

Incidence, outcomes, and mortality of fungal infections in pediatric patients undergoing anticancer therapy or transplantation: a nationwide cohort study

Monika Richert-Przygonska

monika_richert@yahoo.com

Collegium Medicum, Nicolaus Copernicus University Torun

Anna Fraczekiewicz-Jablonska

Collegium Medicum, Nicolaus Copernicus University Torun

Krzysztof Czyzewski

Collegium Medicum, Nicolaus Copernicus University Torun

Patrycja Zalas-Wiecek

Collegium Medicum, Nicolaus Copernicus University

Olga Gryniewicz-Kwiatkowska

Children's Memorial Health Institute

Agnieszka Gietka

Children's Memorial Health Institute

Iwona Zak

University Children's Hospital

Wojciech Czogala

Jagiellonian University

Malgorzata Salamonowicz-Bodzioch

Medical University

Jowita Fraczekiewicz

Medical University

Olga Zajac-Spychala

University of Medical Sciences

Ninela Irga-Jaworska

Medical University

Marcin Plonowski

Medical University

Pawel Wawrykow

Pomeranian Medical University

Magdalena Bartnik

Pomeranian Medical University

Filip Pierlejewski

Medical University

Wanda Badowska

Children Hospital

Weronika Stolpa

Silesian Medical University

Radoslaw Chaber

University of Rzeszow, Clinical Provincial Hospital

Anna Szmydki-Baran

Medical University of Warsaw

Lukasz Hutnik

Medical University of Warsaw

Renata Tomaszewska

Silesian Medical University

Agnieszka Urbanek-Dadela

Children Hospital

Agnieszka Zaucha-Prazmo

Medical University

Joanna Zawitkowska

Medical University

Jolanta Gozdzik

University Children's Hospital, Jagiellonian University Collegium Medicum

Jan Styczynski

Collegium Medicum, Nicolaus Copernicus University Torun

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Abstract

Background

Invasive fungal disease (IFD) is a major cause of mortality and morbidity in immunocompromised children, particularly those with hematological malignancies or undergoing hematopoietic cell transplantation (HCT).

Aim

To analyze the incidence of fungal infections and identify the risk factors for infection-related mortality.

Methods

Clinical and microbiological data were retrospectively evaluated for children treated across 17 pediatric hematology and oncology centers (PHO) and 6 pediatric HCT centers in Poland.

Results

Over the 6-year study period, 846 fungal infections were recorded in 5,564 PHO patients and 158 in 1,064 HCT recipients, with a similar (13.2% vs. 14.5%, $p = 0.179$). Acute leukemia was the strongest independent predictor of fungal infection (OR 3.14, 95% CI: 2.75–3.71; $p < 0.001$), while age > 8 years, disease relapse and allogeneic transplantation were additional risk factors. Infection-related mortality was significantly higher in HCT recipients than in PHO patients (21.3% vs. 6.7%; OR 3.96, 95% CI 2.36–6.65; $p < 0.001$). Most deaths occurred within 30 days of diagnosis, predominantly due to *Aspergillus* spp. and *Candida* spp., despite antifungal prophylaxis.

Conclusions

Although IFD incidence was comparable, HCT recipients had nearly fourfold higher infection-related mortality. This highlights the need of improved supportive care and antifungal prophylaxis.

Introduction

Invasive fungal infections are regarded as one of the most serious complications in patients with primary or secondary immunodeficiencies. In children undergoing anticancer therapy, hematopoietic cell transplantation (HCT) or solid organ transplantation (SOT) invasive fungal disease (IFD) is a major contributor to mortality and morbidity^{1–3}. The highest incidence rates of IFD are consistently observed among children with diagnosis of acute myeloblastic leukemia (AML), high-risk acute lymphoblastic leukemia (ALL), children after HCT or admitted to intensive care units (ICU). Previous pediatric studies

identified number of additional factors commonly associated with an increased risk of IFD, including prolonged and profound neutropenia, the use of steroids, mucosal tissue damage, broad-spectrum antibiotic therapy, central venous access or graft versus host disease^{1,4–7}.

Despite the implementation of risk-adapted antifungal prophylaxis, advances in diagnostic modalities, the introduction of novel antifungal agents, and improvements in clinical management, case-fatality rates associated with IFD have remained largely unchanged². These observations highlight the persistent need to identify individual and population-specific risk factors and to elucidate the biological mechanisms underlying susceptibility to IFD, thereby facilitating more precise risk stratification and targeted preventive strategies.

The objective of the study was to analyze incidence, clinical outcomes, and mortality of fungal infections in pediatric patients undergoing anticancer therapy or hematopoietic cell transplantation.

Patients and Methods

Design of the study

We conducted a nationwide multicenter analysis to investigate the epidemiology, clinical manifestation and outcomes of fungal infections in pediatric patients with malignancy or following hematopoietic cell transplantation.

Patients and data collection

On behalf of the Polish Society of Pediatric Oncology and Hematology, a retrospective evaluation of clinical and microbiological data was performed for children undergoing anticancer therapy or hematopoietic cell transplantation between 2018 and 2023. Data were gathered every two years from 17 pediatric hematology-oncology (PHO) units and 6 pediatric HCT centers throughout Poland.

The study was approved by the Ethics Committee of Collegium Medicum in Bydgoszcz (agreement No. 263/2022). The requirement for informed consent was waived by the Ethics Committee. All patient data were analyzed anonymously, and no individual-level data were published. All methods were carried out in accordance with relevant guidelines and regulations.

Definitions

Level of diagnosis of invasive fungal disease (IFD) was adapted from current expert recommendations^{4,8–10}.

