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MAJOR ARTICLE

Breakthrough Invasive Mold Infections in Hematologic Cases: Relevance of the Host's Factors

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Background. Breakthrough invasive mold infections (bIMIs) are life-threatening complications in hematologic cases. Most previous studies in this field covered the whole spectrum of fungal pathogens, including yeasts, and antifungal agents.

Methods. We conducted a retrospective study including all hematologic cases of patients diagnosed with a bIMI while receiving a mold-active antifungal agent at our center between January 2017 and June 2022.

Results. Overall 37 patients were diagnosed with bIMI: 6 (16.2%) proven, 18 (48.6%) probable, and 13 (35.1%) possible. The highest incidence rate was found for micafungin (1.31 bIMI episodes per 1000 treatment-days), although with no significant differences across antifungal agents. Most patients (90.9%) for whom therapeutic drug monitoring was performed exhibited adequate through levels. Ten (27.0%) patients had undergone allogeneic hematopoietic stem cell transplantation. *Aspergillus* species was the most common pathogen in cases with microbiological identification. Regarding risk factors, 67.6% had severe neutropenia at diagnosis and 40.5% had received high-intensity chemotherapy. Rates of clinical response and attributable mortality by day +30 were 64.9% and 23.3%, respectively. Poorer performance status, higher Charlson Comorbidity index, older age, and higher C-reactive protein by day +7 were associated with 30-day attributable mortality.

Conclusions. *Aspergillus* was the predominant pathogen in our cohort of bIMIs, with a significant proportion of episodes occurring despite adequate triazole levels. Thirty-day attributable mortality was lower than previously reported. Poorer performance status, higher comorbidity burden, and older age had a relevant role in the outcome of bIMI.

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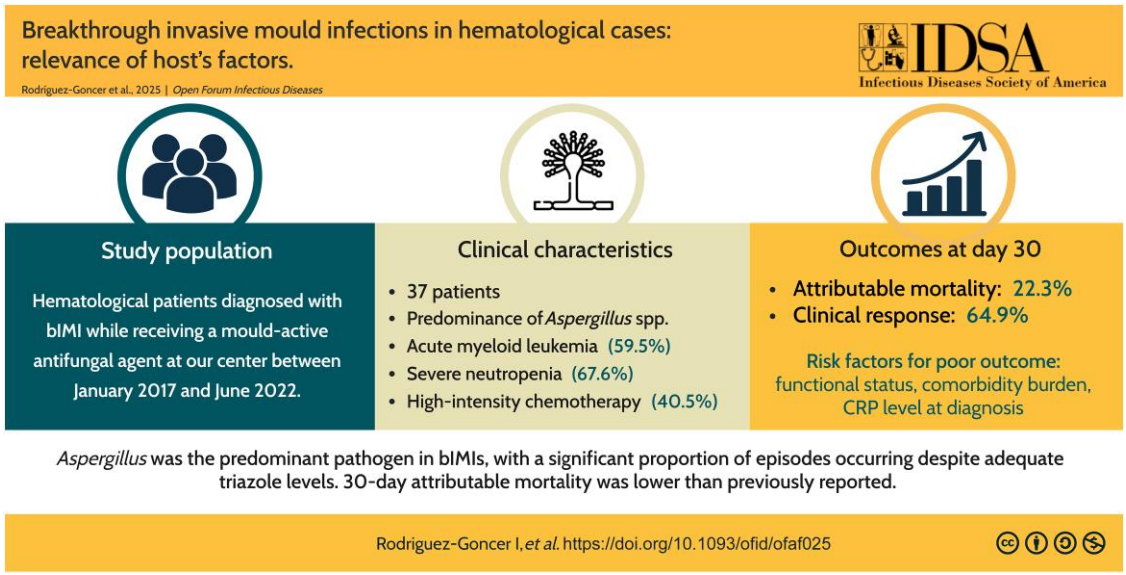
Graphical Abstract



Keywords. breakthrough; hematology; invasive fungal infection; mold; prophylaxis.

Invasive fungal infection (IFI) is a serious complication in high-risk hematologic cases. Multiple risk factors have been implied in the pathogenesis of this complication [1, 2]. Most of them have remained unchanged over the last decades, such as acute myeloid leukemia or high-risk myelodysplastic syndrome during remission-induction chemotherapy, allogeneic hematopoietic stem cell transplantation (HSCT) [3, 4], relapsed or refractory disease, prolonged neutropenia, long-term corticosteroid therapy, presence of mucositis, or previous respiratory tract viral infection [5]. An emerging risk group includes patients treated with chimeric antigen receptor T-cell therapies, especially those with high-grade cytokine release syndrome and prolonged lymphopenia [6].

Patients at increased risk of developing invasive mold infections (IMIs) are frequently exposed to antifungal agents with antimold activity for either prophylaxis or treatment [7–9]. Although the clinical efficacy of extended-spectrum triazoles for the prevention of IMI in high-risk populations is well established [10, 11], the occurrence of breakthrough IMI (bIMI) has emerged as a significant threat [12–14]. Inadequate plasma levels [13, 15–17], in vitro nonsusceptibility to the administered antifungal, and biofilm formation have been identified as frequent drivers [16, 18].

It is to be expected that the widespread use of these extended-spectrum agents has an impact on the epidemiology of bIMIs [19], with increasing roles for non-*fumigatus Aspergillus* species, azole-resistant *Aspergillus*, Mucorales, and rare molds such as *Fusarium* species or *Lomentospora prolificans* [14, 18, 20]. Most previous studies that investigated the characteristics of bIMIs were performed before isavuconazol (ISA) became widely available, or they were subject to potential selection bias due to the inclusion of patients with low comorbidity burden and expected mortality [21–23]. Recent prospective studies were focused on high-risk cases with documented bIFI, although they included episodes that occurred while the patient was taking non–mold-active agents (eg, fluconazole) or they covered the entire spectrum of involved fungi—such as invasive candidiasis and episodes due to rare yeasts—rather than be restricted to molds [24]. The pathogenesis and clinical picture of bIFIs due to mold and yeasts are markedly different, thus preventing a joint undifferentiated analysis.

We have performed a retrospective study to describe the incidence, clinical and microbiological features, therapeutic approaches, and outcome of bIMIs that occurred in hematologic cases of patients exposed to a mold-active antifungal agent.

