REVIEW 10.1111/j.1469-0691.2009.02999.x

Zygomycosis: conventional laboratory diagnosis

C. Lass-Flörl

Department of Hygiene, Microbiology and Social Medicine, Division of Hygiene and Medical Microbiology, Innsbruck Medical University, Innsbruck, Austria

Abstract

A definitive diagnosis of zygomycosis caused by Mucorales can be made by histopathological examination with or without isolation of the fungus from the same site. Histopathological examination of the tissues affected typically shows characteristic broad, hyaline, ribbon-like, irregular fungal hyphae with wide-angle branching, accompanied by tissue necrosis and angioinvasion of the fungi. Tissue invasion by the fungal hyphae as seen by microscopy is essential to establish the diagnosis. Fungal elements can be stained with Gomori methenamine-silver, periodic acid-Schiff or Calcoflour white stain. All Mucorales grow rapidly on most fungal media such as Sabouraud dextrose agar incubated at 25–30 °C. Mucorales from a sterile site or repeated positive cultures of the fungi from a non-sterile site are considered significant in a high-risk patient with predisposing factors for acquisition of zygomycosis. Positive cultures from non-sterile specimens should be interpreted with caution and will require correlation between the finding and the clinical situation.

Keywords: Diagnosis, Mucorales, Zygomycetes, zygomycosis

Clin Microbiol Infect 2009; **15** (Suppl. 5): 60–65

Corresponding author and reprint requests: C. Lass-Flörl, Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University, Fritz Pregl Strasse 3/III, 6020 Innsbruck, Austria
E-mail: cornelia.lass-floerl@i-med.ac.at

Introduction

Although *Aspergillus* species, particularly *Aspergillus fumigatus*, account for the largest proportion of invasive mould infections, the last decade has witnessed the emergence of new opportunistic pathogens, including non-fumigatus *Aspergillus* species, *Fusarium* species, *Scedosporium* species, the dematiaceous fungi (*Alternaria*, *Bipolaris*, *Curvularia*, *Cladosporium* and *Exserohilum* species) and the agents of zygomycosis (mucormycosis) [1–3].

The class Zygomycetes includes a variety of filamentous fungi that may cause human disease and have emerged as an important cause of morbidity and mortality among immunocompromised patients [4,5]. The medically important Zygomycetes encompass two orders of filamentous fungi with distinct morphological, epidemiological and pathogenic characteristics, the Mucorales and the Entomophthorales [5]. The majority of cases of zygomycosis in humans are caused by members of the order Mucorales. Organisms of the genus *Rhizopus* are by far the most common clinical isolates, with *Rhizopus oryzae* occcurring most frequently. Members of the genus *Mucor* are second to *Rhizopus* in order of frequency, whereas Cunninghamella, Apophysomyces, *Absidia*, *Saksenaea*, *Rhizomucor* and other genera each represent a significantly smaller percentage of clinical isolates [4–7]. Zygomycetes are ubiquitous in soil and the environment and occasionally colonize humans.

The diseases produced by these fungi are referred to by the label 'zygomycosis'. Manifestations depend on the location of involvement, but they typically concern rhinocerebral, pulmonary, cutaneous, gastrointestinal and central nervous system (CNS) diseases [4–7].

Zygomycetes are characterized in culture by broad, nonseptate or sparsely septated hyphae and by the presence of sporangiophores supporting sporangia, which contain sporangiospores [5]. During sexual reproduction in culture, zygospores may be produced. Zygomycetes are characterized in tissue by the formation of wide, ribbon-like, hyaline, aseptate or sparsely septated hyphae with wide-angle (approximately 90 °) branching. The substantial differences among these and other structures allow mycology laboratories to diagnose organisms by genus and species [5].

Laboratory Diagnosis

As infections caused by Zygomycetes, and particularly the Mucorales, in humans may be rapidly fatal, timely diagnosis is crucial to avoid treatment delay [8,9]. Although confirmation of the diagnosis and species identification of the causative organism should be pursued, treatment should be initiated as soon as the diagnosis is suspected because of the severity of

these infections. The diagnosis of zygomycosis relies on a constellation of a high index of suspicion, assessment of presenting signs and symptoms, imaging studies, cultures and direct examinations of clinical specimens, and histopathology [10,11].

