

## Infections of the central nervous system by melanized fungi: a review of cases presented between 1999 and 2004

### Infektionen des Zentralnervensystems durch melanisierte Pilze: Eine Übersicht von Fällen präsentiert zwischen 1999 und 2004

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#### Summary

Several types of infections of the central nervous system by melanized fungi can be distinguished: (a) single-organ infection of the cerebrum, (b) extension into the cerebrum from adjacent cavities, (c) fungal presence in the cerebrospinal fluid, or (d) meningitis. The fungal order Chaetothyriales (containing *Exophiala*-like black yeasts and relatives) is particularly rich in fungi causing cerebral infections. Cases by the main agents, *Cladophialophora bantiana*, *Exophiala dermatitidis*, and *Ramichloridium mackenziei*, published during the last 5 years are reviewed. Most of these infections prove to be fatal. Resection of the lesions in combination with antimycotic therapy may reduce mortality.

#### Zusammenfassung

Mehrere Formen von Infektionen des Zentralnervensystems können unterschieden werden: (1) Infektionen limitiert auf das Cerebrum, (2) Ausdehnung der Infektion von anliegenden Höhlen ins Gehirn, (3) Befall der cerebrospinalen Flüssigkeit, oder (4) Meningitis. Die Ordnung der Chaetothyriales (die *Exophiala*-ähnliche schwarze Hefen und Verwandte enthält) sind unter den Verursachern zerebraler Infektionen überdurchschnittlich vertreten. Zu den wichtigsten infektionsverursachenden Arten gehören *Cladophialophora bantiana*, *Exophiala dermatitidis* und *Ramichloridium mackenziei*. Eine Übersicht von Infektionen verursacht von diesen Arten und in den letzten fünf Jahren veröffentlicht, wird präsentiert. Die meisten dieser Infektionen verliefen tödlich. Das Entfernen der Läsionen, kombiniert mit antimykotischer Therapie kann die Mortalität verringern.

**Key words:** *Cladophialophora*, *Exophiala*, *Ramichloridium*, Chaetothyriales, Pleosporales, Dematiaceae, melanized fungi, phaeohyphomycosis, central nervous system infections.

**Schlüsselwörter:** *Cladophialophora*, *Exophiala*, *Ramichloridium*, Chaetothyriales, Pleosporales, Dematiaceae, melanisierte Pilze, Phaeohyphomykose, Zentralnervensysteminfektion.

#### Introduction

Three main clinical types of infection of the central nervous system (CNS) by melanized fungi have been

distinguished:<sup>1</sup> (a) primary cerebral infections, where the first symptoms and localization are of neurologic nature; (b) secondary cerebral infections; extending from adjacent tissues such as the sinus, and (c) extra-cerebral infections, with cells being present in cerebrospinal fluid (CSF). A fourth category (d), meningitis, has been practically absent in melanized fungi but was observed repeatedly in recent years. In main traits the categories (a–b) are caused by phylogenetically different ascomycetous groups. Primary cerebrites (a) are

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predominated by members of the order, Chaetothyriales, comprising the black yeasts and relatives (*Exophiala*, *Cladophialophora* and *Ramichloridium*); occasionally another neurotropic but probably unrelated taxon, *Ochroconis*, is encountered. Secondary cerebrites (b) mostly are complications of chronic sinusitis by grass-inhabiting fungi of the order Pleosporales (*Bipolaris*, *Dissitimurus*, *Exserohilum*). CSF infection (c) is mostly caused by the essentially non-melanized genus *Pseudallescheria*, a member of the order Microascales. Outside these orders, phaeohyphomycotic CNS infections are extremely rare. *Cladophialophora bantiana*, *Exophiala dermatitidis*, *Ramichloridium mackenziei* and *Ochroconis gallopava* are considered to be truly neurotropic melanized fungi, exhibiting a strong predilection for nervous tissue for reasons that are thus far poorly understood. *Bipolaris spicifera*, *B. hawaiiensis* and *Curvularia pallescens* are extremely rarely found in humans, having an entirely different natural ecology as plant pathogens or saprobes.

A review of primary cerebral infections by melanized fungi with an evaluation of their potential pathogenicity was recently published by Horr  and de Hoog.<sup>1</sup> In the present study, additional case reports are listed, and antifungal susceptibility data of the etiologic agents are summarized.

