Incidence and Risk Factors for Invasive Mold Infections in Children During First-Line Chemotherapy for Primary Acute Leukemia: Protocol for a National Retrospective Cohort Study

Rasmus Moeller Duus, MD[1,2,3]

Dorthe Grosen, MD, PhD[1,2]

Flemming Schoenning Rosenvinge, MD[4,5]

Robin Christensen, BSc, MSc, PhD[6,7]

Jesper Bonnet Moeller, MSc, PhD[3,8]

Mathias Rathe, MD, PhD[1,2]

[1]Hans Christian Andersen Children's Hospital, Odense University Hospital, Odense, Denmark;

[2]Research unit of Paediatrics, University of Southern Denmark, Odense, Denmark;

[3]Department of Cancer and Inflammation Research, University of Southern Denmark;

[4]Department of Clinical Microbiology, Odense University Hospital, Odense, Denmark;

[5]Research Unit of Clinical Microbiology, University of Southern Denmark, Odense, Denmark;

[6]Section for Biostatistics and Evidence-Based Research, the Parker Institute, Bispebjerg and Frederiksberg Hospital, Copenhagen

[7]Research Unit of Rheumatology, Department of Clinical Research, University of Southern Denmark, Odense University Hospital, Denmark;

[8]Danish Institute for Advanced Study (D-IAS), University of Southern Denmark.

May 9th, 2023
NCT number (clinicaltrials.gov): NCT05774990

INTRODUCTION

Background

Children treated with chemotherapy are immunocompromised, making them susceptible to infections, including devastating invasive mold infections (IMI), of which Aspergillus spp. are the major pathogenic group. The at-risk population for IMI in children with acquired immunodeficiency is patients with prolonged granulocytopenia due to hematologic malignancies treated with chemotherapy or allogeneic hematopoietic stem cell transplantation (HSCT) recipients and children receiving long-term corticosteroid treatment (1). Incidence in at-risk patients may be influenced by the choice of prophylactic strategy, outbreaks related to local factors such as hospital renovation, or reflecting changing epidemiology of mold infections (2).

Few studies have examined the incidence according to the currently used "*European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group*" (EORTC/MSG) guidelines for a proven, probable, or possible IMI (3). An Australian 10-year epidemiological study, however, indicates an incidence of invasive aspergillosis in patients treated for acute myelogenous leukemia (AML) and acute lymphoblastic leukemia (ALL) of (14/232) 6%, (33/1216) 2.7 %, respectively (4, 5). Only few epidemiologic reports have been performed in Denmark or Scandinavia (6).

Despite progress in the diagnosis, prevention, and management of IMI, the reported mortality rates are often high. In an epidemiologic multicenter study, the main proportion of molds was A. fumigatus, with a mortality rate of 30% (7). A number of risk factors have been associated with IMI, among these, corticosteroid exposure, neutropenia, as well as different chemotherapy and conditioning regimens. However, few studies have addressed this in children with cancer, and knowledge regarding individual risk prediction for developing IMI in immunosuppressed children remains sparse (8, 9). Besides established risk factors for IMI, other treatment-related toxicities, including metabolic changes and gastrointestinal toxicity, may also be related to infectious morbidity (10-12).

Our aim is to obtain an epidemiological overview of IMI in children receiving first-line treatment for ALL or AML in Denmark and identify possible risk factors, including treatment-related adverse effects.

Objectives

1. To determine and compare the incidence of IMI in childhood AML and ALL during first-line chemotherapy in Denmark.
2. To determine and compare the mortality of IMI in childhood AML and ALL during first-line chemotherapy in Denmark.
3. To explore risk factors for IMI in childhood AML and ALL during first-line chemotherapy in Denmark.

METHODS

Study design

A retrospective nationwide multicenter, open cohort study of all children who received first-line chemotherapy against acute leukemia from January 1st, 2008, to December 31st, 2022, in a Danish pediatric oncology unit; this includes data from the four Danish tertiary pediatric oncology units at Rigshospitalet, Aarhus University Hospital, Odense University Hospital, and Aalborg University Hospital.