MATERIALS AND METHODS

Study Population, Setting, and Design

We conducted a retrospective observational cohort study including all adult patients (age ≥18 years) with a hematologic disease who received a mold-active antifungal agent—voriconazole (VCZ), posaconazole (POS), ISA, intravenous (IV) liposomal amphotericin B (L-AmB), or an echinocandin—as either prophylaxis or treatment and developed at least 1 episode of bIMI between January 2017 and June 2022 at the Hospital Universitario 12 de Octubre (Madrid, Spain).

Eligible patients were identified by means of the prescription orders for IV L-AmB provided by the Department of Pharmacy, since it constitutes the cornerstone of the empirical treatment for

suspected bIMI (usually combined with a second mold-active agent) according to our institutional protocols. Additional bIMI episodes were identified by reviewing the diagnostic codes at discharge from the Department of Hematology. Electronic health records were reviewed by 2 researchers (J. B. and R. C.-A.) to ascertain whether the diagnostic criteria for bIMI were met. Disagreements were resolved by a third senior researcher (I. R.-G. or J. M. S.-P.). Clinical data were retrospectively collected with a standardized case report form and included the following: demographics, underlying hematologic disease and associated immunosuppressive therapies, type of HSCT, major comorbidities, performance status according to the Eastern Cooperative Oncology Group (ECOG) score, prior antifungal agents, risk factors for IFI (ie, previous respiratory tract viral infection, neutropenia, previous chemotherapy, concomitant corticosteroid therapy, and graft-vs-host disease), previous IFI episodes, microbiological and histopathologic features, trough plasma levels of azole agents in patients with available therapeutic drug monitoring (TDM), results of computed tomography imaging studies and bronchoscopic examinations, antifungal treatment upon diagnosis of bIMI, occurrence of treatment-emergent adverse events (TEAEs), and patient outcomes (clinical response and all-cause and attributable mortality by days +14, +30, and +100). To estimate incidence rates, information on the overall prescriptions of mold-active antifungal agents during the study period was provided by the Department of Pharmacy.

The study protocol was approved by the local ethics committee, and informed consent was waived due to its retrospective observational nature. All personal data were processed in an anonymized way according to applicable national laws and regulations.

Study Definitions

IFI episodes were classified as "proven," "probable," or "possible" according to the 2019 revised criteria of the European Organization for Research and Treatment of Cancer and the Mycoses Study Group (EORTC/MSG) [25]. bIMI was defined as any bIFI caused by a mold pathogen that occurred in patients receiving a mold-active agent (VCZ, POS, ISA, L-AmB, or an echinocandin), irrespective of whether the intention was prophylactic or therapeutic. Given the differences in half-life and antifungal dosing intervals, the period to define breakthrough was considered according to the 2019 revised definitions for bIFI proposed by the MSG Education and Research Consortium and the European Confederation of Medical Mycology [26], which vary across antifungal classes and take into account the pharmacokinetic properties of each drug. The breakthrough period extends at least until 1 dosing interval after drug discontinuation. Day 0 was the calendar day on which the diagnosis of bIMI was established. Prior fungal infection was considered any previous episode of IFI that was correctly treated. First-line antifungal therapy was defined as that prescribed once the diagnosis of bIMI was established. Combined therapy required the use of 2 antifungal drug classes for >5 days. Clinical response at days +14 and +30 was defined by the complete or partial resolution of attributable signs and symptoms and/or an improvement ≥50% in the size of radiologic lesions from baseline to each point. To adjudicate attributable mortality, we took into account the cause of death established in the electronic health records, autopsy reports, and/or death certificates, as well as the response to antifungal therapy. Neutropenia was defined by an absolute neutrophil count <500 cells/mm^3 at the time of diagnosis of bIMI. Prior corticosteroid therapy required a daily dose >0.3 mg/kg of prednisone equivalent for >3 weeks during the previous 60 days. High-intensity chemotherapy included treatment with high-dose cytarabine (>1 g/m^2/d) or continuous cytarabine plus an anthracycline (3 + 7 regimen), as well as dose-adjusted EPOCH-R (etoposide, prednisone, vincristine, doxorubicin, cyclophosphamide, and rituximab). Disease stage was established according to the International Working Group criteria for each specific condition [27–29]. Clinically significant cytomegalovirus infection was defined as the occurrence of cytomegalovirus disease or any cytomegalovirus viremia that led to the initiation of antiviral therapy. Comorbidity burden was measured with the Charlson Comorbidity Index (CCI) [30].

Microbiological Methods

Microbiological diagnoses were routinely performed at the Department of Microbiology. Respiratory samples were processed for fungal culture in Sabouraud dextrose agar incubated at 30 °C for 5 to 7 days. Fungal isolates were identified by conventional and molecular methods (polymerase chain reaction and sequencing). In vitro antifungal activity was initially assessed by E-test for clinically significant isolates and subsequently confirmed at the Mycology Reference Laboratory of the Spanish National Centre for Microbiology (Majadahonda, Madrid) with microdilution according to the reference methods of the European Committee on Antimicrobial Susceptibility Testing. Available clinical breakpoints were used to define resistance. Galactomannan (GM) antigen testing was performed with the Platelia *Aspergillus* assay (Bio-Rad Laboratories). In accordance with the 2019 EORTC/MSG criteria [25], we considered the following a positive result: a single index ≥1.0 in serum or bronchoalveolar lavage (BAL) or the combination of an index ≥0.7 in serum and ≥0.8 in the BAL fluid.

Statistical Analysis

Quantitative results were expressed as the mean ± SD or median (IQR), as appropriate. Qualitative data were expressed with absolute and relative frequencies. The overall incidence rate of bIMI was calculated for the total number of at-risk patients (ie, those who received at least 1 dose of a mold-active antifungal

Table 1. Demographics, Clinical Characteristics, and Predisposing Factors of the Cohort (N = 37)