### CNS infections due to *Exophiala dermatitidis*

*Exophiala dermatitidis* is the main neurotropic agent in East Asia, although its distribution, possibly as a saprobe in the environment, is worldwide.<sup>1</sup> The regional differences in the occurrence of systemic *E. dermatitidis* infections have thus far not been explained. Since infections that involve the brain appear to occur nearly only in Asian patients, the possibility of race-dependent virulence has been suggested.<sup>1</sup> *Exophiala dermatitidis* seems to be able to affect young, otherwise healthy patients.<sup>2,3</sup> Infections lead to granulomatous lesions with large numbers of multinucleate giant cells, polymorphonuclear neutrophils (PMNs) and local masses of eosinophils.<sup>2</sup> Final brain lesions may become ring-like with advancing hydrocephalus. T-lymphocyte activation of phagocytes play a role in cellular immunity.<sup>4</sup>

In the cerebral *E. dermatitidis* infections listed by Hiruma *et al.*<sup>2</sup> and Matsumoto *et al.*<sup>5</sup> six patients were female, six male. Most of them were otherwise in good health and the outcome was invariably fatal. Early symptoms were not always of neurologic nature, but might concern swelling of cervical lymph nodes and skin rash. Headache occurred only occasionally. Para-

lysis of the limbs resulting from cerebral destruction usually occurred at a later stage of infection. The mycosis is chronic, the time elapsed between first symptoms and death being between 2 months and 4 years.<sup>1</sup>

Five additional cases have been reported as the review of Horr  and de Hoog<sup>1</sup> (Table 1a). The first was in a 28-years-old immunocompetent engineer from Korea.<sup>3</sup> He had a history of severe headache that worsened over 5 days, with nausea and vomiting, but he was afebrile. He had traveled for 3 years to many countries in southeast and central Asia, North and South America, and Europe. The gram and acid-fast *Bacillus* staining and India ink preparation of the CSF remained negative, while a biopsy specimen from the ventricular wall showed fungal elements. Interestingly, his CSF showed pleocytosis with a high eosinophil count, but there was no peripheral blood eosinophilia. Despite amphotericin B (AMB) therapy, patient expired on day 13 after admission. The internal transcribed spacer (ITS) rDNA region of the strain (CBS 109154) has been sequenced; it was attributed to the pathogenic genotype A according to Matos *et al.*<sup>6</sup>

Four cases caused a pseudoepidemic as a result of contaminated injected steroid solution prepared by a compounding pharmacy in the USA.<sup>7</sup> The patients were all female; age ranges were 52–77. Symptoms were progressive diffuse or mild headache, fever (in one patient, 38.0 °C), chills, malaise, vertigo, nausea, and vomiting. Mild neck stiffness was noticed in one patient. All patients had received epidural injections with contaminated methylprednisolone acetate for 34–35 days before hospital admission. Although other factors such as inoculum size are essential parameters in successful infection, this information allows establishing an incubation period of about 1 month for the development of an *E. dermatitidis* cerebral infection after epidural inoculation. One patient died despite AMB therapy which was switched to voriconazole (VRZ) and flucytosine (5FC); another ones' condition improved after 70 days VRZ therapy. Strain CDC B-6450 from the contaminated solution was sequenced and found to belong to the pathogenic genotype A of Matos *et al.*<sup>6</sup> (G. S. de Hoog, unpublished data). In one case the injection of the same solution had been given intra-articularly; this led to a case of sacroiliitis.<sup>7</sup> This patient was a 52-years-old female who received injections 103 and 152 days before hospitalization. An earlier, very similar case by *E. dermatitidis* was reported in a patient with a painful pigmented chronic nodule on the dorsum of the right hand, at the site of intra-articular steroid injections 5 years previously.<sup>8</sup> Nucci *et al.*<sup>9,10</sup> reported a

