Review

Diagnosis and Treatment of Mucormycosis in Patients with Hematological Malignancies [Translated Article]

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ABSTRACT

The risk of invasive fungal infections (IFIs) is extremely high in patients with hematological malignancies due to the prolonged and profound neutropenia and immunosuppression after chemotherapy and hematopoietic stem cell transplantation. There has been increasing interest in mucormycosis despite its relatively uncommon occurrence, because occasional breakthrough infections have been observed under anti-*Aspergillus* prophylaxis. The aggressive nature of mucormycosis easily leads to high mortality because of delays in diagnosis and incorrect treatment decisions, which are due in part to lack of adjunctive diagnostic tools and having similar clinical and radiological features with invasive aspergillosis. The only currently available antifungals against Mucorales in Japan are amphotericin B formulations. Thus, comprehensive therapeutic strategies, including surgery, should be considered to achieve a successful outcome.

Key words : hematological malignancies, invasive fungal infections, liposomal-amphotericin B, mucormycosis

Introduction

Invasive fungal infections (IFIs) are important complications affecting the prognosis of hematological diseases. Remarkable advances in the treatment of hematopoietic stem cell transplantation (HSCT) have expanded the application to the elderly or patients without an HLA (human leukocyte antigen) matched donor, resulting in the increased risks of IFIs. Although advanced diagnostic techniques including high-resolution CT scans and non-culture based diagnosis, and the development of newer drugs with a broad antifungal spectrum and low toxicity, have improved the prevention and treatment of invasive candidiasis (IC) and invasive aspergillosis (IA), other forms of fungal infection are emerging. One of these is mucormycosis. The difficulty in distinguishing between mucormycosis and IA based on their clinical course or imaging results is a matter, because voriconazole (VRCZ: the first drug of choice for IA) is ineffective against mucormycosis. Although guidelines specific to mucormy-

cosis have been published in recent years^{1,2)}, we still lack adjunctive diagnosis techniques for mucormycosis and effective antifungal agents are also limited. As a result, the prognosis of mucormycosis is even very poor now. In this paper, we review the current status of mucormycosis in immunocompromised patients with hematological malignancies, and consider how these problems can be overcome and the future prospects for its management.

Epidemiology of mucormycosis

Mucormycosis, previously known as zygomycosis, was renamed following the elimination of the zygomycota phylum based on a phylogenetic classification using molecular biological techniques. It is now considered to belong to the pathogenic fungi that are mainly in the Mucorales order of the newly classified mucoromycotina subphylum. Although mucormycosis also occurs in immunocompetent patients³⁾ and its reported frequency differs between institutions, the frequency is significantly higher and the prognosis is

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extremely poor in immunocompromised patients.

Most cases of mucormycosis are currently observed in hematological patients, in spite of the common occurrence in diabetes patients previously⁴. A prospective multicenter study conducted for HSCT patients between 2001 and 2006 by the Transplant Associated Infection Surveillance Network (TRANSNET) reported that the frequency of mucormycosis came in third place (8%) after IA (43%) and IC (28%), and the cumulative incidence at one year after transplantation was 0.29%⁵. In another prospective study for HSCT patients between 2008 and 2010 by the Gruppo Italiano Trapianto Midollo Osseo (GITMO), an incidence of mucormycosis was 3.7%, following IA (81.1%) and IC (11%)⁶. A prospective multicenter study for patients with IFIs by the PATH (Prospective Antifungal Therapy) Alliance identified mucormycosis in 18 out of overall 250 IFI cases (7.2%)⁷. Although the incidence of mucormycosis is relative rare even in high-risk hematological patients, the onset might be underestimated due to the difficulty of ante-mortem diagnosis, and therefore observing future trends is very important.

The fungi responsible for mucormycosis in humans include *Rhizopus* spp., *Mucor* spp., *Rhizomucor* spp., *Cunninghamella* spp. and *Lichtheimia* spp. The identification of fungal species is extremely difficult, because mucormycosis is mostly diagnosed by pathological examination and predominantly confirmed by autopsy. The TRANSNET study reported *Rhizopus* spp. as the most common (55 cases = 52.4%), followed by *Mucor* spp. (15.6%) and *Rhizomucor* spp. (9.1%) (Table 1)⁸, and the PATH alliance study also reported *Rhizopus* spp. as the most common (52.1%), followed by *Mucor* spp. (23.1%)⁹. In contrast with the results of these two studies in North America, the *Lichtheimia* spp. is relatively common in Europe^{10, 11}. Although there is no large-scale clinical research of mucormycosis in Japan, the distribution of pathogenic fungi seems to be slightly different from those in Europe and the United States¹².

