Invasive fungal disease and antifungal prophylaxis in children with acute leukaemia: a multicentre retrospective Australian cohort study

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The Lancet Regional Health - Western Pacific 2024;52: 101201

Published Online xxx
https://doi.org/10.1016/j.lanwpc.2024.101201

Summary

Background Invasive fungal disease (IFD) is a significant complication for children receiving treatment for leukaemia, contributing to morbidity and mortality. Recent regional paediatric epidemiological IFD data are lacking. Additionally uncertainty remains regarding the optimal prophylactic approach in this context.

Methods In a multi-centre Australian cohort study of children diagnosed with *de novo* acute leukaemia between 1st January 2017 and 30th June 2020, we characterised antifungal prophylaxis prescribing and IFD prevalence. Impact of antifungal prophylaxis was assessed using Kaplan Meier curves and Cox-proportional hazards regression adjusting for known IFD risk factors.

Findings A total of 434 children were included (47.2% female; median age 5.0 years, median follow-up 240 days). This cohort included 351 children with ALL (214 high-risk [HR-ALL]; 137 standard-risk [SR-ALL]), and 73 with AML. The prevalence of proven/probable IFD was 6.8% for AML, 14.0% for HR-ALL and 4.4% for SR-ALL. A mould was implicated as the causative pathogen in almost two thirds of cases. Antifungal prophylaxis was prescribed in 98.7% of chemotherapy cycles for AML, 56.7% for HR-ALL and 14.9% for SR-ALL. A mould-active agent was used in 77.4% of AML cycles and 21.2% of HR-ALL cycles. Mould-active prophylaxis was associated with a lower risk of IFD overall and increased IFD-free survival in AML.

Interpretation These data demonstrate the persistent high regional burden of IFD in children with HR-ALL, and the potential for mould-active prophylaxis to ameliorate this. Strategies to increase uptake of appropriate prophylaxis are required in this cohort.

Funding This study was supported by a Perth Children's Hospital Foundation grant (PCHF9973).

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Keywords: Acute myeloid leukaemia; Acute lymphoblastic leukaemia; Antifungal prophylaxis; Children; Invasive fungal disease; Invasive fungal infections

Research in context

Evidence before this study

We reviewed paediatric studies regarding invasive fungal disease (IFD) and antifungal prophylaxis in children with leukaemia. PubMed and Scopus were searched using keywords including "invasive fungal disease", "children OR paediatric", "antifungal prophylaxis" and "leukaemia" with articles up to October 2023 considered. A number of paediatric observational studies were identified describing IFD epidemiology in children with leukaemia, many of which assessed paediatric cohorts from single centres, often treated 20 or more years ago. Few studies reported in detail on antifungal prophylaxis prescribing, with very few recent studies assessing the impact of antifungal prophylaxis strategies in preventing IFD in children. Importantly, recent data on IFD epidemiology and antifungal prophylaxis strategies in children in the Western Pacific region were lacking.

Added value of this study

This multi-centre cohort study provides a detailed analysis of regional IFD epidemiology in children with leukaemia, demonstrating the ongoing high burden of disease in children with high-risk acute lymphoblastic leukaemia particularly. Furthermore, our data illustrate the efficacy of mould-active prophylaxis, consistently across analyses, in reducing the burden of IFD in this vulnerable population.

Implications of all the available evidence

The findings of this study affirm recently updated regional guidelines that recommend anti-mould prophylaxis in children with AML and HR-ALL. Importantly, the persistent high prevalence of IFD in children with HR-ALL together with the low uptake of mould-active prophylaxis in this group, highlight the need to explore novel strategies to deliver appropriate prophylaxis to this cohort, including the use of outpatient parenteral antimicrobial therapy (OPAT) services, newer antifungal formulations, and novel antifungal agents.

Introduction

Invasive fungal disease (IFD) is a major cause of morbidity and mortality in children receiving chemotherapy for cancer[1,2] with significant associated costs for health care systems.[3] Antifungal prophylaxis has potential to reduce the burden of IFD in children undergoing chemotherapy, yet paediatric data to inform best practice is required. Optimising prophylaxis is particularly important for children with acute leukaemia who comprise the largest population of children undergoing chemotherapy, including patient subgroups at high risk of IFD.

Regional epidemiological data are key in guiding clinical decisions regarding diagnosis, treatment, and prevention of IFD in immunocompromised children. In Australian national antifungal guidelines published in 2014, fluconazole prophylaxis was recommended for children with acute myeloid leukaemia (AML) but not those with acute lymphoblastic leukaemia (ALL),[4] consistent with contemporaneous international guidelines.[5] In a subsequent national study assessing data from 2003 to 2014, IFD prevalence was high amongst patients with *de novo* high-risk ALL (HR-ALL) (8.7% proven/probable, 14.5% proven/probable/possible/modified possible), and AML (10.3% proven/probable, 20.7% proven/probable/possible/modified possible), with a predominance of mould infections,[6–8] indicating that these groups may benefit from mould-active prophylaxis. More recent data of IFD in children in the region are lacking.

Consistent with recent international guidelines,[9,10] the updated Australasian antifungal guidelines now recommend mould-active prophylaxis for paediatric patients at high risk of IFD, including those with AML and HR-ALL.[11] Although there are paediatric data from randomised controlled trials demonstrating superiority of posaconazole[12] and caspofungin[13] compared to fluconazole in preventing IFD in patients with AML, data in children with HR-ALL are lacking. Moreover, there are numerous practical challenges with administering antifungal prophylaxis in children including drug–drug interactions and poor attainment of therapeutic levels with available formulations (for mould-active triazoles), feasibility of daily intravenous administration (for echinocandins), tolerability, and cost.[14–18] Alternative agents including voriconazole, liposomal amphotericin B, and micafungin, are included in guideline recommendations, yet considerable variation in practice exists, reflecting the lack of studies comparing these approaches in children with acute leukaemia.[5,9–11,19–21]

Across Australian paediatric oncology centres, local antifungal prophylaxis guidelines differ, offering an opportunity to compare the utilisation, tolerability, and efficacy of different prophylactic regimens.[6,22] Furthermore, with likely increased uptake following updated guideline recommendations, we sought to assess the impact of mould-active prophylaxis in children with AML and HR-ALL. The aims of this study were: (i) to

characterise primary antifungal prophylaxis prescribing practices during intensive chemotherapy in children with acute leukaemia across three Australian paediatric oncology centres, (ii) to assess the efficacy and tolerability of different antifungal prophylaxis agents used and (iii) to determine the prevalence of and risk factors for IFD in this cohort.

