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Breakthrough invasive fungal infection among patients with haematologic malignancies: A national, prospective, and multicentre study

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SUMMARY

Objectives: We describe the current epidemiology, causes, and outcomes of breakthrough invasive fungal infections (BtIFI) in patients with haematologic malignancies.
Methods: BtIFI in patients with ≥ 7 days of prior antifungals were prospectively diagnosed (36 months across 13 Spanish hospitals) according to revised EORTC/MSG definitions.
Results: 121 episodes of BtIFI were documented, of which 41 (33.9%) were proven; 53 (43.8%), probable; and 27 (22.3%), possible. The most frequent prior antifungals included posaconazole (32.2%), echinocandins (28.9%) and fluconazole (24.8%)—mainly for primary prophylaxis (81%). The most common haematologic malignancy was acute leukaemia (64.5%), and 59 (48.8%) patients had undergone a hematopoietic stem-cell transplantation. Invasive aspergillosis, principally caused by non-*fumigatus Aspergillus*, was the most frequent BtIFI with 55 (45.5%) episodes recorded, followed by candidemia (23, 19%), mucormycosis (7, 5.8%), other moulds (6, 5%) and other yeasts (5, 4.1%). Azole resistance/non-susceptibility was commonly found. Prior antifungal therapy widely determined BtIFI epidemiology. The most common cause of BtIFI in proven and probable cases was the lack of activity of the prior antifungal (63, 67.0%). At diagnosis, antifungal therapy was mostly changed (90.9%), mainly to liposomal amphotericin-B (48.8%). Overall, 100-day mortality was 47.1%; BtIFI was either the cause or an essential contributing factor to death in 61.4% of cases.
Conclusions: BtIFI are mainly caused by non-*fumigatus Aspergillus*, non-*albicans Candida*, Mucorales and

Abbreviations: BtIFI, Breakthrough invasive fungal infection; ICU, Intensive care unit; SD, Standard deviation; IQR, Interquartile range; CT, Computed tomography
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other rare species of mould and yeast. Prior antifungals determine the epidemiology of BtIFI. The exceedingly high mortality due to BtIFI warrants an aggressive diagnostic approach and early initiation of broad-spectrum antifungals different than those previously used.

Introduction

Breakthrough invasive fungal infections (BtIFI) have increased in patients with haematologic malignancies due to the widespread use of antifungal treatment as prophylaxis, pre-emptive and targeted therapy. However, information on patients with BtIFI is scarce, even though challenges presented by this type of infection are significant.[1–7] For instance, the epidemiology of fungi causing BtIFI has not been well established. It is easy to hypothesise that rare fungi and high antifungal resistance may become predominant in this setting. Secondly, there is a lack of definitive characterisation for why these infections occur. Thirdly, sensitivity of some microbiological diagnostic tests may be significantly lower in patients receiving antifungal treatment, and improved diagnostic strategies have yet to be established. Fourthly, the paucity of randomised clinical trials does not allow for clearer guidance concerning empirical and/or definitive antifungal therapy in this setting. Lastly, outcomes of these infections have been poorly described.

With the aim to address these challenges, we describe the current epidemiology, clinical and diagnostic characteristics, causes of infection, antifungal susceptibility, and outcomes of BtIFI in a large and real-life cohort of patients with haematologic malignancies in Spain.

Methods

Patients, setting, data collection and study design

This is a prospective, multicentre cohort study conducted across 13 Spanish university hospitals. We prospectively recorded all BtIFI episodes in adult patients (aged ≥18 years) with haematologic malignancies during a 36-month period (September 2017 – September 2020). We obtained the following data for all patients: age and sex, pre-existing co-morbidities, baseline haematologic malignancy, prior antifungal therapy, prior surgery (within the last month), immunosuppressive drugs, corticosteroid treatment, leucocyte count, causative agent, intensive care unit (ICU) admission, the need for mechanical ventilation, empirical and definitive antifungal treatment, and mortality. All data gathered were anonymously registered in a specific database designed for this study.

Management regarding fungal infection surveillance (i.e., biomarkers performance) or whenever a BtIFI was suspected (i.e., CT chest and/or bronchoscopy performance, need for invasive procedures, etc.) relied on each centre's standard of care and/or the clinical judgement of the responsible physician.

Definitions

BtIFI was defined as that occurring in patients with ≥ 7 days of current antifungal treatment when there was first clinical suspicion of IFI (due to symptoms, radiological findings, and/or positive biomarkers). IFI was defined according to the revised EORTC/MSG definitions.[8] Empirical antifungal therapy was defined as that initiated when there was clinical suspicion of BtIFI. The source of infection was determined by an infectious disease specialist who had evaluated the patient's medical history, performed a physical examination, and assessed results obtained from microbiological tests and complementary imaging. Neutropenia was defined as an absolute neutrophil count of < 500 cells/mm^3. Prior viral infection, and prior intensive care unit (ICU) admission were defined as that occurring within the 30 days before a BtIFI diagnosis. Prior corticosteroid use was defined as a minimum dose of 0.3 mg/kg/day of prednisone equivalent for > 3 weeks. Prior fungal infection was considered independently of time until current episode. Appropriateness of empirical antifungal therapy was based on international guidelines/consensus.[9–13]

Microbiological methods

The microbiological diagnoses performed throughout the whole cohort were similar. The blood samples were processed using either a BACTEC 9240 system (Becton–Dickinson Microbiology Systems, Franklin Lakes, NJ, USA) or BacTAlert (BioMérieux SA, Marcy L′Etoile, France) for a 5-day incubation period. If fungal cells were observed after microscopic examination of the Gram staining, blood bottles were sub-cultured into Sabouraud agar plates (BD BBL StrackerTM PlatesTM, Heidelberg, Germany) and chromogenic media (ChromAgar BioMerieux SA, Paris, France). Respiratory sample cultures were done using Sabouraud dextrose and BHI (Brain Heart Infusion) agar. Fungal isolates were identified by conventional methods (MALDI-TOF or pan-fungal PCR and sequencing). *In vitro* antifungal activity was studied in some centres by employing either a commercial microdilution method (YeastOne Sensititre, TREK Diagnostic Systems, Independence, Ohio) or an Etest (bioMérieux SA, Marcy L′Etoile, France), and each centre classified the MIC according to their standards. In those strains that were available, antifungal susceptibility was confirmed at the Spanish National Centre for Microbiology by EUCAST reference methods 7.3.2 and 9.4 and available breakpoints were used to define resistance. When breakpoints are not available and in order to ease the interpretation of results, we classified the strains as: resistant (R), when they are considered intrinsically resistant or when there is a breakpoint available for a very closely related species (e.g., *Candida orthopsilosis* and *C. parapsilosis*); and as non-susceptible (NS), when the species has intrinsically intermediate MICs to the drug and/or there is insufficient evidence that the species is a good target for the compound in question (e.g., *Candida glabrata* and azoles).

