Voriconazole-associated zygomycosis: a significant consequence of evolving antifungal prophylaxis and immunosuppression practices?

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Abstract

Mucormycosis (zygomycosis) is an uncommon infection that affects severely immunocompromised patients and those with poorly controlled diabetes mellitus. A recent increase in the incidence of mucormycosis at many transplant centres has been linked to the introduction and widespread use of voriconazole prophylaxis in these high-risk populations. However, it is not known if this association reflects a true epidemiological link or represents a marker of changing immunosuppression occurring in parallel with the evolution of transplant practices and immunosuppression strategies.

Keywords: Immunocompromised, invasive fungal infections, mucormycosis, posaconazole, voriconazole, zygomycosis

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Introduction

Mucormycosis is a unifying term for a group of filamentous fungi in the phylum Mucorales that are capable of causing severe, frequently life-threatening infections in humans. Agents of mucormycosis (e.g. Rhizopus, Mucor, Cunninghamella, Apophysomyces, Absidia, Saksenaea, Rhizomucor) are found worldwide in soil, decaying organic matter and food [1]. These fungi are opportunistic pathogens, causing disease in immunocompromised patients and diabetes patients. A hallmark of invasive mucormycosis is its rapid progression to tissue necrosis because of blood vessel invasion and infarction [2]. The clinical manifestations of mucormycosis include pulmonary, rhinocerebral, gastrointestinal, cutaneous and disseminated infections, although other uncommon presentations can be encountered.

Epidemiology and Risk Factors

Among filamentous fungi, Mucorales have the ability to infect a broader, more heterogeneous population of human hosts than other opportunistic moulds. Historically, diabetes mellitus has been the most common underlying risk factor. However, during the past 30 years there has been an increase in the numbers of mucormycosis patients with malignancy and those undergoing haematopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT) [3]. Additionally, iron overload, ketoacidosis, prolonged high-dose systemic corticosteroid therapy and penetrating trauma or burns are well-documented underlying risk factors for mucormycosis.

Although the number of published reports of mucormycosis cases has steadily increased since the 1940s [4], the incidence of this infection has accelerated in many centres in the last decade. At the University of Texas M. D. Anderson Cancer Center, for example, the incidence of mucormycosis increased from 0.0079/1000 patient-days in 1999 (prior to usage of voriconazole (VRC)) to 0.095/1000 patient-days during 2002–2004 [5,6]. This trend was especially notable in the HSCT population, in which the proportion of all invasive fungal infections (IFIs) represented by this mycosis increased from 0.25% during 1989–1998 to 1.55% during 2002–2004 [5].

The recent increase in mucormycosis cases has been temporally linked in many transplant centres to the introduction and widespread use of VRC, a drug that lacks activity against the Mucorales [7–9]. Not surprisingly, the increase in cases of mucormycosis is often associated with a decrease in the incidence of documented cases of invasive aspergillosis (IA) [7]. These parallel temporal trends raise the question of whether mucormycosis has increased because of the
improved management of more common opportunistic moulds such as Aspergillus, or whether VRC exerts selective pressure on the microbiology and pathogenesis of fungal pneumonia in the immunocompromised host.

Voriconazole Pre-exposure as a Risk Factor for Mucormycosis

Marty et al. [10] first described an increased frequency of mucormycosis after VRC prophylaxis among recipients of allogeneic HSCT. Subsequently, several retrospective case series from geographically distinct transplant centres in the USA suggested an association between prior VRC exposure (as prophylaxis or pre-emptive antifungal therapy) and the subsequent development of mucormycosis (Table I) [5,11–15]. Of the 150 cases of mucormycosis from these combined series, 73 (49%) were encountered in the setting of VRC prophylaxis.

Zygomycetes species distribution (51% Rhizopus), organ involvement (half of the cases were pulmonary infections) and mortality were consistent with earlier reports of mucormycosis prior to the availability of VRC. Although data are scant, mucormycosis should be an important consideration in a patient with presumed fungal infection and adequate VRC serum levels. In a small study, mucormycosis represented 40% of VRC breakthrough infections, typically associated with prolonged exposure and VRC steady state levels >2 mg/L [15].

The association of VRC with breakthrough mucormycosis has been examined in two prospective studies. A prospective survey of invasive fungal infections, a controlled study of 393 transplant recipients (Transplant Associated Infection Surveillance Network (TRANSNET)), demonstrated that VRC was associated more frequently with breakthrough infections caused by Zygomycetes and Fusarium spp. than with breakthrough infections caused by Aspergillus spp. (odds ratio 24.0) [16]. In the only prospective (single-institution) study of mucormycosis risk in the context of the relative frequency of other moulds, Kontoyiannis et al. [5] found that prior VRC prophylaxis was an independent risk factor for developing mucormycosis rather than IA in patients with haematological malignancies.

