Title: 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

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Invasive fungal infections (IFIs) pose a significant risk to patients with haematological malignancies (Pagano *et al*, 2006). However epidemiological data in paediatric acute lymphoblastic leukaemia (ALL) is scarce (Groll *et al*, 2014). Differences in the biology of ALL, pre-existing and treatment-associated co-morbidities, sensitivity and specificity of diagnostics and dosing and pharmacokinetics of anti-fungal (AF) agents preclude direct comparison with adult cohorts. The reported incidence of IFI in paediatric ALL is hugely variable, ranging from 0.01-22% (Afzal *et al*, 2009, Das *et al*, 2018, Hale *et al*), interpretation limited by differing chemotherapy protocols, patient populations and diagnostic strategies. In particular, there is uncertainty on the value of prophylactic AF agents in this cohort and many institutions opt to devise their own clinical guidelines. With this in mind, we conducted a retrospective study in two London centres to explore the incidence and outcome of IFI in children and young people undergoing contemporary ALL therapy and to identify risk factors associated with IFI.

Patients with ALL treated on the UKALL 2011 protocol, a phase 3 randomised controlled trial, at Great Ormond Street Hospital (GOSH) and University College London Hospital (UCLH) between January 1st 2013 and January 1st 2017 were included. In induction, patients were randomised to receive either a short course of dexamethasone (10mg/m^2/day) for 14 days or a standard course of 6mg/m^2/day for 28 days. In the second randomisation, patients received either high dose methotrexate or standard interim maintenance (IM). Medical records and online databases were used to collate data during induction, consolidation, IM and delayed intensification. IFIs were classified as proven, probable and possible according to revised EORTC criteria (De Pauw *et al*, 2008). Risk factors for IFI were analysed using logistic regression (Stata version 15.1, StataCorp, Texas).

Over the 4-year period, 290 patients (Male=159 (55%), Female=131 (45%)) with a median age of 5 years (range 1 -24 years), were treated for ALL (B-lineage n=247 (85%), T-lineage n=43 (15%)) at

GOSH (n=227) and UCLH (n=63). Young adults aged 20 to 24 years (n=15, 5%) who received AF prophylaxis were excluded from our analysis. Routine AF prophylaxis was not otherwise implemented (n=275). Median follow up was 17 months (range 3-41).

In total, 22 (8.0%) patients experienced IFI giving an incidence of proven, probable and possible infection of 7 (2.5%), 4 (1.5%) and 11 (4%) respectively. Of the seven with proven IFI, one had a positive blood culture only, a *candida* subspecies. The remaining 6 patients had disseminated disease, all *candida* subspecies with the exception of one case of *Scedoporium apiospermum*. Four patients had probable IFI, in 3 of these, the mycological criterion was a positive serum beta D glucan. In the 4th case, *aspergillus niger* was identified in the sputum. Eleven patients had possible IFI, all with involvement of the lower respiratory tract (one case of suspected dissemination). Positive mycological findings and the breakdown of IFI by site and treatment phase (induction 50%, consolidation 45%, IM 5%) is demonstrated in Table I.

Univariable and multivariable analyses were performed to identify IFI risk factors. In the univariable analysis the short course of dexamethasone reaches statistical significance (p=0.045). However, in multivariable analysis, this just loses significance (p=0.073). Multivariable analysis showed a significant association with Regimen B, the more intensive 4-drug induction protocol (p=0.046) with an incidence of IFI of 11% versus 5.5% (Regimen A). Baseline characteristics, univariable and multivariable analysis are displayed in Table II.

The majority of patients with IFI (17/22; 77%) had a chemotherapy delay, 6 out of 7 proven (86%), 3 out of 4 probable (75%) and 8 out of 11 possible (73%). Median delay was 3 weeks (range 1-15 weeks). Three patients with proven and one with possible disease had significant chemotherapy omissions and/or dose reductions. At study completion, 20 patients were in remission, though one was lost to follow-up at 3 months. Three patients proceeded to bone marrow transplant (BMT), one with aplasia due to co-existing Wiskott-Aldrich Syndrome, one with a relapse of childhood ALL in adolescence and one with refractory B-ALL post induction. Two patients died, both post BMT, one with respiratory failure with no evidence of IFI. The other died from multi-organ failure and had a positive blood culture for *candida* (same pathogen reported previously) noted at the time of death.