Proven fungal infection was defined as positive histopathologic, cytopathologic or direct microscopic examination of sterile material, positive culture of sterile material (excluding bronchoalveolar lavage fluid, a cranial sinus cavity specimen, and urine).

Probable IFD defined by the presence of a host factors, clinical criteria and a mycological criteria.

Cases that meet the criteria for a host factor and a clinical criterion but for which mycological criteria are absent were considered possible IFD^{8,11}.

Antifungal prophylaxis

In accordance with recommendation of the European Conference on Infections in Leukaemia (ECIL-8) antifungal prophylaxis was applied in all centers⁴. For the high-risk patients (children on chemotherapy for AML, high risk or relapsed ALL, myelodysplastic syndromes (MDS), graft versus host disease (GVHD) after HCT and secondary prophylaxis after HCT) posaconazole or voriconazole was used as the part of national prophylactic program introduced since 2012¹².

Statistical methods

Categorical variables were presented as numbers and percentages, and continuous variables as medians with ranges or interquartile ranges, as appropriate. Group comparisons were performed using the chi-squared test or Fisher's exact test for categorical variables and the Mann–Whitney U test or Kruskal–Wallis test for continuous variables.

Time-to-event analyses were performed using the Kaplan–Meier method at the patient level. The cumulative probability of fungal infection was estimated as 1 minus infection-free survival, with follow-up starting from malignancy diagnosis in PHO patients and from transplantation in HCT recipients. The endpoint was the first fungal infection; patients without infection were censored at last follow-up or at 24 months. Survival after fungal infection was calculated from the date of first fungal infection to death or last follow-up. Differences between curves were assessed using the log-rank test.

Cox proportional hazards regression was used to identify factors associated with fungal infection and infection-related mortality after fungal infection. Univariable analyses were performed for clinically relevant demographic, disease-related, transplant-related, and infection-related variables. Variables selected on the basis of clinical relevance and univariable results were included in multivariable models. Subgroup analyses were performed for HCT recipients. Episode-level analyses were used to describe fungal infection characteristics, prophylaxis, treatment, etiology, and outcomes. Treatment-related analyses were considered exploratory because of potential confounding by indication.

Hazard ratios (HRs) with 95% confidence intervals (CIs) were reported. All tests were two-sided, and p-values < 0.05 were considered statistically significant. Patients with missing data for a given variable were excluded from analyses involving that variable.

Results

Demographics

Between 2018 and 2023, 5,714 children with newly diagnosed malignancies were treated with anticancer therapy at 17 PHO centers. During the same period, 1,064 HCTs were performed at 6 transplant centers, including 799 allogeneic and 265 autologous procedures. Complete datasets for the fungal infection analysis were available for 5,564 patients, comprising 4,631 treated in PHO centers and 933 HCT recipients.

Characteristics of patients with fungal infections

Among the study cohort, 763 patients were diagnosed with fungal infections, including 627 PHO patients and 136 HCT recipients.

Children treated in PHO centers represented a diverse population with hematologic cancers and solid tumors, most frequently ALL, AML, non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), central nervous system neoplasms, neuroblastoma, sarcomas, and other uncommon malignancies. Indications for HCT included both malignant and non-malignant disorders, with acute leukemias, myelodysplastic syndrome/myeloproliferative neoplasms (MDS/MPN), lymphomas, severe aplastic anemia, primary immunodeficiencies, and other bone marrow failure or immune disorders accounting for the majority of cases. Overall, 12 patients (8.8%) underwent auto-HCT and 124 (91.2%) allo-HCT. Among allo-HCT recipients, matched unrelated donor transplantation was the most common donor type (92/124, 74.2%).

The median age was 7.1 years (range, 0.1–17.8) in the PHO cohort and 9.9 years (range, 0.1–19.9) in HCT recipients. The majority of patients in both groups were male, 55.5% in PHO and 63.2% in HCT.

Incidence of infection

Fungal infections occurred in 763 of 5,564 patients included in the analysis. Within 24 months, the cumulative incidence of fungal infection was 13.2% (95% CI: 12.2–14.2) in PHO patients and 14.5% (95% CI: 12.1–16.9) in HCT recipients, with no statistically significant difference between the groups ($p = 0.179$). Among patients with ALL, the 24-month cumulative incidence of fungal infection was higher in the PHO cohort than among HCT recipients, reaching 22.3% (95% CI: 20.1–24.5) and 15.9% (95% CI: 11.4–20.4), respectively ($p = 0.035$). Among patients with AML, the 24-month cumulative incidence was also significantly higher in the PHO cohort than in HCT group, reaching 32.0% (95% CI: 26.5–37.5) and 17.7% (95% CI: 10.6–24.8), respectively ($p = 0.007$). Among patients with MDS/MDS-MPN, the 24-month cumulative incidence was comparable, reaching 15.6% in PHO (95% CI: 3.1–28.1) and 14.5% in HCT cohort (95% CI: 6.3–22.7) ($p = 0.887$). Among patients with NHL, the 24-month cumulative incidence was similar in PHO patients and HCT recipients, reaching 17.9% (95% CI: 13.6–22.2) and 16.2% (95% CI: 4.2–28.2), respectively ($p = 0.799$) (Table 1, Fig. 1a–e).

Table 1. Incidences of fungal infections in relation to the primary diagnosis.