Methods

Study design and population

A retrospective multi-centre cohort study of children diagnosed with *de novo* acute leukaemia at Perth Children's Hospital (PCH), Royal Children's Hospital (RCH) Melbourne, and Queensland Children's Hospital (QCH) between 1st January 2017 to 30th June 2020 was performed, as part of the Prophylaxis and CT imaging for Invasive Fungal Infection in Children with Cancer (PACIFIC) study. Episodes of relapsed leukaemia were excluded. Participants were identified from local oncology databases at each site and followed through to either completion of the intensive phases of chemotherapy (i.e., start of maintenance ALL therapy or end of primary AML therapy), commencement of salvage therapy for refractory disease, or conditioning for haematopoietic stem cell transplant (HSCT). Local antifungal prophylaxis recommendations are summarised in Supplementary Table S1. Administration of prophylaxis was at the discretion of the treating clinician.

Data were entered onto a secure, web-based software platform (REDCap hosted at Murdoch Children's Research Institute). Pharmacy prescribing data, pathology results and imaging reports were comprehensively reviewed to identify episodes of IFD.[7] Baseline demographics, leukaemia diagnosis and treatment protocol, were documented. For each chemotherapy cycle, data on antifungal prophylaxis (agent, dose, therapeutic drug monitoring (TDM), outpatient ambulatory administration, toxicity leading to cessation as documented by treating clinician), risk factors for IFD (neutropenia, lymphopenia, corticosteroid use, diabetes requiring insulin) and characteristics of IFD episodes were collected. For patients with IFD, prophylaxis and risk factor data were included up to the time of IFD diagnosis; secondary antifungal prophylaxis and subsequent IFD episodes were excluded. All study centres had Human Research Ethics Committee approval (coordinating HREC RCH (65,080)). Given the retrospective design and de-identification of data, patient consent was not obtained in accordance with the HREC approval.

Definitions

For ALL, patients treated on the Children's Oncology Group (COG) AALL1131, AALL1231, AALL1732, AALL1122, AALL0434, AALL1521, AALL1631, AALL0631, and AALL15P1 protocols as well as the Interfant-06 protocol were classified as HR-ALL (this comprises treatment protocols for: high-risk B-cell ALL, T-cell ALL, Philadelphia chromosome-positive ALL, and infant ALL). Patients treated on the COG AALL1731, and AALL0932 protocols were classified as standard-risk (SR-ALL) (comprising treatment protocols for standard-risk B-cell ALL). Patients initiated on a SR-ALL protocol who switched to a HR-ALL protocol before the end of consolidation were included in the HR-ALL group.

Mould-active prophylaxis was defined as the receipt of a mould-active triazole (posaconazole, voriconazole, itraconazole, isavuconazole), echinocandin (micafungin, caspofungin, anidulafungin) or liposomal amphotericin B as prophylaxis. Drug levels of >700 ng/mL for posaconazole and between 1000 and 5000 ng/mL for voriconazole were considered therapeutic.[9] For IFD-free survival analysis, AML patients who received a mould-active agent for ≥70% of time at risk were classified as receiving mould-active prophylaxis. For HR-ALL and SR-ALL, a threshold of ≥50% of time at risk on prophylaxis ("any" of "mould-active" respectively) was used to classify antifungal exposure. This threshold was chosen due to the inclusion of less intensive chemotherapy cycles (i.e., interim maintenance) interspersed between intensive cycles, during which antifungal prophylaxis is not indicated.[11]

Lymphopenia was defined as a lymphocyte count $<1.0 \times 10^9$/L, neutropenia as an absolute neutrophil count (ANC) $< 0.5 \times 10^9$/L and severe neutropenia as an ANC $<0.1 \times 10^9$/L.

Invasive fungal disease episodes were classified as proven, probable, and possible according to the European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria.[23] Modified possible IFD cases were also included as previously defined as: (i) the presence of host factors and clinical criteria suggestive of IFD not listed by EORTC criteria (e.g., lesions suggestive of hepatosplenic candidiasis with negative blood cultures) or (ii) host and mycology criteria in the absence of EORTC clinical criteria.[6,7] Breakthrough IFD was defined as IFD with first symptoms occurring from the time to steady state of antifungal (based on dosing and elimination half-life) to one dosing interval after drug discontinuation.[24] The response to therapy was assessed using defined EORTC/MSG criteria.[25]

Statistical analysis

For baseline demographics, antifungal use, and tolerability, descriptive statistics were calculated 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 for continuous variables.

Kaplan Meier curves and log rank test were used to estimate probability of IFD-free survival for proven/probable IFD and "any IFD" (proven/probable/possible/modified possible) for AML, HR-ALL and SR-ALL

patients respectively. For AML, a comparison between patients receiving mould-active prophylaxis and those not receiving mould-active prophylaxis was made. For the HR-ALL and SR-ALL cohorts, comparisons of any prophylaxis versus no prophylaxis and mould-active prophylaxis versus no mould-active prophylaxis was performed. A sensitivity analysis used median proportion of time on prophylaxis as a cut-off to classify antifungal exposure for each comparison.

Cox proportional hazards regression analysis was used to calculate hazard ratios and 95% confidence intervals for antifungal prophylaxis exposure variables and risk factors of interest over the entire cohort. For this analysis the outcomes were proven/probable IFD and "any IFD". The individual patient variables in this model included age, gender, hospital site, exposure to antifungal prophylaxis ("any" and "mould-active"), underlying leukaemia diagnosis (AML, HR-ALL, SR-ALL, other leukaemia), neutropenia, lymphopenia, any steroid exposure, and diabetes requiring insulin.

Role of the funding source

The funders of this study had no role in study design, data collection, data analysis, interpretation, writing of this report. All authors' financial disclosures are listed in detail at the end of the manuscript.

Results

A total of 434 patients were diagnosed with *de novo* acute leukaemia across study sites from 1st January 2017 to 30th June 2020 (Table 1), 73 with AML, 214 with HR-ALL, 137 SR-ALL and 10 with other leukaemia. Median age was 5.0 years (IQR 3.0–9.3 years), and 47.2% (205/434) were female. The median duration of follow up was 240 days (IQR 140.3–263.8 days). Of AML patients, 34.2% (25/73) were subsequently transitioned to salvage therapy and or HSCT. Of HR-ALL patients, 7.0% (15/214) proceeded to salvage therapy and or HSCT. All SR-ALL patients completed intensive chemotherapy and progressed to maintenance.