Mucormycosis includes five disease types: pulmonary, rhino-orbito-cerebral, gastrointestinal, cutaneous and disseminated¹³. The fungus mainly attaches to the nasal passages or respiratory tract, penetrates through the airways and often forms lesions in the lungs or nasal cavity (Fig. 1). It may sometimes enter via the gastrointestinal

tract, or occasionally propagates to multiple organs via the blood stream if the host immunity decreases. The sites of infection vary with underlying disease, the main of which are lungs followed by rhinocranial region in hematological patients^{4, 9, 10}.

Risk factors of mucormycosis

As with IA, risk factors of mucormycosis include prolonged severe neutropenia, high-risk transplantations, graft-versus-host disease (GVHD) and its treatments, prolonged use of high-dose steroids, cytomegalovirus infections and previous IFIs. Iron overload, administration of deferoxamine, persistent hyperglycemia and poorly controlled diabetes, and the use of VRCZ are relatively characteristic of mucormycosis (Fig. 1)^{14–16}. When the level of free iron, normally controlled by transferrin, is increased, excess iron is used for fungal growth¹⁷. Although deferoxamine is the iron chelating agent, it binds with iron to form siderophores which act as iron donors to fungi and promote fungal growth. Diabetes and hyperglycemia are independent risk factors for mucormycosis in patients with or without hematological diseases³. When high-dose steroids are administered, the migratory and fungicidal abilities of phagocytes are diminished, and their ability to prevent infection is suppressed. VRCZ has been regarded as a risk factor, and breakthrough infections while on VRCZ have been reported^{18, 19}. However, careful consideration should be given to the fact that patients receiving VRCZ prophylaxis are generally at high risk for IFIs, and extremely like to have other factors related to an increased risk of IFIs including prolonged neutropenia and the use of steroids. The assessment of detection frequency at each medical facility is important, because the onset of mucormycosis tends to accumulate at same facilities.

Diagnosis of mucormycosis

The first step towards a definitive diagnosis of mucormycosis is “doubt it”. Mucormycosis should be always kept in mind in the differential diagnosis of IFIs, because its clinical symptoms and imaging findings are similar to those of IA, and there are few adjunctive diagnosis methods besides pathological examination.

Table 1 Epidemiology and outcome of mucormycosis in patients with hematological malignancies

	Period	Country	No. of total pts.	No. of pulmonary cases	Species in total pts.	Global Mortality	No. of pts. with HM	No of pulmonary cases	Species in HM pts.	Mortality in HM pts.
Roden <i>et al</i> (2005) ⁴⁾	1885-2003	Global review	929	224 (24.1%)	<i>Rhizopus</i> (47%) <i>Mucor</i> (18%) <i>Cunninghamella</i> (7%)	54% (overall)	HM 147 (15.8%) HSCT 44 (4.7%)	-	-	HM (-) HSCT 91%
Park <i>et al</i> (2011) ⁸⁾	2001-2006	USA	105	52 (53.1%)	<i>Rhizopus</i> (52.4%) <i>Mucor</i> (18.1%) <i>Rhizomucor</i> (6.7%)	58.2% (day90)	HSCT 7 (62.1%)	-	<i>Rhizopus</i> (50.6%) <i>Mucor</i> (15.6%) <i>Rhizomucor</i> (9.1%)	-
Kontoyiannis <i>et al</i> (2014) ⁹⁾	2004-2008	North America	121	46 (38.0%)	<i>Rhizopus</i> (52.1%) <i>Mucor</i> (23.1%) <i>Rhizomucor</i> (7.4%)	59% (12weeks)	74 (61.2%)	32 (43.2%)	<i>Rhizopus</i> (51.4%) <i>Mucor</i> (21.6%) <i>Rhizomucor</i> (9.5%)	-
Skiada <i>et al</i> (2011) ¹⁰⁾	2005-2007	Europe	230	68 (29.6%)	<i>Rhizopus</i> (33.7%) <i>Mucor</i> (19.2%) <i>Lichtheimia</i> (18.6%)	47% (overall) 56% (Pulmonary)	HM 102 (44.3%) HSCT 21 (9.1%)	HM 35 (34.3%)	-	HM 52% HSCT 76%
Lanternier <i>et al</i> (2012) ¹¹⁾	2005-2007	France	101	28 (27.7%)	<i>Rhizopus</i> (51%) <i>Lichtheimia</i> (29%) <i>Rhizomucor</i> (7%)	44% (day90) 48% (Pulmonary) 70% (overall)	50 (49.5%) (inc. HSCT 12)	22 (44.0%)	-	60%
Chakrabarti <i>et al</i> (2006) ³⁾	2000-2004	India	178	12 (6.7%)	<i>Rhizopus</i> (69.8%) <i>Apophysomyces</i> (27.0%) <i>Rhizomucor</i> (1.6%)	28%* 28%* 28%*	2 (1.1%)	-	-	-