**Table 1a** Recent central nervous system (CNS) infections by *Exophiala dermatitidis*.

Reference	Patient characteristics																					
	Age/sex/ geography/ race	Probable portal of entry	Risk factor/ underlying disease	Site of infection/number of abscesses	Specimens studied	Therapy	Outcome															
Chang <i>et al.</i> <sup>3</sup>	28/M/Korea/As	Unknown	None	Lateral ventricle/NS	Biopsy of ventricular wall	AMB (50 mg day <sup>-1</sup> )	Expired (13 day)															
Engemann <i>et al.</i> <sup>7</sup>	77/F/NC, USA/NS	Injection	Steroid	Meningitis	CSF	AMB, VRZ, 5FC	Expired (51 day)															
	61/F/NC, USA/NS							Steroid	Meningitis	CSF	VRZ	Cured										
	71/F/NC, USA/NS												Steroid	Meningitis	CSF	NS	NS					
	65/F/NC, USA/NS																	Steroid	Meningitis	CSF	NS	NS
	52/F/NC, USA/NS																					

F, female; M, male; As, Asian; CSF, cerebrospinal fluid; AMB, amphotericin B; 5FC, 5-flucytosin; VRZ, voriconazole; NS, not specified by the authors.

pseudoepidemic because of *E. jeanselmei* due to injection of a contaminated solution. Black yeast contamination of medical fluids seems to be a recurrent problem.

Kano<sup>11</sup> reported the first fatal animal case of an *E. dermatitidis* infection. A dog had a history of lymphoma and developed subcutaneous nodules on the dorsum of the neck.

### CNS infections due to *Cladophialophora bantiana*

The genus *Cladophialophora* comprises nearly exclusively human-pathogenic species, previously classified in *Cladosporium* or *Xylohypha*.<sup>12</sup> *Cladophialophora bantiana* is an unambiguously neurotropic species that only has rarely been isolated from sources other than living mammal tissue. The species has been found in cases of cerebritis worldwide.<sup>1</sup> A general preference for warmer climates with high average humidity is apparent, although the species is also known from Canada and the UK. Cases from hot, arid climatic zones like the central USA, Northern Africa, the Middle East and Australia are rare.<sup>1</sup>

The fungus is probably introduced by via inhalation and is fatal if untreated.<sup>1,13–15</sup> The infection is often found in immunocompetent young male patients,<sup>16</sup> but in *c.* 40% (20 : 53) of the cases contributing constitutional factors were reported, such as solid organ transplant<sup>17,18</sup> or drug abuse.<sup>19</sup> Nishimura and Miyaji<sup>20</sup> demonstrated that suppression of innate cellular immunity by the use of cortisone promotes *C. bantiana* infection. Infections nearly always provoke granulomatous reactions containing large numbers of giant cells. The fungal elements inside the phagocytes retain their viability.<sup>21</sup> The first line of defense probably includes

complement system and PMNs, but also T cell-mediated immunity plays a role.<sup>22</sup>

Of the previous 53 cases reviewed by Horr  and de Hoog<sup>1</sup> with *C. bantiana* involving the brain the average was 35.8 years (6 months–76 years); 13 were female, 39 male, and the ratio of caucasian : African race was 9 : 16. Remarkably, in none of the cases where the race of the patient was mentioned, an Asian patient was concerned. Pharmacologic immunosuppression was not an important risk factor,<sup>23</sup> whereas cellular immune deficiencies proved to be significant.<sup>1</sup> Occupations with regular exposure to dust such as farming were noticed to be abundantly represented among patients. The overall survival rate in patients with infection caused by *C. bantiana* ranges between 28 and 35%. Acute, severe headache, paralysis of the limbs, seizures and somnolence were the most common clinical manifestations. Although most lesions were located in the cerebrum, a few cases involved the cerebellum and ventricles and their adjacent CSF-containing cavities, suggesting hematogenous spread. Fifteen patients had a solitary, 16 had multiple (ring) lesions. Fever was mostly absent, or could be recognized only at the end of the disease. In about 25% of the cases symptoms indicative of meningitis were noted, such as neck stiffness, despite absence of meningeal involvement in histopathology. Death occurred between 3 weeks and 12 months after the first symptoms were noted.<sup>1</sup>

Six additional cases<sup>18,23–26</sup> caused by *C. bantiana* were reported (Table 1b), one of which was in an otherwise healthy individual.<sup>24</sup> Four were solid organ transplant recipients, repeatedly combined with diabetic problems. One was a pediatric patient<sup>27</sup> with a primary immunodeficiency of unknown origin. Five patients were male and one was female. The age range was