	Mean ± SD, No. (%), or Median (IQR)
Age, y	58.5 ± 15.4
Male gender	18 (48.6)
Chronic comorbidities	
Diabetes mellitus	9 (24.3)
Solid cancer	5 (13.5)
Chronic obstructive pulmonary disease	4 (10.8)
Chronic heart disease	4 (10.8)
Chronic liver disease	4 (10.8)
Chronic kidney disease	1 (2.7)
Charlson Comorbidity Index	2 (2–4)
Underlying hematologic disease	
Acute myeloid leukemia	22 (59.5)
Myelodysplastic syndrome	6 (16.2)
Non-Hodgkin lymphoma	5 (13.5)
Other[a]	4 (10.8)
High-intensity chemotherapy in the previous 30 d	15 (40.5)
No. of prior treatment lines	2 (1–3)
Previous HSCT	15 (40.5)
Allogeneic	10 (66.6)
Autologous	5 (33.3)
Previous CAR-T therapy[b]	2 (5.4)
Disease stage	
Progressive or relapsing disease	15 (40.5)
Complete or partial remission	14 (37.8)
Stable disease	3 (8.1)
Not evaluable	5 (13.5)
ECOG 2–4	8 (21.6)
Clinically significant CMV infection	
In the previous 30 d	6 (18.9)
In the previous 90 d	9 (24.3)
Risk factors for IMI	
Neutropenia	25 (67.6)
High-intensity chemotherapy in the previous 30 d	15 (40.5)
Graft-vs-host disease	4 (10.8)
Corticosteroid therapy	4 (10.8)
Respiratory tract viral infection in the previous 30 d[c]	6 (16.2)
ICU admission in the previous 30 d	6 (16.2)
Previous IMI	8 (21.6)
Probable	2 (25.0)
Possible	6 (75.0)
Interval to the diagnosis of bIMI, d	176.0 (66.5–475.8)
Laboratory values at diagnosis	
ANC, $\times 10^9$/L	0.1 (0.0–1.1)
C-reactive protein, mg/dL	14.4 ± 12.5
Previous mold-active antifungal agent	
Posaconazole	24 (64.9)
Isavuconazole	9 (24.3)
Voriconazole	1 (2.7)
Micafungin	3 (8.1)
Indication for antifungal therapy	
Prophylaxis	37 (100.0)
Primary prophylaxis	33 (89.2)
Secondary prophylaxis	4 (10.8)
Duration of therapy until diagnosis of bIMI, d	16.0 (10.5–66.5)
Therapeutic drug monitoring performed	11 (29.7)
Plasma levels within therapeutic range	10 (90.9)
Trough plasma levels, mg/L	
Posaconazole (n = 6)	3.6 (1.3–4.3)
Isavuconazole (n = 4)	4.9 (3.1–10.1)
Voriconazole (n = 1)	<0.25

Abbreviations: ANC, absolute neutrophil count; bIMI, breakthrough invasive mold infection; CAR-T, chimeric antigen receptors T cell; CMV, cytomegalovirus; ECOG, Eastern Cooperative Oncology Group; HSCT, hematopoietic stem cell transplantation; ICU, intensive care unit.

[a]Includes 1 patient each with medullary aplasia, Hodgkin lymphoma, acute lymphoblastic leukemia, and multiple myeloma.

[b]Both patients received CAR-T therapy for non-Hodgkin lymphoma.

[c]Includes metapneumovirus (2 episodes), influenza, parainfluenza, respiratory syncytial virus, and rhinovirus (1 episode each).

during the study period) and was expressed as number episodes per 1000 treatment-days with 95% CIs. To this end, we computed the cumulative exposure to any mold-active agent and to each agent considered. Patients who were receiving combination therapy at the onset of bIMI contributed separately to the incidence rates of each agent. We also explored clinical factors associated with 30-day all-cause and attributable mortality. To this end, continuous variables were compared with the Student *t* test or Mann-Whitney *U* test, whereas categorical variables were evaluated with the χ^2 test or 2-tailed Fisher exact test. Survival curves were plotted with the Kaplan-Meier method, and differences between groups were compared by the log-rank test. All analyses were performed with SPSS software version 21.0 (IBM Corp).

RESULTS

Patient Characteristics and Risk Factors

During the study period, 37 patients developed an episode of bIMI. Demographics, clinical characteristics, and predisposing factors are detailed in Table 1. Patients were followed up for a median 112 days (IQR, 25–1267). The most prevalent hematologic condition was acute myeloid leukemia (59.5%, 22/37). Regarding the stage according to the International Working Group criteria, most patients had progressive or relapsing disease (40.5%, 15/37), followed by complete or partial remission (37.8%, 14/37). Twenty-five (67.6%) patients had neutropenia at the diagnosis of bIMI and 15 (40.5%) had undergone HSCT, mostly allogeneic. Among the 10 allogeneic HSCT recipients, 5 (50.0%) developed bIMI during the first month after the infusion (mean interval, 16.2 ± 5.4 days); 4 (40.0%) had experienced a relapse of their hematologic disease; and 2 (20.0%) had severe intestinal graft-vs-host disease treated with high-dose corticosteroids. Both chimeric antigen receptor T-cell recipients showed severe neutropenia and had received IV tocilizumab

and high-dose corticosteroids for grade II-III cytokine release syndrome.

Prior Mold-Active Antifungal Therapy

Mold-active antifungal agents administered at the onset of bIMI are summarized in Table 1. The most frequent antifungals were POS (64.9%, 24/37) and ISA (24.3%, 9/37). The median number of days of antifungal exposure before diagnosis was 16.0 (IQR, 10.0–66.5). Eight (21.6%) patients had a previous diagnosis of IMI: 2 and 6 cases of probable and possible invasive aspergillosis, respectively. The median interval between the diagnosis of previous IMI and the index episode of bIMI was 176.0 days (IQR, 66.5–475.8). All the patients had successfully completed the course of antifungal therapy, and 4 of them were still undergoing secondary prophylaxis at the time of diagnosis of bIMI: 3 with ISA and 1 with POS.

Overall, TDM was performed in 11 of 34 patients (29.7%) receiving an azole agent. Of the 11 patients with available TDM, 10 had plasma levels within the therapeutic range, whereas the remaining 1 had infratherapeutic VCZ levels (<0.25 mg/L) at the time of diagnosis.

Incidence Rates According to Previous Antifungal Therapy

The number and duration of courses of mold-active antifungal therapy during the study period, as either outpatient or inpatient prescriptions, in the overall at-risk cohort are shown in Table 2, as well as the incidence rates of bIMIs for each agent (cumulative and per 1000 treatment-days). The overall cumulative incidence was 6.9% (37/539), which accounted for an incidence rate of 0.76 bIMI episodes per 1000 treatment-days (95% CI, .06–1.02). Patients receiving micafungin showed the highest incidence rate (1.31 per 1000 treatment-days), followed by ISA (0.82), POS (0.76), and VCZ (0.25). There were no significant differences in incidence rates among antifungals (data not shown).

