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First reported case of disseminated *Microascus gracilis* infection in a lung transplant patient

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ABSTRACT

Microascus gracilis is a specie of the genus *Microascus* in the family of *Microascaceae* and has been isolated from lung. It has never been reported as the cause of disseminated infection in humans. Herein, we report a fatal case of disseminated *Microascus gracilis* infection in a 65-year-old man with a history of primary idiopathic pulmonary fibrosis, status-post bilateral lung transplant. His course was complicated by donor lung cultures positive for multiple organisms and persistent pleural effusions. Multiple lung biopsy and bronchial lavage specimens were negative for mold. Later, pleural fluid cultures grew *M. gracilis* confirmed by DNA sequencing. Despite aggressive antifungal treatment, the patient continued to deteriorate with altered mental status. Imaging showed scattered hemorrhagic and hypodense lesions in the brain. The patient eventually succumbed to his infections and a restricted autopsy was performed. Autopsy findings included multiple hemorrhagic foci and abscesses involving the whole brain. Numerous punctuate, tan-white circular lesions were on the endocardium and diffuse tan exudates covered the pericardium and lungs. Histologically, similar fungal organisms with septate branching hyphae and short chains of conidia were identified, along with hemorrhage, neutrophilic inflammation, and necrosis in the brain, pleura, peripheral parenchyma of lungs and heart. This is the first reported case of disseminated *M. gracilis* infection in an immunosuppressed human, indicating it can cause localized infections and disseminated infections. This case increases our awareness of such fatal opportunistic infections, particularly in lung transplant patients, and urges earlier aggressive prophylaxis, diagnosis, and treatment.

1 Introduction

Solid organ transplantation has become more and more sophisticated in technique, and is anticipated to increase over time, yet fungal infections leading to high morbidity and mortality remain threatening. Organisms in the genera *Microascus* and *Scopulariopsis* of the family *Microascaceae* are common hyaline molds that cause hyalohyphomycosis and are frequently discussed together regarding rare but severe opportunistic infections in transplant patients. Species of these two genera are usually found in soil, decaying plant material, indoor environments, and even food [1,2], and have been involved in superficial infections as well as localized invasive infections, including cutaneous and brain abscesses, invasive sinusitis and endocarditis [3–8]. Recently, the taxonomy of the Microascaceae was reevaluated based on DNA sequencing for four loci — internal transcribed spacer (ITS), a fragment including the D1/D2 regions of the LSU rDNA gene, fragments of the translation elongation factor 1-alpha (EF-1α), and the beta-tubulin gene (TUB) [1]. *Scopulariopsis gracilis* was re-categorized and designated as *Microascus gracilis* [1]. *M. gracilis* has been isolated from human bronchoalveolar lavage (BAL) fluid, synovial fluid, and sputum samples [1,9] and was recently reported to cause localized bronchopulmonary infection in a lung transplant patient [10]. We report a fatal case of disseminated infection of *M. gracilis* in a lung transplant patient, followed by a literature review on documented infections by *Microascus* spp in solid organ transplant patients.

2 Case presentation

A 65-year-old man with a history of primary idiopathic pulmonary fibrosis underwent lung transplantation and donor cultures were positive for Methicillin-resistant *Staphylococcus aureus* (MRSA), *Candida dubliniensis,* and *Aspergillus niger* after bilateral lung transplant (Day 0). The patient's underlying conditions included coronary artery disease, hypertension, hyperlipidemia, gastroesophageal reflux disease (GERD), and steroid