**Table 1b** Recent central nervous system (CNS) infections by *Cladophialophora bantiana*.

Patient characteristics							
Reference	Age/sex/ geography/ race	Probable portal of entry	Risk factor/ underlying disease	Site of infection/number of abscesses	Specimens studied	Therapy	Outcome
Sood <sup>24</sup>	25/M/India/cau	Inhalation of spores from the soil	None	Left posterior frontal/single	Drained pus	Excision, FLZ (200 mg twice day <sup>-1</sup> )	Cured
Silveira et al. <sup>18</sup> CBS 102586	61/M/Brasil/Afr	NS	Kidney transplant, nephrectomy	Left parieto-temporal/ single	Tissue obtained by surgery	Excision, FLZ (400 mg day <sup>-1</sup> ), relapse, FLZ + AMB (50 mg day <sup>-1</sup> )	Expired (8 months)
Osiyemi et al. <sup>25</sup>	30/F/USA/Afr	NS	Heart transplant, CMV seropositive, diabetic ketoacidosis	Left cingulate gyrus/single	Biopsy	Excision, AMB (renal insufficiency), L-AMB (10–5 mg kg day <sup>-1</sup> ), relaps, L-AMB (427 mg q.o.d.), ITZ (200 mg p.o.b.i.d.)	Expired (10 week)
Keyser et al. <sup>23</sup>	57/M/Germany/cau	NS	Diabetic, heart transplant, excision of atheroma	Right cerebellar hemisphere/single, multiple after relapse	Resected wound lips, stereotactic puncture specimen, endotracheal cultures, necropsy	Excision, L-AMB (200 mg twice day <sup>-1</sup> ) + 5FC, ITZ (200 mg twice day <sup>-1</sup> )	Expired (4 week)
Baddley et al. <sup>28</sup> Al Habib and Bryce <sup>26</sup>	NS/NS/US/NS 72/M/Canada/NS	NS NS	Lung transplant Systemic lupus erythematosus, pulmonary tuberculosis, steroid-induced diabetes	Lung and brain/NS Right hemisphere/multiple	Brain aspirate fluid Biopsy	AMB, ITZ 5FC (IV 0.3 g twice day <sup>-1</sup> ), ITZ (200 mg day <sup>-1</sup> ), L-AMB (5 mg kg <sup>-1</sup> day <sup>-1</sup> )	Expired Expired (8 week)
Trinh et al. <sup>27</sup>	8 /M /USA/Asian	NS	Primary immunodeficiency from unknown origin, recurrent CMV viremia, pneumonitis, neutropenia	Frontal lobe, external capsule, occipital lobe/multiple	Biopsy	VRZ (4 mg kg <sup>-1</sup> day <sup>-1</sup> ) + 5FC (150 mg kg <sup>-1</sup> day <sup>-1</sup> ) 3 months + caspofungin (0.7 mg kg <sup>-1</sup> day <sup>-1</sup> ) 4 week	Expired (4 months)

F, female; M, male; Afr, African; cau, caucasian; CMV, cytomegalo virus; AMB, amphotericin B; L-AMB, lipid amphotericin B; 5FC, 5-flucytosin; FLZ, fluconazole; ITZ, itraconazole; NS, not specified by the authors.