Diagnostic Procedures and Clinical Characteristics. The diagnostic procedures and clinical characteristics of bIMIs are shown in Table 3. Twenty-four episodes (64.8%) were proven or probable infections. Microbiological identification at least to the genus level was obtained in 14 episodes (37.8%). *Aspergillus fumigatus* was the most common species in these microbiologically documented episodes (14.3% [2/14]; Figure 1). According to the baseline antimold agent, *Aspergillus* species was also predominant among patients with a microbiological diagnosis who were receiving ISA (83.3%, 5/6), POS (80.0%, 4/5), or micafungin (100.0%, 2/2). One patient undergoing POS prophylaxis had bIMI caused by 2 fungi, *A calidoustus* and *Fusarium solani*. The only case of mucormycosis was diagnosed in a patient receiving ISA.

Antifungal susceptibility testing was performed in 4 episodes. In 3 of them (75.0%), the isolate was resistant to the agent being administered for prophylaxis: 2 patients receiving ISA who developed bIMI due to *A fumigatus* and *A fumigatiaffinis* and 1 patient receiving POS who developed bIMI due to *A flavus*. The remaining episode occurred in a patient undergoing micafungin prophylaxis who was diagnosed with bIMI due to echinocandin-susceptible *A fumigatus*.

Proven episodes of bIMIs (16.2%, 6/37) were diagnosed on the basis of histopathologic examination of lung biopsy samples, which revealed the presence of hyphae with associated tissue damage. The culture of the lung biopsy specimen was additionally positive for *Aspergillus* species in 4 of these cases, whereas a further patient also had a positive serum GM result. Probable bIMIs were diagnosed mostly by fungal identification in a respiratory tract specimen (55.5%, 10/18), molecular methods (11.1%, 2/18), or a positive GM result in either serum (61.1%, 11/18) or BAL fluid (22.2%, 4/18). Three of these patients had a positive GM result in serum and BAL samples. Bronchoscopic examinations were performed in 27 episodes (72.9%). The median interval from the first result suggestive of bIMI to the bronchoscopic examination was 6 days (IQR, 4.3–9.8). Of 27 examinations performed, 10 (37.0%) were useful for the diagnosis, mainly on the basis of a compatible histopathologic examination of the transbronchial biopsy (85.7%, 6/7).

Therapeutic Approaches. Combination therapy was commonly initiated as the first-line regimen upon the diagnosis of bIMI (83.8%, 31/37) and typically included L-AmB (93.5%, 29/31),

Table 2. Prescriptions of Mold-Active Antifungal Agents During the Study Period and Incidence of bIMI

	Any Mold-Active Antifungal	ISA	VCZ	POS	Micafungin
At-risk patients[a]	539	112	78	252	97
Courses of therapy	586	116	80	276	114
Cumulative					
Duration of therapy, d	48 584	10 963	3937	31 394	2290
Incidence	37 (6.9)	9 (8.0)	1 (1.3)	24 (9.5)	3 (3.1)
Incidence rate per 1000 treatment-days (95% CI)	0.76 (.06–1.02)	0.82 (.38–1.55)	0.25 (.01–1.41)	0.76 (.48–1.14)	1.31 (.27–3.8)

Data are presented as No. (%) unless noted otherwise.

Abbreviations: bIMI, breakthrough invasive mold infection; ISA, isavuconazole; POS, posaconazole; VCZ, voriconazole.

[a]Patients with a hematologic disease who received at least 1 dose during the study period.

Table 3. Diagnostic Procedures and Clinical Characteristics of Episodes of bIMI (N = 37)

	No. (%) or Mean ± SD
EORTC/MSG diagnostic category	
Proven	6 (16.2)
Probable	18 (48.6)
Possible	13 (35.1)
Site of infection	
Pulmonary involvement only	37 (100.0)
Causative fungi[a]	14 (37.8)
Aspergillus species	7 (50.0)
Aspergillus fumigatus	2 (14.3)
Aspergillus fumigatiaffinis	1 (7.1)
Aspergillus terreus	1 (7.1)
Aspergillus flavus	1 (7.1)
Aspergillus calidoustus and *Fusarium solani*	1 (7.1)
Mucorales	1 (7.1)
Fungal biomarkers	
Serum GM antigen tested	37 (100.0)
Positive[b]	12 (32.4)
Index value	3.2 ± 2.1
BAL GM antigen tested	27 (72.9)
Positive[c]	4 (14.8)
Index value	4.3 ± 2.7
Bronchoscopic examination performed	27 (73.0)
Diagnostic results	10 (37.0)
Lung biopsy performed	7 (18.9)
Transbronchial biopsy	3 (42.9)
CT-guided biopsy	4 (57.1)
Diagnostic results	6 (85.7)

Abbreviations: BAL, bronchoalveolar lavage; bIMI, breakthrough invasive mold infection; CT, computed tomography; EORTC/MSG, European Organization for Research and Treatment of Cancer/Mycoses Study Group; GM, galactomannan.

[a]Based on conventional culture (11 episodes), molecular methods (2 episodes), and direct visualization of broad nonseptate hyphae branching at 90° compatible with mucoral (1 episode).

[b]The serum GM assay was positive in 1 and 11 episodes of proven and probable bIMI, respectively.

[c]The BAL GM assay was positive in 4 episodes of probable bIMI.

followed by ISA (41.9%, 13/31) and VCZ (38.7%, 12/31). The median duration of combination therapy was 14.0 days (IQR, 9.0–17.0). The flow of treatment sequence according to the previous antifungal agent administered at bIMI onset is illustrated in Figure 2.

Regarding TEAEs, 2 patients treated with L-AmB (6,8%, 2/29) developed an infusion reaction that led to drug discontinuation. Four patients receiving VCZ (30.8%, 4/13) developed liver function test abnormalities that required drug discontinuation. One patient with POS (20%, 1/5) developed neurotoxicity attributed to the interaction between this azole and quizartinib. No TEAEs led to the discontinuation of ISA. Overall, 5 patients (13.5%) had TEAEs in the course of triazole therapy that required changing to a different triazole agent.