PHO- pediatric hematology oncology, HCT- hematopoietic cell transplantation, AML- acute myeloblastic leukemia, ALL- acute lymphoblastic leukemia, NHL- non-Hodgkin lymphoma, MDS- myelodysplastic syndrome, RMS- rhabdomyosarcoma, NBL- neuroblastoma, HD- Hodgkin disease, OS- osteosarcoma, CNST- central nervous system tumors, WT- Wilms tumor, ES- Ewing sarcoma, ES- Ewing sarcoma, LCH- Langerhans' cell histiocytosis, GCT- germ cell tumor, HLH- hemophagocytic histiocytosis

Other solid tumors refers to: hepatoblastoma, hepatocarcinoma, non-RMS sarcomas, non- Wilms renal tumors, adrenal tumors, melanoma, gastrointestinal stromal tumor, desmoid fibromatosis, pancreatic tumors, mesoblastic nephroma;

Bone marrow failure syndromes refers to: severe aplastic anemia, Fanconi anemia, Blackfan-Diamond anemia, paroxysmal nocturnal hemoglobinuria

Inborn errors of immunity refers to: chronic granulomatous disease, Wiskott-Aldrich syndrome, Nijmegen breakage syndrome, hyper-IgM syndrome, severe congenital neutropenia, X-linked lymphoproliferative disease, combined immune deficiencies

PHO patients		HCT patients	
Total	627/4631 (13.5%)	Total	136/933 (14.6%)
AML	89/274 (32.5%)	Bone marrow failure syndromes	26/125 (20.8%)
ALL	346/1526 (22.7%)	AML	21/115 (18.3%)
NHL	59/325 (18.2%)	Inborn error of immunity	15/86 (17.4%)
MDS	5/30 (16.7%)	ALL	40/256 (15.6%)
RMS	14/203 (6.9%)	NHL	5/33 (15.2%)
NBL	19/383 (5.0%)	MDS	10/68 (14.7%)
HD	17/359 (4.7%)	HLH	2/18 (11.1%)
OS	2/47 (4.3%)	NBL	9/98 (9.2%)
CNST	23/545 (4.2%)	Solid tumors	2/22 (9.1%)
Solid tumors	10/259 (3.9%)	Metabolic diseases	2/26 (7.7%)
WT	9/235 (3.8%)	HD	1/29 (3.4%)
ES	3/81 (3.7%)	Other	3/54 (5.6%)
LCH	2/64 (3.1%)		
GCT	5/172 (2.9%)		
Other	24/111 (21.6%)		

Figure 1. Cumulative incidence of fungal infections in PHO and HCT patients according to cohort, primary diagnosis, transplant type, age, and calendar period.

(a) Overall cohort, PHO vs. HCT; (b) ALL, PHO vs. HCT; (c) AML, PHO vs. HCT; (d) NHL, PHO vs. HCT; (e) MDS/MDS-MPN, PHO vs. HCT; (f) HCT, auto-HSCT vs. allo-HSCT; (g) PHO, age < 8 vs. ≥8 years; (h) HCT, age < 8 vs. ≥8 years; (i) PHO, 2018–2019 vs. 2020–2021 vs. 2022–2023; (j) HCT, 2018–2019 vs. 2020–2021 vs. 2022–2023. Values represent 24-month cumulative incidence estimates ± standard error. *P* values were calculated using the log-rank test.

PHO- pediatric hematology oncology, HCT- hematopoietic cell transplantation, ALL- acute lymphoblastic leukemia, AML- acute myeloblastic leukemia, MDS- myelodysplastic syndrome, autoHSCT- autologous stem cell transplantation, alloHSCT- allogeneic stem cell transplantation

Older age was associated with a significantly higher 24-month cumulative incidence of fungal infection in both cohorts. In PHO patients, the cumulative incidence was 15.0% (95% CI: 13.4–16.6) in patients aged ≥ 8 years compared with 12.1% (95% CI: 10.9–13.3) in those aged < 8 years ($p = 0.004$). In HCT recipients, the corresponding values were 18.1% (95% CI: 14.6–21.6) and 10.9% (95% CI: 8.2–13.6), respectively ($p = 0.002$) (Fig. 1g–h). In the HCT cohort, allogeneic transplantation was associated with a markedly higher cumulative incidence of fungal infection compared with autologous transplantation. The 24-month cumulative incidence was 17.5% (95% CI: 14.8–20.2) after allo-HCT and 5.2% (95% CI: 2.3–8.1) after auto-HCT ($p < 0.001$) (Fig. 1f).

In the PHO cohort, the 24-month cumulative incidence of fungal infection decreased over consecutive calendar periods, from 15.2% (95% CI: 13.2–17.2) in 2018–2019 to 13.5% (95% CI: 11.7–15.3) in 2020–2021 and 11.5% (95% CI: 10.1–12.9) in 2022–2023. The differences between calendar periods were statistically significant ($p = 0.009$). In the HCT cohort, the 24-month cumulative incidence differed significantly across calendar periods in the opposite manner, with the highest in 2022–2023. The cumulative incidence was 15.4% (95% CI: 11.5–19.3) in 2018–2019, 10.1% (95% CI: 6.8–13.4) in 2020–2021, and 17.6% (95% CI: 13.3–21.9) in 2022–2023 ($p = 0.035$) (Fig. 1i–j).

Recurrent fungal infections were observed in both study groups. Two or more fungal infection episodes occurred in 129 of 627 PHO patients (20.6%) and in 15 of 136 HCT recipients (11.0%), indicating a significantly higher frequency of recurrent infections in the PHO cohort ($p = 0.010$). The maximum number of fungal infection episodes recorded in a single patient was 12 in the PHO cohort and 9 in the HCT cohort.

In total, 846 fungal infection episodes were recorded in 627 PHO patients and 158 episodes in 136 HCT recipients. The distribution of fungal pathogens and diagnostic certainty differed markedly between the two groups (Table 2).