**Table 1c** Recent cases of central nervous system (CNS) infections by *Ramichloridium mackenziei*.

Patient characteristics							
Reference	Age/sex/ geography/face	Probable portal of entry	Risk factor/ underlying disease	Site of infection/number of abscesses	Specimens studied	Therapy	Outcome
Podnos <i>et al.</i> <sup>32</sup>	58/F/Kuwait/cau	NS	Chronic renal failure, hemodialysis	Parieto-occipital/multiple	Aspirate from, and biopsy of lesion, postmortem aspirate	AMB (1 mg kg <sup>-1</sup> ), ITZ	Expired (1 day)
Kanj <i>et al.</i> <sup>37</sup>	71/M/S Arabia/cau	NS	Chronic myelomonocytic leukemia (without chemotherapy)	Right parietal/multiple	Biopsy	Craniotomy, AMB (50 mg day <sup>-1</sup> ), ITZ (800 mg day <sup>-1</sup> )	Expired (6 week)
	42/M/S Arabia/cau	NS	None	Right frontal parietal/single	Aspirated fluid	Craniotomy, AMB (30 mg day <sup>-1</sup> ), FLZ (400 mg day <sup>-1</sup> )/AMB, ITZ (200 mg)	Expired
Kashgari <i>et al.</i> <sup>36</sup>	67/F/S Arabia/cau	NS	Diabetes	Left parietal/single	Aspirated pus	AMB	Expired (4 week)
	65/F/S Arabia/cau	NS	Hodgkin's lymphoma	Right parietal/single	Aspirated pus	AMB	Expired (3 week)
Khan <i>et al.</i> <sup>35</sup>	56/M/Egypt immigrant in Kuwait/cau	NS	Chronic Hepatitis B-associated liver disease	Left occipital/single	Aspirated pus	AMB (1 mg kg <sup>-1</sup> day <sup>-1</sup> )	Expired (18 day)
Al-Abdely <i>et al.</i> <sup>34</sup>	62/M/S Arabia/cau	NS	Kidney transplant	Single, later multiple	Biopsy	L-AMB (5 mg kg <sup>-1</sup> day <sup>-1</sup> ), ITZ (200 mg twice day <sup>-1</sup> ), 5FC (25 mg kg <sup>-1</sup> three times day <sup>-1</sup> ), PCZ (800 mg day <sup>-1</sup> ), PCZ (400 mg b.i.d.)	Cured

F, female; M, male; cau, caucasian; AMB, amphotericin B; L-AMB, lipid amphotericin B; 5FC, 5-flucytosin; FLZ, fluconazole; ITZ, itraconazole; PCZ, posaconazole; NS, not specified by the authors.

**Table 1d** Recent secondary/non-Chaetothyrialean cases of central nervous system (CNS) infection.

Species	Reference	Patient characteristics				Specimens studied	Therapy	Outcome
		Age/sex/ geography/race	Probable portal of entry	Risk factor/ underlying disease	Site of infection/ number of abscesses			
<i>Bipolaris spicifera</i>	Latham et al. <sup>38</sup>	18/M/USA/NS	NS	Acoustic necroma resection	Meningitis	AMB	Cured	
<i>Curvularia clavata</i>	Ebright et al. <sup>39</sup>	46/F/USA/NS	Inhalation	None	Invasive sinusitis and cerebritis/single	Sinus surgery specimen Sinus surgery, AMB (6 mg kg <sup>-1</sup> day <sup>-1</sup> ), ITZ (400–200 mg twice day <sup>-1</sup> )	Cured	
<i>Cladosporium cladosporioides</i>	Kantarcioglu et al. <sup>40</sup>	30/M/Turkey/cau	NS	HIV	Brainstem/single	None	Expired <sup>1</sup>	
<i>Nodulisporium</i> sp.	Umbala et al. <sup>42</sup>	55/M/India/cau	NS	None	Right medial temporo-parietal/single	Excision, AMB (0.6 mg kg <sup>-1</sup> day <sup>-1</sup> )	Cured	

F, female; M, male; cau, caucasian; AMB, amphotericin B; ITZ, itraconazole; NS, not specified by the authors; CSF, cerebrospinal fluid; <sup>1</sup>Role of the fungus unclear.

8–72. One fatal case caused by *C. bantiana* in a lung transplant patient was mentioned in a retrospective global report on mycotic brain abscess in solid organ and hematopoietic stem cell transplant recipients,<sup>28</sup> without providing detailed patient data. In most cases, the cerebral lesion was single, once becoming multiple at the same localization after surgery and relapse. One patient had multiple abscesses upon first diagnosis. Among the most prevalent symptoms were headache, nausea, seizures, speech difficulties and right hemiparesis. In one patient motoric dysphasia was observed. In one patient antituberculosis treatment was started, based on an interpretation of a CT scan, but no clinical improvement was obtained after 2 weeks of therapy. Surgical intervention was performed in four but one case. Four cases were fatal despite antimycotic therapy with AMB, L-AMB, ITZ or fluconazole (FLZ), either alone or as combination therapy. The pediatric patient with inoperable cerebral abscess had fatal outcome despite a combination treatment with VRZ, 5FC and caspofungin. One patient responded favorably to FLZ therapy, but as a second patient under combined FLZ and AMB therapy nevertheless expired, survival may have been because of successful surgery. Death occurred between 2 weeks and 4 months after first symptoms were noted. The etiologic agent of one case<sup>18</sup> was deposited in the culture collection of the Centraalbureau voor Schimmelcultures, the Netherlands, with the accession number CBS 102586, and its identity was confirmed by sequencing. The fungus from another case<sup>27</sup> was confirmed by demonstrating the presence of a 558 bp intron considered to be specific for *C. bantiana sensu stricto* using an intron-specific primer and preserved in culture collection of Duke University Mycology Research Unit, USA as DUMC 112.01.

