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Stability in the cumulative incidence, severity, and mortality of 101 cases of invasive mucormycosis in high-risk patients from 1995-2011: A comparison of eras immediately before and after the availability of voriconazole and echinocandin-amphotericin combination therapies

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Abstract

Background—As invasive mucormycosis (IM) numbers rise, clinicians suspect prior voriconazole worsens IM incidence and severity, and believe combination anti-fungal therapy improves IM survival.

Objectives—To compare the cumulative incidence (CI), severity and mortality of IM in eras immediately before and after the commercial availability of voriconazole.

Methods—All IM cases from 1995–2011 were analyzed across four risk-groups (hematologic/oncologic malignancy (H/O), stem cell transplantation (SCT), solid organ transplantation (SOT), and other), and two eras, E1, (1995–2003), and E2, (2004–2011).

Results—Of 101 IM cases, (79 proven, 22 probable): 30 were in E1 (3.3/year) and 71 in E2 (8.9/year). Between eras, the proportion with H/O or SCT rose from 47% to 73%, while “other” dropped from 33% to 11% ($p=0.036$). Between eras, the CI of IM did not significantly increase in SCT ($p=0.27$) or SOT ($p=0.30$), and patterns of anatomic location ($p=0.122$) and surgical debridement ($p=0.200$) were similar. Significantly more patients received amphotericin-echinocandin combination therapy in E2 (31% vs. 5%, $p=.01$); however, 90-day survival did not improve (54% vs. 59%, $p=0.67$).

Conclusions—Since 2003, the rise of IM reflects increasing numbers at risk, not prior use of voriconazole. Frequent combination anti-fungal therapy has not improved survival.

Keywords

mucormycosis; incidence; outcome; prophylaxis; combination therapy; invasive filamentous fungal infections; amphotericin B; echinocandins

Introduction

Invasive Mucormycosis (IM) is an aggressive, highly fatal infection in immune compromised hosts. As the group of patients who now undergo stem cell transplantation (SCT), chemotherapy for malignancy, and solid organ transplantation (SOT) expands, more patients are at risk for IM (1).

According to some experts in IM, a matter of significant and unresolved controversy is whether broad spectrum antifungal agents, in particular voriconazole, have led to increased rates of zygomycosis in immunocompromised hosts. In a matched, case-controlled study of patients with leukemia or allogeneic stem cell transplant recipients, voriconazole prophylaxis was significantly associated with zygomycosis (OR, 1.43 [95% CI, 1.11–1.85]) (2). Additionally, voriconazole enhanced the virulence of zygomycetes in animal models (2). Pre-exposure to voriconazole in mice infected with *Rhizopus oryzae* was associated with higher mortality (3).

Because IM is still relatively rare and still only about 1/10th as common as Invasive Aspergillus (IA) in these groups (4), studying risk factors for IM, and factors that affect IM mortality and morbidity, is difficult. Even more difficult is any prospective randomized trial of drug therapy for IM, because the power requirements would be quite large, and would require a large, multicentered trial.

Recent case-controlled studies evaluating risk and outcome of IM in special populations are also limited because the entire duration of the study periods took place during a time when voriconazole was commercially available (5, 6). Although the studies are referred to as prospective, they actually study clinical data of IM cases after the case took place; therefore, even with their case-controlled and time-linked control cohort design, there is an implicit bias in the exposure of patients to voriconazole.

In real-time clinical practice, voriconazole use can be chosen for patients with various risks for IM that may or may not be captured in post hoc studies. In other words, voriconazole use itself may be a marker for the clinician's judgment that the patient has one or more risk factors of invasive filamentous fungal infections, particularly IA; these same risks for IA also increase the risk for IM.

Post hoc analyses of Voriconazole use are also problematic when treatment protocols for leukemia or transplantation systematically give voriconazole to *all* patients for prophylaxis. This has especially been the case since 2007, when studies showed that routine anti-filamentous antifungal prophylaxis improved survival in neutropenic patients and in patients with GVHD after SCT (7).

In other words, retrospective studies that attempt to analyze the impact of voriconazole on the risk of acquiring IM, or on the severity and outcome of IM, cannot clearly disassociate voriconazole use from the risks that clinicians perceived in their empirical and prophylactic use of voriconazole in high risk patients, when the entire research period takes place in an era when voriconazole is available.

In order to clearly separate and thus compare the impact of voriconazole use, we took an alternative approach, which was: first, to analyze the Kaplan-Meier cumulative incidence of IM within well-defined high-risk populations in whom detailed long-term survival data was available, i.e., the entire group of patients who received SCT and SOT at Mayo Clinic; and to then compare this CI for IM in these populations in two different eras, the era immediately preceding the availability of voriconazole and the era after voriconazole became available.

For similar reasons, we also compared, between these two eras, the survival and overall clinical severity (e.g., rates of dissemination and central nervous system disease, and rates of cases requiring surgery) of IM. This again, enabled us to clearly separate the possible effect that voriconazole had on these aspects of IM.

Another variable that was amenable to this historical-era comparison was the use of combination therapies for IM (e.g., ambisome plus echinocandin) and their impact on IM survival. Again, for reasons cited above, the low frequency and severity of IM make it very

difficult to study treatment regimens for IM in truly prospective, randomized-controlled trials. Using the historical-era comparison design, we were able to compare groups of patients for whom combination therapy was impossible (because the newer drugs were not yet available), i.e., before 2004, with groups for whom such combination therapy was available and frequently used (i.e., after 2004). This approach avoided the bias that is intrinsically problematic in any post hoc study that only studies patients from an era when the newer drugs were available: i.e., it is the patients who are more severely ill who will likely be given combination therapy in the newer era. By contrast, similarly severely ill patients in the earlier historical era could not have been given combination therapy.

