CASE REPORT

Scopulariopsis Pleural Empyema Coinfection With Pneumocystis Jirovecii Pneumonia and Cytomegaloviraemia in an Immunocompromised Patient: A Case Report and Review of the Literature

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Abstract

Microascus gracilis is a rare cause of empyema. A 70s year old male present with worsening dyspnoea who was found to have spontaneous pneumothorax and thoracic empyema of the right lung coinfection with *Pneumocystis jirovecii* pneumonia and cytomegaloviraemia. *M. gracilis* identified from pleural fluids and the removed chest tube had high minimum inhibitory concentration values of currently available antifungal agents. The patient was treated with an antifungal regimen of caspofungin combined with posaconazole and then with terbinafine plus posaconazole for sequential therapy. Thymosin α1 plus immunoglobulin was also administered as adjuvant immunomodulatory therapy. This case highlights the importance of an accurate diagnosis, along with appropriate antifungal therapy in combination with immunomodulatory therapy to achieve complete recovery after *M. gracilis* infection.

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Case presentation

A 70s year old male presented with shortness of breath and wheezing lasting for four months and acute episodes of worsening dyspnoea developed one day prior to admission. On January 2023, the patient was admitted to the emergency agency with spontaneous hydropneumothorax. Thoracentesis was performed, and a chest tube drainage system was maintained. Afterwards, the patient had recurrent pleural infections with *Enterococcus faecalis* and *Streptococcus sanguinis*. The signs and symptoms of were reduced or alleviated after a long-term therapy with multiantibiotic regimens. After 3 months treatment, the patient was discharged home with intercostal drainage *in situ*. The patient's underlying conditions included chronic obstructive pulmonary disease, hypertension, diabetes and nephrotic syndrome, and for which he received treatment with prednisone and cyclophosphamide. On physical examination, the patient was in respiratory distress, accessory muscles were used for respiration, and there were decreased breath sounds over the bilateral chest.

Moreover, the chest tube was found to be unsecured for fixation. His consciousness was clear, body temperature, 37.6°C; blood pressure, 142/88 mmHg; heart rate, 92 beats/min; and respiratory rate, 20 breaths/min; peripheral oxygen saturation in room air, 95%. Lymphocyte count was low (320 cells/mm[1]) and an elevated hsCRP level of 189.3 mg/L. The results of serological tests for the (1–3)–beta–D–glucan level, galactomannan (GM) antigen, syphilis, and acquired immunodeficiency syndrome and the interferon–gamma release assay were normal. The GeneXpert MTB/RIF assay for the pleural fluid (PF) sample was negative. Blood and urine cultures were sterile. A chest CT scan revealed a drainage tube, an air–crescent sign and hydropneumothorax in the right pleural cavity (Fig 1A).

