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INVASIVE FUNGAL DISEASE IN PRETERM NEONATES AND CHILDREN WITH HAEMATOLOGICAL MALIGNANCY: AETIOLOGY, CLINICAL CHARACTERISTICS AND OUTCOME

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ABSTRACT

Background: Invasive fungal disease (IFD) is an important cause of morbidity and mortality in immunocompromised children, particularly preterm neonates and children receiving intensive immunosuppression. Diagnosis is hindered by non-specific clinical features, restricted sample volumes and limited access to fungal biomarkers, and paediatric data from India remain sparse. This study evaluated the risk factors, clinical features, microbiological profile and outcomes of IFD in a mixed cohort of immunocompromised children.

Materials and Methods: A longitudinal observational study was conducted at a tertiary paediatric centre in Kerala, India, between June 2021 and December 2022. Children up to 12 years of age, including preterm neonates, who fulfilled the revised European Organization for Research and Treatment of Cancer / Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) definitions of IFD were enrolled. As fungal biomarkers (galactomannan, (1,3)-β-D-glucan) and protocolised computed tomography were not available, cases could be classified only as proven (fungal elements demonstrated by culture or histopathology) or possible (host factor with compatible clinical features but no mycological confirmation); no case satisfied the criteria for probable IFD. Clinical characteristics, risk factors, fungal isolates and antifungal treatment response were recorded. Associations between risk factors and outcome (cured vs expired) were examined using Pearson's chi-square test, with Fisher's exact test applied to 2 × 2 tables containing sparse expected counts.

Results: Sixty-six children were included, comprising 34 (51.5%) preterm neonates and 32 (48.5%) children with haematological malignancy; 38 (57.6%) were female. Culture positivity was 74.2% (49 proven IFD); the remaining 17 (25.8%) were classified as possible IFD and occurred exclusively within the malignancy subgroup. Non-albicans Candida species were the predominant isolates (32, 48.5%), followed by Candida albicans (12, 18.2%). Overall mortality was 24.2% (16/66) and was higher among preterm neonates (11/34, 32.4%) than among children with malignancy (5/32, 15.6%). Mortality was significantly associated with neutropenia ($p = 0.003$), ICU admission ($p = 0.006$), central venous catheter (CVC) use ($p = 0.007$) and total parenteral nutrition (TPN) ($p = 0.031$). Neither the diagnostic category of IFD ($p = 0.533$) nor the organism isolated ($p = 0.799$) was associated with mortality.

Conclusion: In this mixed paediatric cohort, outcome was determined principally by host vulnerability rather than by fungal species. Because risk factor exposures and pathogen distributions differed substantially between the neonatal and oncology subgroups, cohort-level associations are best regarded as hypothesis-generating. Early risk stratification, expanded diagnostic capability

including fungal biomarkers, and species-directed antifungal therapy remain the practical priorities in resource-limited settings.
Keywords: Invasive fungal disease; Candidaemia; Non-albicans Candida; Preterm neonate; Paediatric oncology; Neutropenia; Antifungal agents.

INTRODUCTION

Invasive fungal disease (IFD) comprises life-threatening opportunistic infections that occur predominantly in immunocompromised children, including those with haematological malignancy, primary or acquired immunodeficiency, prematurity-associated immune immaturity, or prolonged exposure to immunosuppressive therapy. These conditions impair innate and adaptive host defences and render children susceptible to systemic invasion by Candida, Aspergillus and emerging non-albicans species.[1,2] Preterm neonates requiring intensive care, central venous access or total parenteral nutrition constitute a further high-risk group, owing to immature immune responses, thin skin and mucosal barriers, and frequent exposure to broad-spectrum antibiotics.

IFD carries substantial morbidity and mortality in paediatric populations, and early identification of high-risk patients improves outcome, particularly when targeted antifungal prophylaxis is employed.[3] Paediatric IFD differs from adult disease in epidemiology, underlying comorbidity and pharmacokinetic behaviour, and diagnostic practice remains heterogeneous across institutions.[4]

Timely diagnosis in children is complicated by non-specific clinical features, difficulty obtaining adequate sample volumes, limited feasibility of invasive diagnostic procedures, and the variable performance of fungal biomarkers in the paediatric age group.[5] These constraints frequently result in prolonged empirical broad-spectrum antifungal therapy, which in turn contributes to the emergence of resistant strains. [6]

Paediatric IFD data from India are scarce, and are particularly limited for mixed cohorts that include both neonates and older immunocompromised children treated within a single institution. Such combined cohorts reflect the real-world case mix of many Indian tertiary paediatric services, although they also introduce clinical heterogeneity that must be acknowledged in interpretation. This study was therefore undertaken to describe the risk factors, clinical profile, microbiological spectrum and outcomes of IFD among immunocompromised children at a tertiary referral centre, with the aim of informing earlier recognition and more rational antifungal use.

