Comparison With Antigen Testing. *Clin Infect Dis*. 2015;61(8):1293-1303.
18. *R: A language and environment for statistical computing* [computer program]. R Foundation for Statistical Computing; 2013.
19. Larsen EC, Devidas M, Chen S, et al. Dexamethasone and High-Dose Methotrexate Improve Outcome for Children and Young Adults With High-Risk B-Acute Lymphoblastic Leukemia: A Report From Children's Oncology Group Study AALL0232. *J Clin Oncol*. 2016;34(20):2380-2388.

20. Winter SS, Dunsmore KP, Devidas M, et al. Improved Survival for Children and Young Adults With T-Lineage Acute Lymphoblastic Leukemia: Results From the Children's Oncology Group AALL0434 Methotrexate Randomization. *J Clin Oncol*. 2018;36(29):2926-2934.
21. Parker C, Waters R, Leighton C, et al. Effect of mitoxantrone on outcome of children with first relapse of acute lymphoblastic leukaemia (ALL R3): an open-label randomised trial. *Lancet*. 2010;376(9757):2009-2017.
22. Arlachov Y, Ganatra RH. Sedation/anaesthesia in paediatric radiology. *Br J Radiol*. 2012;85(1019):e1018-1031.
23. Ankrah AO, Sathekge MM, Dierckx RA, Glaudemans AW. Imaging fungal infections in children. *Clin Transl Imaging*. 2016;4:57-72.
24. Katragkou A, Fisher BT, Groll AH, Roilides E, Walsh TJ. Diagnostic Imaging and Invasive Fungal Diseases in Children. *J Pediatric Infect Dis Soc*. 2017;6(suppl_1):S22-S31.
25. O'Reilly MA, Govender D, Kirkwood AA, et al. The incidence of invasive fungal infections in children, adolescents and young adults with acute lymphoblastic leukaemia/lymphoma treated with the UKALL2011 protocol: a multicentre retrospective study. *Br J Haematol*. 2019.
26. 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(8):e327-340.

27. Lehrnbecher T. Antifungal prophylaxis in pediatric patients undergoing therapy for cancer: drugs and dosing. *Curr Opin Infect Dis.* 2015;28(6):523-531.
28. Burgos A, Zaoutis TE, Dvorak CC, et al. Pediatric invasive aspergillosis: a multicenter retrospective analysis of 139 contemporary cases. *Pediatrics.* 2008;121(5):e1286-1294.
29. Tragiannidis A, Dokos C, Lehrnbecher T, Groll AH. Antifungal chemoprophylaxis in children and adolescents with haematological malignancies and following allogeneic haematopoietic stem cell transplantation: review of the literature and options for clinical practice. *Drugs.* 2012;72(5):685-704.

FIGURE LEGENDS

Figure 1. IFI diagnoses according to ALL treatment phase, n=94. IFI, invasive fungal infection; ALL, Acute Lymphoblastic Leukaemia; IM, Interim Maintenance; DI, Delayed Intensification; IM2, Interim Maintenance 2.

Figure 2. Overall Survival for patients with Acute Lymphoblastic Leukaemia

TABLE 1 Patient Characteristics per IFI Episode

Total number of IFI episodes	123
Median age, years (IQR)	7 (4-12)
Male sex, n (%)	63 (51.2)
Diagnosis, n (%)	
- Primary B cell ALL	87 (70.7)
→ Standard risk	
→ High risk	35
- Primary T cell ALL	52
- Relapsed/refractory ALL	
→ B cell	7 (5.7)
→ T cell	29 (23.6)
	24
	5
Treatment phase in non-relapsed disease, n (%)	94
Induction	28 (29.8)
Consolidation	26 (27.7)
Interim maintenance	2 (2.1)
Delayed intensification / Reinduction	25 (26.6)
Maintenance	9 (9.6)
Unknown	4 (4.2)
CVAD in situ, n (%)	113 (91.9)
Fungal prophylaxis, n (%)	34 (27.6)
- Yeast-active: Fluconazole	26 (76.5)
- Mould-active	8 (23.5)
- Itraconazole	5
- Liposomal amphotericin	3
Neutropenia (<0.5 µmol/L) within 30 days, n (%)	116 (94.3)
Median duration of neutropenia prior to IFI diagnosis, days (IQR)	16 (11-27)

IFI, Invasive Fungal Infection; IQR, Inter-Quartile Range; ALL, Acute Lymphoblastic Leukaemia; CVAD, Central Venous Access Device