Table 2

Distribution of clinically important fungal infection episodes according to etiology and diagnostic certainty. PHO- pediatric hematology oncology, HCT- hematopoietic cell transplantation

Etiology	PHO				HCT			
	n (%)	possible	probable	proven	n (%)	possible	probable	proven
Unknown	380 (44.9)	298	61	21	35 (22.2)	28	7	0
<i>Aspergillus</i> spp.	219 (25.9)	121	78	20	76 (48.1)	35	29	12
<i>Candida</i> spp.	214 (25.3)	107	37	70	37 (23.4)	12	4	21
Mixed infection	20 (2.4)	9	2	9	2 (1.3)	1	0	1
<i>Mucorales</i>	5 (0.6)	0	0	5	2 (1.3)	0	0	2
<i>Fusarium</i> spp.	3 (0.4)	0	0	3	4 (2.5)	1	0	3
Other rare fungi	5 (0.6)	1	2	2	2 (1.3)	0	0	2
Total	846	536	180	130	158	77	40	41

In PHO patients, the median time from malignancy diagnosis to fungal infection was 3.0 months (range, 0.0–86.5), and the median duration of antifungal treatment was 19 days (range, 3–392). In HCT recipients, fungal infections occurred significantly earlier, with a median time of 1.5 months from transplantation to infection (range, 0.0–22.7; $p < 0.001$). The median duration of antifungal treatment in this group was significantly longer than in PHO patients, reaching 27 days (range, 2–378; $p < 0.001$).

Pulmonary involvement was the most common clinical manifestation in both cohorts, occurring more frequently in HCT recipients than in the PHO cohort (55.1% vs. 34.6%). Fever of unknown origin or an unspecified presentation was the second most common manifestation in both groups, although it was slightly more frequent in the PHO cohort (15.1% vs. 10.8%). Compared with HCT recipients, patients in the PHO cohort more often presented with other specified sites of infection, including mucosal/oropharyngeal involvement (16.2% vs. 1.3%), disseminated or multifocal disease (12.9% vs. 4.4%), and skin or soft tissue involvement (2.4% vs. 0.6%). In contrast, gastrointestinal/hepatosplenic involvement (11.4% vs. 7.0%), bloodstream infection/fungemia (8.2% vs. 6.7%), and sinonasal/ENT involvement (5.7% vs. 2.1%) were relatively more common among HCT recipients.

Typical radiological findings suggestive of IFD on CT, HRCT, MRI, or ultrasound were reported in 422 of 846 PHO episodes (49.9%). The most frequently recorded host factor was underlying leukemia, present in 605 episodes (71.5%). Steroid exposure was documented in 117 episodes (13.8%). Previous IFD and central venous catheter-related risk were reported in 3 episodes (0.4%).

In the HCT cohort, typical radiological findings suggestive of IFD were observed in 100 of 158 episodes (63.3%). The most common recorded host factor was neutropenia or pancytopenia, reported in 99 episodes (62.7%), followed by immunosuppressive therapy in 45 episodes (28.5%). Steroid exposure was documented in 21 episodes (13.3%), graft-versus-host disease in 19 episodes (12.0%), and previous IFD in 13 episodes (8.2%). Less frequently recorded factors included leukemia in 3 episodes (1.9%), central venous catheter-related risk in 2 episodes (1.3%), and fever as an associated clinical factor in 2 episodes (1.3%).

In the multivariable model, acute leukemia emerged as the strongest independent predictor of fungal infection. Age > 8 years, disease relapse and allogeneic transplantation in HCT cohort remained independently associated with increased risk of fungal infection (Table 3).

Table 3

Analysis of risk factors for fungal infection. PHO- pediatric hematology oncology, HCT- hematopoietic cell transplantation, autoHSCT- autologous stem cell transplantation, alloHSCT- allogeneic stem cell transplantation, HR- hazard ratio, CI- confidence interval, p- statistical significance value

Risk factor	Univariable Cox regression HR (95% CI)	p	Multivariable Cox regression HR (95% CI)	p
Age ≥ 8 vs < 8 years	1.33 (1.15–1.53)	< 0.001	1.44 (1.18–1.75)	< 0.001
Female vs male	1.08 (0.94–1.25)	0.270	1.09 (0.94–1.25)	0.263
HCT vs PHO	1.14 (0.95–1.37)	0.168	1.06 (0.88–1.28)	0.526
Acute leukemia vs other diagnoses	3.14 (2.70–3.64)	< 0.001	3.19 (2.75–3.71)	< 0.001
Relapse vs no relapse	1.77 (1.27–2.46)	< 0.001	1.43 (1.02–2.02)	0.039
2020–2021 vs 2018–2019	0.85 (0.71–1.02)	0.081	0.93 (0.76–1.13)	0.460
2022–2023 vs 2018–2019	0.82 (0.69–0.97)	0.019	1.04 (0.82–1.33)	0.737
HCT subgroup				
Allo-HSCT vs auto-HSCT	3.51 (1.94–6.35)	< 0.001	—	—

Prophylaxis and Treatment

Antifungal prophylaxis was documented in 639 of 1004 fungal infection episodes (63.6%), more frequently documented in HCT than in PHO group (88.0% vs. 59.1%).

The distribution of specific antifungal agents used for prophylaxis differed between the two cohorts. In PHO patients, the most commonly used prophylactic agents were fluconazole (18.2%), posaconazole (17.0%) and micafungin (16.5%). In HCT recipients posaconazole was the predominant prophylactic agent

(37.3%), followed by micafungin (17.1%), fluconazole(13.3%), voriconazole(6.3%) and amphotericin B(1.9%).