MATERIALS AND METHODS

Study design, setting and participants: This longitudinal observational study was conducted at the Institute of Maternal and Child Health, Government Medical College, Kozhikode, from June 2021 to December 2022. The study is reported in accordance with the STROBE statement for observational research. All children up to 12 years of age, including preterm neonates, who were diagnosed with IFD and met the eligibility criteria during the study period were enrolled consecutively.

Inclusion criteria were immunocompromised children with primary immunodeficiency, haematological malignancy receiving chemotherapy, nephrotic syndrome on immunosuppressive therapy, HIV infection with severe immunosuppression, and preterm neonates. Children whose immunocompromise arose from surgical causes, and those unwilling to participate, were excluded.

The sample size was calculated using the formula $N = 4pq/d^2$, assuming an expected cure rate of 80% derived from a Japanese paediatric cohort and 10% absolute precision, yielding a required sample of 66 children. It should be noted that this formula was designed to estimate a single proportion with specified precision; it was not powered for the comparison of subgroups or for multivariable modelling (see Limitations).

Data collection and microbiological methods: Following recruitment, demographic details, clinical history and examination findings were recorded on a semi-structured proforma. Specimens — blood (3 mL), urine (3 mL), cerebrospinal fluid (1 mL) and sputum (1 mL) — were collected under aseptic precautions, with volumes finalised in consultation with the microbiology department to accommodate paediatric and neonatal sampling constraints. Samples were transported immediately to the laboratory, where direct microscopy and fungal culture were performed usingSabouraud dextrose agar, corn meal agar and brain heart infusion agar. Imaging was obtained as clinically indicated rather than by protocol. All participants were followed until completion of antifungal therapy, and outcome (cured or expired) was documented at that point.

Diagnostic classification: Diagnosis and classification of IFD were based on the revised EORTC/MSGERC consensus definitions, which categorise cases as proven (fungal elements demonstrated by culture or histopathology), probable (host factor, compatible clinical or radiological features and mycological evidence such as a positive fungal biomarker) and possible (host factor and compatible clinical features without mycological confirmation).[5,7]

Fungal biomarker assays — serum galactomannan and (1,3)-β-D-glucan — and protocolised high-resolution computed tomography were not available for this cohort. Consequently, the mycological criterion required for the probable category could not be satisfied in any patient. Culture-negative children who had a recognised host factor together with

compatible clinical features were therefore classified as possible IFD, and the cohort comprises only proven and possible cases. This represents a deliberate correction of a common misclassification and is discussed further under Limitations.

It should also be acknowledged that the EORTC/MSGERC criteria were developed and validated principally for patients with haematological malignancy and haematopoietic stem cell transplant recipients. Their extension to preterm neonates — in whom host factors, radiological correlates and biomarker performance all differ substantially — is not formally validated and constitutes a methodological limitation of this and comparable neonatal studies.

Statistical analysis: Data were entered in Microsoft Excel 2010 and analysed in jamovi version 2.6.44. All study variables were categorical and are summarised as frequencies and percentages. Associations between risk factors — neutropenia severity, ICU admission, central venous catheter insertion, total parenteral nutrition, chemotherapy phase and underlying immunocompromised state — and treatment outcome (cured vs expired) were assessed using Pearson's chi-square test. Fisher's exact test was substituted for 2 × 2 tables in which expected counts were below five in more than 20% of cells. Yates' continuity correction was not applied, as it is defined only for 2 × 2 contingency tables and is not appropriate for the multi-category variables analysed here; for such variables the uncorrected Pearson statistic is reported, with the caveat that the asymptotic approximation is unreliable where cells are sparse. Effect sizes are reported as the phi coefficient for 2 × 2 tables and Cramér's V for larger tables. A heatmap was used to display the observed clinical response of each organism category to the antifungal agents used under institutional policy; this represents in vivo clinical response and not in vitro susceptibility testing, as antifungal susceptibility testing was not performed. A two-sided p-value < 0.05 was considered statistically significant.