HM: hematological malignancies, HSCT: Hematopoietic stem cell transplantation

* Data on patients who received amphotericin B treatment totaling 1 g or more.

Fig. 1 Clinical manifestations, risk and prognostic factors of mucormycosis. CNS: central nervous system, HSCT: hematopoietic stem cell transplantation, GVHD: graft-versus-host disease, VRCZ: voriconazole

1. Diagnostic imaging

Although distinguishing pulmonary mucormycosis from invasive pulmonary aspergillosis (IPA) is difficult due to the similar clinical features and CT findings, there are a few signs indicative of mucormycosis, such as the presence of multiple nodules at 10 or more sites, pleural effusion, reversed halo sign (RHS), and cellulitis of the thoracic wall adjacent to pulmonary infarctions^{15, 20, 21}. RHS is defined as central ground-glass opacity surrounded by a complete or nearly complete ring of dense consolidation, as demonstrated on high-resolution CT scan. The central ground-glass opacity corresponds to coagulation necrosis which presents as reticular pattern, because the fungus spreads along alveolar lumen without disrupting the blood vessels and air content is remained in alveolar space. The ring-shaped consolidation corresponds to granulomatous tissue, because severe bleeding or inflammation are caused by neutrophils and macrophages attack to the outwardly expanding fungi during immune restoration²². RHS must be useful in the early diagnosis, because it is observed more often in mucormycosis than in IPA and appears in the early stage²⁰.

A sinus CT scan is also recommended in patients with pulmonary lesions suspicious of

mucormycosis, because sinusitis is presented as a complication more frequently than IPA²¹. Mucosal necrosis of the palate and nasal cavity, together with orbital cellulitis, cavernous sinus thrombosis or cerebral abscess is observed in some cases. When sinus lesions are found, a brain CT scan or MRI is preferably performed to screen central nervous system (CNS) lesions. Intensification of treatment is required for CNS mucormycosis¹.

2. Culturing and histopathological diagnosis

Unlike in the case of IA, there are no specific antigen tests for mucormycosis, and the β -D-glucan test results are negative due to its small amount of production. Although a positive β -D-glucan test result can not rule out mucormycosis, a negative result of *Aspergillus* galactomannan antigen suggests the possibility of mucormycosis².

Mucorales is angioinvasive, but hardly ever yields a positive blood culture test result. Although it is important to repeatedly collect clinical specimens such as sputum, bronchoalveolar lavage fluid, pleural fluid, nasal secretions and sinus aspirate, the positive culture rate is not high and therefore diagnosis is principally made by histopathological examination from biopsy samples. Even if the fungus is pathologically confirmed the culture can be still negative in some cases.

Although biopsies are often difficult due to the increased susceptibility of infection or bleeding in hematological patients, the usefulness of CT-guided needle biopsy has been reported²³⁾. Histopathological characteristics have the potential to distinguish Mucorales from *Aspergillus* species. Mucorales produce broad, aseptate hyphae branching irregularly at obtuse angles²⁴⁾, whereas *Aspergillus* species are thinner, septate, with regular branching at acute angles. Along with the difficulty in detecting hyphae on biopsy, coexistence with other fungal infections make it difficult to diagnose mucormycosis in high-risk hematological patients. However, the greatest efforts possible should be made to identify the fungal species, because the differences in treatment response and prognosis depending on the fungal strains have been suggested²⁵⁾. Although advances have been made in molecular identification of fungal species including identification by DNA sequencing, identification by PCR methods specific to the Mucorales, and identification by in situ hybridization using pathology sections²⁴⁾, none of them have been standardized and therefore specialist consultation is necessary.