Methods

Patient Population

Patients diagnosed with invasive mucormycosis from January 1995 through December 2011 at all three Mayo Clinic campuses (Minnesota, Arizona and Florida) were identified through search of microbiology and pathology databases. Data abstraction included patient demographics, co-morbidities, histopathologic findings, genus-level identification of Mucorales organisms, prior antifungal use (within 2 weeks and for more than 3 days) before the diagnosis of mucormycosis, co-infecting fungal organisms, clinical manifestations, graft versus host disease (GVHD), choice of antifungal therapy, surgical intervention and 90 day mortality. Trauma cases were excluded. All patients had consented to use of their medical records for research purposes and the study protocol was approved by Mayo Clinic Institutional Review Board.

Diagnostic Criteria

Invasive mucormycosis (IM) was defined using the diagnostic criteria of the 2008 European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) (8). Only “proven” and “probable” IM cases were included. Date of first documented clinical suspicion for IM was established as the date of diagnosis.

Definitions

At-risk populations for IM were categorized in 4 distinct groups: (1) hematological or oncologic malignancy (H/O) without transplantation; (2) hematopoietic stem cell transplantation (SCT); (3) solid organ transplantation (SOT); or in the absence of the aforementioned (4) “others” including diabetes mellitus. Only one category was assigned to each patient. Patients who had hematologic malignancy but then went on to undergo SCT were only classified as SCT patients; patients with diabetes mellitus who also had either H/O, SCT, or SOT, were classified in one of these categories, not “Other”.

Clinical manifestations of IM were classified as: pulmonary, abdominal, isolated sinus, rhino-cerebral, sino-orbital, cutaneous and disseminated. Disseminated mucormycosis was defined as two or more non-contiguous sites of involvement. In addition to phenotypic observation, DNA sequencing for Mucorales agents was also employed for identification of tissue-based samples. Cases of IM were categorized as “mixed” if one or more other fungi, besides Mucorales, were isolated or identified from the same specimen(s) in which

Mucorales had been found. Associated conditions including neutropenia (granulocyte count < 1000 cells/ μ L) and histopathology-proven graft-versus-host disease (GVHD) were also recorded.

Clinical Era Definitions

The dates of FDA approval of new antifungal agents included: voriconazole, May 2002; caspofungin January 2001; and posaconazole, October 2006. However, voriconazole was initially used for defined cases of invasive aspergillosis. Antifungal protocols that included voriconazole for high risk patients were first adopted at Mayo Clinic Rochester in 2004.

Similarly, caspofungin, initially used for candidiasis and IA, was not used in combination with amphotericin-based agents for IM until several years after its commercial availability. Posaconazole, by contrast, was used soon after its approval in 2006 for treatment of IM, and it was also available as a salvage agent for IM, with an emergency IND, for several years before its FDA approval.

With all of these considerations, we divided the 17 years of the study period into two intervals of 8 to 9 years each, *a priori*, before extracting or analyzing the clinical data: era 1 (E1) from 1995 through 2003, and era 2 (E2) from 2004 through 2011. During the former era, voriconazole could not have been used prophylactically; during the latter era, voriconazole prophylaxis and empirical therapy in high-risk patients had become common, and the newer anti-fungal compounds, i.e., posaconazole and echinocandins, were available for specific IM treatment.

Statistical Analysis

Patient demographics, clinical characteristics, and disease management were summarized with descriptive statistics. To assess temporal trends over the study time period, IM cases were grouped into two intervals according to calendar year of diagnosis: era 1 (E1) from 1995 through 2003 and era 2 (E2) from 2004 through 2011. Baseline data was compared between the two time intervals, and also between the four risk groups, using a Chi-square test (or Fisher's exact test as appropriate) for categorical variables and one-way analysis of variance (ANOVA) for continuous variables.

With the availability of comprehensive follow-up data on all patients who had a Stem Cell Transplantation or Solid Organ Transplantation at Mayo Clinic Rochester, cumulative incidence (CI) of IM was estimated in these two risk groups by the Kaplan-Meier method and tested for a difference between time eras using a log-rank test. However, the diversity of malignancies in the H/O group, and variation in the proportion of care provided at referring institutions, precluded calculation of CI in this subgroup. Calculation of CI for the "Other" category was not feasible for similar reasons.

Survival after diagnosis of IM was also analyzed by the Kaplan-Meier method. Death by any cause that occurred within 90 days of IM diagnosis was treated as an "event", while those known to be alive after 90 days (or those lost to follow up prior to then) were censored. The log-rank test was used to compare survival rates between different at-risk groups and between calendar year intervals. To assess the association between 90-day survival and type

of anti-fungal therapy, we used a landmark approach that conditioned on 14-day survival (i.e., cases that died or were lost to follow-up within 14 days of diagnosis were excluded) with treatment defined from data during this interim period.