In contrast with these studies, in a large prospective study comparing fluconazole and VRC for the prevention of IFIs, Wingard et al. did not find excess numbers of cases of mucormycosis in the VRC-treated group (two mucormycosis cases in 305 VRC-treated patients and three in 295 fluconazole-treated patients) [17]. However, the rate of IFI in both arms of the study was low (10.6% for the fluconazole group and 6.6% for the VRC group at 6 months), perhaps indicating that a relatively lower-risk cohort of HSCT recipients had been enrolled in the clinical trial.

Is there a Causal Link between VRC Exposure and Excess Numbers of Cases of Mucormycosis, or does VRC Make a Pre-existing Trend More Apparent?

Given that the incidence of mucormycosis was increasing prior to the widespread use of VRC [6,18,19], the use of this and other newer antifungal agents may have only accelerated the progress of a pre-existing trend and the increasing incidence may, perhaps, represent a marker of changes in immunosuppression status.

The availability of VRC in oral and parenteral formulations permits extended periods of antifungal exposure in highly immunosuppressed patients. One could hypothesize that HSCT patients, by not developing aspergillosis during the post-transplant period, might subsequently develop mucormycosis in the setting of continuous immunosuppression associated with graft vs. host disease (GvHD) and exposure to high-dose corticosteroids. Single-centre studies of IFI epidemiology may support this hypothesis, where mucormycosis was found to predominate as a ‘very late’ (>100 days after HSCT) infection, primarily in patients who had received non-myeloablative conditioning, had received frequent blood transfusions with evidence of iron overload (ferritin >2000 ng/mL), had chronic GvHD, and had received high-dose (2–3 mg/kg/day) corticosteroids [20]. It is possible that metabolic factors associated with recurrent transfusions and prolonged high-dose corticosteroid therapy (i.e. iron overload, hyperglycaemia), in addition to GvHD and suppressed cell-mediated immunity, may favour the development of mucormycosis independent of VRC exposure, in these chronically immunosuppressed patients.

Other non-mutually exclusive explanations may exist. Lamaris et al. [21] showed that ex vivo exposure of Rhizopus

### TABLE I. Studies of mucormycosis in which voriconazole (VRC) prophylaxis was included as a possible risk factor

<table>
<thead>
<tr>
<th>Authors/year/reference</th>
<th>Cases of mucormycosis, n</th>
<th>Study type</th>
<th>Cases on VRC prophylaxis, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marty et al. 2004 [10]</td>
<td>4</td>
<td>Retrospective</td>
<td>75</td>
</tr>
<tr>
<td>Schalk et al. 2006 [14]</td>
<td>4</td>
<td>Retrospective</td>
<td>75</td>
</tr>
<tr>
<td>TRANSNET 2006 [16]</td>
<td>105</td>
<td>Prospective</td>
<td>45</td>
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</table>
oryzae to VRC increases the virulence of the fungus in a corticosteroid-immunosuppressed mouse model of invasive pulmonary zygomycosis. This switch to a hypervirulent phenotype was specific to VRC pre-exposure because no increase in mortality was observed with other azoles. However, the virulence reverted to baseline when exposure to VRC was terminated, suggesting an unstable epigenetic modification rather than a genetic change. Examples of possible epigenetic modifications following VRC exposure may include upregulation of an efflux pump involved in the secretion of virulence factors (i.e. proteases or iron-scavenging siderophores). Alternatively, changes in sterol composition may result in increased adherence to endothelial surfaces or to the extracellular matrix (Fig. 1). By contrast with *R. oryzae*, pre-exposure of *Aspergillus fumigatus* to VRC did not enhance the virulence of the fungus in a non-vertebrate model of infection [22], suggesting the uniqueness of this phenomenon for fungi of the phyla Mucoraceae.

**Challenges in Diagnosis of VRC-associated Mucormycosis**

As mucormycosis typically occurs in patients treated with VRC for presumed aspergillosis, making an early distinction between these mycoses, the clinical features of which, to some extent, overlap, is critical for the effective management of this devastating infection. Unfortunately, the non-specific clinical presentation of mucormycosis, combined with the intrinsic difficulty in diagnosis, impedes the rapid definitive diagnosis and treatment of the infection.

Definitive diagnosis requires histopathological proof of mycelia in tissue samples, with or without positive culture. However, 30% of histopathology-proven mucormycosis samples are culture-negative and there is a 20% discordance between morphology-based and sequencing-based identification [5]. In addition, laboratory contaminants can accidentally contribute to false positive results [23], and the isolation of fungi causing mucormycosis from sputum or bronchoalveolar lavage specimens is not synonymous with invasive infection, even in cancer patients [6]. Recently, real-time quantitative polymerase chain reaction (qPCR) assays in a rabbit model, performed by targeting the 28S rRNA gene showed promise for the diagnosis of some Mucorales species [24]. However, how much promise this technique holds as a clinical application remains unknown. Another potentially useful diagnostic algorithm is the combination of a computed tomography (CT)-guided percutaneous biopsy and non-culture based diagnostics (Galactomannan (GM), PCR) in patients with invasive pulmonary infections where mucormycosis is included in the differential diagnosis [25].