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induced diabetes. His disease course was further complicated by right chylothorax, acute kidney failure, recurrent dysrhythmias, acute hypoxemic respiratory failure, and co-infections of *Candida albicans*, *Penicillium* spp, *Enterobacter cloacae,* respiratory syncytial virus (RSV), and coronavirus 229E detected in BALs. The patient was treated with multiple antibacterial, antiviral and antifungal agents including trimethoprim/sulfamethoxazole, ribavirin (400 mg, oral, daily, x14 days), voriconazole (300 mg, intravenous (IV), twice daily), and inhaled amphotericin B lipid (50 mg, three times per week). The co-infections and other complications were well controlled; however, persistent, fluctuant pleural effusions through chest tubes were worrisome and difficult to deal with, partially due to chylothorax. The effusion ranged from 200 mL to 1600 mL per 24 h and was varied from serous, serosanguinous to cloudy, concerning for empyema. The patient remained afebrile with occasional dry cough and shortness of breath with exertion. From day 71 to day 81, multiple cultures of pleural fluid from chest tubes were positive for *Enterococcus faecalis* and mold; however, BAL cultures remained negative for fungal organisms. Chest imaging showed bilateral pleural thickening with stable partially loculated bilateral pleural effusions and trace pneumothorax. There was scattered linear atelectasis change in parenchyma without focal airspace consolidation. The mold could not be identified with confidence so the isolate from pleural fluid of day 81 was sent to a reference laboratory for identification. At this time, the patient was treated with ampicillin (2000 mg, IV, three times per day, x 21 days), continuous voriconazole and inhaled amphotericin B lipid. Despite antifungal treatment, the patient continued to have chest wall pain, worsening hypoxic respiratory failure, and weakening. On day 107, the patient became critically ill with hypotension and acute renal failure and was transferred to ICU for intubation. At the same time, the mold isolated in pleural fluid was identified as *M. gracilis* at the Fungus Testing Laboratory, University of Texas Health Science Center, San Antonio, TX, by morphologic characteristics and DNA sequencing of the loci of D1/D2, TUB, and TEF. Micafungin (100 mg, IV, once per 24 h) was added to voriconazole and inhaled amphotericin B lipid in the antifungal regimen. The patient remained intubated and afebrile with normal white blood cell count and normal finding on bronchoscopy. Subsequently, drainage from the chest tubes became copious, thick, and yellow with a foul odor; the chest tubes were removed (day 115). Around that same time, the patient exhibited altered mental status. A chest computed tomography (CT) scan showed no evidence of pneumonia or underlying loculated fluid, although some stable interstitial and airspace opacities without consolidation were present. A transthoracic echocardiogram (TTE) showed no evidence of endocarditis. Blood and cerebrospinal fluid cultures were negative for fungal organisms. However, a brain CT and magnetic resonance imaging (MRI) scan showed scattered hemorrhagic, ischemic, hypodense, and nodular foci involving the brain, raising clinical concern about disseminated fungal infection. Since echinocandins poorly penetrate the brain, isavuconazonium sulfate (372 mg, IV, three times per 24 h), amphotericin B (500 mg, IV, once per 24 h), and terbinafine (250 mg, per nasogastric tube, once daily) were added to micafungin (day 118). The patient then also presented with potential bilateral fungal endophthalmitis and became unresponsive (day 124). Due to declining clinical status, care was withdrawn and an autopsy was ordered. On day131, drug susceptibility testing performed by the Fungus Testing Laboratory indicated that minimal inhibitory concentrations (MICs) were 1 mcg/mL for amphotericin B, >16 mcg/mL for both posaconazole and voriconazole, 2 mcg/mL for isavuconazole, and 2 mcg/mL for terbinafine and the minimum effective concentration (MEC) was 1 mcg/mL for micafungin.

External autopsy findings included multiple skin ulcerations, right sternal wound dehiscence, and ecchymosis around the eyes. Internal exam showed multiple hemorrhagic foci and abscesses involving the cerebral hemispheres from the frontal lobes to occipital lobes, basal ganglia, midbrain, brainstem, and right cerebellum (Fig. 1. A and B). The left ventricle was filled with hemorrhagic and necrotic material. Portions of the brain tissue were submitted for fungal culture which showed growth of *M. gracilis*. (Fig. 3). Numerous punctuate tan-white circular lesions up