Ethical considerations: The study protocol was approved by the Institutional Ethics Committee, and written informed consent was obtained from the parent or legal guardian of each participant prior to enrolment.

RESULTS

Cohort composition and clinical presentation: Sixty-six immunocompromised children with IFD were enrolled. The cohort comprised two clinically distinct populations: 34 preterm neonates (51.5%) and 32 children older than one year with haematological malignancy (48.5%), predominantly B-cell acute lymphoblastic leukaemia (B-ALL; 29, 43.9%) and T-cell ALL (3, 4.5%). Because these two groups differ fundamentally in host physiology, exposures and pathogen risk, results are presented at cohort level but interpreted throughout with reference to subgroup composition.

Overall, 38 children (57.6%) were female. Fever was absent in 33 (50%); where present, it exceeded four days in 32 (48.4%). Respiratory distress occurred in 29 (43.9%), while subcutaneous nodules, apnoea and altered sensorium each affected 12 children (18.2%). Oral thrush was noted in 16 (24.2%), and vomiting and abdominal distension each in 14 (21.2%) [Table 1]. The apparent absence of fever in half the cohort is attributable to the neonatal subgroup, in which sepsis frequently presents with temperature instability or hypothermia rather than pyrexia.

Table 1: Demographic profile and clinical manifestations in children diagnosed with invasive fungal disease (N = 66).

Variable	Category	Count (%)
Patient characteristics		
Age group	Preterm	34 (51.5)
	1–5 years	18 (27.3)
	>5 years	14 (21.2)
Sex	Male	28 (42.4)
	Female	38 (57.6)
Clinical manifestations		
Fever duration	No fever	33 (50.0)
	<4 days	1 (1.5)
	4 days – 1 week	16 (24.2)
	>1 week	16 (24.2)
Abdominal pain	Yes	8 (12.1)
	No	58 (87.9)
Subcutaneous nodule	Yes	12 (18.2)
	No	54 (81.8)
Cough	Yes	9 (13.6)
	No	57 (86.4)
Respiratory distress	Yes	29 (43.9)
	No	37 (56.1)
Apnoea	Yes	12 (18.2)
	No	54 (81.8)
Vomiting	Yes	14 (21.2)
	No	52 (78.8)
Abdominal distension	Yes	14 (21.2)
	No	52 (78.8)
Altered sensorium	Yes	12 (18.2)

	No	54 (81.8)
Other associated symptoms	Oral thrush	16 (24.2)
	Hyperglycaemia	5 (7.6)
	Local pain	2 (3.0)
	Haemoptysis	1 (1.5)
	Disseminated sepsis	1 (1.5)
	Headache	1 (1.5)

Risk factor exposures and their association with outcome: Fifty children (75.8%) survived and 16 (24.2%) died. Most children carried several concurrent risk factors. Prolonged antibiotic exposure was the commonest (54, 81.8%), followed by central venous catheter insertion (49, 74.2%), ICU admission (43, 65.2%), neutropenia of any severity (39, 59.1%) and total parenteral nutrition (19, 28.8%). Within the preterm subgroup, 16 (24.2% of the cohort) were extremely preterm, 13 (19.7%) very preterm and 5 (7.6%) moderately preterm. These exposures were not evenly distributed across the cohort: TPN, CVC dependence and prolonged antibiotic use were concentrated among the preterm neonates, whereas neutropenia and chemotherapy-related immunosuppression were essentially confined to the oncology subgroup. Cohort-level frequencies therefore describe the pooled population rather than any single clinical group.

On bivariate testing [Table 2], mortality was significantly associated with neutropenia ($\chi^2 = 13.6$, df = 3, p = 0.003; Cramér's V ≈ 0.45), ICU admission ($\chi^2 = 7.61$, df = 1, p = 0.006; φ ≈ 0.34), central venous catheter insertion (p = 0.007, Fisher's exact; φ ≈ 0.31) and total parenteral nutrition ($\chi^2 = 4.64$, df = 1, p = 0.031; φ ≈ 0.27). Fifteen of 43 children admitted to intensive care died (34.9%), compared with 1 of 23 (4.3%) never requiring intensive care. Sixteen of 49 children with a CVC died (32.7%), whereas all 17 children managed without a CVC survived. Mortality among children receiving TPN was 42.1% (8/19) versus 17.0% (8/47) in those who did not. In contrast, prolonged antibiotic exposure (p = 0.715), chemotherapy phase (p = 0.319) and the broader category of underlying immunocompromise (p = 0.055) were not significantly associated with mortality.