Prophylaxis prescribing by leukaemia diagnosis

Primary antifungal prophylaxis prescribing varied according to leukaemia diagnosis (Table 2). For AML, antifungal prophylaxis was prescribed in 98.7% (231/234) of cycles with a mould-active agent prescribed in 77.4% (181/234). For HR-ALL, prophylaxis was prescribed in 56.7% (469/827) (mould-active in 21.2% (175/827)) of cycles overall, including 67.3% (403/599) (mould-active in 27.2% (163/599)) of intensive cycles. For SR-ALL, prophylaxis was prescribed in 14.9% (96/644) of cycles overall and 19.8% (78/394) of intensive cycles, with fluconazole as the predominant agent.

The use of mould-active prophylaxis was higher in 2019–2020 compared to 2017–2018 for patients with AML (88.7% (110/124 cycles) vs 64.0% (71/111), $p < 0.001$) and HR-ALL (31.7% (127/401) vs 11.3% (48/426), $p < 0.001$). This finding was observed at all sites.

Characteristic	Total n = 434
Age in years—median (IQR)	5.0 (3.0–9.3)
Gender—female (%)	205 (47.2%)
Study site	
PCH (%)	99 (22.8%)
QCH (%)	181 (41.7%)
RCH (%)	154 (35.5%)
Leukaemia diagnosis & treatment protocol	
AML (%)	73 (16.8%)
AAML 1031 (% of AML)	33 (45.2%)
MyeChild 01 (% of AML)	30 (41.1%)
AAML 1531 (% of AML)	6 (8.2%)
other (% of AML)	4 (5.5%)
ALL (total)[a]	351 (80.9%)
pre B-cell ALL (% of ALL)	290 (82.6%)
T-cell ALL (% of ALL)	60 (17.1%)
High-risk (HR) ALL[c]	214 (49.3%)
AALL 1131 (% of HR ALL)	131 (61.2%)
AALL 1231 (% of HR ALL)	55 (25.7%)
Interfant 06 (% of HR ALL)	6 (2.8%)
other (% of HR ALL)	22 (10.3%)
Standard-risk (SR) ALL[c]	137 (31.6%)
AALL 0932 (% of SR ALL)	115 (83.9%)
AALL 1731 (% of SR ALL)	18 (13.1%)
other (% of SR ALL)	4 (2.9%)
Other leukemia[b]	10 (2.3%)
Other demographics/exposures	
Trisomy 21	19 (4.4%)
Corticosteroids (any exposure)	363[d] (83.6%)
Diabetes requiring insulin	34 (7.8%)

[a]"Mixed phenotype" infant ALL (n = 1). [b]Bi-phenotypic leukaemia (n = 3), juvenile myelomonocytic leukaemia (n = 2), Burkitt leukaemia (n = 2), acute leukaemia not otherwise specified (n = 1), myeloid leukaemia associated with Down Syndrome (n = 1), Blastic plasmacytoid dendritic cell neoplasm (n = 1). [c]Risk stratification at end of consolidation (at diagnosis 180 (41.5%) SR-ALL and 171 (39.4%) HR-ALL). [d]Steroid use was in accordance with chemotherapy treatment protocol in 345 (95.0%) or in addition to treatment protocol in 22 (5.0%).

Table 1: **Patient characteristics and leukemia diagnosis details.**

Antifungal prophylaxis agents, monitoring and toxicity

The age range of patients prescribed individual antifungal agents varied (Table 3), most notably, those who received posaconazole were older than those who received voriconazole (median age 11.3 vs 3.5 years, $p < 0.001$). Cessation due to toxicity was rare overall and was lowest with micafungin (0%) and highest with voriconazole (8.1% (8/99) of cycles in which voriconazole was given) (Table 3). At least one TDM level was performed in 81.9% (68/83) of posaconazole cycles and 69.7% (69/99) of voriconazole cycles. Itraconazole, isavuconazole and anidulafungin were not prescribed to any patients as prophylaxis.

	AML	HR ALL	SR ALL	Other leukaemia
Total cycles of chemotherapy	234	827	644	37
Any prophylactic agent given—n (%)	231 (98.7%)	469 (56.7%)	96 (14.9%)	28 (75.7%)
Any mould-active agent given—n (%)	181 (77.4%)	175 (21.2%)	10 (1.6%)	16 (43.2%)
Intensive cycles of chemotherapy[a]	N/A	599	394	N/A
Any prophylactic agent given—n (%)	–	403 (67.3%)[c]	78 (19.8%)	–
Any mould-active agent given—n (%)	–	163 (27.2%)	10 (2.5%)	–
Antifungal prophylactic agent prescribed				
Fluconazole—n (% of cycles with prophylaxis)	73 (31.6%)	340 (72.5%)	91 (94.8%)	14 (50.0%)
Posaconazole—n (% of cycles with prophylaxis)	71 (30.7%)	9 (1.9%)	–	3 (10.7%)
Voriconazole—n (% of cycles with prophylaxis)	71 (30.7%)	24 (5.1%)	–	4 (14.3%)
Echinocandin[b]—n (% of cycles with prophylaxis)	46 (19.9%)	55 (11.7%)	5 (5.2%)	8 (28.6%)
Liposomal amphotericin—n (% of cycles with prophylaxis)	38 (16.5%)	121 (25.8%)	5 (5.2%)	6 (21.4%)
Mixed—n (% of cycles with prophylaxis)	62 (26.8%)[d]	73 (15.6%)[e]	5 (5.2%)	6 (21.4%)

[a]Excluding interim maintenance cycles. [b]Predominantly micafungin (112/114 cycles); caspofungin used in 2/114 cycles (AML patients). [c]Prophylaxis was given in 129/214 (60.3%) of HR-ALL induction cycles, including 47/214 (22.0%) who received a mould-active agent. [d]Most common combinations: micafungin/posaconazole (n = 19 cycles), fluconazole/amphotericin (n = 11), amphotericin/voriconazole (n = 10), amphotericin/posaconazole (n = 10). [e]Most common combinations: fluconazole/amphotericin (n = 31), fluconazole/micafungin (n = 19), amphotericin/voriconazole (n = 9).