Results

We reviewed all patients with cultures positive for Mucorales during the study time period from microbiology databases at the Mayo Clinic campuses in Minnesota (991 cases), Florida (35 cases) and Arizona (13 cases). Histopathology records from all sites contributed an additional 43 cases. After applying EORTC criteria, and exclusion of duplicates cases, a total of 101 cases of “proven” and “probable” IM were identified. Using the *a priori* definition of two clinically distinct eras, as described above, based on the availability of newer antifungals over time, thirty of these 101 cases were observed during the earlier era (E1), and 71 cases during the latter era (E2), summarized in Table 1. In E1, only one patient had received voriconazole, (before the diagnosis of IM), and only one patient received caspofungin, (in combination with liposomal-amphotericin, for treatment of IM), both as investigational agents; these single exceptions demonstrate that the *a priori* definitions of E1 and E2, based on a review of FDA release and practice protocols, did successfully demarcate two distinct eras of clinical practice.

Patient Characteristics

Of the 101 cases of IM, 78 percent were “proven” and 22% were “probable”. The mean ($\pm$ SD) age at diagnosis of IM was 52.4 ($\pm$ 15.6) years; the majority were male (59%). Malignancies (n=48) included acute leukemias (myeloid, lymphoid), lymphoma, and non-hematologic malignancies. Acute myeloid leukemia was the most common hematologic malignancy (n=17). Eighteen patients had hematopoietic SCT: allogeneic SCT (n=13) and autologous SCT (n=5). Seventeen IM cases were in SOT recipients (liver 5, kidney 4, lung 5, heart 1, combined heart-lung 1, and combined kidney-pancreas 1). Patients without an underlying malignancy or transplant were grouped into “others (Non H/O)”. These included patients with diabetes mellitus (n=12), end stage liver disease (n=2), lupus (n=2), ulcerative colitis (n=1), and Crohn’s disease (n=1).

The 30 cases of IM in E1, a 9-year era, represents an average of 3.3 cases per year; the 71 cases in E2, an 8-year era, is an average of 8.9 cases per year. The absolute number of IM cases, both overall and in H/O and SCT patients, rose noticeably between eras. While the absolute number from the “other” risk category did not change appreciably, there were proportionally fewer IM cases from this group in E2 (11%) compared to E1 (33%, $p=0.036$).

The cumulative incidence (CI, Kaplan-Meier) of IM was compared between the two time eras among all SCT and SOT transplant patients at Mayo Clinic Rochester (MCR) (Figure 1). Among these SCT patients, the CI of IM did not differ significantly between E1 and E2 ($p=0.266$). Across both eras, the frequency of IM was significantly higher in allogeneic SCT compared to autologous SCT (3-year CI: 1.6% vs. 0.2%; $p<0.001$). In the subgroup of allogeneic SCT patients, the 3-year CI of mucormycosis was 0.6% for transplants that occurred in E1, compared to 1.9% in E2 ($p=0.223$). In SOT patients, the CI rates of IM also did not differ significantly between E1 and E2 ($p=0.299$). As previously noted, the diversity

of malignancies in the H/O group, and variation in the proportion of care provided at referring institutions, precluded calculation of CI in this subgroup; calculation of CI for the “Other” category was not feasible for similar reasons.

A significantly larger proportion of cases in E2 were neutropenic at the time of diagnosis of IM (44%, including 6 of 9 SCT and 12 of 37 H/O patients), compared with those in E1 (21%, including 1 of 3 with SCT and 5 of 11 H/O; $p=0.031$). However, there was no significant difference between E1 and E2 in the overall severity of IM at the time of diagnosis, based on patterns in anatomic location of IM at presentation ($p=0.122$).

Prior antifungal exposure (for more than 3 days and within 2 weeks of diagnosis of IM) differed significantly between eras (Table 1) with a larger portion of cases in E2 having such exposure (77% in E2 versus 50% in E1; $p=0.006$). Moreover, the type of prior antifungal also changed significantly ($p<0.001$): in E1, 80% of those who had prior anti-fungal exposure received fluconazole; in E2, 73% of those who had prior anti-fungal exposure received either voriconazole or an echinocandin ($p<0.001$).

In E2, a large portion (20 of 31, 65%) of the cases of IM that were neutropenic at the time of diagnosis had received prior voriconazole (i.e., within 2 weeks and for more than 3 days before the diagnosis of IM). By contrast, in E1, prior voriconazole (being only available as an investigational agent during E1) had been given to only 1 of the 6 patients (17%) who had neutropenia at the time of diagnosis of IM ($p=0.030$).

Mucorales genus involved

During the study period, there were no changes in the laboratory methods of cultivation of Mucorales. Genus identification was available for 77 cases. The remaining 24 cases were identified based upon histopathologic findings of hyphae resembling Mucorales agents with evidence of angioinvasion and tissue damage. The 3 main Mucorales genera identified were *Rhizopus* ($n=39$), *Mucor* ($n=27$), and *Lictheimia*, formerly known as *Absidia*, ($n=5$). The remaining isolates were identified as *Rhizomucor* ($n=2$), *Cunninghamella* ($n=1$), *Syncephalastrum* ($n=1$), *Apophysomyces* ($n=1$), and *Blakeslea trispora* ($n=1$).

Grouped according to isolated cases of Mucorales ($n=75$), Mucorales in combination with *Aspergillus* spp. ($n=23$), and Mucorales in combination with *Alternaria* ($n=3$). the distribution across pure and mixed categories of fungal infection was not significantly different between eras ($p=0.115$).