Recent animal infections comprise a pyogranulomatous meningoencephalitis in a domestic cat<sup>29</sup> and a fatal systemic phaeohyphomycosis in a cat.<sup>30</sup>

### CNS infections due to *Ramichloridium mackenziei*

Thus far, *R. mackenziei* has only been isolated from cases of cerebral phaeohyphomycosis in patients living in or originating from the Middle East.<sup>23</sup> Cases diagnosed in the USA concerned visitors or immigrants from Saudi Arabia and Kuwait.<sup>31,32</sup> Infections were probably contracted in that region, although the fungus has neither been recorded in aereomycological studies,<sup>23</sup> nor has it been isolated from environmental sources. Restriction of human infections to the Middle East suggests a preference of the saprobic phase, if existent, for hot arid

climates.<sup>1</sup> It is the only neurotropic fungus reported from this region;<sup>1</sup> *E. dermatitidis* and *C. bantiana* are missing. In Saudi Arabia, it is the most common agent of infectious brain disease.<sup>33</sup>

Eleven cases have been reported between 1983 and 1999. All cases were located in the cerebrum, often in otherwise healthy hosts. About half the patients were previously subjected to major surgery. The male-to-female ratio was 1 : 1. Mortality was 100% within 1 year after diagnosis for all reported cases, despite surgery and antifungal therapy.<sup>34</sup> None of the reported cases of brain abscess showed manifestations of pulmonary or other organ involvement.<sup>35</sup> Tissue morphology of the fungus is similar to that of other dematiaceous filamentous fungi, therefore, an unequivocal diagnosis is dependent upon evidence from culture.<sup>35</sup>

Seven additional cerebral infections were reported since 1998 in patients mostly with underlying disease, all originating from or resident in the Middle East (Table 1c). One case occurred in a patient with renal failure,<sup>31</sup> one in a patient with diabetes mellitus,<sup>36</sup> one in a patient with chronic myelomonocytic leukemia,<sup>37</sup> one with myelofibrosis and Hodgkin's lymphoma,<sup>36</sup> one in a patient with chronic liver disease,<sup>35</sup> one in a kidney transplant recipient<sup>34</sup> and one in an otherwise healthy host.<sup>37</sup> Age range was 42–71; four were male and three were female. Early symptoms were headache, blurry vision or loss of vision, seizures, dizziness, convulsions, fever, and sometimes loss of consciousness, slurred speech and drowsiness. In one case, direct microscopic examination of the aspirate revealed many moniliform hyphal elements, with long, slender, septate hyphae branching at acute angles and with conidia projecting from both sides. This was thought to be consistent with *Aspergillus* species. *Ramichloridium mackenziei* was subsequently cultured from biopsy material of the lesion and from a second brain aspirate.

Six of the seven cases were fatal. Four patients expired despite AMB therapy; two cases had a fatal outcome despite aggressive antifungal therapy with AMB and/or ITZ with or without 5FC along with surgical intervention. One case showed radiographic and physical improvement by switching the therapy from AMB and ITZ to salvage therapy with posaconazole (PSZ).

Most strains from human cases have been preserved in major culture collections and showed a high degree of sequence similarity (G. S. de Hoog, unpublished data). Additionally, an agent from an unpublished case from Pakistan is held in the CBS culture collection with accession number CBS 102592.

## CNS infections due to other melanized fungi

Two secondary CNS infections as a result of members of the ascomycete order Pleosporales, *B. spicifera* and *Curvularia clavata*, have been reported (Table 1d). *Bipolaris spicifera* was observed as the etiological agent of a meningitis in a 18-years-old male,<sup>38</sup> probably secondary to preceding acoustic neuroma resection. A sinusitis followed by cerebritis caused by *Cur. clavata* in a 46-year-old immunocompetent female was reported by Ebright *et al.*<sup>39</sup> In the latter case, the patient responded to complete surgical removal of the infected sinus tissue with subsequent AMB therapy followed by ITZ.