The gradient across neutropenia categories was non-monotonic and requires cautious reading. Mortality was 22.2% with no neutropenia, 83.3% with mild neutropenia, 7.7% with moderate neutropenia and 20.0% with severe neutropenia. The strikingly high figure in the mild neutropenia stratum is a small-number statistical artefact rather than a biological gradient: the stratum contained only six children (five deaths), all of whom were preterm neonates carrying multiple concurrent risk factors including intensive care admission, CVC dependence, TPN and prolonged antibiotic exposure. The estimate is correspondingly unstable, and the overall significance of the neutropenia variable should not be read as evidence that milder neutropenia confers greater risk than severe neutropenia.

Table 2: Association between risk factors and outcome in children diagnosed with invasive fungal disease (N = 66).

Risk factor	Category	Curedn (%)	Expiredn (%)	Statistic (df),p-value
Prolonged antibiotics	Yes	40 (74.1)	14 (25.9)	0.715*
	No	10 (83.3)	2 (16.7)	
Neutropenia	None	21 (77.8)	6 (22.2)	13.6 (3), 0.003
	Mild	1 (16.7)	5 (83.3)	
	Moderate	12 (92.3)	1 (7.7)	
	Severe	16 (80.0)	4 (20.0)	
ICU admission	Yes	28 (65.1)	15 (34.9)	7.61 (1), 0.006
	No	22 (95.7)	1 (4.3)	
CVC insertion	Yes	33 (67.3)	16 (32.7)	0.007*
	No	17 (100.0)	0 (0.0)	
Total parenteral nutrition	Yes	11 (57.9)	8 (42.1)	4.64 (1), 0.031
	No	39 (83.0)	8 (17.0)	
Cancer chemotherapy	No chemotherapy	23 (67.6)	11 (32.4)	3.52 (3), 0.319
	Induction	14 (77.8)	4 (22.2)	
	Re-induction	12 (92.3)	1 (7.7)	
	Maintenance	1 (100.0)	0 (0.0)	
Underlying immuno-compromised state	Extreme preterm	8 (50.0)	8 (50.0)	9.25 (4), 0.055
	Very preterm	10 (76.9)	3 (23.1)	
	Moderate preterm	5 (100.0)	0 (0.0)	
	B-ALL	25 (86.2)	4 (13.8)	
	T-ALL	2 (66.7)	1 (33.3)	

*Fisher's exact test (2 × 2 tables with sparse expected counts); all other tests are uncorrected Pearson chi-square. Yates' continuity correction is defined only for 2 × 2 tables and was therefore not applied to any multi-category variable. Where expected cell counts are small (notably the mild neutropenia and maintenance chemotherapy strata), the chi-square approximation is unstable and the corresponding estimates should be interpreted with caution. CVC, central venous catheter; ALL, acute lymphoblastic leukaemia.

Microbiological profile: Fungal culture was positive in 49 children (74.2%), who were classified as proven IFD. Non-albicans Candida (NAC) species were the commonest isolates (32, 48.5%), followed by Candida albicans (12, 18.2%). Within the NAC group, 16 isolates (50%) were reported only to genus level as non-albicans Candida species, while identified species comprised Candida tropicalis (10, 31.3%), Candida krusei (3, 9.4%), Candida parapsilosis (2, 6.3%) and Candida auris (1, 3.1%). Other isolates were Fusarium (2, 3.0%), Cryptococcus (2, 3.0%) and Absidia (1, 1.5%) [Figure 1]. The remaining 17 children (25.8%) were culture-negative and were classified as possible IFD. The distribution of isolates differed significantly by underlying immunocompromised state ($\chi^2 = 31.4$, df = 5, $p < 0.001$) (Table 3). All 17 culture-negative (possible IFD) cases occurred in children with malignancy, whereas C. albicans and NAC species were recovered predominantly from preterm neonates (83.3% and 71.9% of isolates respectively). Cryptococcus and Absidia were identified only in the malignancy subgroup, and the two Fusarium isolates were divided equally between groups. This pattern is expected and largely reflects the underlying case mix: catheter- and gut-associated Candida disease dominates in the neonatal intensive care setting, whereas mould and cryptococcal disease, together with culture-negative presentations following empirical antifungal exposure, cluster in profoundly neutropenic oncology patients. The association should therefore be read as a description of two co-resident but epidemiologically separate populations rather than as a within-population risk relationship. Given the number of zero and single-count cells, the chi-square approximation for this table is unstable and the p-value should be treated as indicative only.