Study population

Any patient (<18 years) who initiated first-line chemotherapy against primary ALL or AML at any of the four Danish departments of pediatric oncology from January 1st, 2008, to December 31st, 2022 (**Figure 1**). The eligible sample of children has been estimated to correspond to approximately 800 individuals who will be included in the cohort and subsequently be referred to as the intention-to-survey (ITS) population.

Data sources

Patients from the four Danish departments of pediatric oncology treating pediatric ALL and AML will be identified by searching the Danish Childhood Cancer Registry. The register holds data since 1985 on all children < 15 years with cancer and all children 15-17 years of age treated at a pediatric oncology department (13).

After cohort inclusion, data will be collected from the databases below, including the patient's medical records, hospital administrative databases, and national databases when applicable. The data for all cohort patients will be collected as listed in **Table 2**. For IMI patients only, additional data for a qualitative synthesis is collected, as detailed in **Table 3**.

- **The Danish Microbiology Database** is a nationwide database carrying all microbiology test results. We will search the database to identify factors that fulfill the criteria of direct or indirect proof of mold infection (Table 1).
- **The Danish Pathology Register is** a nationwide database carrying all pathology test results. We will search the database to identify histopathologic proof of mold infection.
- We will search the regional radiology databases for proof of mold infection, as this is not a nationwide database. We will explore the databases for radiologic interpretations of computed tomographies and magnetic resonance imaging to identify the IMI criteria (Table 1).
- Regional databases of blood test results will be searched to identify the neutrophilic count at the time of mold diagnosis in the IMI group and further to find proof of pancreatitis or hyperlipidemia.
- Patient records will be scrutinized to identify pre-leukemia comorbidities, leukemia diagnosis, and risk group, among other information, as listed in table 2 and table 3.

Data collection will start in April 2023.

Outcome variables

The primary outcome is defined as IMI. To identify patients who develop IMI, all cohort patients will have their pathology, microbiology, and radiology records reviewed for documentation, qualifying for EORTC/MSG criteria for either proven or probable disease. As recommended for epidemiological studies, the possible disease category has been left out (3). In brief, IMI is classified as proven when there is tissue damage and histopathologic, cytopathologic, or microscopic documentation of infection; a fungal DNA amplification by polymerase chain reaction (PCR) in paraffin-embedded tissue; or culture samples from a normally sterile site suspicious of infection.

The probable IMI requires the presence of a host factor (hematologic malignancy, among others), clinical features, and mycological evidence. Clinical features comprise specific clinical, bronchoscopic, and/or radiologic findings showing signs of tracheobronchitis, pulmonary, sino-nasal, or central nervous system (CNS) infection. The mycological documentation is either direct, usual cultures from a non-sterile site (i.e., mold found in sputum) or indirect detection of Aspergillus galactomannan antigen. The criteria for "possible disease" are identical to "probable disease" but without the presence of mycological evidence (3) (see table 1). The outcome variable is binary (i.e., IMI yes or no), but an additional analysis will be made for the IMI subgroups (i.e., IMI proven or probable).