Galactomannan antigen (GM) testing was performed using Platelia™ Aspergillus (Bio-Rad Laboratories), with a cut-off value of ≥ 0.5 in serum and ≥ 1.0 in bronchoalveolar lavage.

Statistical analysis

Categorical variables were described as counts and percentages, whereas continuous variables were expressed as either means and standard deviations (SD) or medians and interquartile ranges (IQRs) as appropriate. The chi-squared Pearson test and either the Mann-Whitney U-test or t-student test were used to compare the categorical and continuous variable distributions, respectively. Kaplan Meier survival curves compared mortality regarding different variables using the log rank test. All analyses were performed with SPSS software (version 25.0; SPSS, Inc., Chicago, IL).

Ethics approval

This observational study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee Board of Hospital Clinic of Barcelona (HCB/2017/0532). To protect personal privacy, identifiable information in the electronic database was encrypted for each patient. Informed consent was waived, as no intervention was involved, and no patient-identifiable information was included.

Results

Cohort characteristics and prior antifungal therapy

We identified 121 BtIFI episodes during the study period. Table 1 shows patients' demographic characteristics and predisposing factors for fungal infection. Table 2 details characteristics of prior antifungal therapy. The most frequent prior antifungals were posaconazole (32.2%), echinocandins (28.9%) and fluconazole (24.8%).

Table 1
Patient demographic characteristics and predisposing factors for fungal infections.

	ALL EPISODES N = 121 (%)
Demographics	
Age, median (IQR) years	59 (47.5–64)
Male sex	68 (56.2)
Underlying haematologic disease	
Acute myeloid leukaemia	67 (55.4)
Myelodysplastic syndrome	13 (10.7)
Non-Hodgkin's lymphoma	12 (9.9)
Acute lymphoblastic leukaemia	11 (9.1)
Other[a]	18 (14.9)
Hematopoietic stem cell transplantation	59 (48.8)
Allogenic	51 (42.1)
Autologous	8 (6.6)
Comorbidities	
Diabetes mellitus	9 (7.4)
Chronic heart disease	9 (7.4)
Chronic kidney disease	11 (9.1)
Chronic pulmonary disease	11 (9.1)
Solid organ transplantation	2 (1.7)
Solid neoplasm[b]	12 (9.9)
Any comorbidity	43 (35.5)
Predisposing factors	
Central venous catheter	103 (85.1)
Total parenteral nutrition (the last three months)	37 (30.6)
ICU admission (the last 30 days)	24 (19.8)
Prior documented viral infection (the last 30 days)[c]	28 (23.1)
Neutropenia (< 500/mm^3)	83 (68.6)
Prior chemotherapy (the last 30 days)	84 (69.4)
Prior corticosteroid use	66 (54.5)
Other immunosuppressive agents	66 (54.5)
Graft-vs-host disease	17 (14)
Grade III/IV graft-vs-host disease	11 (9.1)
Prior fungal infection[d]	14 (11.6)

Abbreviations. IQR: interquartile range; ICU: intensive care unit.
[a] Including four patients with chronic lymphocytic leukaemia, three with Hodgkin's lymphoma, three with hemophagocytic lymphohistiocytosis, two with chronic myeloid leukaemia, two with plasmatic cells leukaemia, two with aplastic anaemia, one with multiple myeloma, and one with amyloidosis.
[b] Only one of the twelve patients with solid neoplasm had an active oncological disease, while all others experienced a complete response after treatment.
[c] Including 11 cases of cytomegalovirus; four, herpes simplex virus; three, influenza virus; two, Epstein-Barr virus; two, syncytial respiratory virus; one, BK virus; and five, non-specified.
[d] Including eight cases of previous invasive aspergillosis; four, candidemia; one, mucormycosis; and one, non-specified fungal infections.

Table 2
Prior antifungal therapy.

	ALL EPISODES N = 121 (%)
Prior antifungal[a]	
Posaconazole[b]	39 (32.2)
Echinocandins	35 (28.9)
Micafungin	21 (17.4)
Anidulafungin	8 (6.7)
Caspofungin	6 (5)
Fluconazole	30 (24.8)
Amphotericin B regimen[c]	12 (9.9)
Isavuconazole	6 (5)
Voriconazole	6 (5)
Inhaled amphotericin-B[d]	3 (2.4)
Indication for prior antifungal therapy	
Primary prophylaxis	98 (81)
Pre-emptive treatment	11 (9.1)
Secondary prophylaxis	12 (9.9)
Median (IQR) days of prior antifungal treatment	20 (11–30.5)
Therapeutic drug monitoring when prior antifungal was posaconazole or voriconazole (n = 45)	16 (35.6)

Abbreviations. IQR: interquartile range; ICU: intensive care unit.
[a] Ten (8.1%) patients received an antifungal combination. 7 patients received an echinocandin combined with inhaled amphotericin-B (3), amphotericin lipid complex (1), isavuconazole (1), voriconazole (1), and posaconazole (1). 3 additional patients received liposomal amphotericin-B combined with posaconazole (2), and voriconazole (1).
[b] Including 33 patients receiving oral posaconazole tablets and 6 patients receiving intravenous formulations. All received a loading dose of 300 mg/12 h followed by 300 mg daily.
[c] Including eight patients who received liposomal amphotericin-B and four patients who received amphotericin lipid complex. Doses for liposomal amphotericin B were: 3 mg/kg/d in four patients; 1 mg/kg/d, two patients; 1 mg/kg every 48 h, one patient; and 1.5 mg/kg three times a week, one patient.
[d] In all three cases, inhaled amphotericin-B was combined with an echinocandin.

BtIFI diagnosis and epidemiology

Table 3 describes the epidemiology of BtIFI episodes. Invasive aspergillosis was the most frequently diagnosed BtIFI (45.5% of all episodes), followed by invasive candidiasis (19%) and mucormycosis (5.8%). There were four BtIFI caused by two different mould species. Supplementary Table S1 details isolated species and antifungal susceptibility to prior antifungal. Remarkably, 62.1% of isolated *Aspergillus* were non-*fumigatus*, and 86.9% of *Candida* species were non-*albicans*. A total of 41 (33.9%) episodes fulfilled criteria for proven BtIFI, 53 (43.8%) for probable, and 27 (22.3%) for possible. Proven BtIFI diagnosis was performed by one or more of the following: fungal isolation in blood culture in 28 (68.3%) cases (23 *Candida* spp., 2 *Geotrichum* spp., 1 *Trichosporon asahii*, 1 *Rhodotorula mucilaginosa*, and 1 *Magnusiomyces capitatus*); positive culture of a sterile site with clinical or radiological significance in 8 (19.5%) cases (2 *F. solani*, 1 *Rhizopus* spp., 1 *A. flavus*, 1 *Cunninghamella* spp., 1 *A. niger*, 1 *C. krusei*, and 1 *C. guilliermondii*); and histopathological findings of a sterile specimen in 7 (17.1%) cases (4 Mucorales, 1 *A. flavus*, 1 *A. fumigatus* [later identified through molecular techniques], and 1 unidentified mould). All 53 probable BtIFI were aspergillosis except for two cases of scedosporiosis and paecilomycosis, one each, and one mixed *A. niger* and *Purpureocillium lilacinum* infection. Supplementary Table S2 refers to the diagnostic characteristics of probable BtIFI episodes. In 27 (52.9%) probable invasive aspergillosis cases, microbiological diagnosis relied on a positive galactomannan, but cultures and/or molecular diagnosis were negative. Finally, most episodes of possible BtIFI (23 of 27, 85.2%) had a suggestive thoracic CT scan with no microbiological findings.