Table 2: **Primary antifungal prophylaxis prescribing according to leukemia diagnosis.**

For posaconazole, the liquid formulation was used in 63.9% (53/83) of cycles, and tablets in 37.3% (31/83) (both formulations in 1.2% (1/83)). The most common dosing regimen was 4 mg/kg (maximum dose 200 mg) three times daily for liquid (79.2%) and 300 mg daily for tablets (83.9%). In cycles where TDM was performed, initial posaconazole levels were therapeutic in 66.2%, (45/68) with better target attainment observed with tablets compared to liquid (95.7% (n = 22/23) vs 51.1% (n = 23/45); OR 21.0 (95% CI 2.6–169.7), p < 0.001).

For voriconazole, dosing was predominantly weight based according to age (children: 9 mg/kg twice daily; adolescents: 200 mg twice daily). Oral voriconazole was used in 96.0% (95/99) of cycles and intravenous in 16.2% (16/99). Of cycles where TDM was performed, initial voriconazole levels were therapeutic in 34.8% (24/69), sub-therapeutic in 39.1% (27/69) and supra-therapeutic in 26.1% (18/69).

For micafungin, a dose of 1 mg/kg (maximum 50 mg) daily was used in 96.4% (108/112) of cycles. At least one dose was administered via an outpatient parenteral antimicrobial therapy (OPAT) service in 26.8% (30/112) of cycles. Liposomal amphotericin B was most commonly dosed at 3 mg/kg thrice weekly (67.1%) or 1 mg/kg daily (27.1%). At least one dose was administered via OPAT in 46.5% (79/170) of cycles. Hypokalaemia complicated 50.0% (85/170) of cycles requiring oral (n = 23/170, 13.5%) or intravenous (n = 61/170, 35.9%) potassium supplementation, and led to cessation in one patient.

Invasive fungal disease prevalence, microbiology, and diagnostic workup

Overall, there were 95 episodes of IFD including 32 proven, 10 probable, 50 possible and 3 modified possible episodes (Table 4). Overall prevalence of

Prophylactic agent	Total cycles (pts)	Site (% of patients on agent)			Age years (IQR)	TDM during cycle	Initial TDM within target	Breakthrough Prov/Prob IFD (% of pts)	Cessation due to toxicity (% of cycles)
		PCH	QCH	RCH					
Fluconazole	518 (212)	72 (34.0%)	54 (25.5%)	86 (40.6%)	5.1 (3.0–9.5)	–	–	12 (5.7%)	8[a] (1.5%)
Posaconazole	83 (35)	17 (48.6%)	11 (31.4%)	7 (20.0%)	11.3 (4.9–13.7)	68 (81.9%)	45 (66.2%)	0	2[b] (2.4%)
Voriconazole	99 (40)	0	14 (35.0%)	26 (65.0%)	3.5 (0.9–7.2)	69 (69.7%)	24 (34.8%)	0	8[c] (8.1%)
Micafungin	112 (58)	30 (51.7%)	5 (8.6%)	23 (39.7%)	8.3 (3.0–13.4)	–	–	3 (5.2%)	0
Liposomal Amphotericin	170 (97)	5 (5.2%)	39 (40.2%)	53 (54.6%)	4.5 (2.4–10.2)	–	–	2 (2.1%)	5[d] (2.9%)

IFD, invasive fungal disease; prov/prob, proven/probable; PCH, Perth Children's Hospital; QCH, Queensland Children's Hospital; RCH, Royal Children's Hospital; TDM, therapeutic drug monitoring. [a]Nausea (n = 2), hepatotoxicity (n = 6). [b]Vomiting (n = 1), visual disturbance (n = 1). [c]Hepatotoxicity (n = 3), visual disturbance (n = 3), allergy (n = 1), cardiac dysfunction (n = 1). [d]Hypokalaemia (n = 1), allergy (n = 2), hepatotoxicity (n = 1), malaise (n = 1).

Table 3: **Primary antifungal prophylaxis agents, monitoring and toxicity.**

	Proven/probable	Possible[a]
Total cases (% total)	42 (44.2%)	53 (55.8%)
Primary site of infection[d]		
Respiratory	21 (50.0%)	45 (84.9%)
Blood	13 (31.0%)	0
Musculoskeletal/soft tissue	4 (9.5%)	0
Hepatosplenic	2 (4.8%)	5 (9.4%)
Sino-nasal	3 (7.1%)	0
Other	1 (2.4%)	3 (5.7%)
Pathogen[d]		
Mould[e]	**27 (64.3%)**	–
Aspergillus fumigatus	6 (14.3%)	–
Other *Aspergillus* spp.[b]	5 (11.9%)	–
Aspergillus spp. (GM/PCR +ve only)	8 (19.0%)	–
Mucorales[c]	3 (7.1%)	–
Other mold[f]	6 (14.3%)	–
Yeast	**17 (40.5%)**	–
Candida albicans	4 (9.5%)	–
Candida tropicalis	5 (11.9%)	–
Candida krusei/Pichia kudriavzevii	3 (7.1%)	–
Other yeasts[g]	5 (11.9%)	–
Microbiological testing[k]		
Bronchoalveolar lavage performed	20 (58.8%)	22 (41.5%)
Microscopy/culture positive (%)	7 (35.0%)	0
Galactomannan/PCR positive[h] (%)	13 (65.0%)	0
Lung biopsy taken	7 (20.6%)	3 (5.7%)
Microbiology positive (%)	4 (57.1%)	0
Histopathology positive (%)	6 (85.7%)	0
Serum galactomannan taken	15 (44.1%)	24 (45.2%)
Positive (%)	3 (20.0%)	0
Other biopsy/invasive sample taken	21[j] (61.7%)	6[i] (11.3%)
Outcome at 6 months		
Complete response	23 (54.8%)	36 (67.9%)
Partial response	14 (33.3%)	15 (28.3%)
Stable disease	1 (2.4%)	0
Progressive disease	1 (2.4%)	0
Death	3 (7.1%)	2 (3.8%)