If the results of *Aspergillus* PCR and/or GM enzyme immunoassay (EIA) are negative, the physician should have an even higher level of suspicion of mucormycosis and request a PCR test specific for mucormycosis and should change therapy to drugs with activity against it [25].

Regarding the clinical presentation of mucormycosis in patients with haematological malignancies, a significant overlap exists with invasive pulmonary aspergillosis (IPA). The presence of sinusitis, VRC pre-exposure and multiple nodules (>10) with micronodules (<1 cm) in chest CT favour the diagnosis of mucormycosis [26].

*FIG. 1.* Possible epigenetic mechanisms of increased virulence in *Rhizopus oryzae* following voriconazole exposure.

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Any delay in differentiating between pulmonary mucormycosis and IPA can have a devastating consequence for patient survival [27]. For example, in a study of patients with haematological malignancies, Chamilos et al. [27] showed that delaying amphotericin B (AmB)-based treatment for >6 days after the onset of symptoms of mucormycosis can result in a two-fold increase in mortality rate, compared with early treatment, and a <20% survival rate at 12 weeks after diagnosis. This delay, along with severity of the active malignancy and monocytopenia, was independently associated with a poor outcome.

**Treatment**

Voriconazole-associated mucormycosis appears to have a poor outcome, perhaps reflecting the advanced immunosuppressive state of infected patients along with the aggressiveness of the infection. In a multicentre retrospective study, Trifilio et al. [28] described a 73% mortality rate in patients with this type of infection, but found no particular association between the causative *Mucorales* and the type of infection.

There is no systematic clinical experience with the treatment of VRC-associated mucormycosis, a disease in which therapeutic options are limited. AmB has been the standard therapy against mucormycosis [29], but over the last decade, because of the nephrotoxicity problems associated with the high doses required to treat this refractory infection, lipid formulations of AmB have become the preferred therapy [30, 31].

Well-tolerated, orally administered agents with activity against *Mucorales* are clearly needed for the long-term management of mucormycosis. Posaconazole is an extended-spectrum, orally administered triazole with proven activity *in vitro* against *Mucorales*, both in an immunosuppressed mouse model of mucormycosis and in humans as salvage therapy against mucormycosis [32,33]. Whether prior exposure of *Mucorales* to VRC ‘devitalizes’ the subsequent activity of posaconazole remains an important, as yet unresolved, question as triazole cross-resistance has been described in *Aspergillus* species. However, in view of its activity against IA and mucormycosis, posaconazole may represent a preferable treatment option because it may be able to prevent both of these IFIs.

**Conclusions**

Voriconazole prophylaxis has been associated in retrospective case series and prospective epidemiological surveillance programmes with increased numbers of reports of mucormycosis. Although emerging preclinical data suggest that VRC exposure can modulate the virulence of some *Mucorales*, the clinical implications of these findings remain unknown. Many questions concerning the host and immunosuppression-specific risk factors that may be driving an increase in the incidence of mucormycosis remain answered, as do questions about the optimal approach towards diagnosing and treating the increasingly complex cases of this devastating infection (Table 2). Future experimental and clinical studies are required to clarify causality and to establish better diagnostic, therapeutic and prophylactic algorithms for VRC-associated mucormycosis.

**Table 2.** Unanswered questions regarding voriconazole (VRC)-associated mucormycosis

<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
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<tbody>
<tr>
<td>Why is this syndrome described only with VRC and not with other triazoles?</td>
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<tr>
<td>What is the role of suboptimal or elevated VRC exposures (i.e. plasma drug levels) in mucormycosis risk?</td>
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<tr>
<td>Is this an associated entity seen only in allo-HSCT recipients and leukemias patients?</td>
<td></td>
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<tr>
<td>What do we know about VRC breakthrough mucormycosis in other host groups (e.g. SOT recipients, chronic granulomatous disease patients)?</td>
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<tr>
<td>Are diabetes, hyperglycaemia, tumour necrosis factor inhibition or iron overload in high-risk haematology patients modulators for increased risk, specifically for mucormycosis in the setting of VRC exposure?</td>
<td></td>
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<tr>
<td>How can the infection be diagnosed earlier, based on computed tomography characteristics or use of polymerase chain reaction?</td>
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<tr>
<td>Is this entity more frequent when VRC is used as primary or secondary prophylaxis?</td>
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<tr>
<td>Is VRC-associated mucormycosis a ‘late’ infection (seen typically after the period of onset of invasive aspergillosis post-SCT in patients effectively ‘protected’ from <em>Candida</em> and <em>Aspergillus</em> or does VRC create selective pressure for mucormycosis rather than Aspergillus-associated infection)?</td>
<td></td>
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<tr>
<td>Would posaconazole prophylaxis decrease the frequency of, or even eliminate, mucormycosis?</td>
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</table>

**Transparency Declaration**

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**References**


17. Wingard et al. 49th Annual Meeting of the American Society of Hematology, Abstract 163.