Treatment and survival curves

There were significant differences between E1 and E2 in the anti-fungal regimens used to treat IM. A similar proportion (80 vs. 82%) of cases received liposomal-amphotericin based agents (L-ABA) as part of their antifungal regimen; however, there was a significant difference in the proportions that received L-AMB as monotherapy (77% vs. 51%, in E1 vs. E2, respectively) versus in combination with other therapies such as caspofungin and/or posaconazole (3% vs. 28%, in E1 vs. E2, respectively; $p=0.001$). This association of anti-fungal regimen with clinical era remained significant when using the refined landmark analysis definitions of therapy (i.e., received at least 1 week of treatment) on the subgroup

who survived at least 14 days ($p=0.041$). Ten patients in the latter time era (E2) received therapy without any L-AMB, including 4 patients who received posaconazole. There was no significant difference in the rate of surgical debridement between the two clinical eras (70% in E1 vs. 56% in E2, $P=0.200$).

Survival analyses were performed on the group of cases surviving at least one day after mucormycosis diagnosis; therefore, 5 IM cases who were diagnosed at autopsy were excluded. There was no significant difference in 90-day survival across at-risk groups of IM ($p=0.513$; Figure 2), or between the two clinical eras ($p=0.667$; Figure 3). Among 14-day survivors, there was no significant difference in 90-day survival between therapy groups including those on Ambisome monotherapy versus combination therapy ($p=0.243$; Figure 4).

When comparing 90-day survival curves for pure mucormycosis vs. mixed mold (*Mucorales* with *Aspergillus* or *Alternaria*) infections, there was a trend towards better survival in the mixed group ($p=0.136$; Figure 5). The 90-day rate of survival was 50% in neutropenic patients, compared to 60% in non-neutropenic patients ($p=0.204$; Figure 6).

Discussion

This study documents the clinical evolution of invasive mucormycosis during a 17 year epoch that included significant changes in annual rates of IM, risk-group demographics, and use of antifungal agents. This is also one of the largest series (101 cases) of IM in contemporary practice from a single institution, and includes an estimation of the cumulative incidence (CI) of IM in both stem cell and organ transplant recipients across two consecutive clinical eras of 8 to 9 years each.

Contrary to prior published observations that voriconazole use in high risk populations can increase the risk of acquiring IM, our analysis suggests that the CI of IM did not increase significantly in either SCT and SOT recipients in the second half of our study period. Moreover, despite more frequent use of voriconazole for prophylaxis in the later era, the severity of IM at presentation, (based on the frequency of disseminated and rhinocerebral disease) was not different either.

These findings are notable because of the intrinsic differences between the two consecutive eras: for the first era, voriconazole prophylaxis was impossible (because it was not commercially available); for the second era, it was frequently used. As noted earlier, antifungal protocols that included voriconazole for high risk patients were first adopted at Mayo Clinic Rochester in 2004. Voriconazole prophylaxis in allo-SCT patients with GVHD requiring systemic immune suppression (typically corticosteroids) and in neutropenia with AML began in 2007. By 2009–2010, approximately one-third of allogeneic SCT patients would have a prior history of possible, probable, or proven mold infection that warranted secondary prophylaxis during the SCT, typically with voriconazole. For the majority of allo-SCT (the other two-thirds of patients) with no prior therapy or no prior fungal history, approximately 60% (40% of total allogeneic patients) received fluconazole and 40% received voriconazole. No fluconazole was used as prophylaxis in AML cases. The

separation of the 17 year interval in this study, into two eras to assess a pre-voriconazole era versus a post-voriconazole era, i.e., 1995–2003 for Era 1 and 2004–2011 for Era 2, also aligns well with another similar analysis (9) which had analyzed published reports from 1970–2005 and compared those to published reports from 2006–2008, to assess voriconazole effects in transplant populations.

The design of the current study prevents the intrinsic selection bias that arises in any non-randomized study confined to the latter (voriconazole-available) era: i.e., that more severely ill patients in an era where voriconazole is available will have been given voriconazole.

Finally, even with frequent combination of L-ABA with an echinocandin and/or posaconazole in the second era, we did not observe any significant improvement in overall survival in IM.

There were significantly more IM patients with neutropenia at diagnosis during the later era, a factor that could have worsened survival. However, the overall survival between neutropenic and non-neutropenic patients with IM was not significantly different, making it unlikely that neutropenia was a confounding variable in comparative survival between eras that could have offset treatment benefits of newer antifungal combination regimes.

Invasive mucormycosis in SCT and SOT recipients is uncommon but is associated with high mortality (10–12). In the TRANSNET study, the 12-month CI of IM in SCT recipients was 0.29% in the 6-year interval from 2001 through 2006 in the United States. This was noted to be higher in patients with allogeneic stem cell transplants from HLA –matched, unrelated donors (1). In the present study, across a 17-year interval, 1995–2011, as well as within each of the 8–9 year eras within it, the cumulative incidence for IM in SCT at 12-months was similar to that of the TRANSNET study. The most common presentations in SCT recipients, in our study, also agreed with the published literature: pulmonary followed by para-nasal sinuses and central nervous system involvement, especially with disseminated disease (13).

In SOT recipients, the TRANSNET study found that the 1-year CI of mucormycosis was 0.07% and mucormycosis represented only 2% of the invasive fungal infections (1). Our study data show a similar 1-year CI of IM in SOT recipients, and a similar anatomic distribution of IM at presentation, to what was reported in the TRANSNET studies (4). However, our data indicate that SOT patients continue to be at risk for IM beyond one year (6 of the 17 IM cases in SOT recipients occurred more than 1 year following transplant); this additional period of risk of IM in SOT was not addressed in the TRANSNET study. Similarly, about one-third of IM cases from SCT recipients developed mucormycosis more than 1 year beyond transplant.