Patient Outcomes. Clinical response by days +14 and +30 was observed in 21.6% (8/37) and 64.9% (24/37) of patients. Rates of 14-, 30-, and 100-day bIMI-attributable mortality were 18.9% (7/37), 24.3% (9/37), and 27.0% (10/37), respectively (Table 4). We investigated clinical predictors of 30-day attributable mortality (Supplementary Table 1). Due to the low number of events, multivariate adjustment was not performed, and only the results of univariate analysis are provided. Attributable mortality was significantly higher in patients with a CCI >3 as compared with those below (88.9% [8/9] vs 32.1% [9/28], $P = .006$; Figure 3), as well as in those with poorer performance status as reflected by an ECOG score of 2 to 4 (66.7% [6/9] vs 7.1% [2/28], $P < .001$). Median (IQR) C-reactive protein levels by day +7 were higher in nonsurvivors than survivors (30.9 mg/dL [9.7–38.5] vs 4.6 [1.2–15.6], $P = .017$). Older age was also borderline associated with an increased attributable mortality (66.1 ± 11.1 years vs 55.9 ± 15.9 in nonsurvivors and survivors, $P = .062$). Finally, patients who had received >2 prior lines of treatment (55.6% [5/9] vs 21.4% [6/28], $P = .091$) and those with a proven or probable diagnosis per the EORTC criteria (88.9% [8/9] vs 57.1% [16/28], $P = .087$) showed a trend toward a higher attributable mortality at day 30.

DISCUSSION

The occurrence of bIFI represents a major clinical challenge in the management of hematologic cases, as illustrated by the growing amount of research in this field [4, 12–18, 31]. In contrast to previous studies, we have exclusively considered bIMI in patients receiving mold-active agents, applied the latest consensus definitions of the MSG Education and Research Consortium and European Confederation of Medical Mycology [26], and estimated agent-specific incidence rates. As the most relevant findings of the present experience, there were no significant differences in the incidence of bIMI across mold-active agents, although micafungin had a numerically higher rate; patients who developed bIMI had a significant burden of comorbidity and immunosuppression; and most episodes were due to *Aspergillus* species with a significant proportion occurring despite adequate triazole levels.

The incidence and microbiological spectrum of bIMI vary depending on local epidemiology, host characteristics, and specific antifungal protocols at each institution. Several studies describing the landscape of bIFI in the setting of broad-spectrum prophylaxis have reported a shift toward an increase role of non-*Aspergillus* molds, including triazole-resistant strains, and uncommon and difficult-to-treat fungi, such as Mucorales or *Fusarium* [14, 23]. These studies included episodes caused by yeasts (*Candida* and rare non-*Candida* yeasts) and molds and covered a large range of antifungal agents, including those with no antimold activity such as fluconazole [24, 32–35]. Therefore, these results may not be fully representative of the widespread use of broad-spectrum antifungals in high-risk populations. In fact, the majority of POS-associated bIMIs in

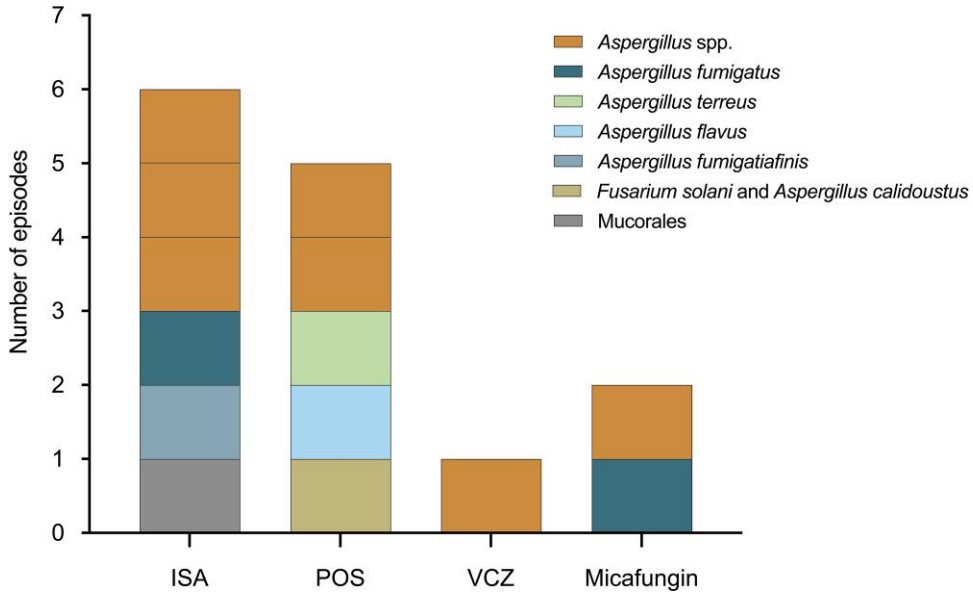


Figure 1. Distribution of causative fungi according to the previous mold-active antifungal agent in episodes of breakthrough invasive mold infection with microbiological documentation (n = 14). Abbreviations: ISA, isavuconazole; POS, posaconazole; VCZ, voriconazole.