A case by a member of the order Dothideales, *Cladosporium cladosporioides* was reported in a 30-year-old caucasian male.<sup>40</sup> Otherwise *Cl. cladosporioides* is an extremely common airborne saprobe. The non-melanized fungus *Paecilomyces variotii* (order Sordariales) was encountered in a 60-year-old female cancer patient.<sup>41</sup> The case was fatal. Strain identities were confirmed by DNA sequencing and have been deposited in the collection of the Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands, with accession numbers CBS 109501 and CBS 110036, respectively.

A very remarkable infection by a *Nodulisporium* species was reported in an 55 year old, otherwise healthy male from India.<sup>42</sup> There was no sinusitis, no underlying disease prior to the infection, and no lesion outside the CNS. An earlier case by *Nodulisporium* sp. concerned just an allergic sinusitis without tissue invasion.<sup>43</sup> *Nodulisporium* belongs to an order of strictly wood-inhabiting fungi, the Xylariales,<sup>44</sup> thus far without any medical representative known.<sup>12</sup>

## In vitro antifungal susceptibility data of melanized fungi

Data on activities of antifungal agents against melanized fungi are still scant. An overview of published susceptibility data for *E. dermatitidis*, *C. bantiana*, *R. mackenziei*, *B. spicifera*, *Cur. clavata* and *Cl. cladosporioides* was also performed. Testing methods differed slightly among published studies. Standard M38-A document by the US National Committee for Clinical Laboratory Standards is proposed as a reproducible procedure for the antifungal susceptibility testing of molds.<sup>45</sup> In a recent article<sup>46</sup> modifications were proposed for determining MICs and minimum fungicidal concentrations (MFCs) for some species. Optimal testing condition was found to be the combination of RPMI 1640 broth with 48–72 h of

incubation (72 h appeared the most reliable incubation time for *C. bantiana*) and 100% growth inhibition (MIC-0). Because black yeasts are rather slow growing fungi, some investigators prefer to incubate somewhat longer. Particularly for *R. mackenziei* it is preferred to read the MICs at the first 24 h interval and 48 h when growth was observed in the drug-free growth control.<sup>31,34,35,47</sup> Several investigators reported separate results for each of the test conditions.<sup>46,48,49</sup> Variations in medium, inoculum size and incubation time or temperature have significant impact on reproducibility of MIC data. The numbers of isolates tested to date is rather low. Thus, the susceptibility profiles of species of melanized fungi as yet have limited predictive value.

In main traits, melanized Chaetothyriales such as *Exophiala* and *Cladophialophora* appear susceptible to AMB *in vitro*, as well as to triazole antifungals, such as ITZ and VRZ.<sup>46,48,50–55</sup> Terbinafine was also found to be active *in vitro* against *E. dermatitidis*<sup>49,52</sup> although it appeared inactive in an experimental mice model.<sup>56</sup> There is no clinical experience with this drug in treatment of patients with CNS infections. The susceptibility for *R. mackenziei* against AMB appeared to be strain-dependent, MICs for FLZ were high, while isolates yielded low-MIC values against ITZ and the newer triazoles VRZ and PSZ.<sup>31,34,35,47,53,57</sup> Some strain-dependent AMB susceptibility was also noted in other black yeasts by Vitale *et al.*<sup>58</sup> Patients may expire despite therapy with AMB, a compound to which strains mostly showed *in vitro* susceptibility. This indicates a marked absence of correlation between *in vitro* results and *in vivo* response, possibly because of pharmacokinetics of the drug, general host factors, site and progression of the infection at onset of therapy, combined with virulence of the pathogen.

## Conclusions

The CNS infections caused by melanized fungi have a high mortality. These infections are nearly always caused by members of the order Chaetothyriales, underlining the clinical significance of the black yeasts and related fungi. Infections where *C. bantiana* or *R. mackenziei* is the etiologic agent are preponderantly cerebral and thus these fungi are associated with very low survival rates. Early recognition is highly significant. The requirements for successful therapy are complete resection of the lesion, supplementary to adequate antifungal treatment. In these species, mortality is 100% without surgery, but 65% when the lesion is excised.

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