The main finding in this study is that the Kaplan-Meier calculation for CI in well-defined, longitudinally followed high risk patient populations, and the mortality and severity of IM, have remained stable over the past 17 years. With the CI and survival remaining stable between historical eras, there was no feasible basis to analyze what factors might have made the CI or survival of IM change between eras, because the CI and survival of IM had not significantly changed.

The influence of voriconazole on the cumulative incidence of IM in SCT recipients remains controversial (5, 13–17). The TRANSNET study reported only several cases of IM in patients who had previously received voriconazole (1). Kontoyiannis et al also described prior voriconazole use as a risk factor for IM in hematology patients and SCT recipients in a case-control study (5). Two other observational studies have also implicated prior voriconazole use as a risk factor for IM (6, 18). However, in a randomized trial comparing voriconazole versus fluconazole prophylaxis in SCT recipients, the CI of mucormycosis was not higher in patients who received voriconazole for prophylaxis compared to those who did not (19).

Our study indicates that the widespread use of voriconazole in high risk populations, including its use as prophylaxis in some groups, such as those with neutropenia and GVHD, has not led to an increased rate of IM in these populations.

Similarly, our study indicates that the relatively frequent use of combination therapy for IM (i.e., over 30 percent of IM cases in the newer era, presumably those who are most severe), has not improved overall survival IM compared to an earlier era when combination therapy was not available (but which was still an era that had a similarly high proportion of patients with severe illness).

Because of the high mortality associated with IM, combination anti-fungal therapy has been evaluated in *in-vitro*, experimental and clinical research. *Rhizopus oryzae* expresses the target enzyme for echinocandins (20). Murine infection models (21), and a clinical retrospective study in patients with rhinocerebral mucormycosis (22), have shown improved survival with combination therapy with a polyene agent and echinocandin.

Some experts recommend combination anti-fungal in patients with mucormycosis (23). However, recent ECIL3 (Third European Conference on Infections in Leukemia) guidelines find insufficient data to support combination anti-fungal treatment as first-line therapy for IM (24). Higher dose of L-AMB (10mg/kg/day) for management of IM is now being evaluated in clinical studies (25).

Given the rapid growth rate of most Mucorales *in vivo*, the “window of opportunity” to intervene and provide effective treatment, i.e. before extensive angioinvasion and dissemination sets in, is described to be much shorter for patients with mucormycosis than for patients with aspergillosis (26, 27). The emphasis is clearly on quickly loading the infected tissue with L-AMB formulations so that this slows fungal proliferation and reduces the risk of further fungal angioinvasion (27). Delays in starting L-ABA therapy for IM have been associated with a 2-fold increase in mortality rate (28). Therefore, initial empirical therapy of suspected filamentous fungal infection should include L-AMB until a definite fungal organism can be identified, and every effort should be made to attain a definite fungal species etiology as soon as possible. Studies done in animal models have shown that higher amphotericin B tissue concentrations may be required for effective treatment of mucormycosis as compared to aspergillosis (29–31). Thus earlier initiation of high-dose L-AMB (5mg/kg/day) is perhaps more important than use of combination therapy in these patients (28, 32, 33).

Overall, IM remains a formidable, but still uncommon invasive fungal infections in immunocompromised hosts. Assessing the role of voriconazole on increasing the frequency or severity of IM, or the role of new empirical antifungal practices on the survival of IM, is a complex process, especially if all analyzed cases occur within a era when clinicians have access to voriconazole and newer agents (17). When we take a longer historical perspective, we do not find evidence that voriconazole use can be invoked as a risk factor for IM; instead, voriconazole appears to be primarily a marker for physicians' clinical judgment that certain patients are at risk for filamentous IFI (including both IA and IM).

Efforts to reduce the risk of IM should focus on other areas rather than reducing the use of voriconazole. It is possible that intrinsic risk factors for IM could be used to identify sub-populations who would benefit from posaconazole rather than voriconazole for IFI prophylaxis, e.g., recurring neutropenia, neutropenia plus diabetes mellitus, or neutropenia plus renal disease. Environmental factors also need to be addressed, such as exposure to environments with high mold content, particularly as neutropenic patients are given voriconazole prophylaxis but are otherwise permitted to return to general outpatient settings (34).

Similarly, efforts to improve the survival and outcome of IM should focus on other interventions rather than combination therapy. such as earlier therapy and higher dose therapy with Ambisome, earlier and more extensive surgery, earlier recovery of neutrophils, and reversal of reversible risks such as glucose, steroids, and renal dysfunction.

Our study has certain limitations. The retrospective design has inherent confounders, such as selection and recall bias. To limit these biases, we relied on objective data and used standardized and reproducible case definitions using EORTC criteria. Another limitation was the inability to calculate CI in patients with hematologic malignancy who did not undergo SCT. This study also omitted cases of suspected filamentous fungal infection that responded to empirical therapy without getting a definitive diagnosis. Ideally, prospective monitoring of all patients at risk for IFI would include these empirically treated cases. Finally, despite three geographically diverse Mayo Clinic campuses, our cohort remained predominantly caucasian.