Prevention of mucormycosis

Antifungal prophylaxis is primarily targeted at IC and IA, and prophylaxis against mucormycosis is not generally performed. However, species of Mucorales widely distributed in the world, despite being not as common as *Aspergillus* spp. Careful environment management is therefore essential, based on several reports of outbreaks during medical implements and rebuilding works²⁶⁾. Posaconazole is expected to be effective as prophylaxis of mucormycosis, which is not currently available in Japan. Two prospective studies comparing posaconazole with fluconazole (FLCZ) or itraconazole (ITCZ) for prophylaxis against IFIs in allogeneic HSCT recipients with severe GVHD and in prolonged neutropenic patients with acute myelogenous leukemia or myelodysplastic syndrome reported one case of mucormycosis in the control (FLCZ or ITCZ) group, compared with none in the posaconazole group^{27, 28)}. However, there has even been a report of a breakthrough infection while on posaconazole²⁹⁾, and therefore the efficacy of prophylactic posaconazole is difficult to discuss solely on the basis of these results.

Treatment of mucormycosis

There are three approaches that can be considered for the treatment of mucormycosis: (1) elimination of risk factors, (2) treatment with antifungal drugs, and (3) surgical excision.

1. Elimination of risk factors

Neutropenia and immunosuppression are key risk factors in hematological patients, which can be reduced by protected environment (high efficiency particulate air (HEPA) filters), the use of granulocyte-colony stimulating factor (G-CSF)²⁾, and tapering of immunosuppressants and steroids if possible. Attention to a breakthrough infection under VRCZ administration should be paid, because VRCZ reportedly has the potential to enhance the pathogenicity of Mucorales³⁰⁾. Management of transfusion-induced iron overload (by administering deferasirox) and hyperglycemia is also important.

2. Treatment with antifungal drugs

Immediate initiation of high-dose amphotericin B formulations is essential in cases where mucormycosis cannot be ruled out based on the clinical course and laboratory findings, because mucormycosis is difficult to distinguish from IA and to diagnose ante-mortem, and its causative agents are resistant to VRCZ. Amphotericin B and its liposomal formulation called liposomal amphotericin B (L-AMB) are currently available in Japan, the latter of which is recommended due to the lower renal toxicity and better CNS penetration.

The treatment with L-AMB at 5 mg/kg or more is recommended for mucormycosis, because of the poorer response compared with IA^{1, 2, 15)}. The use of high-dose L-AMB should not be hesitated even in patients receiving nephrotoxic drugs, with adequate replacement fluids and strict electrolyte management. We have experienced a fatal case of pulmonary mucormycosis that was hardly distinguishable from IPA and firstly treated with 3 mg/kg of L-AMB²²⁾. Although currently available dose of L-AMB is 5 mg/kg in Japan and the superiority of initial treatment with L-AMB at 10 mg/kg was not shown in a recent prospective preliminary study³¹⁾, an increased dose of 7.5-10 mg/kg is recommended as an expert opinion¹⁴⁾. The use of L-AMB at 10 mg/kg is recommended for the treatment of CNS mucormycosis by the European guidelines^{1, 2)}. The dosage of L-AMB

should be therefore individualized for each patient, by reference to patient's background, organ functions including renal function, and the progress of mucormycosis.

The initiation timing of L-AMB is also important^{9, 32}. Chamilos *et al.* reported a 2-fold increase in mortality rate at 12 weeks after diagnosis in the delayed treatment group (initiating treatment ≥ 6 days after diagnosis) compared to the early treatment group (82.9% versus 48.6%)³². A significant association between the timing of initiation and the prognosis has been also reported by Kontoyiannis *et al.*, based on the result that the treatment initiation within 3 days led to better survival⁹. Prompt initiation of treatment is highly desirable in cases where mucormycosis cannot be ruled out, because even a slight delay in treatment potentially result in a lethal outcome.

Although a treatment duration with L-AMB has been controversial, it is recommended to continue until the image findings are completely improved and all risk factors have been eliminated (e. g., until immunosuppressants are no longer being administered), and at least for a continuous period of 6-8 weeks. For L-AMB intolerant patients, or as maintenance therapy after an initial treatment of L-AMB, the administration of posaconazole is recommended in Europe and the United States^{33, 34}, and the effectiveness of isavuconazole against mucormycosis has been recently reported³⁵. However, neither of these has yet been approved in Japan.