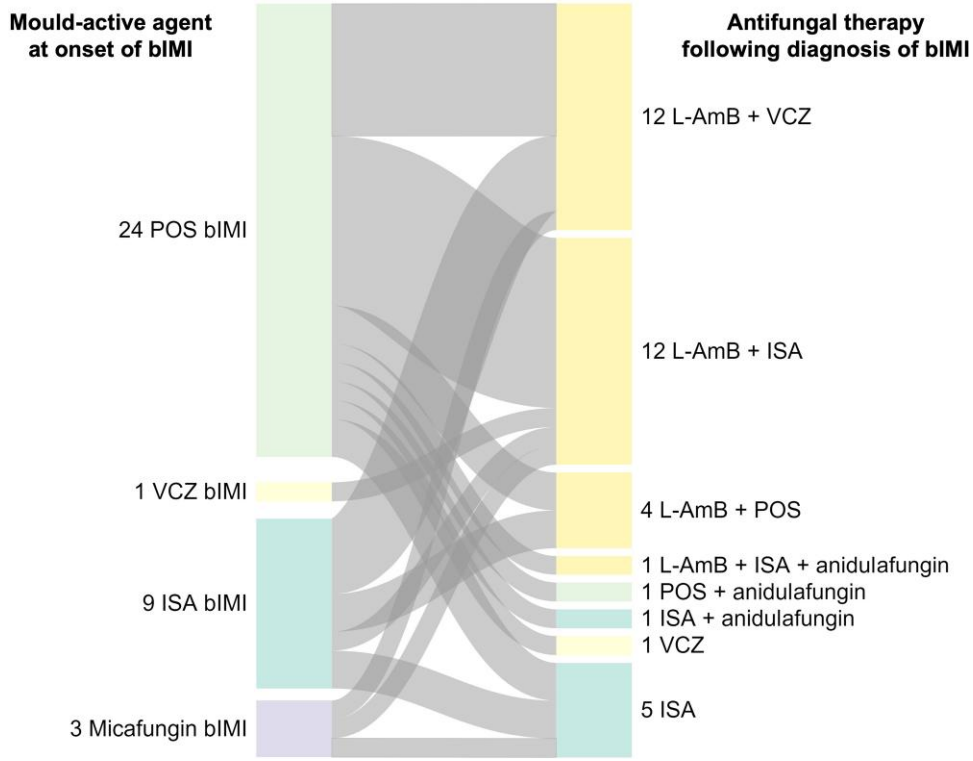


Figure 2. Sankey diagram depicting sequential modifications in antifungal therapy once bIMI was diagnosed according to the previous mold-active antifungal agent. Abbreviations: bIMI, breakthrough invasive mold infection; ISA, isavuconazole; L-AmB, liposomal amphotericin B; POS, posaconazole; VCZ, voriconazole.

pivotal randomized clinical trials were invasive aspergillosis, with a few cases of scedosporiosis [10, 11]. The type of antifungal exposure appears to determine the epidemiology of bIFI, with invasive aspergillosis due to cryptic species being more frequent with POS and mucormycosis with VCZ [24, 36]. Although some cryptic species within the *Fumigati* section show

Table 4. Patient Outcomes in the Study Cohort (n = 37)

	No. (%)
Clinical response	
By day +14	8 (21.6)
By day +30	24 (64.9)
All-cause mortality	
By day +14	7 (18.9)
By day +30	12 (32.4)
By day +100	17 (45.9)
Attributable mortality	
By day +14	7 (18.9)
By day +30	9 (24.3)
By day +100	10 (27.0)

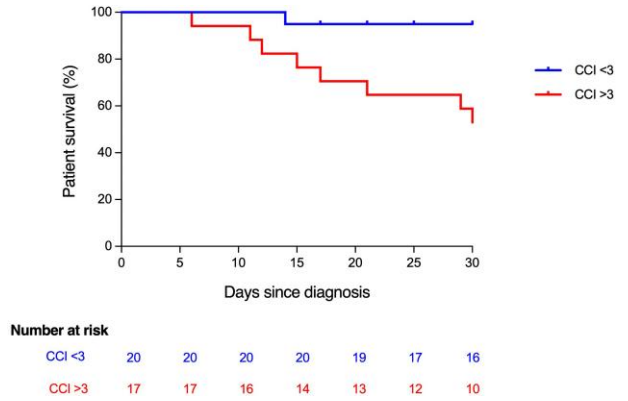


Figure 3. Kaplan-Meier curves for 30-day mortality attributable to breakthrough invasive mold infection according to the Charlson Comorbidity Index (CCI; log-rank test $P = .004$).

resistance to triazoles [37, 38], *A fumigatus* was predominant in the present cohort.

The diagnostic approach to bIFI is challenging, since the performance of cultures, serologic tests, and polymerase chain reaction–based assays in patients exposed to broad-spectrum antifungals is decreased [39]. In our cohort, only 32.4% of serum samples and 14.8% of BAL samples yielded a positive GM result. It should be highlighted that lung biopsy was usually diagnostic in the few cases in which this procedure was performed. A recent study also revealed a good diagnostic yield for lung biopsy [24]. In view of the difficulties for diagnosis of bIMI, especially in cases of mucormycosis or fusariosis, these results should encourage efforts aimed at obtaining histopathologic samples when possible.

The efficacy of POS-based prophylactic strategies is well established, with reported bIMI rates of 2.0% to 5.3% [10, 11]. In line with our results, the figures derived from observational studies ranged from 3.0% to 25% [13, 15, 21, 40]. However, VCZ, ISA, and equinocandins have been shown to be safe and effective second-line options in patients in which POS is hindered by pharmacokinetic issues, drug-drug interactions, or toxicity [41]. The incidence of bIFI with VCZ and ISA prophylaxis in observational series varies from 2.0% to 18% [12, 33, 42, 43]. In contrast to these triazoles, equinocandins exert a fungistatic action against *Aspergillus* but no activity against other molds. Proven or probable bIFIs during echinocandin prophylaxis have been reported in up to ~7.5% of patients [44]. A retrospective study found a significantly higher incidence rate of bIFIs with equinocandins as compared with mold-active triazoles (2.4 vs 1.1 cases per 1000 prophylaxis-days) [45]. In our cohort, the incidence of bIMI, taking into account the duration of exposure (ie, number of days of therapy), was numerically higher for micafungin, although the differences did not reach statistical significance.

Our results showed that patients with bIMI had a relevant burden of immunosuppression, such as high-intensity chemotherapy, previous allogeneic HSCT, and neutropenia. In fact, only 37.8% of the patients had complete or partial remission of their underlying hematologic disease at the time of bIMI. These findings point to an increase in the host's susceptibility due to impaired immune responses. Matsuo et al highlighted the importance of host factors, such as neutrophil recovery or achievement of complete remission, on the outcome of fusariosis [46]. Although we were not able to demonstrate the impact of absolute neutrophil count on outcome at day +7, higher ECOG score and CCI—2 variables reflecting increased patient frailty—were significantly associated with a poorer survival at day +30.

Attributable mortality by day +30 was 24.3%, whereas other studies have reported even higher mortality rates [33]. Interestingly, this rate did not substantially increase by day +100 (27.0%), in contrast to the clear rise in all-cause mortality between points (from 32.4% to 45.9%). This discordance may be explained by the fact that the outcome associated with such a severe complication as bIMI is better captured in the short term, whereas the effect of the underlying hematologic malignancy and the common delay or temporary discontinuation of chemotherapy upon diagnosis is more evident in the medium and long term.

Prompt initiation of appropriate antifungal treatment has a critical impact on the odds of successful outcome. Current guidelines and experts' opinion recommend switching the antifungal class after the diagnosis of bIFI, as this event should be considered a treatment failure. This approach is challenged by the fact that a high proportion of identified pathogens exhibit in vitro susceptibility to the baseline antifungal agent. Thus, some authors propose that maintaining the triazole used as prophylaxis may be also acceptable [13]. Nevertheless, as the prevalence of azole-resistant *Aspergillus* strains has increased in many centers worldwide, it seems reasonable to provide a broader spectrum of activity against azole-susceptible and

resistant fungi. We considered that combining empirical IV L-AmB with a switched broad-spectrum mold-active triazole might be the most suitable first-line empirical therapy when bIMI is suspected [14, 47]. It is tempting to hypothesize that this approach would at least partially account for the relatively good outcome observed in our cohort as compared with previous studies. We found no differences in mortality according to the therapy administered, although our study was likely underpowered due to the diversity of regimens and the low numbers in each group.