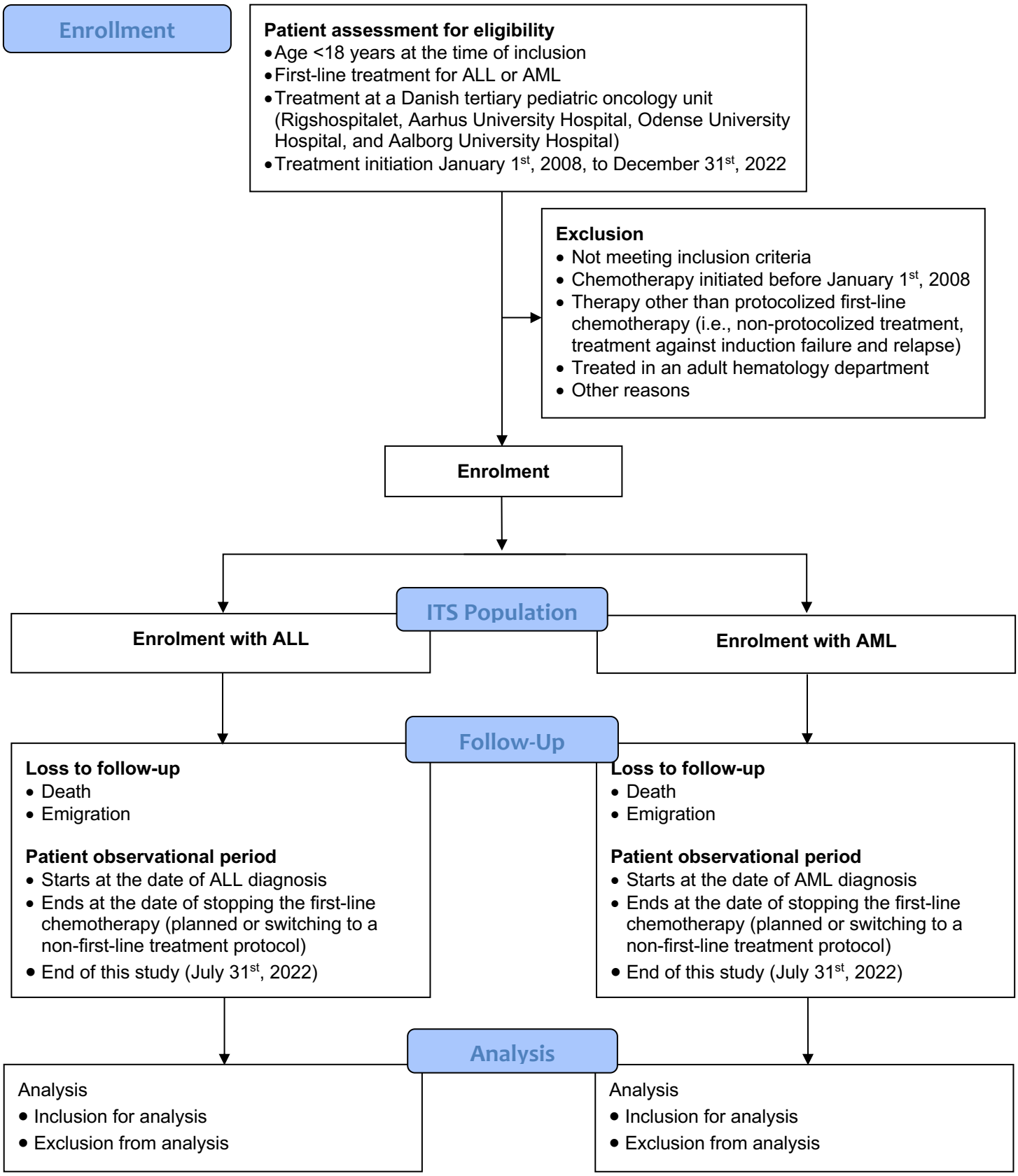
Exposure variables

The putative risk factors, referred to as 'exposures', are obtained from the patient's medical charts and corresponding paraclinical databases. These include antifungal medication, its type, dosing, and duration. It will be registered whether this was given as primary prophylaxis, treatment, or as secondary prophylaxis (after treated IMI). Also, preleukemic morbidities, treatment protocol, neutrophil granulocyte count at leukemia diagnosis, concomitant infections, and blood transfusion history, as a means of generalized toxicity, will be registered. Furthermore, therapy-related adverse effects, including gastrointestinal toxicity, neutropenic colitis, and treatment-related metabolic changes, including hyperglycosemia, pancreatitis, and hyperlipidemia (Table 2).

Observational period

The period of IMI risk is defined as the period of active anticancer treatment. Exposure data from cohort subjects are collected from the date of cancer diagnosis until finished first-line chemotherapy, IMI occurrence, death, emigration, switching to a non-first-line treatment (e.g., treatment for induction failure or relapse), or 31st December 2022 (end of the study period), whichever comes first. For the IMI patients, we will also collect IMI outcome (cured, deceased, or emigrated) data after the IMI diagnosis for a narrative description of this group and to calculate the fatality rate.