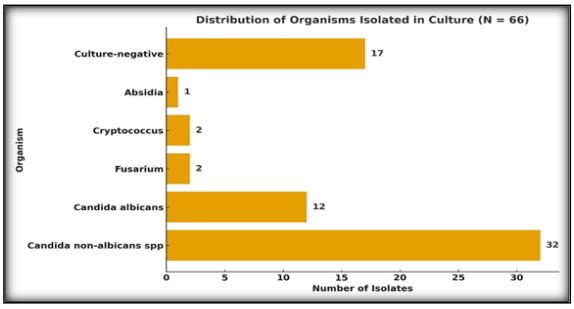

Figure 1: Organisms isolated in culture (N = 66). Reproduce at full page width.

Table 3: Association between culture result and underlying immunocompromised state in children diagnosed with invasive fungal disease (N = 66).

Culture result	Pretermn (%)	Malignancyn (%)	Statistic (df),p-value
Culture negative (possible IFD)	0 (0.0)	17 (100.0)	31.4 (5), <0.001
Candida albicans	10 (83.3)	2 (16.7)	
Candida non-albicans spp.	23 (71.9)	9 (28.1)	
Fusarium	1 (50.0)	1 (50.0)	
Cryptococcus	0 (0.0)	2 (100.0)	
Absidia	0 (0.0)	1 (100.0)	

Uncorrected Pearson chi-square test. Yates' continuity correction was not applied, as it is defined only for 2 × 2 tables. Several cells contain zero or single observations; the asymptotic approximation is therefore unreliable and the p-value is presented as indicative. Percentages are row percentages. IFD, invasive fungal disease.

Diagnostic category, organism and outcome: Neither the diagnostic category of IFD (proven vs possible; p = 0.533) nor the specific organism isolated (p = 0.799) was significantly associated with mortality [Table 4]. Survival was comparable across C. albicans (75.0%), NAC species (71.9%) and possible (culture-negative) IFD (82.4%). All children with Cryptococcus (n = 2) or Absidia (n = 1) survived, and one of the two children with Fusarium died. Organism-specific survival estimates based on one or two patients carry no meaningful precision and are reported for completeness only.

Table 4: Association between diagnostic category of invasive fungal disease, culture result and clinical outcome (N = 66).

Variable	Category	Curedn (%)	Expiredn (%)	Statistic (df),p-value
Type of IFD	Proven	36 (73.5)	13 (26.5)	0.533*
	Possible	14 (82.4)	3 (17.6)	
Culture result	Culture negative (possible IFD)	14 (82.4)	3 (17.6)	2.35 (5), 0.799
	Candida albicans	9 (75.0)	3 (25.0)	
	Candida non-albicans spp.	23 (71.9)	9 (28.1)	
	Fusarium	1 (50.0)	1 (50.0)	
	Absidia	1 (100.0)	0 (0.0)	
	Cryptococcus	2 (100.0)	0 (0.0)	

*Fisher's exact test; the culture result comparison used the uncorrected Pearson chi-square test. Yates' continuity correction was not applied, as it is defined only for 2 × 2 tables. Cells containing one or two observations preclude reliable inference for the individual mould categories. IFD, invasive fungal disease.

All 34 preterm infants presented with neonatal sepsis, accounting for 51.5% of the cohort. The remaining

diagnoses, occurring in children older than one year, are listed in the Supplementary Table.

Clinical response to antifungal agents: Amphotericin B was the most frequently prescribed antifungal agent, followed by fluconazole, voriconazole, caspofungin and flucytosine. [Figure 2] displays the proportion of children within each organism category who showed a favourable clinical response to each agent received. It is important to emphasise that this figure represents in vivo clinical response and not in vitro antifungal susceptibility: minimum inhibitory concentrations were not determined, and no isolate underwent formal susceptibility testing.

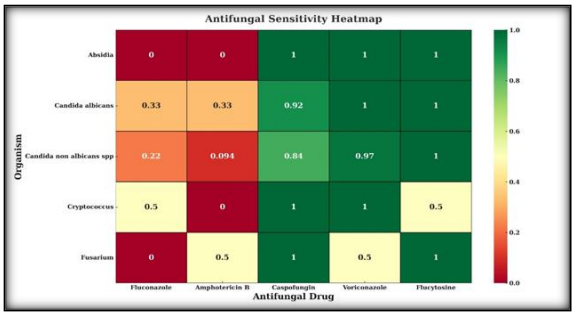

Figure 2: Observed clinical response of isolated organisms to the antifungal agents received (N = 49 culture-positive children). Values are the percentage of children in each organism category who showed a favourable clinical response to the agent concerned; they do not represent in vitro susceptibility. Reproduce at full page width.