Antifungal treatment duration was highly variable and right-skewed; therefore, median values were used for descriptive comparisons. In the PHO cohort, treatment duration increased with diagnostic certainty: median treatment duration was 14 days for possible IFD, 31 days for probable IFD, and 26.5 days for proven IFD. By etiology, treatment was longest in mold infections, particularly *Aspergillus* spp. infections, with a median duration of 31 days, compared with 15 days in both *Candida* spp. infections and infections without pathogen identification.

In HCT recipients, antifungal treatment duration was generally longer and more comparable across diagnostic certainty categories, with median durations of 27.5 days for possible IFD, 35 days for probable IFD, and 33 days for proven IFD. By etiology, median treatment duration was longer for *Aspergillus* spp. infections than for *Candida* spp. infections, 32 versus 23 days, respectively. Longer median treatment durations observed in rare etiological groups, including *Fusarium* spp. and mixed fungal infections, should be interpreted cautiously because of the small number of cases.

Antifungal treatment patterns differed regarding the etiology and analyzed cohorts (Table 4).

Table 4

Antifungal treatment according to fungal etiology in PHO and HCT episodes. PHO- pediatric hematology oncology, HCT- hematopoietic cell transplantation

Antifungal treatment	Unknown/no pathogen		Aspergillus		Candida		Mixed/rare	
	PHO	HCT	PHO	HCT	PHO	HCT	PHO	HCT
Amphotericin B	113 (29.7%)	19 (54.3%)	99 (45.4%)	54 (71.1%)	53 (24.8%)	16 (43.2%)	19 (55.9%)	9 (90.0%)
Voriconazole	134 (35.3%)	9 (25.7%)	126 (57.8%)	27 (35.5%)	30 (14.0%)	3 (8.1%)	14 (41.2%)	4 (40.0%)
Micafungin	117 (30.8%)	8 (22.9%)	17 (7.8%)	9 (11.8%)	71 (33.2%)	10 (27.0%)	8 (23.5%)	0 (0.0%)
Caspofungin	58 (15.3%)	3 (8.6%)	27 (12.4%)	16 (21.1%)	46 (21.5%)	12 (32.4%)	4 (11.8%)	1 (10.0%)
Fluconazole	35 (9.2%)	0 (0.0%)	2 (0.9%)	1 (1.3%)	66 (30.8%)	4 (10.8%)	3 (8.8%)	0 (0.0%)
Posaconazole	7 (1.8%)	3 (8.6%)	6 (2.8%)	7 (9.2%)	8 (3.7%)	4 (10.8%)	5 (14.7%)	4 (40.0%)
Isavuconazole	5 (1.3%)	0 (0.0%)	8 (3.7%)	2 (2.6%)	3 (1.4%)	1 (2.7%)	5 (14.7%)	2 (20.0%)
Combination therapy	49 (12.9%)	2 (5.7%)	44 (20.2%)	23 (30.3%)	25 (11.7%)	3 (8.1%)	10 (29.4%)	8 (80.0%)

Exploratory analyses of antifungal treatment showed no agent could be clearly interpreted as protective against infection-related mortality. Micafungin and fluconazole were associated with non-significant trends toward lower mortality, with HRs of 0.52 (95% CI: 0.26–1.05, $p = 0.069$) and 0.25 (95% CI: 0.06–1.01, $p = 0.052$), respectively. Voriconazole and posaconazole use were not significantly associated with mortality. In contrast, amphotericin B, caspofungin, and isavuconazole use were associated with higher mortality in univariable analyses.

Outcome

Among patients with fungal infections, infection-related mortality was 42 of 627 (6.7%) in the PHO group and 29 of 136 (21.3%) among HCT recipients. HCT recipients had almost fourfold higher odds of infection-related death compared with PHO patients (OR 3.96; 95% CI: 2.36–6.65; $p < 0.001$).

Among patients who died due to fungal infection, 42 fatal episodes were recorded in the PHO cohort and 29 in the HCT cohort. Most infection-related deaths occurred within the first 30 days after fungal infection, accounting for 28 of 42 deaths (66.7%) in PHO patients and 15 of 29 deaths (51.7%) in HCT recipients (Table 5).

Table 5

Timing of infection-related deaths after fungal infection. PHO- pediatric hematology oncology, HCT- hematopoietic cell transplantation

Group	0–30 days	31–60 days	61–90 days	91–120 days	> 120 days	Total
Cohort						
PHO	28 (66.7%)	6 (14.3%)	4 (9.5%)	2 (4.8%)	2 (4.8%)	42
HCT	15 (51.7%)	8 (27.6%)	2 (6.9%)	1 (3.4%)	3 (10.3%)	29
Fungal infection group						
Unknown pathogen	10 (55.6%)	4 (22.2%)	2 (11.1%)	1 (5.6%)	1 (5.6%)	18
<i>Aspergillus</i> spp.	16 (53.3%)	5 (16.7%)	4 (13.3%)	2 (6.7%)	3 (10.0%)	30
<i>Candida</i> spp.	14 (87.5%)	1 (6.3%)	0	0	1 (6.3%)	16
<i>Fusarium</i> spp.	1 (50.0%)	1 (50.0%)	0	0	0	2
Mucorales	2 (50.0%)	2 (50.0%)	0	0	0	4
Mixed fungal infection	0	1 (100.0%)	0	0	0	1
Total	43 (60.6%)	14 (19.7%)	6 (8.5%)	3 (4.2%)	5 (7.0%)	71

In PHO, fatal infections occurred predominantly in patients with ALL, which accounted for 21 of 42 deaths (50.0%), followed by AML in 6 cases (14.3%). The etiological spectrum was heterogeneous. *Aspergillus* spp. was identified in 14 fatal episodes, including one mixed *Aspergillus/Candida* infection, while *Candida* spp. was diagnosed in 13 cases. Rare molds were less frequent and included *Mucorales* in 2 cases and *Fusarium oxysporum* in 1 case. No pathogen was identified in 13 fatal episodes (31.0%).