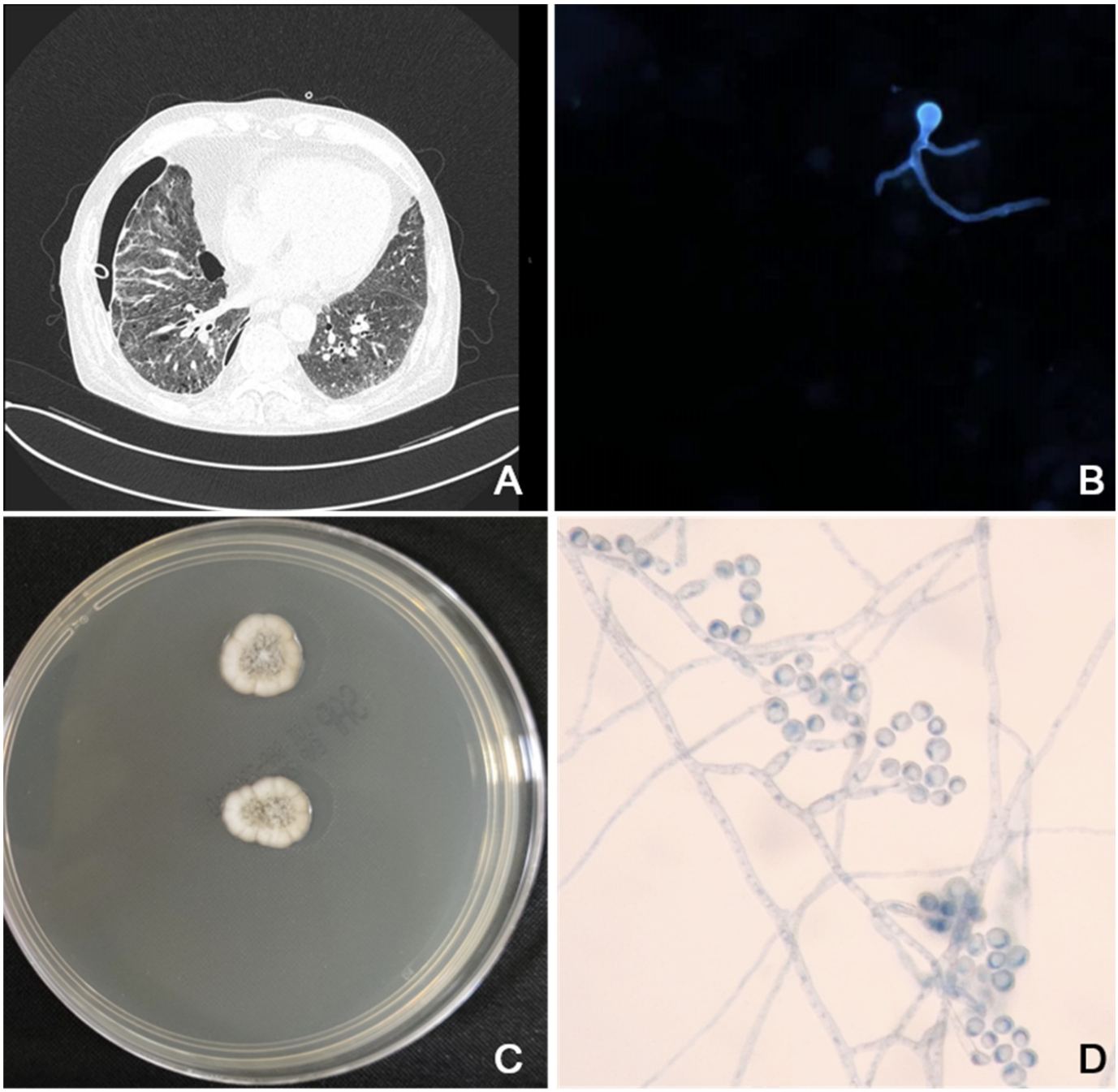

Figure 1. (A) Chest CT scan revealed an air–crescent sign and hydropneumothorax in the right pleural cavity. (B) Calcofluor white staining revealed pigmented, septate fungal hyphae in the pleural fluid at 400× original magnification. (C) Morphology of *M. gracilis* colonies on Sabouraud agar after 7 days of incubation at 35°C. (D) Microscopic examination showing hyphae with bottle–shaped conidiogenous cells along with obovate–shaped,

smooth conidia arranged in short chains (lactophenol cotton blue stain, 400× original magnification).