TABLE 2 IFI episodes according to study site and ALL risk status

		Melbourne	Brisbane	Perth	Sydney	Total*
Patients diagnosed with ALL during study period	TOTAL	617	375	224	NA	1216
	SR	545	200	113	NA	1099
	HR		153	88	NA	
	Relapsed/refractory	62	NA	23	NA	85
	Infant	10	NA**	NA**	NA	10
	Unknown	-	22	-	NA	22
Proven	TOTAL	14	29	11	2	54
	SR	4	14	3	0	21
	HR	3	8	5	1	16
	Relapsed/refractory	5	7	2	0	14
	Infant	2	0	1	1	3
Probable	TOTAL	12	7	2	1	21
	SR	3	1	0	1	4
	HR	6	5	1	0	12
	Relapsed/refractory	3	0	1	0	4
	Infant	0	1	0	0	1
Possible	TOTAL	21	11	5	2	37
	SR	3	3	1	1	7
	HR	9	7	4	1	20
	Relapsed/refractory	9	1	0	0	10
	Infant	0	0	0	0	0
Modified Possible	TOTAL	1	5	0	0	6
	SR	0	2	0	0	2
	HR	1	2	0	0	3
	Relapsed/refractory	0	1	0	0	1

	Infant	0	0	0	0	0
--	--------	---	---	---	---	---

ALL, Acute Lymphoblastic Leukaemia; NA, Not Available; SR, Standard Risk; HR, High Risk

*data from Sydney not included in Total; **included in the high risk group

TABLE 3 Details of proven and probable IFI

	Relapsed disease		Non relapsed disease	
	Proven	Probable	Proven	Probable
Mould				
- <i>Aspergillus fumigatus</i> complex	3	1	3	4
- <i>Aspergillus flavus</i>	2	-	1	1
- <i>Aspergillus niger</i>	-	-	1	-
- <i>Aspergillus</i> spp	1	3^	2	11^^
- <i>Exserohilum</i> spp	-	-	4	-
-Zycomycetes			2	-
- <i>Lomentospora prolificans</i>	1*	-	3	-
- <i>Fusarium</i> spp	-	-	1	-
- <i>Acremonium</i> spp	-	-	1	-
- <i>Penicillium</i> spp	-	-	1	-
- <i>Paecilomyces lilacinus</i>	-	-	1	-

-Hyphomycete	-	-	-	1
-Hyphae on tissue biopsy	3	NA	2	NA
Non-mould				
- <i>Candida albicans</i>	3	-	7	-
- <i>Candida parapsilosis</i>	1	-	6	-
- <i>Pichia kudriavzevii</i>	1	-	1	-
- <i>Candida guilliermondii</i>	-	-	2	-
- <i>Candida tropicalis</i>	-	-	2	-
- <i>Candida</i> spp.	-	-	1	-
- <i>Candida rugosa</i>	-	-	1	-
- <i>Cryptococcus neoformans</i>	-	-	1	-
- <i>Trichosporon asahii</i>	-	-	1**	-
- <i>Rhodotorula</i> spp	-	-	1	-

*1 episode had 2 pathogens (*A. fumigatus* and *L. prolificans*)

**1 episode had 2 pathogens (*T. asahii* and *C. rugosa*)

^3 identified on Polymerase Chain Reaction (PCR); ^^10 identified by PCR and 1 by galactomannan on Bronchoalveolar Lavage (BAL) specimen

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Wang, SS;Kotecha, RS;Bernard, A;Blyth, CC;McMullan, BJ;Cann, MP;Yeoh, DK;Bartlett, AW;Ryan, AL;Moore, A;Bryant, PA;Clark, J;Haeusler, GM

Title:

Invasive fungal infections in children with acute lymphoblastic leukaemia: Results from four Australian centres, 2003-2013

Date:

2019-07-16

Citation:

Wang, S. S., Kotecha, R. S., Bernard, A., Blyth, C. C., McMullan, B. J., Cann, M. P., Yeoh, D. K., Bartlett, A. W., Ryan, A. L., Moore, A., Bryant, P. A., Clark, J. & Haeusler, G. M. (2019). Invasive fungal infections in children with acute lymphoblastic leukaemia: Results from four Australian centres, 2003-2013. PEDIATRIC BLOOD & CANCER, 66 (10), <https://doi.org/10.1002/pbc.27915>.

Persistent Link:

<http://hdl.handle.net/11343/286152>