In summary, the rising absolute number of IM cases at Mayo Clinic over the last 17 years can be at least partly explained by a higher absolute number of at-risk patients (Figure 7). During the latter era, which was associated with frequent prophylaxis and empirical and preemptive therapy using voriconazole, we did not see a significant increase in the CI of IM in high risk groups, compared to the immediately preceding era when voriconazole was not commercially available. Finally, despite significantly more frequent use of combination anti-fungal therapy in the latter era (2004–2011), we did not see any significant change in short-term survival after diagnosis of IM. The trends that are increasing the numbers of patients at risk for IM show no signs of abating. More research is needed to protect and improve the outcome of these ever-increasing groups of immune compromised hosts.

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References

1. Park BJ, Pappas PG, Wannemuehler KA, Alexander BD, Anaissie EJ, Andes DR, et al. Invasive non-Aspergillus mold infections in transplant recipients, United States, 2001–2006. *Emerg Infect Dis.* 2011; 17(10):1855–1864.
2. Garcia-Vidal C, Upton A, Kirby KA, Marr KA. Epidemiology of invasive mold infections in allogeneic stem cell transplant recipients: biological risk factors for infection according to time after transplantation. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2008; 47(8):1041–1050. [PubMed: 18781877]
3. Lamaris GA, Ben-Ami R, Lewis RE, Chamilos G, Samonis G, Kontoyiannis DP. Increased virulence of Zygomycetes organisms following exposure to voriconazole: a study involving fly and murine models of zygomycosis. *J Infect Dis.* 2009; 199(9):1399–1406. [PubMed: 19358672]
4. Pappas PG, Alexander BD, Andes DR, Hadley S, Kauffman CA, Freifeld A, et al. Invasive fungal infections among organ transplant recipients: results of the Transplant-Associated Infection Surveillance Network (TRANSNET). *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2010; 50(8):1101–1111. [PubMed: 20218876]
5. Kontoyiannis DP, Lionakis MS, Lewis RE, Chamilos G, Healy M, Perego C, et al. Zygomycosis in a tertiary-care cancer center in the era of Aspergillus-active antifungal therapy: a case-control observational study of 27 recent cases. *J Infect Dis.* 2005; 191(8):1350–1360. [PubMed: 15776383]
6. Singh N, Aguado JM, Bonatti H, Forrest G, Gupta KL, Safdar N, et al. Zygomycosis in solid organ transplant recipients: a prospective, matched case-control study to assess risks for disease and outcome. *J Infect Dis.* 2009; 200(6):1002–1011. [PubMed: 19659439]
7. Cornely OA, Maertens J, Winston DJ, Perfect J, Ullmann AJ, Walsh TJ, et al. Posaconazole vs. fluconazole or itraconazole prophylaxis in patients with neutropenia. *The New England journal of medicine.* 2007; 356(4):348–359. [PubMed: 17251531]
8. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, 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) Consensus Group. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2008; 46(12):1813–1821. [PubMed: 18462102]
9. Cuenca-Estrella M, Bernal-Martinez L, Isla G, Gomez-Lopez A, Alcazar-Fuoli L, Buitrago MJ. Incidence of zygomycosis in transplant recipients. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases.* 2009; 15(Suppl 5):37–40.
10. Lanternier F, Dannaoui E, Morizot G, Elie C, Garcia-Hermoso D, Huerre M, et al. A global analysis of mucormycosis in France: the RetroZygo Study (2005–2007). *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2012; 54(Suppl 1):S35–S43. [PubMed: 22247443]
11. Petrikos G, Skiada A, Lortholary O, Roilides E, Walsh TJ, Kontoyiannis DP. Epidemiology and clinical manifestations of mucormycosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2012; 54(Suppl 1):S23–S34. [PubMed: 22247442]
12. Skiada A, Pagano L, Groll A, Zimmerli S, Dupont B, Lagrou K, et al. Zygomycosis in Europe: analysis of 230 cases accrued by the registry of the European Confederation of Medical Mycology (ECMM) Working Group on Zygomycosis between 2005 and 2007. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases.* 2011; 17(12):1859–1867.
13. Lanternier F, Sun HY, Ribaud P, Singh N, Kontoyiannis DP, Lortholary O. Mucormycosis in organ and stem cell transplant recipients. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America.* 2012; 54(11):1629–1636. [PubMed: 22431807]