Among BtIFI episodes caused by mould species, 81.6% were pulmonary infections; 6.9%, sino-nasal; and 11.5%, disseminated. Supplementary Table S3 outlines the radiological characteristics of mould-causing episodes with pulmonary involvement (n = 86).

Table 3
BtIFI diagnosis, site of infection, and microbiological results.

	ALL EPISODES N = 121 (%)
IFI classification	
Proven	41 (33.9)
Probable	53 (43.8)
Possible	27 (22.3)
Diagnosed IFI classified as Proven or Probable[a]	94 (77.7)
Invasive aspergillosis[b]	55 (45.5)
Candidemia[c]	23 (19)
Mucormycosis[d]	7 (5.8)
Other mould infections[e]	6 (5)
Other fungemias[f]	5 (4.1)
IFI site	
Pulmonary	71 (58.7)
Disseminated	43 (35.5)
Sinonasal infection	6 (5)
CNS infection	1 (0.8)
Source of fungemia (n = 30)	
Unknown source	13 (43.3)
Catheter-related	11 (36.7)
Abdominal source	5 (16.7)
Urinary source	1 (3.3)
Microbiological results	
Positive galactomannan antigen in plasma	32 (26.4)
Mean (SD) galactomannan value in plasma[g]	3.14 (2.40)
Positive galactomannan antigen in bronchoalveolar lavage	33 (27.3)
Mean (SD) galactomannan value in bronchoalveolar lavage[g]	4.09 (2.95)
Positive culture or PCR	65 (53.7)
Positive pan-fungal PCR	5 (4.1)
Antifungal susceptibility to a prior antifungal in isolated species (n = 48)	
Susceptible	8 (16.7)
Resistant/Non-susceptible	40 (83.3)

Abbreviations. IQR: Abbreviations: interquartile range; ICU: intensive care unit.
[a] Including four mixed BtIFI: one, *A. fumigatus* + *A. niger*; one, *A. fumigatus* + *A. alliaceus*; one, *A. fumigatus* + *Lichtheimia* spp.; and one, *A. niger* + *Purpureocillium lilacinum*.
[b] Including eleven cases of *A. fumigatus*; seven, *A. terreus*; four, *A. flavus*; four, *A. niger*; one, *A. ustus*; one, *A. alliaceus*; and one *A. hiratsukae*. The other probable aspergillosis cases were diagnosed following positive galactomannan antigen but had no microbiological isolation.
[c] Including six cases of *C. krusei*; five, *C. parapsilosis*; four, *C. glabrata*; three, *C. albicans*; two, *C. guilliermondii*; one, *C. tropicalis*; one, *C. orthopsilosis*; and one, *C. kefyr*.
[d] Including two cases of *Lichtheimia* spp.; two, *Rhizopus* spp.; two, *Rhizomucor* spp.; and one, *Cunninghamella* spp.
[e] Including two patients with *Fusarium solani*; one, *Paecilomyces* spp; one, *Purpureocillium lilacinum*; one, *Scedosporium* spp.; and one, non-identified mould.
[f] Including two cases of *Geotrichum* spp.; one, *Trichosporon asahii*; one, *Rhodotorula mucilaginosa*; and one, *Magnusiomyces capitatus*.
[g] Among those that were positive.

Macronodule (54.2%), consolidation or mass (51.8%), halo sign (45.8%) and ground-glass opacities (67.5%) were the most common findings.

Fig. 1 details BtIFI epidemiology per prior antifungal treatment in those episodes classified as proven or probable (n = 94). Remarkably, invasive aspergillosis was commonly found after posaconazole; mucormycosis was mainly observed among patients receiving voriconazole previously.

Causes of BtIFI

The most frequent cause of fungal disease in the 94 proven or probable cases of BtIFI was the poor activity of the administered antifungal (63 cases, 67.0%). Of those, 48 (51.0%) patients had an infection caused by a fungus either resistant or non-susceptible to the antifungal drug given to the patient. Specifically, the diagnosed fungi were intrinsically resistant to the prior antifungal in 27 (28.7%) cases; antifungal non-susceptibility or resistance was documented in 9 (9.6%) and 12 (12.8%) additional cases, respectively, following a positive culture. Furthermore, echinocandins have limited activity against *Aspergillus* spp. We documented 15 (16%) patients receiving these drugs and presenting breakthrough aspergillosis, despite no documented *in vitro* antifungal resistance. We did not document any case of azole-resistant *A. fumigatus.*

The presence of a factor favouring/perpetuating the infection—in particular, an intravenous catheter—was the cause of 7 (7.4%) episodes of breakthrough fungemia. Inappropriate antifungal dosage/levels were the potential cause of BtIFI in 4 (4.3%) patients, of which three received 1 mg/kg/day of amphotericin-B and one, had documented sub-therapeutic voriconazole levels.

The potential cause of BtIFI could not be confirmed in 20 (21.3%) patients; 15 had received posaconazole; 3, isavuconazole; 1, liposomal amphotericin-B; and 1, liposomal amphotericin-B combined with posaconazole. In four patients who received prior posaconazole, two episodes of invasive aspergillosis caused by *A. niger* and *A. terreus,* and one paecilomycosis, and one mucormycosis caused by *Rhizopus* spp., were documented. None had antifungal susceptibility testing available. The other 11 patients receiving posaconazole were diagnosed with probable invasive aspergillosis following positive galactomannan results and a compatible CT scan; however, no fungi were identified. Five of these patients had optimal serum drug levels documented (all were ≥0.85 mg/L), while therapeutic drug monitoring was not performed in the other 10 cases. Two patients receiving prior isavuconazole developed probable infections with positive culture by *Aspergillus fumigatus* complex and *Scedosporium* spp., respectively; no antifungal susceptibility testing was available. One additional patient receiving isavuconazole was diagnosed with probable aspergillosis following positive galactomannan results and a compatible CT scan; however, no fungi were identified. One patient receiving prior liposomal amphotericin-B developed *A. flavus*-causing invasive aspergillosis, identifiable by culture and without antifungal susceptibility testing. Finally, one patient receiving a combination of liposomal amphotericin-B and posaconazole presented probable aspergillosis following positive galactomannan results and a compatible CT scan. No posaconazole levels were available.