Antifungal prophylaxis had been documented in 32 fatal PHO episodes (76.2%), and combination antifungal therapy was used in 15 episodes (35.7%). The median duration of antifungal treatment before death was 15 days, with a wide range from 0 to 383 days.

In the HCT cohort, fatal infections occurred most frequently in patients transplanted for ALL, accounting for 9 of 29 deaths (31.0%), bone marrow failure syndromes in 6 cases (20.7%), MDS/MDS-MPN in 5 cases (17.2%), AML in 3 cases (10.3%), and inborn errors of immunity in 3 cases (10.3%). *Aspergillus* spp. predominated among fatal HCT infections and was identified in 16 episodes. *Candida* spp. in 5 episodes, while no pathogen was identified in 5 cases (17.2%). Rare molds included *Fusarium solani*, *Mucorales*, and *Rhizopus* spp.

Antifungal prophylaxis had been documented in 25 of 29 fatal HCT episodes (86.2%). In the available dataset, combination antifungal therapy was not recorded among fatal HCT episodes. The median duration of antifungal treatment before death was 28 days, ranging from 5 to 230 days.

Survival estimates are shown in Fig. 2a–g.

Figure 2. Survival after fungal infection in PHO and HCT patients according to cohort, primary diagnosis, age, diagnostic certainty, transplant-related factors, and calendar period.

(a) Total cohort, PHO vs. HCT; (b) ALL, PHO vs. HCT; (c) AML, PHO vs. HCT; (d) PHO, age < 8 vs. ≥8 years; (e) HCT, age < 8 vs. ≥8 years; (f) PHO, possible vs. probable vs. proven IFD; (g) HCT, possible vs. probable vs. proven IFD; (h) HCT, auto-HSCT vs. allo-HSCT; (i) HCT, GvHD vs. non-GvHD; (j) PHO, 2018–2019 vs. 2020–2021 vs. 2022–2023; (k) HCT, 2018–2019 vs. 2020–2021 vs. 2022–2023. Values represent survival estimates after fungal infection ± standard error. P values were calculated using the log-rank test.

PHO- pediatric hematology oncology, HCT- hematopoietic cell transplantation, ALL- acute lymphoblastic leukemia, AML- acute myeloblastic leukemia, MDS- myelodysplastic syndrome, autoHSCT- autologous stem cell transplantation, alloHSCT- allogeneic stem cell transplantation, GvHD- graft versus host disease

Discussion

Regarding the epidemiology of fungal infections, historically allogeneic HCT has consistently been considered as the highest-risk pediatric population^{4–6,13,14}. Our data suggest a different picture. Despite HCT remains a classical high-risk group, cumulative incidence of fungal infections was similar in both PHO and HCT group. In our analysis HCT was not an independent predictor for infection, while leukemia was one of the strongest determinants. Patients with acute leukemia had more than a threefold higher risk

of developing fungal infection compared with patients with other diagnoses. This implicates the modern transplant supportive care, especially mold-active prophylaxis, standardize surveillance and earlier imaging may have narrowed the historical risk group.

Moreover, our multivariable model revealed the strongest determinant of fungal infection is disease biology. We found acute leukemia, older age, disease relapse to be the most significant. These findings would argue for a conceptual shift from the treatment-based risk assessment to the disease-risk assessment and the future prophylaxis algorithms prioritizing leukemia subtype, disease relapse or cumulative chemotherapy dosing.

Although the fungal infection incidence was similar, mortality differed significantly between both groups. HCT remained independently associated with infection-related mortality, with more than a threefold higher risk of death compared with PHO patients. This indicates that acquiring the infection and surviving the infection depends on different mechanisms. Children after HCT who have impaired immune reconstitution, persistent immunosuppression, acute or chronic GVHD and impaired organ reserve may present delayed recovery from infection¹⁵.

Among fatal infections, most patients in our analysis had already received prophylaxis. Breakthrough infections may reveal the need of comprehensive understanding of the antifungal resistance, possible drug interactions or therapeutic drug monitoring algorithms.

Clinically important is the fact that mortality remains concentrated during the first month after diagnosis of fungal infection. Although since 2012 national prophylactic program was introduced in all Polish PHO and HCT centers¹², approximately two-thirds of PHO deaths and half of HCT fatal cases occurred within 30 days. This arises that the critical therapeutical window is very short and indicates the rapid diagnosis, imaging, therapeutical interventions and multidisciplinary approach.

Persistent diagnostic uncertainty remains one of the greatest unmet needs. In our analysis almost 45% of PHO episodes and 22% of HCT episodes had no identified pathogen, most were categorized as possible IFD. Recently major advances have been made to define and optimize early detection of IFD in children, but some recommendations are suboptimal. Current ECIL guidelines report favorable predictive performance of fungal antigen testing but only limited diagnostic utility of standardized PCR-based detection of fungal nucleic acids in pediatric patients. In addition, although systematic chest CT screening facilitates early diagnosis and has been associated with improved outcomes in adults, pediatric patients frequently lack the characteristic radiological manifestations of IFD⁴. These limitations highlight the need for further studies evaluating the role of these diagnostic modalities across different clinical phenotypes of pediatric IFD.

We found that proven IFD independently predicted mortality. Importantly probable disease did not. This underlines the positive microbiological results confirm higher fungal burden, advance tissue invasion and disseminated disease. Paradoxically waiting for microbiological confirmation may identify patients at a later stage of IFD.