Figure 1. STROBE flow diagram presenting the study design. ITS, intention to survey; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia (AML); IMI, invasive aspergillus.



Bias and limitations

As the cohort study is observational and retrospective, the study relies on data extracted from medical charts. To avoid imprecise documentation, the predominant amount of data is exact (i.e., blood values), whereas more loosely medical chart statements will be avoided as far as possible. There is no risk of 'recall bias,' as no patients or health care professionals will be contacted.

Another limitation in our study could be a small number of IMI patients and exposures; this would yield a low power and could be the reason for inconclusive findings. To avoid this and type 1 errors, we will report measures of association with confidence intervals; if these exclude measures of clinical interest, we will assume that our study is not limited by sample size and number of events (say, if the 95% confidence intervals around the Hazard Ratio reasonably precise [95%CI: 0.80 to 1.25]).

During the 15 years of study, the treatment protocols have changed. We will apply the analyses to the whole population and do subgroup analyses for patients according to their specific treatment protocols.

Statistical methods

The primary analyses will be based on the Intention to Survey (ITS) population, i.e., based on the Full Analysis Set (see Figure 1 illustrated above). To assess distributions and check for errors, variables will be summarized before analysis. The frequency of IMI in the cohort will be determined by calculating the cumulative incidence.

Hazard ratios (HR) for ALL relative to AML will be estimated, with two-sided 95% confidence intervals, based on a Cox proportional-hazards model, with AL-type as the primary covariate. Equivalence will be shown if the limits of the two-sided 95% confidence interval exclude (less than) 0.80 and (more than) 1.25. Crude incidence rates will be expressed in patients with IMI events per 100 patient-years, with two-sided 95% confidence intervals. No multiplicity adjustments will be applied. P values, without adjustment for multiplicity, will be produced for all listed objectives. To identify independent risk factors for the development of IMI, regression models will be applied to explore which children were more likely to develop IMI (e.g., AL-type, sex, age at diagnosis, and related toxicities, see table 2).

All *P* values and 95% confidence intervals will be two sided. We will not apply explicit adjustments for multiplicity, rather we will analyze the secondary objectives in a prioritized order: The analyses of these will be performed in sequence until one of the analyses fails to show the statistically significant difference, or until all analyses have been completed at a statistical significance level of 0.05. The key secondary statistical tests will be reported with *P* values for hypothesis tests and claims of statistical significance.

Analyses will be performed using STATA software version 17 (StataCorp. 2021. Stata Statistical Software: Release 17. College Station, TX: StataCorp LLC.).

Patient and public involvement statement

As all data will be retrieved from patients no longer in active treatment, patients are not actively involved and were unfortunately not included in designing this study.

Ethics and dissemination

The relevant Danish authorities have approved the study, including the regional research legislation office "Regionssekretariat og jura, Syddanmark" (journal no. 22/7692), "Region Syddanmarks Interne Fortegnelse" (journal no. 22/15850), and by the involved departments. Other ethics approval has not been required for this study.

The study will be registered at Clinical.Trials.gov. The study acts under the Declaration of Helsinki. No patient will be contacted and is not exposed to any inconvenience. All data are confidential and will

be processed in accordance with the General Data Protection Regulation (GDPR) and The Danish Data Protection Act.

Data management is carried out in OPEN Odense Patient Data Explorative Network, Odense University Hospital, Clinical Institute, University of Southern Denmark https://www.sdu.dk/da/ki/open. Data is stored in an electronic database in RedCap, http://www.project-redcap.org., according to the EU GDPR https://www.eugdpr.org/. The findings are reported according to the guidelines for reporting observational studies as outlined in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement (14).

Anonymized raw data will be published as an appendix to the finished article. After five years, the data will be offered to The Danish National Archives.