Other limitations must be acknowledged, the most significant being the retrospective and single-center design and the lack of a control group with non-bIMI or similar hematologic cases that did not include this complication. The small sample size and reduced number of attributable deaths prevented us from performing multivariate analysis. The lack of microbiological documentation in most episodes limits drawing definitive conclusions on the local epidemiology. This circumstance, however, reflects real-life practice. In the same line, TDM was not systematically performed, and antifungal susceptibility testing was available in only 4 episodes.

In conclusion, susceptible *Aspergillus* sp continues to be the predominant cause of bIMI among high-risk hematologic cases. When TDM was performed, triazole plasma levels were usually within the therapeutic range. The deleterious impact on outcomes shown for comorbidity burden, performance status, and stage of the underlying disease would point to host factors and impaired immune response as essential contributors to the pathogenesis of bIMI. When feasible, efforts should be made to accelerate immune reconstitution by tapering immunosuppression (ie, corticosteroids), as well as to carry out an aggressive diagnostic workup with prompt initiation of broad-spectrum antifungal therapy.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. All authors contributed to study conception and design. Material preparation, data collection, and analysis were performed by I. R.-G., J. B., R. C.-A., J. M. S.-P., M. L., and M. F.-R. Patient management was performed by I. R.-G., J. M. S.-P., M. L., M. F.-R., R. S.-J., F. L.-M., A. P.-A., J. M. C.-T., J. M.-L., and M. C. The first draft of the manuscript was written by I. R.-G. All the authors read and approved the final manuscript.

Data availability statement. The data that support the findings of this study are available upon reasonable request to the corresponding author.

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References

1. Pagano L, Busca A, Candoni A, et al. Risk stratification for invasive fungal infections in patients with hematological malignancies: SEIFEM recommendations. Blood Rev **2017**; 31:17–29.
2. Mori G, Diotallevi S, Farina F, et al. High-risk neutropenic fever and invasive fungal diseases in patients with hematological malignancies. Microorganisms **2024**; 12:117.
3. van de Peppel RJ, Dekkers OM, von dem Borne PA, de Boer MG. Relapsed and secondary disease drive the risk profile for invasive aspergillosis prior to stem cell transplantation in patients with acute myeloid leukemia or myelodysplastic syndrome. Med Mycol **2014**; 52:699–705.
4. Jenks JD, Cornely OA, Chen SC, Thompson GR 3rd, Hoenigl M. Breakthrough invasive fungal infections: who is at risk? Mycoses **2020**; 63:1021–32.
5. Rodriguez-Goncer I, Thomas S, Foden P, et al. Invasive pulmonary aspergillosis is associated with adverse clinical outcomes in critically ill patients receiving veno-venous extracorporeal membrane oxygenation. Eur J Clin Microbiol Infect Dis **2018**; 37:1251–7.
6. Yang J, Zhang J, Wei J, et al. Prolonged duration of lymphocyte deficiency, high-grade CRS, and ventilation are linked to fungal breakthrough in patients with hematologic malignancies 60 days after CAR-T infusion: a single center case-control study. J Infect Public Health **2022**; 15:1521–30.
7. Maertens JA, Girmenia C, Bruggemann RJ, et al. European guidelines for primary antifungal prophylaxis in adult haematology patients: summary of the updated recommendations from the European Conference on Infections in Leukaemia. J Antimicrob Chemother **2018**; 73:3221–30.
8. Halpern AB, Lyman GH, Walsh TJ, Kontoyiannis DP, Walter RB. Primary antifungal prophylaxis during curative-intent therapy for acute myeloid leukemia. Blood **2015**; 126:2790–7.
9. Bose P, McCue D, Wurster S, et al. Isavuconazole as primary antifungal prophylaxis in patients with acute myeloid leukemia or myelodysplastic syndrome: an open-label, prospective, phase 2 study. Clin Infect Dis **2021**; 72:1755–63.
10. Cornely OA, Maertens J, Winston DJ, et al. Posaconazole vs fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med **2007**; 356:348–59.
11. Ullmann AJ, Lipton JH, Vesole DH, et al. Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med **2007**; 356:335–47.
12. Khatri AM, Natori Y, Anderson A, et al. Breakthrough invasive fungal infections on isavuconazole prophylaxis in hematologic malignancy and hematopoietic stem cell transplant patients. Transpl Infect Dis **2023**; 25(suppl 1):e14162.
13. Scott SA, Perry C, Mahmoudjafari Z, et al. Incidence of breakthrough fungal infections on isavuconazole prophylaxis compared to posaconazole and voriconazole. Transpl Infect Dis **2023**; 25:e14045.
14. Lamoth F, Chung SJ, Damonti L, Alexander BD. Changing epidemiology of invasive mold infections in patients receiving azole prophylaxis. Clin Infect Dis **2017**; 64:1619–21.
15. Lerolle N, Raffoux E, Socie G, et al. Breakthrough invasive fungal disease in patients receiving posaconazole primary prophylaxis: a 4-year study. Clin Microbiol Infect **2014**; 20:O952–9.
16. Hong JY, Kang CI, Yang J, et al. Breakthrough invasive fungal infection in patients with myeloid malignancy receiving posaconazole tablet prophylaxis: clinical features, risk factors, and posaconazole profiles. Med Mycol **2023**; 61:myad046.
17. Chen X, Wang J, Wang S, et al. Real-world assessment of the effectiveness of posaconazole for the prophylaxis and treatment of invasive fungal infections in hematological patients: a retrospective observational study. Medicine (Baltimore) **2021**; 100:e26772.
18. Jenks JD, Reed SL, Seidel D, et al. Rare mould infections caused by *Mucorales, Lomentospora prolificans* and *Fusarium*, in San Diego, CA: the role of antifungal combination therapy. Int J Antimicrob Agents **2018**; 52:706–12.
19. Weeraphon B, Nakaranurack C, Jutivorakool K, Puttilerpong C. Epidemiology and factors associated with treatment success of invasive fungal infections among newly hematologic malignancy patients receiving chemotherapy or hematopoietic stem cell transplant in Thailand. Infect Drug Resist **2023**; 16:2029–42.
20. Jenks JD, Seidel D, Cornely OA, et al. Voriconazole plus terbinafine combination antifungal therapy for invasive *Lomentospora prolificans* infections: analysis of 41 patients from the FungiScope registry 2008–2019. Clin Microbiol Infect **2020**; 26: 784.e1–5.
21. Auberger J, Lass-Florl C, Aigner M, Clausen J, Gastl G, Nachbaur D. Invasive fungal breakthrough infections, fungal colonization and emergence of resistant