BtIFI treatment and outcomes

Table 4 shows antifungal therapy and outcomes of BtIFI episodes. Fifteen (12.4%) episodes received inappropriate empirical antifungal therapy (IEAT). There was a trend for higher prevalence of IEAT in patients receiving empirical echinocandins (21.9% vs 9%, p = 0.058). In patients receiving amphotericin-B-based therapies, IEAT tended to be less frequent (8.5% vs 16.1%, p = 0.202). There were no differences in mortality regarding the change in the antifungal family.

Additionally, 100-day mortality was 47.1%, with BtIFI either being the cause of or playing an essential role in the death of 61.4% of cases. We observed a higher mortality trend in those episodes receiving IEAT (66.7% vs 44.3%, p = 0.105). Fig. 2 displays Kaplan-Meier survival curves at 180 days following BtIFI diagnosis. The highest mortality was seen in cases of mucormycosis and BtIFI caused by other species of rare yeast.

Discussion

The current study describes the epidemiology of BtIFI in a large cohort of patients with haematologic malignancies and focuses on the potential causes underlying this type of breakthrough infection to prior antifungals. The most important findings were: 1) posaconazole and echinocandins comprised the most frequent prior antifungals in current patients with haematologic malignancies presenting BtIFI; 2) invasive aspergillosis remains the most common BtIFI, followed by candidiasis and mucormycosis; however, other rare species of moulds and yeasts are commonly found; 3) prior

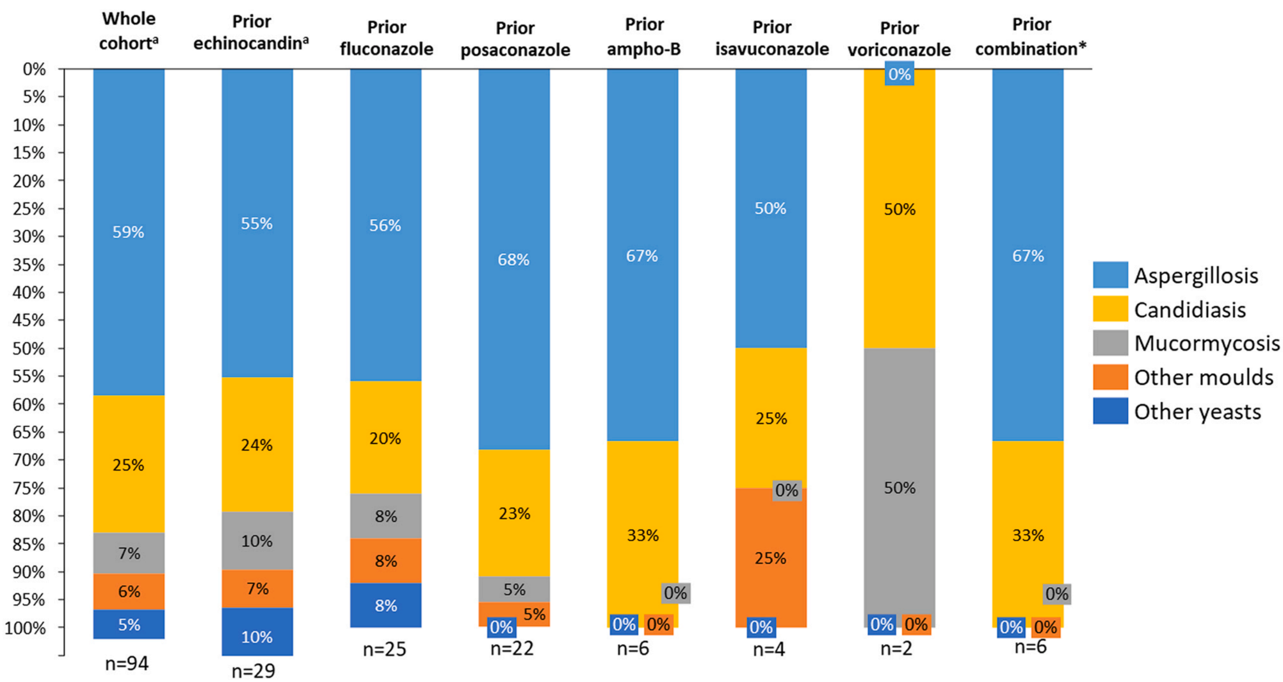

Fig. 1. Breakthrough fungal infection epidemiology per prior antifungal treatment in proven and probable cases. The patients receiving prior combinations presented the following breakthrough infections. Echinocandin plus posaconale (n = 1) = 1 invasive candidiasis; echinocandin plus liposomal amphotericin-B (n = 1), isavuconazole (n = 1) or voriconazole (n = 1) = 3 invasive aspergillosis; liposomal amphotericin B plus posaconazole (n = 1) = 1 invasive aspergillosis; and liposomal amphotericin B plus voriconazole (n = 1) = invasive candidiasis. [a]These percentages add up to more than 100 due to the presence of mixed infections: 1 mixed aspergillosis + mucormycosis, and 1 mixed aspergillosis + *Purpureocillium lilacinum* infection.

antifungal therapy widely determines BtIFI epidemiology; 4) molecular testing of biopsy samples identifies a high number of non-*Aspergillus* moulds, yet positive culture and/or galactomannan results are still the basis for a high number of diagnoses; 5) most BtIFI episodes occur due to a lack of activity of the prior antifungal, either intrinsic or acquired; 6) remarkably, in our series, we could not isolate any fungus susceptible to the prior administered antifungal administered at good therapeutic levels, except for some catheter-related fungemia; 7) 100-day mortality is exceedingly high in patients suffering a BtIFI, especially in proven cases and mucormycosis episodes.

Currently, most patients with high-risk haematologic malignancies receive antifungal prophylaxis with posaconazole and echinocandins when treated with intensive chemotherapies. This is due to the fact that some trials showed a decrease in IFI rates.[14–17] Consequently, most BtIFI episodes occur after the use of these drugs. Of note, prior antifungal conditioned BtIFI epidemiology, with invasive aspergillosis and mucormycosis being more frequent after posaconazole and voriconazole, respectively.

In our cohort, invasive aspergillosis was the most frequent BtIFI. Remarkably, over 80% of breakthrough aspergillosis cases had positive galactomannan results, even though fungal biomarker sensitivity has been reported to be possibly lower in patients receiving prior antifungals.[18,19] Of the 27 patients with a positive *Aspergillus* culture, 62% had an infection caused by a non-*fumigatus* species. These results represent a notable change in the aspergillosis species epidemiology compared with previous studies.[20,21] Additionally, moulds different from *Aspergillus* spp. caused a high percentage of proven infections in our series. Similar results have been previously reported in small case series.[4,5,22–26]

Regarding invasive candidiasis (mostly candidemia), approximately 90% of the isolated species in our study were non-*albicans* and most of them were azole resistant/non-susceptible. Some reports have previously shown this shift to non-*albicans* species in relation to the widespread introduction of antifungal prophylaxis.[27,28] Nevertheless, none of such studies found the high rates of azole non-susceptibility, and non-*albicans* species, described in the current cohort. Finally, it is not surprising to have found many fungemia due to rare yeasts (e.g., *Geotrichum* spp., *Trichosporon asahii,* etc.) since these are intrinsically resistant to commonly used echinocandins.