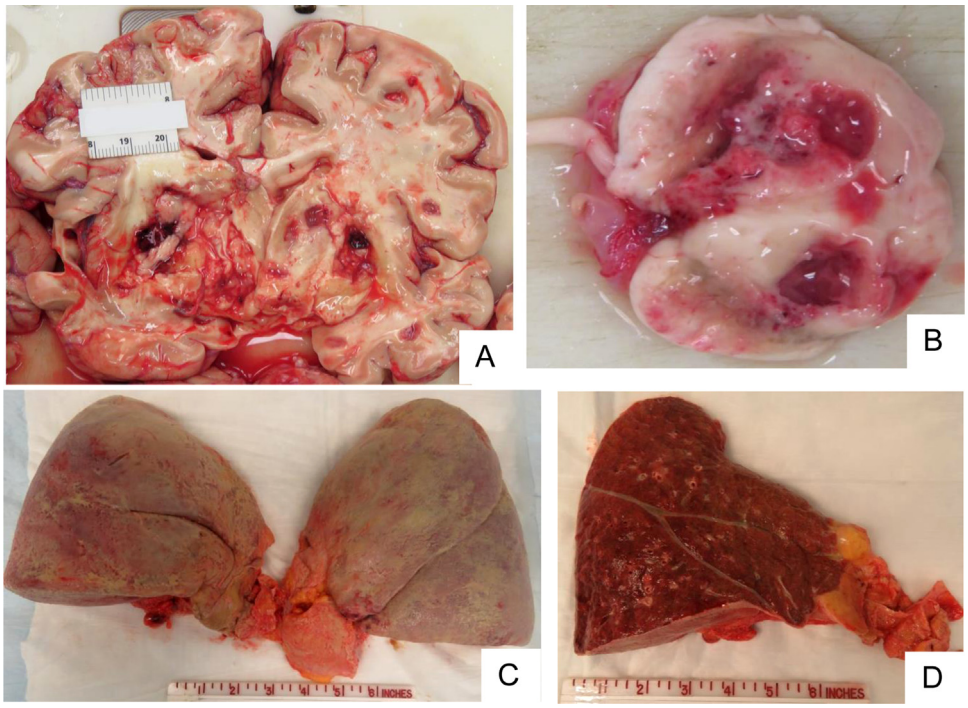

Fig. 1. Gross findings in brain and lungs: **A** and **B**, multiple hemorrhagic foci and abscesses in brain (**A)** and midbrain (**B**); **C**, Diffuse tan-yellow exudate on pleural surface of bilateral lungs; **D**, Rubbery texture and edema in lung parenchyma with interlobular septa covered with tan-yellow exudates.