strains in high-risk patients receiving antifungal prophylaxis with posaconazole: real-life data from a single-centre institutional retrospective observational study. J Antimicrob Chemother **2012**; 67:2268–73.
22. Biehl LM, Vehreschild JJ, Liss B, et al. A cohort study on breakthrough invasive fungal infections in high-risk patients receiving antifungal prophylaxis. J Antimicrob Chemother **2016**; 71:2634–41.
23. Corzo-Leon DE, Satlin MJ, Soave R, et al. Epidemiology and outcomes of invasive fungal infections in allogeneic haematopoietic stem cell transplant recipients in the era of antifungal prophylaxis: a single-centre study with focus on emerging pathogens. Mycoses **2015**; 58:325–36.
24. Puerta-Alcalde P, Monzo-Gallo P, Aguilar-Guisado M, et al. Breakthrough invasive fungal infection among patients with haematologic malignancies: a national, prospective, and multicentre study. J Infect **2023**; 87:46–53.
25. Donnelly JP, Chen SC, Kauffman CA, 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**; 71:1367–76.
26. Cornely OA, Hoenigl M, Lass-Florl C, et al. Defining breakthrough invasive fungal infection: position paper of the Mycoses Study Group Education and Research Consortium and the European Confederation of Medical Mycology. Mycoses **2019**; 62:716–29.
27. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol **2003**; 21:4642–9.
28. Younes A, Hilden P, Coiffier B, et al. International Working Group consensus response evaluation criteria in lymphoma (RECIL 2017). Ann Oncol **2017**; 28: 1436–47.
29. Kumar S, Paiva B, Anderson KC, et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol **2016**; 17:e328–46.
30. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. J Chronic Dis **1987**; 40:373–83.
31. Verweij PE, Chowdhary A, Melchers WJ, Meis JF. Azole resistance in *Aspergillus fumigatus:* can we retain the clinical use of mold-active antifungal azoles? Clin Infect Dis **2016**; 62:362–8.
32. Winston DJ, Bartoni K, Territo MC, Schiller GJ. Efficacy, safety, and breakthrough infections associated with standard long-term posaconazole antifungal prophylaxis in allogeneic stem cell transplantation recipients. Biol Blood Marrow Transplant **2011**; 17:507–15.
33. Kim SB, Cho SY, Lee DG, et al. Breakthrough invasive fungal diseases during voriconazole treatment for aspergillosis: a 5-year retrospective cohort study. Med Mycol **2017**; 55:237–45.
34. Fontana L, Perlin DS, Zhao Y, et al. Isavuconazole prophylaxis in patients with hematologic malignancies and hematopoietic cell transplant recipients. Clin Infect Dis **2020**; 70:723–30.
35. Chan TS, Gill H, Hwang YY, et al. Breakthrough invasive fungal diseases during echinocandin treatment in high-risk hospitalized hematologic patients. Ann Hematol **2014**; 93:493–8.
36. Imbert S, Cassaing S, Bonnal C, et al. Invasive aspergillosis due to *Aspergillus* cryptic species: a prospective multicentre study. Mycoses **2021**; 64:1346–53.
37. Imbert S, Normand AC, Cassaing S, et al. Multicentric analysis of the species distribution and antifungal susceptibility of cryptic isolates from *Aspergillus* section *Fumigati*. Antimicrob Agents Chemother **2020**; 64:e01374-20.
38. Escribano P, Rodriguez-Sanchez B, Diaz-Garcia J, et al. Azole resistance survey on clinical *Aspergillus fumigatus* isolates in Spain. Clin Microbiol Infect **2021**; 27: 1170.e1–7.
39. Duarte RF, Sanchez-Ortega I, Cuesta I, et al. Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. Clin Infect Dis **2014**; 59:1696–702.
40. Pagano L, Caira M, Candoni A, et al. Evaluation of the practice of antifungal prophylaxis use in patients with newly diagnosed acute myeloid leukemia: results from the SEIFEM 2010-B registry. Clin Infect Dis **2012**; 55:1515–21.
41. van Burik JA, Ratanatharathorn V, Stepan DE, et al. Micafungin versus fluconazole for prophylaxis against invasive fungal infections during neutropenia in patients undergoing hematopoietic stem cell transplantation. Clin Infect Dis **2004**; 39:1407–16.
42. Trifilio S, Singhal S, Williams S, et al. Breakthrough fungal infections after allogeneic hematopoietic stem cell transplantation in patients on prophylactic voriconazole. Bone Marrow Transplant **2007**; 40:451–6.
43. Nguyen MH, Ostrosky-Zeichner L, Pappas PG, et al. Real-world use of mold-active triazole prophylaxis in the prevention of invasive fungal diseases: results from a subgroup analysis of a multicenter national registry. Open Forum Infect Dis **2023**; 10:ofad424.
44. Morris SK, Allen UD, Gupta S, Richardson SE. Breakthrough filamentous fungal infections in pediatric hematopoetic stem cell transplant and oncology patients receiving caspofungin. Can J Infect Dis Med Microbiol **2012**; 23:179–82.
45. Gomes MZ, Mulanovich VE, Jiang Y, Lewis RE, Kontoyiannis DP. Incidence density of invasive fungal infections during primary antifungal prophylaxis in newly diagnosed acute myeloid leukemia patients in a tertiary cancer center, 2009 to 2011. Antimicrob Agents Chemother **2014**; 58:865–73.
46. Matsuo T, Wurster S, Kontoyiannis DP. Good outcomes in salvage therapy of fusariosis in patients with leukemia: is it the host or the drug? Clin Infect Dis **2024**; 79:571–2.
47. Lionakis MS, Lewis RE, Kontoyiannis DP. Breakthrough invasive mold infections in the hematology patient: current concepts and future directions. Clin Infect Dis **2018**; 67:1621–30.