14. Imhof A, Balajee SA, Fredricks DN, Englund JA, Marr KA. Breakthrough fungal infections in stem cell transplant recipients receiving voriconazole. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2004; 39(5):743–746. [PubMed: 15356792]
15. Siwek GT, Dodgson KJ, de Magalhaes-Silverman M, Bartelt LA, Kilborn SB, Hoth PL, et al. Invasive zygomycosis in hematopoietic stem cell transplant recipients receiving voriconazole prophylaxis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2004; 39(4):584–587. [PubMed: 15356827]
16. Marty FM, Cosimi LA, Baden LR. Breakthrough zygomycosis after voriconazole treatment in recipients of hematopoietic stem-cell transplants. *The New England journal of medicine*. 2004; 350(9):950–502. [PubMed: 14985500]
17. Pongas GN, Lewis RE, Samonis G, Kontoyiannis DP. Voriconazole-associated zygomycosis: a significant consequence of evolving antifungal prophylaxis and immunosuppression practices? *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2009; 15(Suppl 5):93–97.
18. Xhaard A, Lanternier F, Porcher R, Dannaoui E, Bergeron A, Clement L, et al. Mucormycosis after allogeneic haematopoietic stem cell transplantation: a French Multicentre Cohort Study (2003–2008). *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2012; 18(10):E396–E400.
19. Wingard JR, Carter SL, Walsh TJ, Kurtzberg J, Small TN, Baden LR, et al. Randomized, double-blind trial of fluconazole versus voriconazole for prevention of invasive fungal infection after allogeneic hematopoietic cell transplantation. *Blood*. 2010; 116(24):5111–5118. [PubMed: 20826719]
20. Ibrahim AS, Bowman JC, Avanesian V, Brown K, Spellberg B, Edwards JE Jr. et al. Caspofungin inhibits *Rhizopus oryzae* 1,3-beta-D-glucan synthase, lowers burden in brain measured by quantitative PCR, and improves survival at a low but not a high dose during murine disseminated zygomycosis. *Antimicrobial agents and chemotherapy*. 2005; 49(2):721–727. [PubMed: 15673756]
21. Ibrahim AS, Gebremariam T, Fu Y, Edwards JE Jr. Spellberg B. Combination echinocandin-polyene treatment of murine mucormycosis. *Antimicrobial agents and chemotherapy*. 2008; 52(4):1556–1558. [PubMed: 18212099]
22. Reed C, Bryant R, Ibrahim AS, Edwards J Jr. Filler SG, Goldberg R, et al. Combination polyene-caspofungin treatment of rhino-orbital-cerebral mucormycosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2008; 47(3):364–371. [PubMed: 18558882]
23. Kontoyiannis DP, Lewis RE. How I treat mucormycosis. *Blood*. 2011; 118(5):1216–1224. [PubMed: 21622653]
24. Skiada A, Lanternier F, Groll AH, Pagano L, Zimmerli S, Herbrecht R, et al. Diagnosis and treatment of mucormycosis in patients with haematological malignancies: guidelines from the 3rd European Conference on Infections in Leukemia (ECIL 3). *Haematologica*. 2012
25. Lanternier F, Lortholary O. [AMBIZYGO: phase II study of high dose liposomal amphotericin B (AmBisome) [10 mg/kg/j] efficacy against zygomycosis]. *Med Mal Infect*. 2008; 38(Suppl 2):S90–S91. [PubMed: 18598882]
26. Katragkou A, Walsh TJ, Roilides E. Why is mucormycosis more difficult to cure than more common mycoses? *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2013
27. Lewis RE, Lortholary O, Spellberg B, Roilides E, Kontoyiannis DP, Walsh TJ. How does antifungal pharmacology differ for mucormycosis versus aspergillosis? *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2012; 54(Suppl 1):S67–S72. [PubMed: 22247448]
28. Chamilos G, Lewis RE, Kontoyiannis DP. Delaying amphotericin B-based frontline therapy significantly increases mortality among patients with hematologic malignancy who have zygomycosis. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2008; 47(4):503–509. [PubMed: 18611163]

29. Ibrahim AS, Gebremariam T, Hussein MI, Stevens DA, Fu Y, Edwards JE Jr. et al. Comparison of lipid amphotericin B preparations in treating murine zygomycosis. *Antimicrobial agents and chemotherapy*. 2008; 52(4):1573–1576. [PubMed: 18227182]
30. Lewis RE, Albert ND, Liao G, Hou J, Prince RA, Kontoyiannis DP. Comparative pharmacodynamics of amphotericin B lipid complex and liposomal amphotericin B in a murine model of pulmonary mucormycosis. *Antimicrobial agents and chemotherapy*. 2010; 54(3):1298–1304. [PubMed: 20038620]
31. Lewis RE, Liao G, Hou J, Chamilos G, Prince RA, Kontoyiannis DP. Comparative analysis of amphotericin B lipid complex and liposomal amphotericin B kinetics of lung accumulation and fungal clearance in a murine model of acute invasive pulmonary aspergillosis. *Antimicrobial agents and chemotherapy*. 2007; 51(4):1253–1258. [PubMed: 17261624]
32. Kontoyiannis DP, Azie N, Franks B, Horn DL. Prospective antifungal therapy (PATH) alliance : focus on mucormycosis. *Mycoses*. 2013
33. Hammond SP, Baden LR, Marty FM. Mortality in hematologic malignancy and hematopoietic stem cell transplant patients with mucormycosis, 2001 to 2009. *Antimicrobial agents and chemotherapy*. 2011; 55(11):5018–5021. [PubMed: 21876046]
34. Richardson M. The ecology of the Zygomycetes and its impact on environmental exposure. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases*. 2009; 15(Suppl 5):2–9.

Figure 1. Cumulative Incidence of Mucormycosis in SOT and SCT Patients Across Two Sets of Calendar Years, 1995–2003 and 2004–2011

Legend for Figure 1: Cumulative incidence rates of IM among MCR patients who underwent SCT and SOT did not increase significantly in the latter time period, suggesting that observed increases in the absolute number of transplant patients with IM merely reflects increases in SCT and SOT procedures performed.

Figure 2. 90-day survival Across At-Risk Groups

Legend for Figure 2: There was no significant association between IM risk group and 90-day survival from diagnosis

Figure 3. 90-Day Survival Across Calendar Year Eras

Legend for Figure 3: There was no significant difference in 90-day survival between mucormycosis cases in the two time eras.

Figure 4. 90-Day Survival Across Treatment Groups (Conditional on 14-Day Survival)

Legend for Figure 4: Among 14-day survivors, there was no significant difference in 90-day survival between the three therapy groups including those on Ambisome monotherapy versus combination therapy (Amphotericin B in combination with either Caspofungin or Posaconazole); only 1 of the 18 combination therapy cases occurred in the earlier era (E1). The Ambisome monotherapy group included 14 cases from the earlier era (E1) and 22 cases from the latter era (E2).