Among children with Candida infection, caspofungin, voriconazole and flucytosine were associated with high rates of favourable clinical response, whereas fluconazole and amphotericin B were associated with lower response rates, particularly against NAC species (22.0% and 9.4% respectively) and Cryptococcus (0% for amphotericin B).

Several cells in [Figure 2] must not be read as evidence of monotherapy efficacy, and three cautions apply. First, the mould categories are extremely small — Absidia (n = 1), Fusarium (n = 2) and Cryptococcus (n = 2) — so each cell represents one or two patients and a single event moves the value between 0% and 100%. Second, and more importantly, the apparent 100% favourable response of Absidia and Fusarium to caspofungin and flucytosine is not biologically interpretable as drug activity. Absidia (Mucorales) is intrinsically resistant to echinocandins, flucytosine and voriconazole, and Fusarium species are intrinsically resistant to echinocandins and flucytosine and are frequently resistant to amphotericin B. These children were receiving combination empirical antifungal regimens, and the recorded response reflects the outcome of the regimen as a whole — in practice attributable to the active partner agent, source control and catheter removal — rather than the activity of the agent in that cell. Third, the low apparent response of amphotericin B against Cryptococcus (0%, n = 2) is similarly uninformative, since amphotericin B combined with flucytosine remains the established induction regimen for cryptococcal disease and both children in this category survived. [Figure 2] should therefore be read as a descriptive audit of prescribing and observed outcome, not as a susceptibility surrogate, and it should not be used to guide agent selection against moulds.

DISCUSSION

Invasive fungal disease remains a substantial cause of morbidity and mortality among immunocompromised children worldwide, with reported case fatality ranging from 6% to 80% despite advances in diagnosis and therapy.[1,8,9] In this study of 66 immunocompromised children, risk factor distribution, aetiology, clinical features, treatment response and outcome were assessed at the completion of antifungal therapy. Overall mortality was 24.2%, and the principal determinants of outcome were host-related — neutropenia, intensive care admission, central venous catheter dependence and total parenteral nutrition — rather than the infecting organism.

Population heterogeneity and its effect on interpretation: The most important interpretive caveat concerns the composition of the cohort. Preterm neonates (51.5%) and children with haematological malignancy (48.5%) were pooled into a single analytic population, yet these groups share almost none of the determinants that drive IFD risk. Neonatal IFD is predominantly catheter- and gut-associated candidaemia arising from immature barrier function, prolonged parenteral nutrition and broad-spectrum antibiotic exposure; oncological IFD arises principally from chemotherapy-induced neutropenia and mucosal injury, and carries a substantially higher probability of mould disease and of culture-negative, biomarker-dependent presentations.

This structural heterogeneity generates confounding by indication throughout the analysis. TPN and CVC exposure were overwhelmingly neonatal, and prematurity independently predicts death; the observed associations of TPN and CVC with mortality are therefore partly a restatement of the mortality difference between the neonatal and oncology subgroups (32.4% vs 15.6%) rather than independent catheter- or nutrition-attributable risk. Conversely, neutropenia and chemotherapy phase were almost entirely confined to the oncology subgroup. The same applies to pathogen distribution: the highly significant association between organism and underlying state ($p < 0.001$) largely reflects the fact that Candida disease was neonatal and mould and cryptococcal disease were oncological, which is a description of the two constituent populations rather than a within-population finding. The cohort-level estimates presented here should accordingly be

regarded as descriptive and hypothesis-generating. Stratified or adjusted analysis would be required to separate exposure effects from subgroup membership, and the available sample size did not permit this.