The projects' results (whether positive, negative, or inconclusive) are submitted for publication in a scientific peer-reviewed journal; alternatively, results are published another way (possibly on www.clinicalstudyresults.org). Data are published according to the Act on Processing of Personal Data.

Table 1. Criteria for proven, probable, and possible mold infections.
Modified from the EORTC/MSG guidelines (4).

CRITERIA FOR PROVEN DISEASE - *molds*[a]: one of the following
Microscopic analysis (sterile material): Histopathologic, cytopathologic, or direct microscopic examination[b] of a specimen obtained by needle aspiration or biopsy in which hyphae or melanized yeast-like forms are seen accompanied by evidence of associated tissue damage
Culture (sterile material): Recovery of a hyaline or pigmented mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process, excluding BAL fluid, a paranasal or mastoid sinus cavity specimen, and urine
Blood: Blood culture that yields a mold[c] (eg, *Fusarium* species) in the context of a compatible infectious disease process
Tissue Nucleic Acid Diagnosis: Amplification of fungal DNA by PCR combined with DNA sequencing when molds are seen in formalin-fixed paraffin-embedded tissue
CRITERIA FOR PROBABLE DISEASE: Host factors + clinical features + mycological evidence
Host factors
Hematologic malignancy[d]
Clinical features
Pulmonary aspergillosis
The presence of 1 of the following 4 patterns on CT:
Dense, well-circumscribed lesions(s) with or without a halo sign
Air crescent sign
Cavity
Wedge-shaped and segmental or lobar consolidation
Other pulmonary mold diseases
As for pulmonary aspergillosis but also including a reverse halo sign
Tracheobronchitis
Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis
Sino-nasal diseases
Acute localized pain (including pain radiating to the eye)
Nasal ulcer with black eschar
Extension from the paranasal sinus across bony barriers, including into the orbit
Central nervous system infection
1 of the following 2 signs:
Focal lesions on imaging
Meningeal enhancement on magnetic resonance imaging or CT
Mycological evidence
Any mold, for example, *Aspergillus*, *Fusarium*, *Scedosporium* species or Mucorales recovered by culture from sputum, BAL, bronchial brush, or aspirate
Microscopical detection of fungal elements in sputum, BAL, bronchial brush, or aspirate indicating a mold
Tracheobronchitis
Aspergillus recovered by culture of BAL or bronchial brush
Microscopic detection of fungal elements in BAL or bronchial brush indicating a mold
Sino-nasal diseases
Mold recovered by culture of sinus aspirate samples
Microscopic detection of fungal elements in sinus aspirate samples indicating a mold
Aspergillosis only
Galactomannan antigen
Antigen detected in plasma, serum, BAL, or CSF
Any 1 of the following:
Single serum or plasma: ≥1.0
BAL fluid: ≥1.0
Single serum or plasma: ≥0.7 and BAL fluid ≥0.8
CSF: ≥1.0
Aspergillus PCR
Any 1 of the following:
Plasma, serum, or whole blood 2 or more consecutive PCR tests positive
BAL fluid 2 or more duplicate PCR tests positive
At least 1 PCR test positive in plasma, serum, or whole blood and 1 PCR test positive in BAL fluid
Aspergillus species recovered by culture from sputum, BAL, bronchial brush, or aspirate
CRITERIA FOR POSSIBLE DISEASE: Host factors + clinical features + no mycological evidence
Probable invasive mold infection (IMI) requires the presence of at least 1 host factor, a clinical feature, and mycologic evidence and is proposed for immunocompromised patients only, whereas proven IMI can apply to any patient, regardless of whether the patient is immunocompromised. Probable IMI requires the presence of a host factor, a clinical feature, and mycologic evidence. Cases that meet the criteria for a host factor and a clinical feature but for which mycological evidence has not been found are considered possible IFD. (1,3)-beta-D glucan was not considered to provide mycological evidence of any invasive mold disease. Abbreviations: BAL, bronchoalveolar lavage; CSF, cerebrospinal fluid; CT, computed tomography; PCR, polymerase chain reaction; IMI, invasive mold infection. [a]If culture is available, append the identification at the genus or species level from the culture results. [b]Tissue and cells submitted for histopathologic or cytopathologic studies should be stained using Grocott-Gomori methenamine silver stain or periodic acid Schiff stain to facilitate inspection of fungal structures. Whenever possible, wet mounts of specimens from foci related to invasive fungal disease should be stained with a fluorescent dye (eg, calcofluor or blankophor). [c]Recovery of *Aspergillus* species from blood cultures rarely indicates endovascular disease and almost always represents contamination. [d]Hematologic malignancy refers to active malignancy, in receipt of treatment for this malignancy, and those in remission in the recent past. These patients would comprise largely acute leukemias and lymphomas, as well as multiple myeloma, whereas patients with aplastic anemia represent a more heterogeneous group of individuals and are not included. The list of host factors has been shortened.