Suspicion should be based on knowledge of the underlying conditions that predispose to zygomycosis and the usual presentation of the infection in each of these conditions [12,13]. A common scenario concerns the development of zygomycosis in oncological patients or transplant recipients who receive antifungal therapy for prophylaxis or treatment of other opportunistic fungal infections [9,14–18]. In such cases the antifungal agents administered are not active against Zygomycetes, such as fluconazole, voriconazole and the echinocandins. Most of the signs and symptoms associated with the clinical manifestations of zygomycosis are non-specific. However, their diagnostic significance may increase if they are interpreted in relation to the patient's underlying condition. Early diagnosis is the cornerstone of successful treatment of zygomycosis; indeed, a direct correlation has been established between early tissue diagnosis and survival [10]. However, as is the case with many fungal infections, diagnosis is often not possible until autopsy [1,10].

Clinical specimens

The reference standard for the definite diagnosis of zygomycosis concerns histopathological, cytopathological or direct microscopic examination from affected organs [1,5,19,20]. The diagnosis relies on the evidence of tissue invasion. Thus, specimens obtained should be processed for fungal stains, cultures and any other procedures (e.g. molecular-based analyses) appropriate for ruling out differential diagnoses (Fig. 1). Combining microscopy and culture will increase the diagnostic yield by 15–20% [4,10,21].

Adequate specimens are skin scrapings from cutaneous lesions, nasal discharges, scrapings and aspirates from sinuses in patients with rhinocerebral lesions, bronchoalevolar lavages and needle biopsies from pulmonary lesions, and biopsy tissue from patients with gastrointestinal and/or disseminated disease. Blood cultures are of no benefit. If CNS abnormalities are present, brain biopsy may be helpful along with analyses of the cerebrospinal fluid. Unfortunately, even in brain involvement, fungal stains and culture results are rarely positive. Overall, it is important to collect many proper clinical specimens in order to obtain a high-yield result because Mucorales are sometimes difficult to distinguish from other filamentous fungi in histopathological examination [19,20]. Zygomycetous fungi have primitive coenocytic hyphae, which become easily damaged during biopsy procedures or tissue grinding in the laboratory. Thus, they are not suitable for growing in culture despite their presence in microscopic or histopathological examinations; in fact, fungal cultures are positive in only 15–25% of cases [6, 22].

Direct microscopic examination

Aspirated material from sinuses, sputum in pulmonary disease and biopsy material should be analysed using 10% potassium hydroxide (KOH) or optical brighteners such as Fungi-Fluor™ (Calcofluor white staining solution; Polysciences, Inc., Warrington, PA, USA) or Blancophor® (Bayer AG, Leverkusen, Germany) [10, 23]. Demonstration of fungal elements from cytological preparations (i.e. sputa, inflammatory

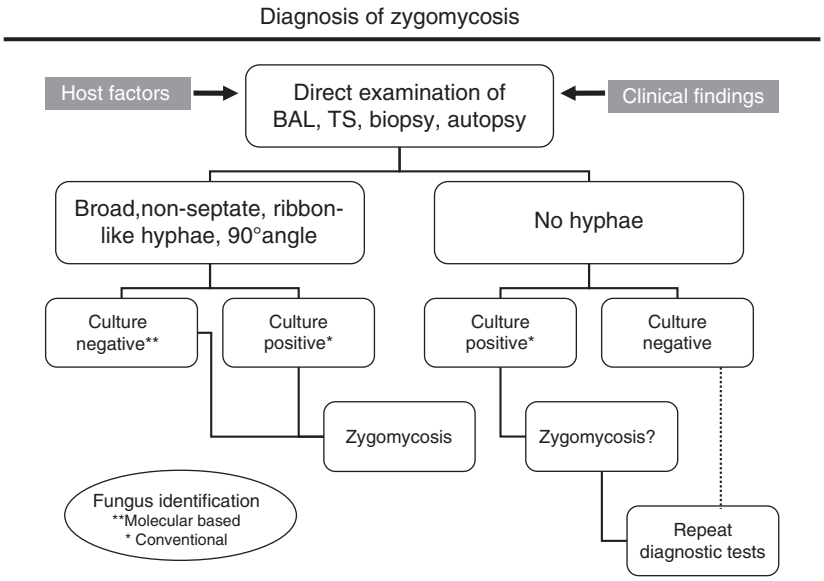

FIG. 1. Zygomycosis: diagnostic approach using microscopic and culture techniques. BAL, Broncho-alveolar lavage; TS, Tracheal secretions.