GM, galactomannan; PCR, polymerase chain reaction. [a]Including modified possible episodes (n = 3). [b]*A. terreus* (n = 2), *A. flavus* (n = 2), *A. niger* (n = 1). [c]*Mucor indicus* (n = 1), *Rhizopus arrhizus* (n = 1), *Rhizomucor* spp. (n = 1). [d]Two cases of concomitant candidaemia and aspergillosis (1 probable pulmonary IFD, 1 proven sinonasal IFD). [e]One episode of pulmonary mold infection with two pathogens (*Exserohilum rostratum* & *Aspergillus* spp. (GM + ve)). [f]*Exserohilum rostratum* (n = 3), mold (histopathological changes only) (n = 2), *Fusarium solani* (n = 1). [g]*Candida lusitaniae/Clavispora lusitaniae* (n = 2), *Candida parapsilosis* (n = 1), *Candida glabrata/Nakaseomyces glabrata* (n = 1), yeast—unable to identify on ITS sequencing (n = 1). [h]BAL GM done on 18/22 BAL samples from possible and 19/21 probable/proven cases (GM positive in 12 + 1 PCR positive). [i]Skin/soft tissue biopsy (n = 3), pleural fluid (n = 2), liver biopsy (n = 1). [j]24 samples from 21 patients (skin/soft tissue (n = 9), sinus (n = 4), liver (n = 3), pleural fluid (n = 2), ascites (n = 1), cardiac (n = 1) brain (n = 1) oesophageal (n = 1), parotid (n = 1), eye (n = 1)). [k]Excluding proven isolated candidaemia cases (n = 8).

Table 4: **Causative pathogens and diagnostic testing—Invasive fungal disease episodes.**

proven/probable IFD was 9.7% (95% CI 7.1%–12.9%) and possible/modified possible IFD was 12.2% (95% CI 9.3%–15.7%). Proven/probable IFD prevalence was similar across study years and sites (Supplementary Appendix S2). Outcome at six months post-IFD diagnosis was favourable (partial/complete response) in 88.1% of proven/probable and 96.2% of possible/modified possible episodes; there was a single death attributable to proven/probable IFD (2.4% mortality).

A mould was implicated in 64.3% (27/42) of proven/probable IFD episodes, comprising Aspergillosis in 70.4% (19/27) and a non-*Aspergillus* mould in 25.9% (7/27) (Table 4). Of 40.5% (17/42) episodes of proven/probable yeast infection, *Candida albicans* comprised 23.5% (4/17) and non-*C. albicans* yeasts 76.5% (13/17). There were two episodes of concomitant candidemia and aspergillosis.

In the diagnostic workup of possible/modified possible IFD episodes, lung biopsy (5.7% vs 20.6%, p = 0.033) and biopsy of another site (11.3% vs 61.7%, p < 0.001) were less frequently performed compared with proven/probable cases (excluding episodes of isolated proven candidemia) (Table 4). Bronchoalveolar lavage was performed in only 41.5% (22/53) of possible/modified IFD episodes, and in 33.9% (18/53) possible/modified possible episodes neither invasive sampling nor serum galactomannan were performed.

For AML, proven/probable IFD prevalence was 6.8% (95% CI 2.3%–15.3%; Supplementary Appendix S2)). Proven/probable IFD episodes occurred in cycle 1 (n = 1), 2 (n = 3), and 3 (n = 1) of AML therapy. A mould was the implicated pathogen in 80% (4/5) of proven/probable episodes in AML. Possible/modified possible IFD prevalence was 31.5% (95% CI 21.1%–43.4%) with episodes diagnosed during cycle 1 (n = 10), 2 (n = 5), 3 (n = 4), and 4 (n = 4) respectively.

For ALL, proven/probable IFD prevalence was 10.3% (95% CI 7.3%–13.9%) overall, 14.0% (95% CI 9.7%–19.4%) for HR-ALL, and 4.4% (95% CI 1.6%–9.3%) for SR-ALL. Proven/probable IFD episodes occurred during intensive chemotherapy cycles in 91.7% of cases (33/36: induction (n = 16) consolidation (n = 11) delayed intensification (n = 6)). Four proven/probable IFD episodes occurred in patients with HR-ALL who were initially commenced on a SR-ALL protocol but subsequently switched to HR-ALL therapy. A mould was implicated in 63.9% (23/36) of proven/probable IFD episodes in patients with ALL. Possible/modified possible IFD was diagnosed in 12.6% (95% CI 8.5%–17.8%) of HR-ALL and 1.5% (95% CI 0.2%–5.2%) of SR-ALL patients.

There were 57 episodes of breakthrough IFD including 17 proven/probable episodes (Fig. 1). Proven/probable breakthrough IFD episodes with mould-active prophylaxis were rare, occurring in 5.2% (3/58) of patients receiving micafungin, 2.1% (2/97) of those receiving liposomal amphotericin B and 0% of patients receiving posaconazole or voriconazole (Table 3). The proportion of proven/probable breakthrough IFD caused by moulds was not significantly higher in patients receiving fluconazole prophylaxis compared with those on mould-active prophylaxis although overall numbers were small (75.0% (9/12) vs 40.0% (2/5), p = 0.6). In patients receiving mould-active triazole

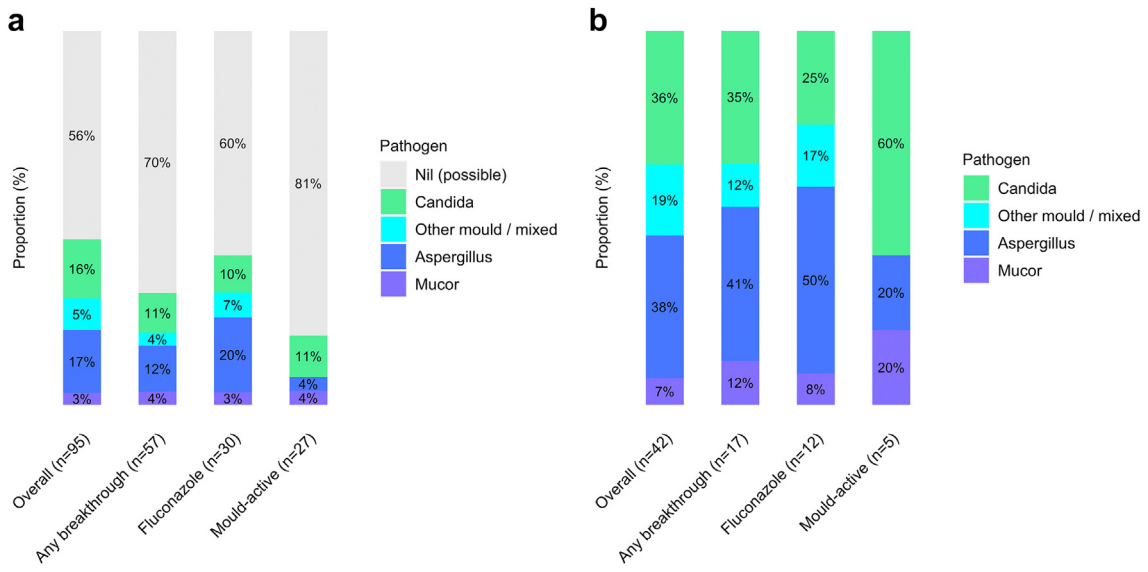

Fig. 1: **Breakthrough invasive fungal disease microbiology according to prophylactic strategy: (a) any IFD and (b) proven/probable IFD.**

prophylaxis, possible breakthrough IFD episodes predominantly occurred in the context of therapeutic levels (posaconazole 100% (4/4), voriconazole 75% (3/4)).