The use of diagnostic tests in BtIFI is challenging. In this cohort of patients, proven infections caused by yeasts were diagnosed by positive blood cultures. Remarkably, 7 of the 11 proven mould infections diagnosed following a tissue biopsy (≈64%) were caused by a Mucoral. In our opinion, despite the risk of tissue biopsy in patients commonly unstable and thrombocytopenic, these results suggest that the puncture of lung nodes plays an extremely important role in establishing the causative agent of a breakthrough episode. Considering information from proven BtIFI episodes, real BtIFI epidemiology might be substantially different should a more aggressive diagnostic approach be conducted. Also, the advent of molecular microbiological diagnoses[29,30] and novel immunological markers[31,32] will help us to better understand the complex landscape of BtIFI epidemiology.

The main cause of BtIFI was the lack of activity of prior antifungals, either intrinsic or acquired. For this reason, it is essential to try to reach an aetiological diagnosis of BtIFI, and perform antifungal susceptibility testing, to be able to offer the best possible, subsequent early treatment. This fact reinforces the change in antifungal family in case of BtIFI suspicion.[9] Other frequent causes of BtIFI were the lack of source control, mainly in yeasts breakthroughs, and presumably low antifungal levels. It is worth noting that even in the framework of a study project, azole therapeutic drug monitoring was hardly performed despite potential treatment failure and subsequent BtIFI. In most of the cases determined, though, the levels were correct.

Prognosis for BtIFI episodes was very poor; 100-day mortality reached 47.1%, and most deaths were secondary to the fungal infection. Similar mortality rates have been reported in some other

Table 4
Antifungal therapy and outcomes of invasive fungal infection episodes.

	ALL EPISODESN= 121 (%)
Antifungal change after BtIFI suspicion/ diagnosis	110 (90.9)
Change of antifungal class after BtIFI suspicion/diagnosis[a]	97 (80.2)
Empirical antifungal therapy	
Liposomal amphotericin-B	59 (48.8)
Voriconazole	36 (29.8)
Echinocandins	32 (26.4)
Posaconazole	9 (7.4)
Isavuconazole	7 (5.8)
Fluconazole	1 (0.8)
Empirical antifungal combination	23 (19)
Definitive antifungal therapy[b]	
Voriconazole-containing regimen[c]	52 (43)
Liposomal amphotericin-B-containing regimen[d]	44 (36.4)
Echinocandin-containing regimen[e]	37 (30.6)
Isavuconazole-containing regimen[f]	22 (18.2)
Posaconazole-containing regimen[g]	15 (12.4)
Fluconazole-containing regimen[h]	5 (4)
Amphotericin-B lipid complex	1 (0.8)
Definitive antifungal combination	34 (28.1)
Management/evolution of patients with fungemia (n = 30)	
Persistent fungemia at 48 h	8 (26.7)
Ophthalmoscopic evaluation	14 (46.7)
Secondary septic metastases	4 (13.3)
Catheter removal	28 (93.3)
Other source control procedures	3 (10)
Echocardiography performance	11 (36.7)
Overall outcomes	
Inappropriate empirical antifungal therapy	15 (12.4)
ICU requirement	33 (27.3)
Invasive mechanical ventilation requirement	24 (19.8)
Clinical IFI response at 100 days[i]	
Complete response	48 (39.7)
Partial response	23 (19)
Stable infection	13 (10.7)
Fungal infection progression	37 (30.6)
100-day mortality	57 (47.1)
IFI was the cause of the death	16 (28.1)
IFI had an essential role in the death	19 (33.3)
IFI had a secondary role in the death	12 (21.1)
Death was unrelated to the IFI	10 (17.5)

Abbreviations. IQR: interquartile range; ICU: intensive care unit.

[a] There where 8 additional patients in which fluconazole was changed to a broader spectrum azole (i.e.; posaconazole, voriconazole, or isavuconazole).

[b] Only those drugs used over seven days were considered as "definitive treatment". Patients who died within the first seven days were not included in any group.

[c] Voriconazole-containing regimens: 19 patients received voriconazole alone; 8 received voriconazole after initial treatment with liposomal amphotericin-B; 8 received initial voriconazole and echinocandin combination and later monotherapy with voriconazole (7) or isavuconazole (1); 4 initially received voriconazole and liposomal amphotericin-B combination and later monotherapy with liposomal amphotericin-B (1), isavuconazole (1), posaconazole (1) or voriconazole (1); 4 received a voriconazole and echinocandin combination; 4 initially received voriconazole and later isavuconazole; 3 received a voriconazole and liposomal amphotericin-B combination; 1 patient initially received voriconazole and later liposomal amphotericin-B; 1 received a voriconazole and terbinafine combination.

[d] Liposomal amphotericin-B-containing regimens: 10 patients received liposomal amphotericin-B alone; 8 initially received liposomal amphotericin-B and later voriconazole; 4 initially received liposomal amphotericin-B and later isavuconazole; 4 received liposomal amphotericin-B and echinocandin combination; 4 initially received liposomal amphotericin-B and voriconazole combination and later monotherapy with liposomal amphotericin-B (1), isavuconazole (1), posaconazole (1), or voriconazole (1); 3 received liposomal amphotericin-B and voriconazole combination; 2 initially received a liposomal amphotericin-B and echinocandin combination and later monotherapy with echinocandin (1) or isavuconazole (1); 2 initially received liposomal amphotericin-B and later posaconazole; 2 initially received a liposomal amphotericin-B and isavuconazole combination and later isavuconazole; 1 initially received liposomal amphotericin-B, later posaconazole, and later isavuconazole; 1 initially received a liposomal amphotericin-B and posaconazole combination and later a liposomal amphotericin-B and isavuconazole combination; 1 received a liposomal amphotericin-B and posaconazole combination; 1 initially received voriconazole and later liposomal amphotericin-B.

[e] Echinocandin-containing regimens: 15 patients received echinocandins alone; 4 received a liposomal amphotericin-B and echinocandin combination; 2 initially received a liposomal amphotericin-B and echinocandin combination and later monotherapy with echinocandin (1) or isavuconazole (1); 1 initially received echinocandin and later fluconazole; 2 received a isavuconazole and echinocandin combination; 1 initially received an echinocandin and isavuconazole combination and later monotherapy with isavuconazole; 4 received a voriconazole and echinocandin combination; 8 initially received voriconazole and echinocandin combination and later monotherapy with voriconazole (7) or isavuconazole (1).