Risk factors and outcome: A broad range of well-established risk factors was identified. Prolonged antibiotic exposure was the commonest, concordant with Roumani et al,[10] and reflects the recognised relationship between repeated broad-spectrum antimicrobial exposure and fungal overgrowth. Central venous catheters were present in 74.2% of cases, closely paralleling NICU-based findings from Baptista et al,[11] and remain a key modifiable exposure. Neutropenia, TPN use and chemotherapy-induced immunosuppression were concentrated in specific subgroups, which explains divergence from haematology-only cohorts such as that of Nathani et al.[12]

Four exposures showed significant bivariate associations with mortality. ICU admission was strongly associated with death ($p = 0.006$), with mortality of 34.9% among children requiring intensive care compared with 4.3% among those who did not, although this almost certainly indexes underlying illness severity rather than a hazard of intensive care itself. CVC insertion was significantly associated with mortality ($p = 0.007$), and the observation that all 17 children managed without a catheter survived reinforces the longstanding recommendation for timely catheter removal in suspected or confirmed IFD.[13] TPN use was similarly associated with mortality ($p = 0.031$), reflecting the clinical fragility of children dependent on prolonged parenteral nutritional support. Neutropenia severity was significantly associated with survival ($p = 0.003$), but the direction of this association is not monotonic and should not be over-interpreted: the 83.3% mortality observed in the mild neutropenia stratum is a small-sample artefact generated by six patients, all preterm neonates carrying multiple concurrent risk exposures, and does not indicate that mild neutropenia is more dangerous than severe neutropenia. By contrast, prolonged antibiotic therapy, chemotherapy phase and the broader category of immunocompromise were not significantly associated with outcome, suggesting that although these variables predispose to IFD, they do not independently determine survival once infection is established.

Aetiological spectrum: Culture positivity was high (74.2%), with NAC species emerging as the predominant pathogens (48.5%), followed by C. albicans (18.2%). This contrasts with the earlier findings of Nathani et al,[12] and Baptista et al,[11] in which C. albicans predominated, but aligns with accumulating evidence of a global shift towards NAC organisms in paediatric and neonatal candidaemia.[14–16] The recovery of a single Candida auris isolate is of particular concern given its capacity for nosocomial transmission and multidrug resistance, and supports the case for routine species-level identification and infection control surveillance in Indian neonatal units.[17]

Species-level mortality patterns — C. auris 100%, C. krusei 33% and C. tropicalis 30%, with no deaths among C. parapsilosis cases — are directionally consistent with reported virulence and resistance trends,[13] but rest on denominators of one to ten patients and should be regarded as descriptive rather than comparative.

Clinical presentation: Fever was the most frequent presenting feature, though its prevalence (50%) was lower than in malignancy-focused studies such as that of Nathani et al,[12] most plausibly because preterm neonates frequently manifest sepsis with temperature instability or hypothermia rather than pyrexia. Respiratory distress (43.9%) was the second most frequent finding, but in the neonatal subgroup this is likely to reflect underlying pulmonary disease of prematurity rather than fungal invasion. Gastrointestinal symptoms, subcutaneous nodules, apnoea and altered sensorium illustrate the heterogeneous presentation of IFD in children, consistent with prior descriptions of neonatal and paediatric fungal sepsis.17 Blood cultures yielded the highest positivity (89.7% of positive cultures), a figure comparable with NICU-based reports,[11] whereas culture-negative disease appeared exclusively in malignancy patients and may reflect deep-seated infection, prior antifungal exposure or restricted diagnostic sampling.

Antifungal therapy and treatment response: Amphotericin B was the most frequently used agent, followed by fluconazole, voriconazole, caspofungin and flucytosine, differing from the fluconazole-dominant regimens described in earlier reports.[11,18] Among Candida infections, caspofungin, voriconazole and flucytosine were associated with higher rates of favourable clinical response than fluconazole, which is clinically relevant in settings where empirical fluconazole remains common practice and supports early transition to a fungicidal or broader-spectrum agent when NAC infection is suspected.

These response data require careful qualification and should not be interpreted as comparative efficacy. No antifungal susceptibility testing was performed, so [Figure 2] describes observed clinical outcome among children who received each agent, not in vitro activity. Children were treated with combination and sequential empirical regimens alongside source control measures such as catheter removal, and outcome cannot be attributed to any single component. This is most evident in the mould categories, where the recorded 100% favourable response of Absidia and Fusarium to caspofungin and flucytosine is biologically implausible as a statement of drug activity: Mucorales are intrinsically resistant to echinocandins, flucytosine and voriconazole, and Fusarium species are intrinsically resistant to echinocandins and flucytosine. These values reflect the survival of one or two patients who were concurrently receiving an active agent — in practice

a lipid formulation of amphotericin B, with or without surgical or catheter source control — and must not be read as support for echinocandin or flucytosine monotherapy against these organisms. Any therapeutic inference from this figure must be made against known intrinsic resistance profiles, and mould-directed therapy should continue to follow established guidance rather than the response percentages shown here.