Impact of prophylaxis and risk factors for IFD

For patients with AML, proven/probable IFD prevalence was 2.4% (1/42) with predominantly mould-active prophylaxis compared to 12.9% (4/31) without. In survival analysis, for AML patients, receipt of mould-active prophylaxis was associated with higher proven/probable IFD-free survival and "any IFD"-free survival (Fig. 2(a) and (b)). For HR-ALL patients, proven/probable IFD prevalence was 4.5% (1/22) with predominantly mould-active prophylaxis compared to 15.1% (29/192) without. In survival analysis for HR-ALL however, there was no significant association between mould-active prophylaxis and proven/probable IFD-free survival or "any IFD"-free survival, with few patients receiving predominantly mould-active prophylaxis (Fig. 2(c) and (d)). In both SR-ALL patients (Fig. 2(e) and (f)) and HR-ALL patients (Supplementary Appendix S4) there was no significant association between receipt of any prophylaxis and proven/probable or "any IFD"-free survival. Sensitivity analyses produced similar results (Supplementary Appendix S5). Excluding episodes of proven/probable IFD, mould-active prophylaxis was associated with higher possible IFD-free survival for patients with AML but not HR-ALL (Supplementary Appendix S6).

In the adjusted Cox regression analysis, neutropenia and steroid exposure were associated with increased risk of proven/probable IFD, whilst SR-ALL (compared with AML) and receipt of anti-mould prophylaxis were associated with decreased risk (Table 5). When possible IFD episodes were included, increasing age, and diabetes requiring insulin were associated with increased IFD risk, whilst a diagnosis of SR-ALL or HR-ALL (compared with AML) was associated with decreased risk (Supplementary Appendix S3).

Discussion

In this multi-centre study of children with *de novo* acute leukaemia in Australia, IFD was a common complication in children undergoing treatment for HR-ALL particularly. Mould-active prophylaxis was associated with a reduced risk of proven/probable IFD. Individual mould-active agents were generally well tolerated and prevalence of breakthrough IFD was low. However, mould-active prophylaxis was only prescribed in a minority of children with HR-ALL and IFD remained a frequent complication in this cohort. This highlights the challenges with antifungal prescribing in this group and the need for improved approaches for these high-risk patients. Possible IFD was frequently diagnosed, with incomplete microbiological workup in most cases, reflecting the difficulty of definitive IFD diagnosis in immunocompromised children.

We found that exposure to mould-active prophylaxis was associated with an overall lower risk of proven/probable IFD and for children with AML, improved IFD-free survival. Furthermore, a mould was implicated as the causative pathogen in the majority of proven/probable IFD cases, consistent with regional data in which haematological malignancy remained the predominant risk factor for invasive aspergillosis in adults.[26] Notably, the prevalence of proven/probable IFD for patients with AML was lower in our cohort (6.8%)