[f] Isavuconazole-containing regimens: 4 patients initially received liposomal amphotericin-B and later isavuconazole; 4 received isavuconazole alone; 4 initially received voriconazole and later isavuconazole; 2 received isavuconazole and echinocandin combination; 2 initially received a liposomal amphotericin-B and isavuconazole combination and later isavuconazole; 1 initially received a liposomal amphotericin-B and echinocandin combination and later monotherapy with isavuconazole; 1 initially received a liposomal amphotericin-B, later posaconazole, and later isavuconazole; 1 received initial liposomal amphotericin-B and posaconazole combination and later a liposomal amphotericin-B and isavuconazole combination; 1 initially received a liposomal amphotericin-B and voriconazole combination and later isavuconazole monotherapy; 1 initially received an echinocandin and isavuconazole combination and later isavuconazole monotherapy; 1 initially received an echinocandin and voriconazole combination and later isavuconazole monotherapy.

[g] Posaconazole-containing regimens: 8 patients received posaconazole alone; 2 initially received liposomal amphotericin-B and later posaconazole; 1 initially received a liposomal amphotericin-B and voriconazole combination and later posaconazole monotherapy; 1 initially received liposomal amphotericin-B, later posaconazole, and later isavuconazole; 1 initially received a liposomal amphotericin-B and posaconazole combination and later a liposomal amphotericin-B and isavuconazole combination; 1 received initial posaconazole and later liposomal amphotericin-B; 1 received a liposomal amphotericin-B and posaconazole combination.

[h] Fluconazole-containing regimens: 4 patients received fluconazole alone; 1 initially received echinocandin and later fluconazole.

[i] For those patients who died within the first 100 days, clinical response was evaluated at the time of death.

cohorts of BtIFI.[4,24,26,27,33,34] Interestingly, Biehl et al.[35] reported that no differences in mortality were found with respect to whether antifungal prophylaxis was maintained or the antifungal class was switched following BtIFI diagnosis per guideline recommendations.[9] However, these findings were hindered by the fact that most patients continuing with prophylaxis had possible BtIFI episodes. In this regard, the highest mortality was found in proven episodes, followed by probable cases, especially in those patients receiving IEAT. Our data supports the use of amphotericin-B as first-line empirical treatment for BtIFI. This drug was the most active one against the identified fungal species.

This study reports a large, prospective, real-life, and detailed cohort of patients with haematologic malignancies and BtIFI. However, this study has some limitations that should be acknowledged. First, this is a non-interventional study. Consequently, diagnostic approach and antifungal therapy after BtIFI consisted of several different schemes with a varying number of combinations and lengths. Also, this differed widely per the diagnosed BtIFI. Second, we analysed yeast and mould infections together, although pathophysiology and clinical pictures of both groups are markedly different. Third, therapeutic drug monitoring was not performed in many patients, limiting our capacity to find a potential cause for the BtIFI, and also restricted a potential analysis about its cost-effectiveness. Fourth, the incidence of BtIFI could not be obtained because the denominator of patients treated with each antifungal was unknown. Fifth, antifungal susceptibility testing was not available in many cases, so we could have missed cases caused by fungus susceptible to prior antifungal. Sixth, as long as biomarkers and culture yields are lower in patients receiving prior antifungal, it is likely that some BtIFI episodes were underdiagnosed or classified as possible cases due to a lack of mycological evidence. Finally, a consensus definition for BtIFI was proposed by the European Confederation of Medical Mycology (ECMM) in 2019.[36] The authors propose that the period to diagnose a BtIFI should start at the time the drug steady

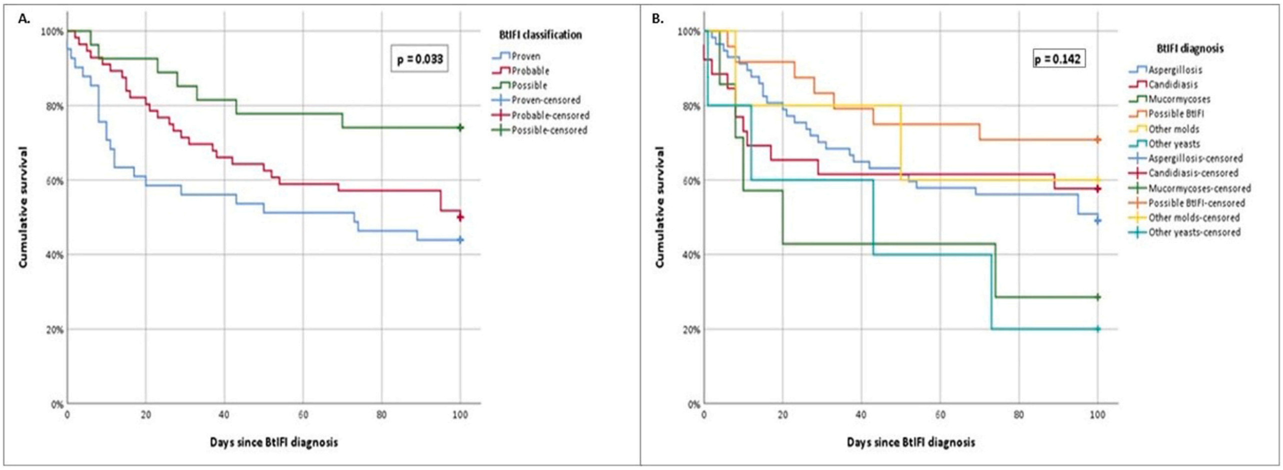

Fig. 2. Kaplan-Meier survival curves at 180 days based on breakthrough fungal infection classification (A) and diagnosis (B).

state has been reached, and should extend beyond the last dose depending on the half-life of the antifungal. Considering the definition of our study, which started two years before the ECMM consensus, we may have underdiagnosed some BtIFI episodes.

In conclusion, non-*fumigatus Aspergillus*, non-*albicans Candida*, Mucorales and other rare moulds and yeasts are commonly found in BtIFI. An aggressive diagnostic approach appears essential in guiding antifungal therapy, especially as it regards identifying the causative fungi and performing antifungal susceptibility. While these results are pending, early initiation of broad-spectrum antifungals different than those previously used is recommended. Current mortality of patients with BtIFI is extremely high. Consequently, improved management of these infections is mandatory.