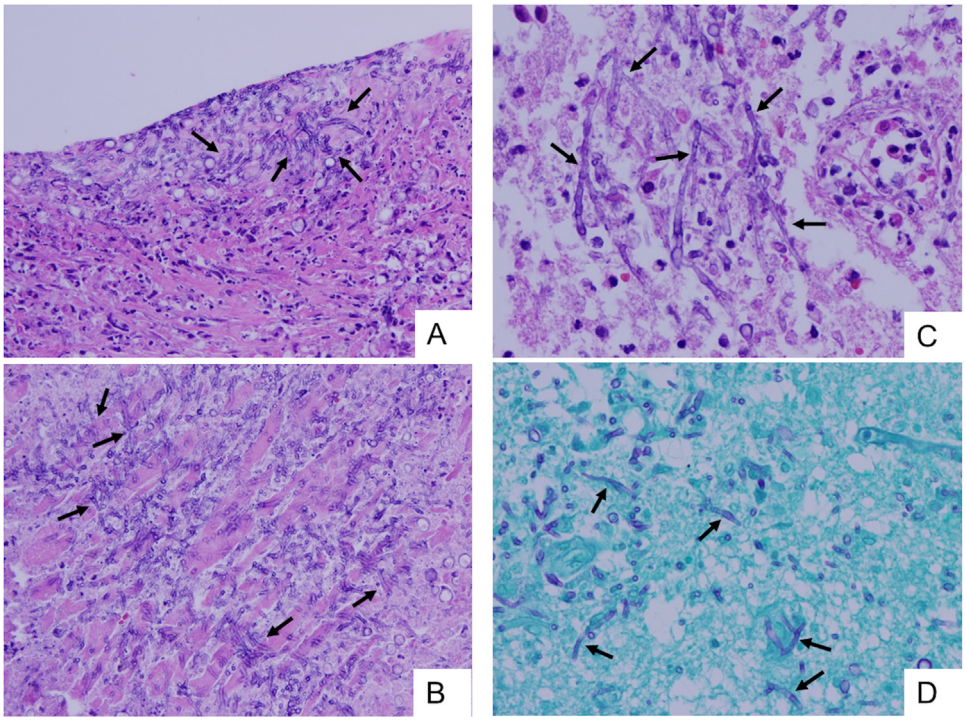

Fig. 2. Histologic findings of invasive fungi infections in multiple organs and tissue: Similar appearing fungal organisms with branching septate hyphae and conidiogenous cells as well as single or short chains of conidia, along with hemorrhage, neutrophilic inflammation, and necrosis in the pleura (**A**, HE, 200x), heart (**B**, HE, 200x), and brain (**C**, HE, 400x; **D**, CAS-F-D stain, 400x). Arrows are pointing to hyphae in the tissue. HE: hematoxylin and eosin stain; CAS-F-D: Chromic Acid-Schiff-diastase.

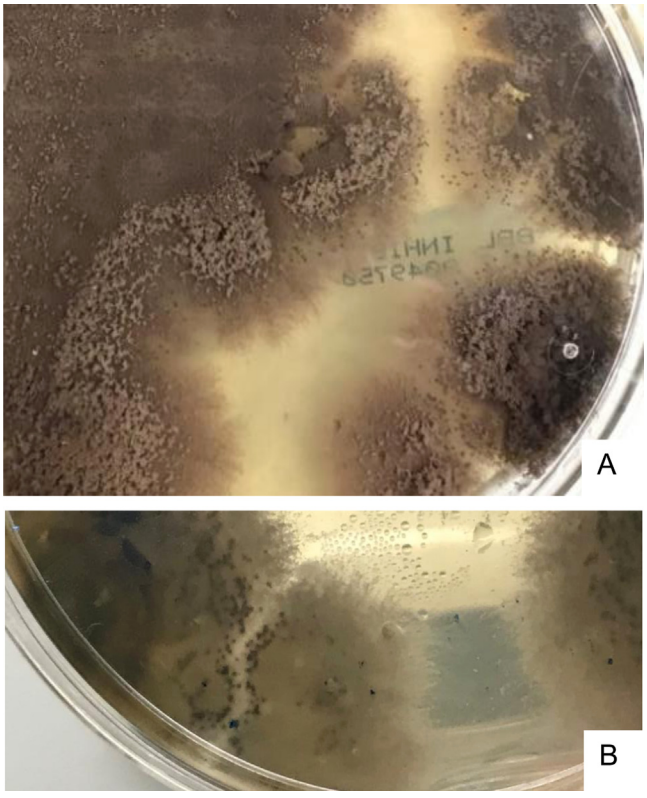

Fig. 3. Fungal culture of brain tissue in inhibitory mold agar grew brown-purple velvety colonies on surface (**A),** with light gray discoloration on reverse side (**B).** The fungus was identified as *M. gracilis.*
Please note: Color should be used for all figures in print

to 0.3 cm were on the endocardium and a tan exudate and adhesion were on the pericardium. There were 200 mL of red-tan fluid in the left pleural cavity and the pleural surfaces were covered by tan-yellow exudates (Fig. 1. C). The pulmonary parenchyma was dark red-purple with increased edema and a rubbery texture and the interlobular septa were covered with tan-yellow exudates (Fig. 1. D).

On histological examination, sections of the brain demonstrated scattered necrotic foci with fungal organisms, numerous neutrophils, and hemorrhage, consistent with abscess formation. Parenchymal edema, congestion, perivascular inflammatory infiltrates, and hemorrhage were also present. Similar changes were found through the cerebral hemispheres, basal ganglia, mid brain, and cerebellum. The fungi presented as branching septate hyphae that were confirmed by Chromic Acid-Schiff-diastase (CAS-F-D) special staining (Fig. 2); occasionally, conidiogenous cells on branching aerial hyphae and short chain of conidia were found amongst the necrotic material. Similar appearing fungal organisms along with hemorrhage, neutrophilic inflammation, and necrosis were identified in heart, and pleura (Fig. 2), with rare fungal elements in the peripheral parenchyma of lungs. Fungal culture of brain tissue on inhibitory mold agar incubated at both 27 °C and 37 °C yielded dark brown, velvety mold colonies with mostly brown mycelia (Fig. 3). Microscopic exam showed hyaline septate hyphae with bottle-shaped conidiogenous cells along with obovate-shaped, yellow-brown, smooth conidia singly or arranged in short chains (image not shown). The final postmortem diagnosis was disseminated infection of *M. gracilis* involving pleura, lung, heart, and brain.