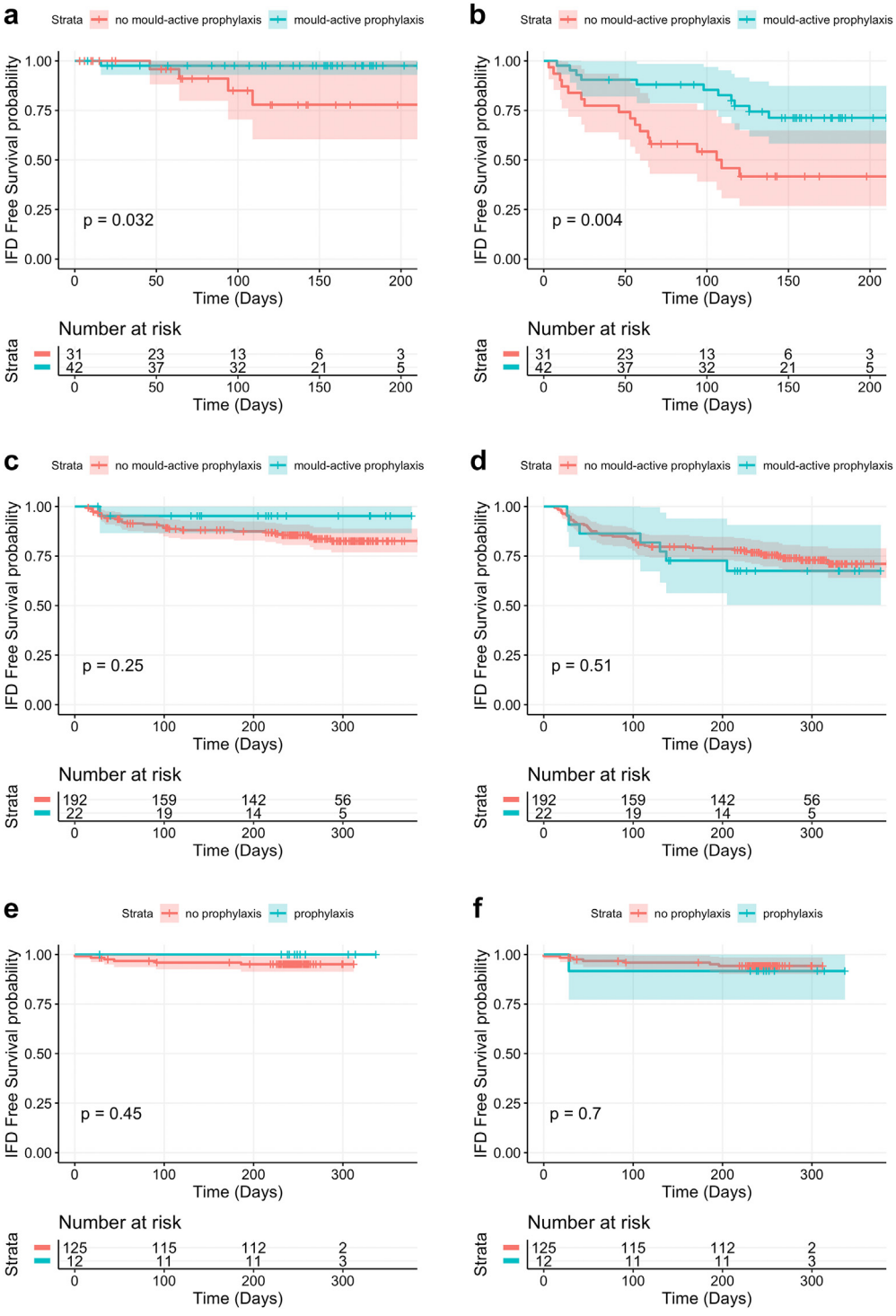

Fig. 2: **Kaplan–Meier Curves for Invasive Fungal Disease (IFD) free survival for children with: Acute myeloid leukaemia (AML) according to mould-active prophylaxis exposure for: (a) proven/probable IFD and (b) all IFD. High-risk acute lymphoblastic leukaemia (HR-ALL) according to mould-active prophylaxis exposure for: (c) proven/probable IFD and (d) all IFD. Standard-risk acute lymphoblastic leukaemia (SR-ALL) according to any prophylaxis exposure for: (e) proven/probable IFD and (f) all IFD.**

Factor	Category	Unadjusted hazard ratio (95% CI) p-value	Adjusted hazard ratio (95% CI) p-value
Age at cycle start (years)		**1.07 (1.00, 1.14) 0.042**	1.04 (0.96, 1.12) 0.331
Gender	Female	1.18 (0.65, 2.17) 0.587	–
	Male	Reference	–
Site	1 (PCH)	0.88 (0.41, 1.87) 0.731	–
	2 (QCH)	Reference	–
	3 (RCH)	0.66 (0.32, 1.34) 0.247	–
Leukemia diagnosis	AML	Reference	Reference
	High risk ALL	1.07 (0.40, 2.80) 0.897	0.48 (0.15, 1.50) 0.205
	Standard risk ALL	0.35 (0.10, 1.14) 0.080	**0.20 (0.05, 0.86) 0.031**
	JMML	Insufficient numbers	Insufficient event numbers
	Other	1.41 (0.16, 12.11) 0.755	0.88 (0.08, 10.28) 0.920
Corticosteroids during cycle	Yes	**2.06 (1.11, 3.85) 0.023**	**1.98 (1.01, 3.91) 0.047**
	No	Reference	Reference
Diabetes requiring insulin	Yes	**4.53 (1.61, 12.73) 0.004**	2.62 (0.93, 7.38) 0.068
	No	Reference	Reference
Neutropenia (<0.5) - weeks		**1.27 (1.14, 1.43) <0.001**	**1.23 (1.05, 1.44) 0.012**
Severe neutropenia (<0.1) - weeks		**1.28 (1.14, 1.44) <0.001**	–
Prolonged neutropenia >10 days (<0.5)		1.76 (0.84, 3.68) 0.131	–
Lymphopenia (<1.0) - weeks		**0.93 (0.88, 0.99) 0.024**	–
Proportion of time at risk on any prophylaxis		0.82 (0.41, 1.65) 0.579	–
Proportion of time at risk on mould-active prophylaxis		**0.64 (0.20, 2.00) 0.442**	**0.20 (0.05, 0.89) 0.034**

Bold font indicates statistically significant results.

Table 5: **Cox proportional regression for proven/probable invasive fungal disease.**

compared with previously local (10.3%)[8] and international cohorts (10.0–12.0%)[27,28] in whom fluconazole prophylaxis was predominantly used. This is consistent with previous randomised controlled trial data that favour caspofungin[13] and posaconazole[12] over fluconazole in children and adolescents with AML. It also validates recent guideline recommendations for mould-active prophylaxis in this cohort.[9–11] Similarly, for SR-ALL, the low IFD prevalence and lack of impact of prophylaxis confirm that routine prophylaxis is not indicated in this context.[9–11] For HR-ALL, the prevalence of proven/probable IFD remained high, compared with previous local[6] and international reports,[29,30] with a predominance of moulds in the context of few patients receiving mould-active prophylaxis. In a recent analysis of children enrolled in an ALL-treatment trial, high bone marrow blast count at day 15 and older age were independently associated with increased proven/probable IFD risk.[29] For our analysis, we stratified HR-ALL according to treatment protocol at the end of consolidation, incorporating patients with high white cell count or older age at baseline, as well as those with unfavourable cytogenetics or poor response to induction therapy. As many IFD episodes occur early in HR-ALL treatment (induction/consolidation), prophylaxis should ideally be initiated as soon as any high-risk criteria for ALL are met. Notably, a number IFD episodes also occurred in later cycles for both HR-ALL (delayed intensification) and AML. This is in keeping with previous Australian paediatric data[6,8] and indicates that prophylaxis should be continued throughout intensive chemotherapy cycles in these groups.

Although mould-active agents were well tolerated overall and their uptake improved over time, mould-active prophylaxis was prescribed in only a minority of HR-ALL patients. Furthermore, the variation between centres in choice of agent and dosing likely reflects the practical challenges with antifungal prescribing in paediatric acute leukaemia, as well as the uncertainty regarding the optimal approach in children. For mould-active triazoles, use in HR-ALL is limited due to the inhibition of vincristine metabolism through cytochrome P450 (CYP3A4).[14,31–33] Furthermore, target attainment for voriconazole and liquid posaconazole was poor in our cohort, which is consistent with previous paediatric studies[15–18] and may potentially be amplified by chemotherapy induced mucositis.[34] For echinocandins, daily intravenous administration may not be feasible, particularly for patients with HR-ALL for whom chemotherapy is largely administered in the outpatient setting and data for intermittent echinocandin dosing are insufficient to support widespread implementation.[35] Finally, for liposomal amphotericin B, although intermittent dosing is generally accepted, efficacy data are limited to observational studies[36,37] and hypokalaemia is a common adverse event, requiring

intravenous potassium supplementation in one third of our cohort.