Figure 5. 90-day survival for pure mucorales vs. mixed mold infections

Legend for Figure 5: There was a slightly worse survival noted in the pure Mucorales group compared to the group with mixed mold infections.

Figure 6. 90-day survival for neutropenic vs. non-neutropenic patients

Legend for Figure 6: Non-neutropenic patients were associated with a slightly better survival compared to the neutropenic patients.

Figure 7. Total annual SOT and SCT procedures and cases of Mucorales

Legend for Figure 7: Illustration of growth of in the total number of patients undergoing their first SOT and SCT procedure at one of the 3 sites (Mayo Rochester) each year, from 1995–2010, and corresponding number of Mucorales cases in in this population.

Table 1

Comparison of patient demographics and clinical presentation of invasive mucormycosis cases across calendar year eras

Variable	1995–2003 (n=30)	2004–2011 (n=71)	P-value
Age at time of IM diagnosis	49.7±17.3	53.3±15.4	0.307
Gender			0.090
•Male	14 (47%)	46 (65%)	
•Female	16 (53%)	25 (35%)	
At-Risk Group			0.036
•Heme Onc	11 (37%)	37 (52%)	
•SCT	3 (10%)	15 (21%)	
•SOT	6 (20%)	11 (15%)	
•Other	10 (33%)	8 (11%)	
Site			0.254
•MCR	25 (83%)	50 (70%)	
•MCA	3 (10%)	7 (10%)	
•MCF	2 (7%)	14 (20%)	
Mucorales Site of infection			0.122
•Rhino/Orbital/Cerebral	6 (20%)	16 (23%)	
•Pulmonary/Lung	9 (30%)	36 (51%)	
•Abdominal/Other	10 (33%)	11 (15%)	
•Multiple Sites	5 (17%)	8 (11%)	
Certainty of Mucorales			0.181
•Proven	26 (87%)	53 (75%)	
•Probable	4 (13%)	18 (25%)	
Prior antifungal use	15 (50%)	55 (77%)	0.006
Antifungal Exposure (N=70)			<.001*
•fluconazole	12 / 15 (80%)	14 / 55 (25%)	
•voriconazole/echinocandin	1 / 15 (7%)	40 / 55 (73%)	
•amphotericin/posaconazole	2 / 15 (13%)	1 / 55 (2%)	
Mucorales with other fungi in cultures			0.115*
•Mucorales only	24 (80%)	51 (72%)	
•Mucorales + <i>Aspergillus</i>	4 (13%)	19 (27%)	
•Mucorales + other fungi	2 (7%)	1 (1%)	
GVHD			0.650*
•none	28 (93%)	60 (85%)	
•chronic	2 (7%)	10 (14%)	
•acute GVHD	0 (0%)	1 (1%)	
Neutropenic at diagnosis of IM	6 / 29 (21%)	31 / 71 (44%)	0.031

Abbreviations: GVHD, graft vs. host disease; Heme-Onc, hematology oncology; IFI, invasive filamentous fungal infection; IM, invasive mucormycosis; MCA, Mayo Clinic Arizona; MCF, Mayo Clinic Florida; MCR, Mayo Clinic Rochester; SCT, stem cell transplant; SOT indicates solid organ transplant.

Table 2

Comparison of treatment and outcome of invasive mucormycosis cases across calendar year eras

Variable	E1 1995–2003 (n=30)	E2 2004–2011 (n=71)	P-value
Surgical debridement	21 (70%)	40 (56%)	0.200
Primary Post-Dx Treatment:			0.001
•None	6 (20%)	5 (7%)	
•AMB alone	23 (77%)	36 (51%)	
•AMB in comb. w/ CASPO and/or POSA	1 (3%)	20 (28%)	
•Other (CASPO/POSA/VORI comb, w/o AMB)	0 (0%)	10 (14%)	
Any combination of AMB in Post-Dx Treatments	24 (80%)	58 (82%)	0.843
Any combination of POSA in Post-Dx Treatments	0 (0%)	23 (32%)	<.001
Any combination of CASPO in Post-Dx Treatments	1 (3%)	18 (25%)	0.010
Post-Dx Treatment (at 14 day follow-up [^]):			0.041 *
•0–6 days of therapy	5 / 20 (25%)	13 / 54 (24%)	
•AMB alone	14 / 20 (70%)	22 / 54 (41%)	
•AMB in combination w/ CASPO and/or POSA	1 / 20 (5%)	17 / 54 (31%)	
•CASPO/POSA/VORI combinations w/o AMB	0 / 20 (0%)	2 / 54 (4%)	
Deaths, # events (K-M) ⁺			
•at 30-day follow-up	9 (32%)	21 (30%)	0.892
•at 90-day follow-up	13 (46%)	28 (41%)	0.667
•at 1-year follow-up	15 (54%)	40 (58%)	0.926

⁺ Cumulative # events (cumulative incidence % based on Kaplan-Meier); p-value from log-rank test

* p-value from Fisher's exact test due to low cell counts

[^] based on subgroup (N=64) who survived at least 14 days

Abbreviations: AMB indicates amphotericin B products; Caspo, caspofungin; Dx, diagnosis; Posa, posaconazole; Vori, voriconazole.