After one week, the patient was reintubated with a new pleural catheter (PC) that was placed away from the original region. A total of 120 mL of grossly turbid and yellowish fluid was drained from the pleural cavity. The abnormal laboratory findings included persistent low lymphocyte counts and an elevated blood CMV nucleic acid concentration of 1,070 copies/mL. Ganciclovir (900 mg IV) was added every 12 hours for two weeks. Calcofluor white staining of the PF revealed septate fungal hyphae (Fig 1B). Filamentous fungal growth was observed in the cultures from the removed tube and PF after three days of incubation at 35°C (Fig 1C). *Microascus.gracilis* was identified by its mycological characteristics (Fig 1D), matrix–assisted laser desorption/ionization time–of–flight mass spectrometry (Bruker Daltonics, log score 2.07) and ITS–based sequencing (accession no. OR131328). From day 2 to day 5, several fungal cultures from the PF were positive, and *M. gracilis* (accession no. PP178150–PP178151) was identified according to the abovementioned protocols. According to the guidelines presented in document M38–A_3[2], the minimal inhibitory concentration values against *M. gracilis* were 4 μg/mL for amphotericin B (AMB), 8 μg/ mL for voriconazole (VOC), >8 μg/ mL for posaconazole (POS), >16 μg/ mL for itraconazole, >256 μg/ml for fluconazole, and 4 μg/ mL for caspofungin (CAS). We added CAS 50 mg/day IV and POS 300 mg/day po for 30 days. On hospital day 15, flow cytometric analyses of the $CD4^+$ T–cell subset and programmed cell death protein 1 (PD–1) expression were performed via an automated AQUIOS cytometer (Beckman Coulter), which revealed a decreased $CD4^+$ T–cell count of 277 cells/mm[1] and 355 cells/mm[1], an increased $CD4^+PD–1^+$ T–cell proportion of 86.6% and 75.8% in the peripheral blood and PF, respectively. The BALF GM test was positive, whereas blood GM and PF GM tests were negative. The CMV nucleic acid load in the peripheral blood was 2589 copies/mL. Metagenomic next–generation sequencing (mNGS) of the collected BALF revealed high sequence numbers and relative abundances of PJ and CMV. Therefore, treatment with ganciclovir (900 mg IV) every 12 hours was reinitiated. Sulfamethoxazole/trimethoprim (2,400 mg/480 mg per day) was used to treat PJP for 30 days. Adjuvant immunomodulatory therapy with thymosin α1 (Tα1, 4.8 mg/week) combined with intravenous immunoglobulin (40 mg/day) for 3 weeks was also administered. On hospital day 37, the abnormal symptoms almost resolved. The PC was removed, and cultures of the PC and PF were all negative. Flow cytometric analysis revealed an increased CD4+ T–cell count of 481.5 cells/mm[1] and 2,818 cells/mm[1], a decreased CD4+PD–1+ T–cell proportion of 14.2% and 36.1% in the peripheral blood and PF, respectively. On hospital day 51, repeated mNGS revealed negative results for PJP and CMV in the BALF. The coinfections and other complications were well controlled, and peripheral blood examination revealed a normal lymphocyte count and a regular hsCRP level. The patient was discharged with no chief complaints and was switched to terbinafine (TEB) 250 mg/d po combined with POS 300 mg/day po for 4 weeks. After 6 months of follow–up, the patient experienced no obvious discomfort.

Discussion

The increasing incidence of pleural infections might be associated with an ageing population, diabetes mellitus, the widespread application of immunosuppressive agents, an increased incidence of severe disease and the

immunosuppressed status[3]. Fungal pleurisy is infrequent and insidious, tends to occur in immunocompromised patients, and is most often caused by *Candida* spp., followed by *Aspergillus* spp[4]. Infections by *Scopulariopsis*/*Microascus* spp. are very rare and, as previously reviewed, are associated with high morbidity and mortality in clinical settings because of delayed diagnoses and intrinsic resistance to currently available antifungal agents[1]. These ubiquitous saprophytic fungi are commonly isolated from soil, air, decaying organic material, dung, insects and moist indoor environments[5]. *Pneumocystis jirovecii* (PJ) is a common opportunistic and life–threatening infection that leads to respiratory failure, PJ and cytomegalovirus (CMV) coinfection is a risk factor for 30–day mortality in patients treated with corticosteroids and immunosuppressants[6]. Herein, we present a case of *Scopulariopsis* pleural empyema coinfection with PJ pneumonia and cytomegaloviraemia in an immunocompromised patient. To our knowledge, this is the first report of *Scopulariopsis* pleural empyema in China.