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CRediT authorship contribution statement

Pedro Puerta-Alcalde: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Patricia Monzó-Gallo:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Manuela Aguilar-Guisado:** Investigation, Resources, Data curation, Writing – review & editing, Visualization, Supervision. **Juan Carlos Ramos:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Júlia Laporte-Amargós:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Marina Machado:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Pilar Martin-Davila:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Mireia Franch-Sarto:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Isabel Sánchez-Romero:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Jon Badiola:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Lucia Gómez:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Isabel Ruiz-Camps:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Lucrecia Yáñez:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Lourdes Vázquez:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Mariana Chumbita:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Francesc Marco:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Alex Soriano:** Formal analysis, Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Pedro González:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Ana Fernández-Cruz:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Montserrat Batlle:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Jesús Fortún:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Jesús Guinea:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Carlota Gudiol:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Julio García:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Maite Ruiz Pérez de Pipaón:** Investigation, Resources, Data curation, Writing – review & editing, Visualization. **Ana Alastruey-Izquierdo:** Software, Investigation, Resources, Data curation, Writing – review & editing, Visualization, Supervision. **Carolina Garcia-Vidal:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Pedro Puerta-Alcalde has received honoraria for talks on behalf of Merck Sharp and Dohme, Lilly, ViiV Healthcare and Gilead Science. Pedro Puerta-Alcalde has participated in advisory boards for Gilead Science. Lucrecia Yáñez has received honoraria for talks on behalf of Gilead, Kite, Merck Sharp and Dohme, Pfizer, Abbvie, Roche, Jannsen and Novartis a grant support from Janssen. Jesús Fortún has received honoraria for talks on behalf of Gilead Science, Pfizer, Merck Sharp and Dohme, and Astellas. Carlota Gudiol has received honoraria for lectures from Pfizer, Gilead and Merck Sharp and Dohme. Ana Alastruey-Izquierdo has received honoraria for educational talks on behalf of Pfizer and Gilead Science. Carolina Garcia-Vidal has received honoraria for talks on behalf of Gilead Science, Merck Sharp and Dohme, Pfizer, Jannsen, Novartis, Lilly and a grant support from Gilead Science and Merck Sharp and Dohme.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jinf.2023.05.005.