3 Discussion

This is the first reported case of disseminated infection by *M. gracilis* in an immunosuppressed human, illustrating that *M. gracilis* is another opportunistic pathogen in the family

Table 1
Clinical and mycology features of infections by Microascus species reported in solid organ transplant patients.

Cases	Report year (Ref)	Age/ gender	Underlying disease	Transplant type	Systemic antifungal agents before infection	Infections type	Infection species		Co-infection	Treatment	Outcome
							current name	original name			
1 (Patel)	1994 (3)	37/M	Primary sclerosing cholangitis	Liver	none	Disseminated (skin, brain, lungs)	*M. paisii*	*S. brumptii*	none	AmB/MIC	Death
2 (Miossec)	2011 (12)	36/M	CF/Renal failure/ DM	Heart/ bilateral lungs	FCZ after dialysis	Disseminated (pleura, blood)	*M. cirrosus*	*M. cirrosus*	none	VOR/ CAS	Death
3 (Shaver)	2014 (13)	56/M	IPF/DM	Bilateral lungs	none	Disseminated (pulmonary pleura, Pleural fluid, skin, ribs, sternum)	*M. paisii*	*S. brumptii*	none	MIC/VOR/AmB/ TER	Death
4 (Schoeppler)	2015 (14)	64/M	IPF/PH	Lungs	VOR	Right lower lobe bronchus and left main stem bronchus	*M. trigonosporus*	*M. trigonosporus*	Aspergillus spp.	AmB/POS	Death
5 (Pate)	2016 (15)	53/M	End-stage liver disease/HCC/ hepatitis C virus infections/alcohol abuse	Liver	none	Lungs	*M. paisii*	*S. brumptii*	Parainfluenza I	AmB/MIC/POS/ TER	Cure
6 (Taton)	2018 (11)	60/F	Severe emphysema/ invasive lung aspergillosis	Lung	Oral VOR (200 mg twice a day)	Left upper lobe bronchus and intermediate bronchus	*M. cirrosus*	*M. cirrosus*	none	AmB/CAS/VOR/ TER	Cure
7 (Huang)	2019 (10)	61/M	COPD/CHD	Bilateral lungs	VOR	Invasive bronchial-pulmonary infection	*M. gracilis*	*S. gracilis*	Enterobacter cloacae, Serratia marcescens	TER/MIC/ POS	Cure
8 (current case)	2020	65/M	IPF/PH/DM/HTN/ CAD/HLD	Bilateral lungs	Inhaled AmB/ VOR	Disseminated (Lung, pleura, heart, brain, potential eyes)	*M. gracilis*	*S. gracilis*	Enterococcus faecalis, Candida albicans, Penicillium spp, Enterobacter cloacae, RSV and coronavirus229E	AmB/MIC/ ISO/ TER	Death

AmB, amphotericin; CAS, caspofungin; CAD, Coronary artery disease; CF, cystic fibrosis; CHD, chronic heart disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; F, female; FCZ, fluconazole; HCC: hepatocellular carcinoma; HLD, hyperlipidemia; HTN, hypertension; ISO, isavuconazole; IPF, idiopathic pulmonary fibrosis; M, male; MIC, micafungin; POS, posaconazole; PH, pulmonary hypertension; Ref, Reference; RSV, Respiratory Syncytial Virus; TER, terbinafine; VOR, voriconazole.

Microascaceae. Presence of these opportunistic fungi may represent colonization or may cause localized infections at the usual residential location of the lungs. Rarely, the infection can spread and cause aggressive fatal disseminated infections in other organs such as the heart, brain, and eyes, potentially, as in the current case.