Fungal aetiologies of pleural infections are less common and more challenging. Numerous randomized controlled trials have demonstrated that an indwelling PC (IPC) substantially reduces the need for further invasive pleural interventions and reduces hospitalization days while providing equivalent benefits in relieving breathlessness and improving the quality of life[7]. Despite the advantages of the IPC, complications occur in up to 20% of cases, and IPC–related infections commonly occur in the pleural cavity/fluid, catheter tract, and skin at the exit site. The incidence of IPC–related infections varies among studies and has been reported to be as high as 25%[7].

A review of the English–language literature since 1962 has identified 5 cases that were reported as proven *Scopulariopsis* empyema (Table 1)[8][9][10][11][12]. All patients were male, and 66.7% underwent solid organ transplantation (SOT). A total of 83.3% of the patients were treated with antifungals for prophylaxis. Combined therapies were performed when diagnostic clues were found, but the therapeutic outcomes were poor, as 83.3% of the patients died. The most commonly identified species was *M. gracilis*, followed by *S. brumptii* and *S. acremonium*. However, because of the isolate morphologically identified as *Microascus cinereus* was found after sequencing to be *M. gracilis*[1].

Table 1. Reported cases of *Scopulariopsis* empyema

Year	Age/Sex	Underlying disease	Risk factors	Antifungal prophylaxis	Involved sites	Pathogen	Systemic antifungal treatment	Outcomes	Reference
2005	63/M	COPD	SOT (single-lung); CMV; IPC	AMB	Heart, thyroid gland, stomach, kidneys, lungs	*S. acremonium*	VOR/CAS	Expired (1 day)	[8]
2007	27/M	AIDS	Anti-retroviral therapy	None	Pleural fluid	*S. brumptii*	None	Expired (14 days)	[9]
2011	36/M	CF/RF/DM	SOT (heart and lungs); IPC	FCZ	Pleural fluid, intrapericardial fluid, blood clots, bronchial secretions	*M. cirrosus*	VOR/CAS	Expired (9 days)	[10]
2014	56/M	IPF/DM	SOT (lungs); IPC	ITC	Pulmonary pleura, pleural fluid, skin, ribs, sternum	*S. brumptii*	MIC/VOR/AmB/TER	Expired (48 days)	[11]
2020	65/M	IPF/PH/DM/HTN/CAD/HLD	SOT (lungs); IPC; RSV	AmB/VOR	Lung, pleura, heart, brain, potential eyes	*M. gracilis*	AmB/MIC/ ISO/TER	Expired (18 days)	[12]
2023	70s/M	NS/ILD/DM	IPC; CMV; PJP	VOR	Pleural fluid	*M. gracilis*	CAS/POS/TEB	Cured	This case

M, male; COPD, chronic obstructive pulmonary disease; SOT, solid organ transplant; CMV, cytomegalovirus; IPC, indwelling pleural catheter; AmB, amphotericin; VOR, voriconazole; CAS, caspofungin; AIDS, Acquired Immune Deficiency Syndrome; CF, cystic fibrosis; RF, renal failure; DM, diabetes mellitus; FCZ, fluconazole; IPF, idiopathic pulmonary fibrosis; ITC, itraconazole; MIC, micafungin; TER, terbinafine; PH, pulmonary hypertension; HTN, hypertension; CAD, Coronary artery disease; HLD, hyperlipidemia; RSV, respiratory syncytial virus; ISO, isavuconazole; NS, nephrotic syndrome; ILD, Interstitial lung Disease; PJP, Pneumocystis jiroveci pneumonia; POS, posaconazole.