For combination therapy, Pagano *et al.* reported the beneficial effect of posaconazole in addition to L-AMB in hematological patients failing to response to L-AMB monotherapy³⁶. Concomitant use of echinocandins with amphotericin B formulations has also been reported to show improved efficacy, because synergetic effect was obtained in spite of little *in vivo* activity of echinocandins against Mucorales³⁷. However, the effectiveness of combination therapy with echinocandins and amphotericin B formulations is not confirmed in other studies^{38, 39}, and therefore it is still controversial. At least in the cases where the first-line therapy is ineffective, the combination therapy is worthy of consideration.

Deferasirox is a new iron chelator which does not act as a siderophore, and suppresses the proliferation of Mucorales by depriving it of iron. However, in a recent prospective randomized controlled trial comparing the treatment with L-

AMB plus deferasirox with the treatment with L-AMB plus placebo, the effectiveness of concomitant deferasirox with L-AMB was not demonstrated, and the mortality rate was higher in the deferasirox group⁴⁰. A definitive conclusion has not yet been reached about concomitant use of deferasirox and L-AMB, because the deferasirox group has included more patients with hematological malignancy or neutropenia. Concomitant deferasirox therapy is discouraged in the European guidelines on mucormycosis^{1, 2}.

3. Surgical excision

Infected tissues should be surgically removed if feasible. Especially in cases with rhino-orbito-cerebral mucormycosis, surgical debridement is recommended for local control and elimination of necrotic tissue which are poorly penetrated by antifungal agents^{1, 2, 41}.

On the other hand, pulmonary mucormycosis, mainly seen in hematological patients, are difficult to excise in most cases due to increased susceptibility of infection and bleeding, and therefore surgical resection is made available as an option in less-severely ill patients with unifocal lesion^{42, 43}. Surgical resection for pulmonary mucormycosis has been reported to lead to a better prognosis^{10, 11}, and we have also experienced successful cases by a combination of surgical excision and antifungal therapy with high-dose L-AMB despite profound immunosuppression and severe neutropenia in the early period after allogeneic HSCT⁴⁴. Surgical intervention is crucial and should be considered under antifungal therapy coupled with ongoing treatment for underlying disease and enhancement of immune recovery.

Prognosis of mucormycosis

Mortality depends on factors including underlying disease, site of infection, fungal species, and therapeutic regimen. The prognosis is poor in patients with active malignancy or neutropenia⁴⁵. The mortality rate of pulmonary mucormycosis (the most common type) in hematological patients reaches approximately 75%, and can be even higher in highly immunosuppressed patients with disseminated diseases^{4, 45}. The detection of *Cunninghamella* spp. is an independent factor for a poor prognosis^{4, 25}. Although a poor prognosis of breakthrough infections while on caspofungin and/or VRCZ has been also reported^{9, 10}, several questions remain unanswered, such as whether

these antifungal agents cause an increase in pathogenicity³⁰⁾, or whether the frequent use of these agents leads to delay of diagnosis in high-risk patients.

Conclusion

Although consensus recommendations have been gradually established and practical guidelines have recently published for the diagnosis and treatment of mucormycosis, there is still insufficient evidence to yield therapeutic success compared with other fungal infections. Despite greater risks for mucormycosis in hematological patients, ante-mortem diagnosis is not feasible due to increased bleeding risk and susceptibility of infections. There are no antifungal agents approved for prophylaxis and only a limited number of choice for treatment. The timing for surgical intervention, the proper doses of L-AMB depending on fungal species, and the pros and cons of combination therapy still remain as unsolved issues.

The most important things are to consider mucormycosis constantly in the differential diagnosis of IFIs, and to start high-dose L-AMB immediately when mucormycosis cannot be ruled out based on the clinical course and findings. A major step towards overcoming mucormycosis would be the comprehensive diagnosis and treatment in close cooperation with respiratory physicians, thoracic surgeons, infectious disease specialists and pathologists, for definite diagnosis, identification of fungal strains, and prompt surgical intervention.

Conflict of interest

None.

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This article is based on a study first reported in the Medical Mycology Journal Vol.57 No.4, J155-J162, with full reference.