Table 2. Characteristics and putative risk factors to be collected from all cohort patients.

	VARIABLE
Age (number in years)	Numerical
Sex (male/female)	Binary
Treating facility (Copenhagen/Århus/Odense/Aalborg)	Categorical
Pre-leukemia comorbidities	Categorical
ALL diagnosis: treatment protocol, risk group and CNS-status	Categorical
AML diagnosis: treatment protocol, risk group, FAB, and CNS-status	Categorical
Date of malignancy diagnosis	-
Name of treatment protocol	Categorical
Criteria met for IMI (proven/probable)	Categorical
Localization of IMI	Categorical
Neutrophil granulocyte count at leukemia diagnosis	Numerical
Anemia and red blood cell transfusion requirement (quantity)	Numerical
Thrombocytopenia and platelet transfusion requirement (quantity)	Numerical
Primary antifungal prophylaxis (type/dose/duration)	Categorical
Fungal infection other than IMI (fungi in a sterile site) (localization and type)	Categorical
Concomitant infections (bacteria in a sterile site) (localization and type)	Categorical
Neutropenic colitis (only radiological evidence: MRI, CT, plain film of the abdomen, US: bowel wall thickening > 4 mm, mesenteric stranding, bowel dilatation, mucosal enhancement, or pneumatosis) (yes/no)	Binary
Pancreatitis (> x3 upper limit of amylase/lipase) (yes/no)	Binary
Therapy related hyperlipidemia (triglycerides/cholesterol blood concentrations greater than upper normal limit (UNL). Grading: Mild: triglycerides/cholesterol <10 times UNL; Moderate: triglycerides/cholesterol 10–20 times UNL; Severe: triglycerides/cholesterol >20 times UNL.) (mild/moderate/severe)	Categorical
Therapy related diabetes (insulin dependent) (yes/no)	Binary
Therapy related diabetes (insulin dependent) (duration)	Numerical
Outcome cancer (finishing anticancer therapy, death, hematopoietic stem-cell transplantation (HSCT), emigration, or 31st December 2022 (end of the study period)) (cured, recurrence, deceased)	Categorical

Table 3. Qualitative description of identified invasive aspergillus patients.

Patient data collection
Type of mold
Mold resistance pattern
Treatment day (days since the date of malignancy diagnosis)
Corticosteroid therapy (duration, type, mg/m^2, time since)
Immunosuppressive therapy type/treatment phase
Neutropenia (severe: < 0,5 x 10^9/l) (days prior to IMI diagnosis/neutrophilic count at day of diagnosis)
Primary antifungal prophylaxis before IA (type/dose/duration)
Antifungal IA treatment (type/dose/duration)
Secondary antifungal prophylaxis after IA treatment (type/dose/duration)
Preceding and concomitant antimicrobial therapy (type / duration prior to IMI)
Diagnostic procedures (culture, galactomannan assay, radiologic findings, etc.)
Intravenous access at time of diagnosis (central venous / port-a-cath)
Anticancer treatment delays/modifications
ICU stay (yes/no/duration)
Outcome fungal (cured, ongoing, deceased)