Opportunistic fungal infections remain a big challenge for successful recovery from transplant surgery. Opportunistic infections of *Microascus* spp have been reported mostly in immunocompromised patients, with most severe infections occurring in solid organ transplant patients, particularly lung transplant patients. A summary of previously reported infections by *Microascus* spp in solid organ transplant patients [3,10–15] is shown in Table 1. Of the total eight cases, six occurred in lung transplant patients. Four of eight cases (including the current case) were disseminated infections that all led to death. The other four cases were localized invasive infections in the lungs and three of the four (75 %) were cured with combined antifungal therapy. Similar to *Scopulariopsis* spp [9,16,17], *Microascus* spp are resistant to a variety of antifungal agents demonstrated by *in vitro* susceptibility testing. Because there seems to be some discrepancy between *in vitro* susceptibilities and *in vivo* antifungal effects, perhaps *in vitro* synergy testing would be more useful than MICs and MECs. The *in vitro* synergy study by Cuenca-Estrella et al. on ten *Scopulariopsis brevicaulis*isolates demonstrates that the combination of posaconazole plus terbinafine achieves the highest rate of synergistic effect, followed by amphotericin B plus caspofungin, posaconazole plus caspofungin, and voriconazole plus caspofungin [18]. In fact, as shown in Table 1 for *Microascus*spp, the combination of posaconazole plus terbinafine (with amphotericin B and/or micafungin) cured two of the three infections, while the combination of voriconazole, terbinafine, amphotericin B, and caspofungin was effective in the third case [10,11,15]. Therefore, for multidrug resistant molds like *Microascus* spp, in addition to *in vitro* susceptibility testing, knowledge of *in vitro* synergy studies would provide more data for clinical decision making. Because echinocandins have poor central nervous system (CNS) penetration, they usually are not reliable in the treatment of brain infections. This should be taken into consideration when interpreting the results of susceptibility tests and synergy studies. Unfortunately, in the current case, although combined antifungal agent regimen was eventually employed, the disseminated *M. gracilis* infection was uncontrollable. Hence, early antifungal therapy with a combination of 3–4 agents appears critical to prevent infection spread by *M gracilis* and promote survival.

A persistent and significant pleural effusion following lung transplant surgery should immediately call for a thorough investigation for bacterial and fungal infections. A lesson from the current case is that early infection of *M. gracilis* can localize in the pleura without detection *via* BAL, blood testing, and lung imaging, and the patient may remain afebrile without an increased white blood cell count. Pleural fungal infection is commonly caused by extension of lung parenchymal infection. However, in the current case, it is likely that the pleural infection originated from the persistent colonization of fungus on the chest tube, considering 1) the long term indwelling bilateral chest tubes, 2) the nonspecific findings in lung parenchyma by imaging, 3) the absence of fungal growth in BALs, and 4) the presence of rare fungal elements identified histologically in the peripheral lung parenchyma near the pleura or interlobular septa, in contrast with the more abundant fungal elements seen in the pleura. Therefore, early removal of chest tubes, if possible, may prevent fungal infections originating from these foreign bodies. Once fungal isolation occurs, it is critical to distinguish invasive infection considering clinical picture, although it may be difficult to differentiate between true infection and persistent colonization in certain situations. In those situations, treatment as a true infection may be desired to prevent irreversible disseminated infection. Furthermore, once the fungal isolation is determined to be an invasive infection or taken as true infection, expedited fungus identification and susceptibility testing is another challenging but critical step for prompt and appropriate clinical management.

As solid organ transplantation continues to increase, opportunistic infections by rare pathogens like *Microascus* spp will become more common. Hopefully, this case increases our awareness of such opportunistic infections, particularly in lung transplant patients, and urges earlier aggressive prophylaxis, diagnosis, and treatment.

Consent

This is a case report on an autopsy case using unidentified gross organ pictures, histopathology pictures and fungal culture pictures. The case description in the paper is also deidentified. The deceased's family is not contactable.

Disclosure of grants or other funding

Nothing to declare

Author statement

All authors read the revised manuscript and accepted all the changes to the original manuscript.

Yanna Ding collected and analyzed data, wrote and revised the manuscript.

Lisa L Steed and Nicholas Batalis analyzed the data and revised the manuscript.

Declaration of Competing Interest

All authors declare that there is no conflict of interest.

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None.

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