Conclusions

The epidemiology of pediatric IFD is changing in the era of optimized prophylaxis and modern supportive care. While the relative incidence of IFD after HCT have been reduced, the outcomes remain substantially worse once fungal infection is diagnosed. This provides a strong rationale for future research focused not only on preventing fungal infection, but also improving early diagnosis and management of breakthrough IFD in the highest-risk pediatric population.

Declarations

Institutional Review Board Statement:

The study was approved by the Ethics Committee of Collegium Medicum in Bydgoszcz (agreement No. 263/2022). The requirement for informed consent was waived by the Ethics Committee. All patient data were analyzed anonymously, and no individual-level data were published. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication:

All authors approved the detailed content to be published.

Competing interests:

The authors declare no competing interest.

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Author Contribution

The conception and design of the study: MRP,JS,KC; collection of data: MRP, KC, MD, PZW, OGK, AG, ZM, KS, LCW, IZ, MS, JF, OZS, EB, NIJ, MP, PW, MB, FP, ZG, WB, WS, JM, ASB, LH, RT, AUD, AZP, JG; statistics and interpretation of data: MRP, AFJ; drafting the article: MRP, AFJ; critical review: all authors; final approval: MRP, JS.

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Data Availability

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding author.

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Figures

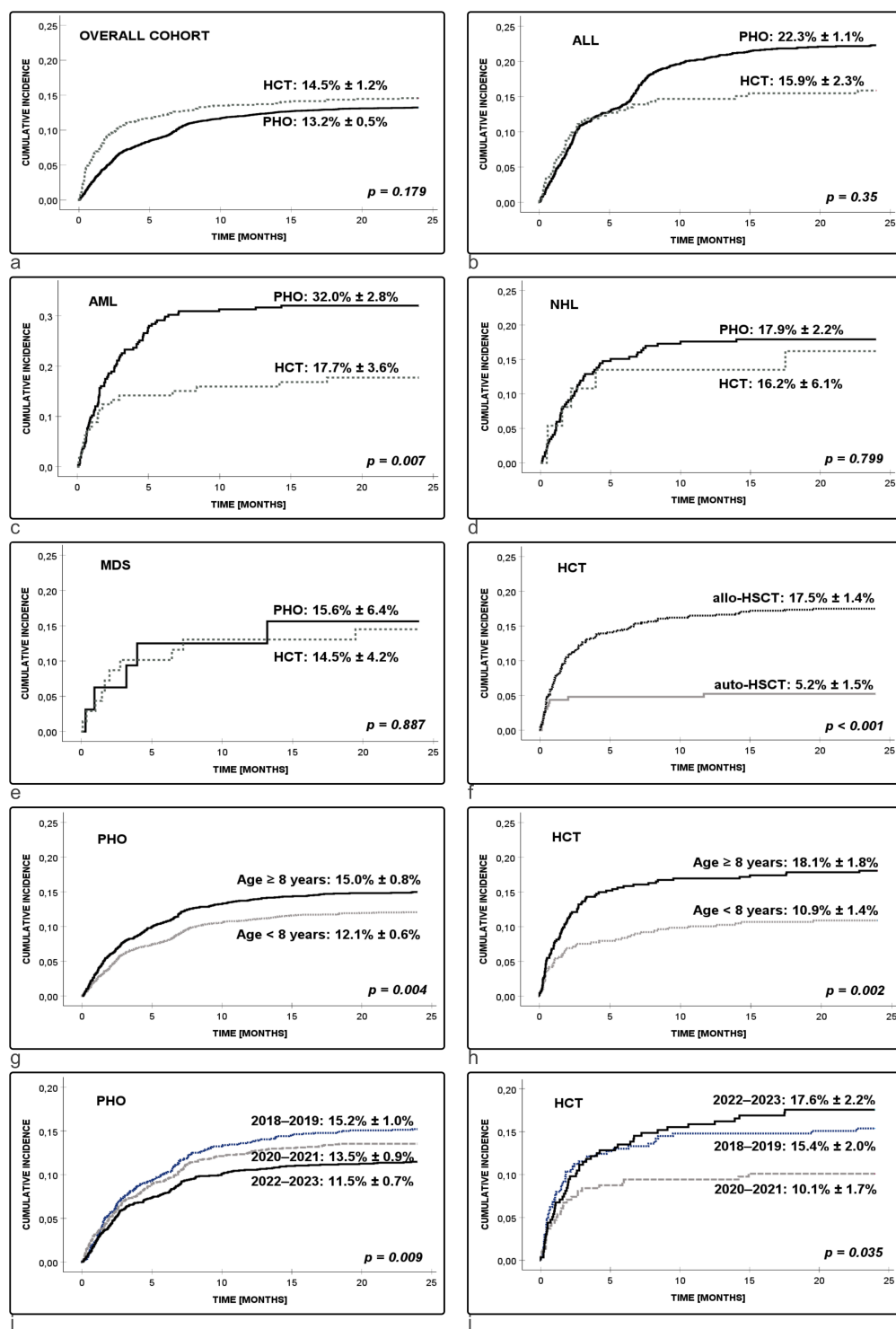


Figure 1

Cumulative incidence of fungal infections in PHO and HCT patients according to cohort, primary diagnosis, transplant type, age, and calendar period.

(a) Overall cohort, PHO vs. HCT; (b) ALL, PHO vs. HCT; (c) AML, PHO vs. HCT; (d) NHL, PHO vs. HCT; (e) MDS/MDS-MPN, PHO vs. HCT; (f) HCT, auto-HSCT vs. allo-HSCT; (g) PHO, age $<$ 8 vs. $\geq$ 8 years; (h) HCT, age $<$ 8 vs. $\geq$ 8 years; (i) PHO, 2018–2019 vs. 2020–2021 vs. 2022–2023; (j) HCT, 2018–2019 vs. 2020–2021 vs.