References

1. Wattier RL, Ramirez-Avila L. Pediatric Invasive Aspergillosis. J Fungi (Basel). 2016;2(2).
2. Vissing NH, Lausen B, Hutchings Hoffmann M, Als-Nielsen B, Schmiegelow K, Helweg-Larsen J, et al. Aspergillus flavus Infections in Children With Leukemia Despite Liposomal Amphotericin-B Prophylaxis. Pediatr Infect Dis J. 2021;40(8):749-52.
3. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, et al. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. Clin Infect Dis. 2020;71(6):1367-76.
4. Yeoh DK, Moore AS, Kotecha RS, Bartlett AW, Ryan AL, Cann MP, et al. Invasive fungal disease in children with acute myeloid leukaemia: An Australian multicentre 10-year review. Pediatr Blood Cancer. 2021:e29275.
5. Wang SS, Kotecha RS, Bernard A, Blyth CC, McMullan BJ, Cann MP, et al. Invasive fungal infections in children with acute lymphoblastic leukaemia: Results from four Australian centres, 2003-2013. Pediatr Blood Cancer. 2019;66(10):e27915.
6. Hovi L, Saxen H, Saarinen-Pihkala UM, Vettenranta K, Meri T, Richardson M. Prevention and monitoring of invasive fungal infections in pediatric patients with cancer and hematologic disorders. Pediatr Blood Cancer. 2007;48(1):28-34.

7. Wattier RL, Dvorak CC, Hoffman JA, Brozovich AA, Bin-Hussain I, Groll AH, et al. A Prospective, International Cohort Study of Invasive Mold Infections in Children. J Pediatric Infect Dis Soc. 2015;4(4):313-22.
8. Bartlett AW, Cann MP, Yeoh DK, Bernard A, Ryan AL, Blyth CC, et al. Epidemiology of invasive fungal infections in immunocompromised children; an Australian national 10-year review. Pediatr Blood Cancer. 2019;66(4):e27564.
9. Fisher BT, Robinson PD, Lehrnbecher T, Steinbach WJ, Zaoutis TE, Phillips B, et al. Risk Factors for Invasive Fungal Disease in Pediatric Cancer and Hematopoietic Stem Cell Transplantation: A Systematic Review. J Pediatric Infect Dis Soc. 2018;7(3):191-8.
10. Schmiegelow K, Attarbaschi A, Barzilai S, Escherich G, Frandsen TL, Halsey C, et al. Consensus definitions of 14 severe acute toxic effects for childhood lymphoblastic leukaemia treatment: a Delphi consensus. Lancet Oncol. 2016;17(6):e231-e9.
11. Sonabend RY, McKay SV, Okcu MF, Yan J, Haymond MW, Margolin JF. Hyperglycemia during induction therapy is associated with increased infectious complications in childhood acute lymphocytic leukemia. Pediatric Blood and Cancer. 2008;51(3):387-92.
12. Szalontay L, Shad AT. Pediatric Acute Myeloid Leukemia: How to Improve Outcome? Current Pediatrics Reports. 2014;2(1):26-37.
13. Hjalgrim LL, Mikkelsen TS, Rosthøj S, Schmiegelow K, Wehner PS, Møller K, et al. Dansk Børnecancer Register (DBCR) Resumé af årsrapport 2020-2021. 2022.
14. von Elm E, Altman DG, Egger M, Pocock SJ, Gotzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies. Int J Surg. 2014;12(12):1495-9.

Footnotes

Authors' contributions

The principal investigator (RD) is the first author in all publications. RD, MR, and RC designed the study. RD, MR, RC, SH, JB, DG, and UH will participate in conducting and analyzing the research and manuscript writing. The first author assumes responsibility for all practical issues, applications, and first drafts of all articles. Articles are written, and authorship is decided according to guidelines by the International Committee of Medical Journal Editors http://www.icmje.org/recommendations/browse/roles-and-responsibilities/.

Funding statement

This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Competing interests

All authors report no conflicts of interest.