In summary, the incidence of IFI on the UKALL2011 protocol is low (8%). While the risk of infection-related mortality is also low, mirroring the UKALL2003 study (O'Connor, *et al*, 2014), there is the potential to compromise the timely delivery of chemotherapy. To date, none of the 17 patients with a chemotherapy delay/omission has suffered a disease relapse. However, we await a longer follow-up. We acknowledge that this study has limitations. We strictly adhered to the EORTC criteria in all cases, however, due to its poor positive predictive value, beta D-glucan testing is of questionable utility in children (Lehrnbecher *et al*, 2016). It is also not standard practice in the UK to perform invasive diagnostic procedures, limiting our ability to establish a histological diagnosis. Additionally, the classical radiological features may be absent on CT imaging in children under the age of 5 (45% of our patient cohort) (Lehrnbecher *et al*, 2012). Therefore, it is difficult to accurately classify these infections and further work is needed to define paediatric-specific criteria. While there is no international consensus, an incidence of IFI of ≥10% is often considered high risk (Tragiannidis, *et al*, 2012, Groll, *et al*, 2014) and an indication for consideration of AF prophylaxis. The incidence in our cohort was only 8%. However, importantly, the incidence was 11% in patients receiving the more intensive induction protocol, Regimen B, and therefore prophylaxis could be considered in this higher risk population. This association with an increased risk of IFI must first be confirmed prospectively in a larger cohort. In the interim, the risk should be assessed on an individual basis. Our conclusions are based on our rates of IFI, the lack of randomised data and the toxicity, interaction profile and administration routes of the currently available AF drugs. Pending the results

of a prospective randomised study, the advent of an oral agent that can be easily administered in children receiving vincristine, with a favourable toxicity and interaction profile, may prompt us to change our practice.

Authors Contributions:

All authors critically appraised drafts of the manuscript and approved final submitted version. MOR, DG, AV, RH and DOC made contributions to study concept and design. MOR, DG, SS, AK, VG, AR, PA, DC, VP, BC and SD assisted with data acquisition. AAK led data analyses and interpretation with MOR, RH and DOC. MOR prepared the manuscript.

Table I. Proven, probable and possible IFI by site, treatment phase and positive mycological results

	Proven	Probable	Possible
	N=7	**N=4**	**N=11**
Site of involvement			
Blood	1	0	0
Disseminated (including CNS)	6	1	1
Lungs only	0	3	10
Treatment phase			
Induction	5	3	3
Consolidation	2	1	7
IM	0	0	1
DI	0	0	0
Mycological Isolates			**N/A**
Candida utilis	1	0	-
Candida albicans	3	0	-
Candida lusitaniae	2	0	-
Scedosporium apiospermum	1	0	-
Aspergillus niger	0	1*	-

*Sputum

Table II. Baseline characteristics, univariable and multivariable analysis of patients with proven, probable or possible IFI during treatment

	IFI*/N	Univariable**		Multivariable**	
		OR (95% CI)	p-value	OR (95% CI)	p-value
Sex					
Male	13/148	1.00	0.65	1.00	0.73
Female	9/124	0.81 (0.34 – 1.97)		0.85 (0.34 – 2.12)	
Age					
1-9	18/208	1.00	0.44	1.00	0.16
10-14	1/33	0.33 (0.04 – 2.56)		0.18 (0.02 – 1.53)	
15-19	3/31	1.13 (0.31 – 4.09)		0.67 (0.17 – 2.71)	
Induction regimen					
Reg A	8/145	1.00	0.10	1.00	0.046
Reg B	14/127	2.12 (0.86 – 5.24)		2.78 (1.02 – 7.57)	
Induction Dexamethasone					
Short	3/93	1.00	0.045	1.00	0.073
Standard	19/179	3.56 (1.03 – 12.37)		3.17 (0.90 – 11.19)	

***Proven, probable and possible IFI combined**

****Includes patients with a value for all variables only**

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