References

1. Marr Kieren A, Seidel Kristy, White Theodore C, Bowden Raleigh A. *Candidemia in allogeneic blood and marrow transplant recipients: evolution of risk factors after the adoption of prophylactic fluconazole. J Infect Dis* 2000;**181**(1):309–16. https://doi.org/10.1086/315193
2. Cornely Oliver A, Maertens Johan, Winston Drew J, Perfect John, Ullmann Andrew J, Walsh Thomas J, et al. *Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. N Engl J Med* 2007;**356**(4):348–59. https://doi.org/10.1056/nejmoa061094
3. Ullmann Andrew J, Lipton Jeffrey H, Vesole David H, Chandrasekar Pranatharthi, Langston Amelia, Tarantolo Stefano R, et al. *Posaconazole or fluconazole for prophylaxis in severe graft-versus-host disease. N Engl J Med* 2007;**356**(4):335–47. https://doi.org/10.1056/nejmoa061098
4. Auberger Jutta, Lass-Flörl Cornelia, Aigner Maria, Clausen Johannes, Gastl Günther, Nachbaur David. *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**(9):2268–73. https://doi.org/10.1093/jac/dks189
5. Lerolle N, Raffoux E, Socie G, Touratier S, Sauvageon H, Porcher R, et al. *Breakthrough invasive fungal disease in patients receiving posaconazole primary prophylaxis: a 4-year study. Clin Microbiol Infect* 2014;**20**(11):O952–9. https://doi.org/10.1111/1469-0691.12688
6. Lionakis Michail S, Lewis Russell E, Kontoyiannis Dimitrios P. *Breakthrough invasive mold infections in the hematology patient: current concepts and future directions. Clin Infect Dis* 2018;**67**(10):1621–30. https://doi.org/10.1093/cid/ciy473
7. Rausch Caitlin R, DiPippo Adam J, Bose Prithviraj, Kontoyiannis Dimitrios P. *Breakthrough fungal infections in patients with leukemia receiving isavuconazole. Clin Infect Dis* 2018;**67**(10):1610–3. https://doi.org/10.1093/cid/ciy406
8. De Pauw Ben, Walsh Thomas J, Donnelly JPeter, Stevens David A, Edwards John E, Calandra Thierry, et al. *Revised definitions of invasive fungal disease from the 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) C. Clin Infect Dis* 2008;**46**(12):1813–21. https://doi.org/10.1086/588660
9. Patterson Thomas F, Thompson George R, Denning David W, Fishman Jay A, Hadley Susan, Herbrecht Raoul, et al. *Practice guidelines for the diagnosis and management of aspergillosis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis* 2016;**63**(4):e1–60. https://doi.org/10.1093/cid/ciw326
10. Pappas Peter G, Kauffman Carol A, Andes David R, Clancy Cornelius J, Marr Kieren A, Ostrosky-Zeichner Luis, et al. *Clinical practice guideline for the management of candidiasis: 2016 update by the Infectious Diseases Society of America. Clin Infect Dis* 2015;**62**(4):e1–50. https://doi.org/10.1093/cid/civ933
11. Cornely Oliver A, Alastruey-Izquierdo Ana, Arenz Dorothee, Chen Sharon CA, Dannaoui Eric, Hochhegger Bruno, et al. *Global guideline for the diagnosis and management of mucormycosis: an initiative of the European Confederation of Medical Mycology in cooperation with the Mycoses Study Group Education and Research Consortium. Lancet Infect Dis* 2019;**19**(12):e405–21. https://doi.org/10.1016/S1473-3099(19)30312-3
12. Hoenigl Martin, Salmanton-García Jon, Walsh Thomas J, Nucci Marcio, Neoh Chin Fen, Jenks Jeffrey D, et al. *Global guideline for the diagnosis and management of rare mould infections: an initiative of the European Confederation of Medical Mycology in cooperation with the International Society for Human and Animal Mycology and the American Society for Microbiology. Lancet Infect Dis* 2021;**21**(8):e246–57. https://doi.org/10.1016/S1473-3099(20)30784-2
13. Chen Sharon CA, Perfect John, Colombo Arnaldo L, Cornely Oliver A, Groll Andreas H, Seidel Danila, et al. *Global guideline for the diagnosis and management of rare yeast infections: an initiative of the ECMM in cooperation with ISHAM and ASM. Lancet Infect Dis* 2021;**21**(12):e375–86. https://doi.org/10.1016/S1473-3099(21)00203-6
14. Freifeld Alison G, Bow Eric J, Sepkowitz Kent A, Boeckh Michael J, Ito James I, Mullen Craig A, et al. *Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 update by the Infectious Diseases Society of America. Clin Infect Dis* 2011;**52**(5):352–3. https://doi.org/10.1093/cid/cir073
15. Maertens Johan A, Girmenia Corrado, Brüggemann Roger J, Duarte Rafael F, Kibbler Christopher C, Ljungman Per, 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**(12):3221–30. https://doi.org/10.1093/jac/dky286
16. Wang JF, Xue Y, Zhu XB, Fan H. *Efficacy and safety of echinocandins versus triazoles for the prophylaxis and treatment of fungal infections: a meta-analysis of RCTs. Eur J Clin Microbiol Infect Dis* 2015;**34**(4):651–9. https://doi.org/10.1007/s10096-014-2287-4
17. Fisher Brian T, Zaoutis Theoklis, Dvorak Christopher C, Nieder Michael, Zerr Danielle, Wingard John R, et al. *Effect of caspofungin vs fluconazole prophylaxis on invasive fungal disease among children and young adults with acute myeloid leukemia: a randomized clinical trial. JAMA J Am Med Assoc* 2019;**322**(17):1673–81. https://doi.org/10.1001/jama.2019.15702
18. Marr Kieren A, Laverdiere Michel, Gugel Anja, Leisenring Wendy. *Antifungal therapy decreases sensitivity of the Aspergillus galactomannan enzyme immunoassay. Clin Infect Dis* 2005;**40**(12):1762–9. https://doi.org/10.1086/429921
19. Duarte Rafael F, Sánchez-Ortega Isabel, Cuesta Isabel, Arnan Montserrat, Patiño Beatriz, Fernández De Sevilla Alberto, et al. *Serum galactomannan-based early detection of invasive aspergillosis in hematology patients receiving effective antimold prophylaxis. Clin Infect Dis* 2014;**59**(12):1696–702. https://doi.org/10.1093/cid/ciu673
20. Garcia-Vidal Carol, Upton Arlo, Kirby Katharine A, Marr Kieren A. *Epidemiology of invasive mold infections in allogeneic stem cell transplant recipients: biological risk factors for infection according to time after transplantation. Clin Infect Dis* 2008;**47**(8):1041–50. https://doi.org/10.1086/591969
21. Kontoyiannis Dimitrios P, Marr Kieren A, Park Benjamin J, Alexander Barbara D, Anaissie Elias J, Walsh Thomas J, et al. *Prospective surveillance for invasive fungal infections in hematopoietic stem cell transplant recipients, 2001-2006: overview of the transplant-associated infection surveillance network (TRANSNET) database. Clin Infect Dis* 2010;**50**(8):1091–100. https://doi.org/10.1086/651263
22. Girmenia Corrado, Busca Alessandro, Candoni Anna, Cesaro Simone, Luppi Mario, Nosari Anna Maria, et al. *Breakthrough invasive fungal diseases in acute myeloid leukemia patients receiving mould active triazole primary prophylaxis after intensive chemotherapy: an Italian consensus agreement on definitions and management. Med Mycol* 2019;**57**(Supplement_2):S127–37. https://doi.org/10.1093/mmy/myy091
23. Imhof Alexander, Balajee SArunmozhi, Fredricks David N, England Janet A, Marr Kieren A. *Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. Clin Infect Dis* 2004;**39**(5):743–6. https://doi.org/10.1086/423274
24. Lamoth Frederic, Chung Shimin J, Damonti Lauro, Alexander Barbara D. *Changing epidemiology of invasive mold infections in patients receiving azole prophylaxis. Clin Infect Dis* 2017;**64**(11):1619–21. https://doi.org/10.1093/cid/cix130
25. Phai Pang Katy Anna, Godet Cendrine, Fekkar Arnaud, Scholler Julie, Nivoix Yasmine, Letscher-Bru Valérie, et al. *Breakthrough invasive mould infections in patients treated with caspofungin. J Infect* 2012;**64**(4):424–9. https://doi.org/10.1016/j.jinf.2011.12.015
26. Kimura Muneyoshi, Araoka Hideki, Yamamoto Hisashi, Nakamura Shigeki, Nagi Minoru, Yamagoe Satoshi, et al. *Micafungin breakthrough fungemia in patients with hematological disorders. Antimicrob Agents Chemother* 2018;**62**(5). https://doi.org/10.1128/AAC.02183-17
27. Cuervo G, Garcia-Vidal C, Nucci M, Puchades F, Fernández-Ruiz M, Obed M, et al. *Breakthrough candidaemia in the era of broad-spectrum antifungal therapies. Clin Microbiol Infect* 2016;**22**(2):181–8. https://doi.org/10.1016/j.cmi.2015.09.029
28. Lortholary Olivier, Desnos-Ollivier Marie, Sitbon Karine, Fontanet Arnaud, Bretagne Stéphane, Dromer Françoise, et al. *Recent exposure to caspofungin or fluconazole influences the epidemiology of candidemia: a prospective multicenter study involving 2,441 patients. Antimicrob Agents Chemother* 2011;**55**(2):532–8. https://doi.org/10.1128/AAC.01128-10
29. Lackner Nina, Posch Wilfried, Lass-Flörl Cornelia. *Microbiological and molecular diagnosis of mucormycosis: from old to new. Microorganisms* 2021;**9**(7):1518. https://doi.org/10.3390/microorganisms9071518
30. Millon Laurence, Caillot Denis, Berceanu Ana, Bretagne Stéphane, Lanternier Fanny, Morio Florent, et al. *Evaluation of serum mucorales polymerase chain reaction (PCR) for the diagnosis of mucormycoses: the MODIMUCOR prospective trial. Clin Infect Dis* 2022;**75**(5). https://doi.org/10.1093/cid/ciab1066
31. Puerta-Alcalde Pedro, Ruiz-Camps Isabel, Gudiol Carlota, Salavert Miquel, Barba Pere, Morandeira Francisco, et al. *Cytokine response as a biomarker for early diagnosis and outcome prediction of stem cell transplant recipients and acute leukemia patients with invasive aspergillosis. Med Mycol* 2022;**60**(7). https://doi.org/10.1093/mmy/myac038
32. Gonçalves Samuel M, Lagrou Katrien, Rodrigues Cláudia S, Campos Cláudia F, Bernal-Martínez Leticia, Rodrigues Fernando, et al. *Evaluation of bronchoalveolar lavage fluid cytokines as biomarkers for invasive pulmonary aspergillosis in at-risk patients. Front Microbiol* 2017;**8**:2362. https://doi.org/10.3389/fmicb.2017.02362
33. Kim Sun Bean, Cho Sung Yeon, Lee Dong Gun, Choi Jae Ki, Lee Hyo Jin, Kim Si Hyun, et al. *Breakthrough invasive fungal diseases during voriconazole treatment for aspergillosis: a 5-year retrospective cohort study. Med Mycol* 2017;**55**(3):237–45. https://doi.org/10.1093/mmy/myw067
34. Orasch Christina, Mertz Dominik, Garbino Jorge, van Delden Christian, Emonet Stephane, Schrenzel Jacques, et al. *Fluconazole non-susceptible breakthrough candidemia after prolonged low-dose prophylaxis: a prospective FUNGINOS study. J Infect* 2018;**76**(5):489–95. https://doi.org/10.1016/j.jinf.2017.12.018
35. Biehl Lena M, Vehreschild JJanne, Liss Blasius, Franke Bernd, Markiefka Birgid, Persigehl Thorsten, et al. *A cohort study on breakthrough invasive fungal infections in high-risk patients receiving antifungal prophylaxis. J Antimicrob Chemother* 2016;**71**(9):2634–41. https://doi.org/10.1093/jac/dkw199
36. Cornely Oliver A, Hoenigl Martin, Lass-Flörl Cornelia, Chen Sharon CA, Kontoyiannis Dimitrios P, Morrissey C Orla, 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**(9):716–29. https://doi.org/10.1111/myc.12960