2022–2023. Values represent 24-month cumulative incidence estimates $\pm$ standard error. *P* values were calculated using the log-rank test.

PHO- pediatric hematology oncology, HCT- hematopoietic cell transplantation, ALL- acute lymphoblastic leukemia, AML- acute myeloblastic leukemia, MDS- myelodysplastic syndrome, autoHSCT- autologous stem cell transplantation, alloHSCT- allogeneic stem cell transplantation

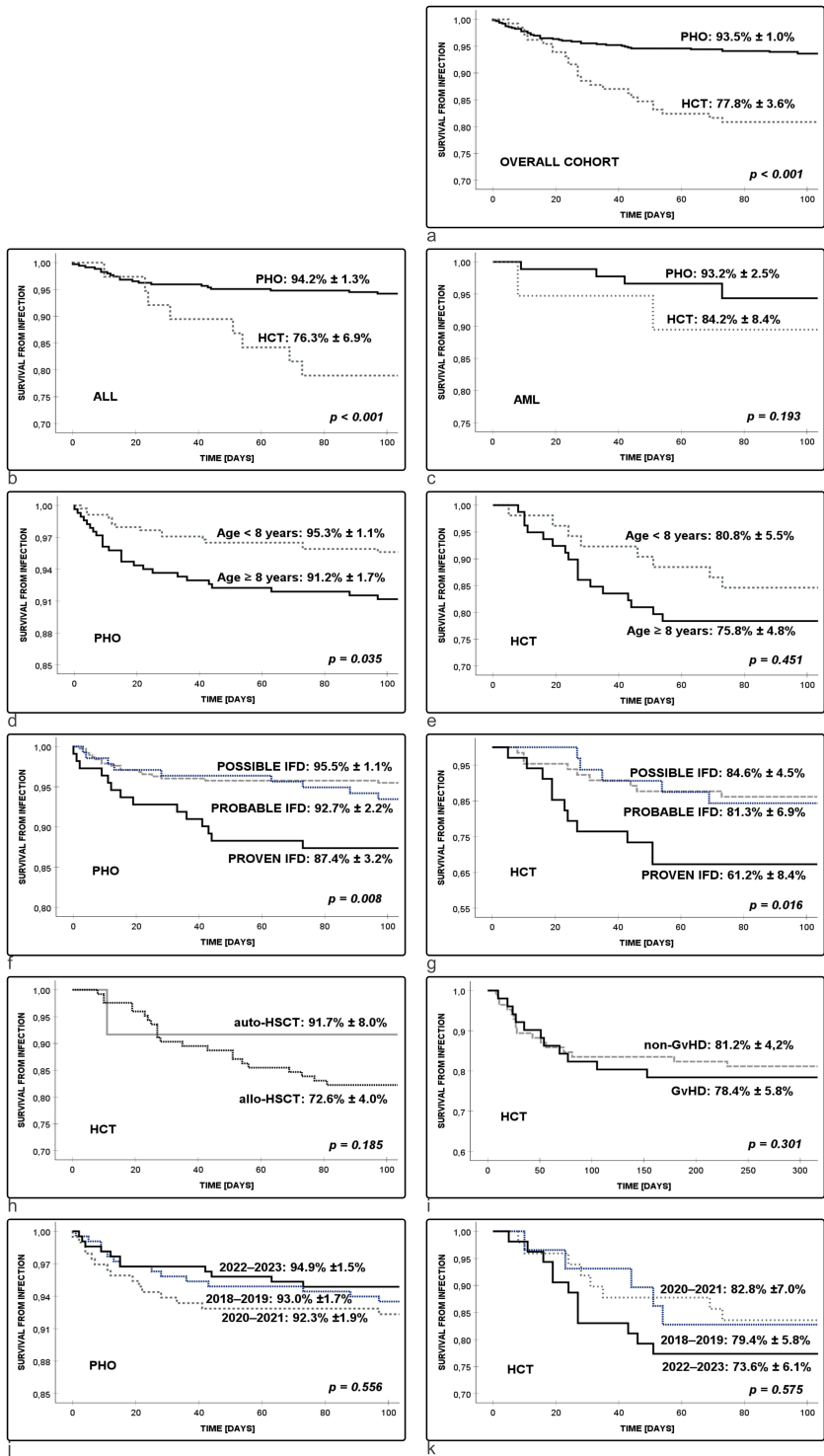


Figure 2

Survival after fungal infection in PHO and HCT patients according to cohort, primary diagnosis, age, diagnostic certainty, transplant-related factors, and calendar period.

(a) Total cohort, PHO vs. HCT; (b) ALL, PHO vs. HCT; (c) AML, PHO vs. HCT; (d) PHO, age <8 vs. ≥8 years; (e) HCT, age <8 vs. ≥8 years; (f) PHO, possible vs. probable vs. proven IFD; (g) HCT, possible vs. probable vs. proven IFD; (h) HCT, auto-HSCT vs. allo-HSCT; (i) HCT, GvHD vs. non-GvHD; (j) PHO, 2018–2019 vs. 2020–2021 vs. 2022–2023; (k) HCT, 2018–2019 vs. 2020–2021 vs. 2022–2023. Values represent survival estimates after fungal infection ± standard error. P values were calculated using the log-rank test.

PHO- pediatric hematology oncology, HCT- hematopoietic cell transplantation, ALL- acute lymphoblastic leukemia, AML- acute myeloblastic leukemia, MDS- myelodysplastic syndrome, autoHSCT- autologous stem cell transplantation, alloHSCT- allogeneic stem cell transplantation, GvHD- graft versus host disease