Mortality in context: Overall mortality of 24.2%, with higher mortality among preterm neonates (32.4%) than children with malignancy (15.6%), is comparable with global reports19–21 and higher than documented by Gülhan et al.[18] This disparity plausibly reflects differences in baseline illness severity, the neonatal predominance of the present cohort and resource constraints on diagnostics and antifungal availability. Mortality did not differ significantly by diagnostic category (proven vs possible) or organism, again supporting the primacy of host factors over pathogen determinants in this population — although the absence of a difference may equally reflect limited statistical power.

Strengths and limitations: The strengths of this study include prospective enrolment with complete follow-up to the end of antifungal therapy, high culture positivity, species-level identification of Candida isolates, and an integrated organism–treatment response analysis in a population for which Indian data are scarce.

Several limitations must be acknowledged. First, the cohort pooled two clinically distinct populations — preterm neonates and paediatric oncology patients — which introduces substantial confounding; cohort-level associations should not be transferred to either subgroup individually.

Second, the sample size was derived from the formula $N = 4pq/d^2$, which estimates a single proportion (cure rate) with specified precision. It was not calculated to detect differences between subgroups, and with only 16 outcome events the study is markedly underpowered for multivariable logistic regression. Adjusted analysis was therefore not attempted, and all reported associations are unadjusted and susceptible to residual confounding. Interpretation as association rather than causation is essential.

Third, fungal biomarkers (galactomannan, (1,3)-β-D-glucan) and protocolised computed tomography were unavailable, so no case could satisfy the mycological criterion for probable IFD. Culture-negative cases have been classified as possible IFD accordingly. This is diagnostically conservative but means the possible IFD group is heterogeneous and may include children with non-fungal illness, which would bias outcome comparisons between diagnostic categories towards the null. It should also be noted that, in the 2020 revision of the EORTC/MSGERC definitions, the possible category is formally reserved for invasive mould disease; its application to culture-negative candidal presentations, as here, is a pragmatic extension rather than a strict application of the criteria.

Fourth, the EORTC/MSGERC framework was developed and validated for patients with haematological malignancy and stem cell transplant recipients. Its application to preterm neonates — for whom host factor definitions, radiological criteria and biomarker thresholds are not established — is a recognised methodological weakness affecting roughly half of this cohort. Related to this, proven IFD in the present study rests principally on culture positivity, and a minority of isolates were recovered from non-sterile sites (urine, sputum), where growth may represent colonisation rather than invasive disease.

Fifth, antifungal susceptibility testing was not performed, so treatment response could be assessed only clinically and in the context of combination empirical regimens; the response data in Figure 2 cannot substitute for susceptibility data and are subject to the intrinsic resistance caveats detailed above.

Finally, the study was conducted at a single tertiary centre, lacked a comparison group of at-risk children without IFD, and species-level subgroups were too small to permit meaningful comparison. Generalisability beyond comparable tertiary settings in southern India is therefore limited.

Implications and future directions: These findings support three practical priorities. Access to fungal biomarkers and molecular diagnostics should be expanded so that probable IFD can be identified and empirical therapy rationalised; without them, a quarter of paediatric IFD in this setting remains diagnostically unresolved. Species-level identification with routine antifungal susceptibility testing should be standard, given NAC predominance and the appearance of C. auris. Catheter stewardship — early removal in suspected IFD and minimisation of dwell time — warrants prospective evaluation as a modifiable determinant of outcome. Future work should be prospectively stratified by underlying condition, with neonatal and oncology cohorts analysed separately and with sufficient events to permit multivariable adjustment; multicentre collaboration will be necessary to achieve this in the Indian context.

CONCLUSION

Invasive fungal disease remains a major clinical challenge in immunocompromised children, particularly where diagnostic resources are constrained. This study demonstrates the predominance of non-albicans Candida species and identifies neutropenia, intensive care admission, central venous catheter dependence and total parenteral nutrition as exposures associated with poor outcome. Mortality appeared to be driven more by host vulnerability than by organism type. These associations were derived from a heterogeneous

cohort of preterm neonates and paediatric oncology patients without adjustment for confounding, and are therefore hypothesis-generating rather than definitive. Strengthening paediatric fungal surveillance, expanding biomarker-based diagnostics and generating locally relevant, condition-stratified evidence remain essential to improving diagnostic precision and survival.

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