The high prevalence of possible IFD episodes, particularly in the AML and HR-ALL cohorts, warrants discussion. Most possible cases involved patients with compatible clinical features and CT imaging changes but no microbiological confirmation of IFD, with diagnostic testing not performed in many cases. Moreover, preceding mould-active prophylaxis may have impacted on microbiological test sensitivity in possible IFD episodes.[38] The survival benefit conferred by anti-mould prophylaxis for possible IFD in AML suggest that at least some possible episodes were likely to represent "true" IFD, however many possible IFD episodes may not, as CT imaging in children is not well validated for IFD diagnosis and alternate causes may be responsible for similar radiological findings. Despite the uncertainties, possible IFD episodes still contribute to prolonged antifungal courses and treatment delays. A better understanding of CT imaging changes in children with "true" or confirmed IFD is required to improve diagnostic certainty, along with exploration of potentially more specific imaging modalities including positron-emission tomography scanning.[39] Similarly, increased utilisation of available microbiological testing as well as incorporation of emerging non-invasive testing into diagnostic algorithms could improve diagnostic certainty in pulmonary IFD.[39] Importantly, treatment informed by microbiological respiratory sampling may lead to improved outcomes of IFD in this context.[40] However, clinical decisions regarding respiratory sampling in suspected IFD should include consideration of the anticipated yield, which may be impacted by the nature and location of imaging changes,[41] as well as potential complications, particularly with lung biopsy.[42,43] Ideally a combination of imaging and microbiological findings with high negative predictive value for IFD could facilitate earlier cessation of unnecessary antifungals in this setting.

Developments in antifungal agents have potential to impact on IFD prevention in the future. For posaconazole, as seen in this study, oral tablets achieve therapeutic levels far more reliably compared to the liquid formulation.[44] For younger children unable to swallow tablets, an improved powder-for-suspension posaconazole formulation has been approved for use in Europe and the United States (but not as yet in Australia), based on supportive pharmacokinetic data.[45] For intravenous agents, in our cohort, a quarter of echinocandin and almost half of liposomal amphotericin B courses utilised OPAT services and expansion of similar services could facilitate increased uptake of these agents.[14] Notably, a reduction in proven/probable *Aspergillus* observed in a single-centre study using twice-weekly prophylactic high-dose micafungin (9 mg/kg) in children with ALL, (1.2% vs 5.8% in a historic cohort).[46] Wider assessment of this regimen in children with ALL is required. Finally, oral amphotericin formulations[47,48] and the next generation once-a-week echinocandin, rezafungin[49] have potential to overcome many antifungal administration challenges, and warrant further investigation as prophylactic agents in children.

This study has several limitations. Firstly, due to the retrospective observational design, unmeasured confounders may impact the assessment of prophylaxis exposure effect on IFD. However, in contrast with previous studies,[28,29] we captured detailed data on prophylaxis and known IFD risk factors throughout intensive chemotherapy cycles in the entire cohort, allowing adjusted analyses accounting for known exposures associated with IFD risk. Furthermore, all cancer care was completed at the respective study sites, therefore limiting the possibility of loss to follow up and missed IFD episodes. Secondly, IFD diagnostic workup was not consistent across cases, possibly leading to under ascertainment of proven/probable IFD cases. Similarly, the high proportion of possible IFD in the AML cohort raises uncertainty about the true impact of prophylaxis in this cohort. Notably, in the previous COG randomised controlled trial, diagnostic workup was also performed according to clinician discretion with <2% of patients undergoing BAL, despite 11.0% of participants being diagnosed with possible IFD.[13] This highlights the challenge in obtaining optimal diagnostic samples in children with suspected IFD and the need for improved tests and diagnostic algorithms. Thirdly, the thresholds used to define antifungal exposure in the survival analysis were arbitrary, based on pragmatic estimates in the absence of specific definitions. Notably the results of the sensitivity analysis and the proportional hazards model were consistent with the survival analysis in demonstrating the benefit of mould-active prophylaxis. Finally, few patients in this study received novel therapeutic approaches for leukaemia and consequently, our findings may not be generalisable to children enrolled in trials incorporating targeted molecular and immunological therapies. With the evolving landscape of acute leukaemia treatment, an ongoing assessment of IFD risk together with any new drug–drug interactions will be required when considering antifungal prophylaxis in this context.[14,50]

Conclusion

Our multicentre study shows that for children with acute leukaemia in Australia, a wide range of antifungal agents are prescribed for prophylaxis, with exposure to anti-mould agents associated with a reduced risk of IFD. Proven/probable IFD prevalence was lower in children with AML in this cohort compared with previous studies, potentially attributable to increased uptake of mould-active prophylaxis. In contrast, for HR-ALL IFD prevalence remains high in the region, in the context of suboptimal uptake of mould-active prophylaxis. This affirms the recommendation for mould-active

prophylaxis in HR-ALL, notwithstanding the challenges with antifungal prescribing in this group. Possible IFD was diagnosed frequently in AML and HR-ALL indicating that improved diagnostic processes, incorporating existing and emerging diagnostic strategies specific to the paediatric context, are required.

Contributors

DKY was responsible for leading study design, co-ordinating data collection, extracting and analysing data, and drafting and revising the manuscript. CCB, MAS, KAT and GMH were responsible for supervision and review of study design, and revision of the manuscript. RSK and SW contributed to study design and revision of the manuscript. SC, JA and CC contributed to data collection and revision of the manuscript. TS contributed to data analysis and revision of the manuscript. All authors approve the final version of the manuscript. The corresponding author (DKY) verified the data, had full access to all the raw data in the study, and had final responsibility for the decision to submit for publication.

Data sharing statement

Deidentified patient data and data dictionary may be provided upon reasonable request via email to the corresponding author.

Declaration of interests

All authors declare no conflicts of interest associated with this publication. MS has received honoraria from Gilead and F2G, outside of this work and participates on a Data Safety and Monitoring Board for Basilea, Pfizer, Roche and Merck. All other authors declare no competing interests.

Acknowledgements

The PACIFIC study was supported by a grant from the Perth Children's Hospital Foundation (PCHF Grant 9973). DKY is supported by an Australian Government Research Training Program Postgraduate Scholarship. The authors were not precluded from accessing data in the study, and they accept responsibility to submit for publication.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanwpc.2024.101201.

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