M. gracilis, the teleomorph of *Scopulariopsis*/*Microascus* spp., is a rare opportunistic fungus associated with human disease.[1] Among the ninety–seven clinical strains morphologically identified as *Scopulariopsis/Microascus* spp., *M. gracilis* was the second most commonly isolated species and was most frequently isolated from respiratory tract samples. Most patients with these infections were determined to have fungal colonization according to the clinical features of their disease.[5] However, to date, only six proven cases of *M. gracilis* infection have been reported, including two cases of disseminated infection.[10][12] two cases of invasive bronchopulmonary infection[13][14] one case of primary subcutaneous infection[15] and one case of keratitis.[16]

PJP most often occurs in immunocompromised hosts with intensified or prolonged immunosuppression and is especially notable in patients taking corticosteroids and in those who have subsequent CMV infection.[17] Cytomegaloviraemia is associated with a heightened risk for developing invasive mycosis due to common clinical risk factors, such as immunosuppressant and corticosteroid usage, possibly because CMV can affect several components of the defence system and, therefore, can increase the pathogenicity of other infectious agents.[13] In the present case, treatment with corticosteroids for nephrotic syndrome might have transiently suppressed immunity, resulting in an opportunistic infection. Furthermore, the incorrect disposal of an IPC may contribute to the occurrence of rare invasive fungal infections caused

by moulds that are often intrinsically resistant to some classes of antifungals.[18] The spread of pulmonary *Scopulariopsis* to the pleura was unlikely in the present case because only pleuritis was detected, without apparent pulmonary *Scopulariopsis*.

Notably, flow cytometry analysis of lymphocyte subsets in the peripheral blood and PF revealed that the number of T lymphocytes was significantly decreased and that T–cell exhaustion occurred. The host could not control infections effectively because of T–cell exhaustion, while the upregulation of PD–1, an immunosuppressive receptor, in exhausted T cells was observed during the progression of symptomatic stages of infection, which contributed to the development of severe symptoms.[19] Elevated PD–1 levels in $CD4^+$ T cells were associated with the inhibition of cell proliferation and decreased effector functions. In contrast, downregulating PD–1 reversed T–cell exhaustion and the immunosuppressive status, resulting in the recovery and enhancement of host innate immunity. The prognosis largely depends on the immune status of the patient, and immune reconstitution is considered essential when combating invasive fungal infections.[5]

Currently, there is no standard protocol for fungal empyema therapy, and combinations that include available agents can be used, as there is variable penetration of systemically administered antifungals into the pleural cavity[20]. The lack of correlation between in vitro and in vivo studies and the intrinsic resistance of fungal pathogens to many of the available antifungals limit successful therapeutic options[21]. Nevertheless, in vitro synergy has been demonstrated for combinations of POS and TEB, POS and CAS, and AMB and CAS and for three drugs in combination, which appears to be promising, and these drugs have a synergistic effect on *M. gracilis*[22]. In particular, intravenous immunoglobulin is essential for reversing immunological crises in immunocompromised patients and is associated with highly effective treatment and good clinical outcomes[13]. Although there is limited information on the efficacy and safety of Tα1 as an adjuvant immunomodulatory therapy, Tα1 may be a treatment option for invasive fungal infections[13]. Our immunocompromised patient was initially treated with CAS combined with POS; when the symptoms and abnormalities in the patient's critical biological indices had resolved, the combination of POS and TRB was started as a sequential therapy until he was cured. Moreover, the combination of antifungal therapy with immunomodulatory therapy was shown to be an effective intervention.

The patient's immunosuppressive status and inappropriate disposal of the IPC may have played the key role in the pleural seeding of *M. gracilis*. Fortunately, multimodal management, including early administration of suitable antifungal agents, and adjuvant immunomodulatory treatment, was beneficial, as the patient condition did not progress to *Scopulariopsis* dissemination. Although infection with *M. gracilis* is rarely pathogenic in the pleural cavity, clinicians and microbiologists should be aware of the potential *M. gracilis* infection in immunocompromised individuals at risk of opportunistic infections.

Statements and Declarations

Author's contribution

Conceptualization: ZMH, DX, LNM; Data curation; ZMH, DX; Formal analysis: ZMH, LNM; Funding acquisition: ZMH,

TYD; Investigation: ZMH, LNM, TYD; Methodology: ZMH, SY; Project administration: ZMH, SY; Writing-original draft: ZMH, DX, SY; Writing-review and editing: ZMH, SY. All authors have read and agreed to the published version of the manuscript.

Conflicts of interest

The authors declare that they have no conflicts of interest.

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