Invasive *Trichoderma longibrachiatum* infection in a neutropaenic patient

Penelope J. Teoh [a,*], Emma McGuire [a], Andrew M. Borman [c], Rebecca Gorton [d], Andrew J. Wilson [b], Chloe Merrion [b], Vanya Gant [b]

[a] *Hospital for Tropical Diseases, University College London Hospital NHS Foundation Trust, Capper Street, London, WC1E 6JB, United Kingdom*
[b] *University College London Hospital NHS Foundation Trust, University College Hospital, 235 Euston Road, London, NW1 2BU, United Kingdom*
[c] *UKHSA National Mycology Reference Laboratory, Bristol, BS10 5NB, UK and Medical Research Council Centre for Medical Mycology, University of Exeter, Exeter, EX4 4QD, United Kingdom*
[d] *Department of Infection Sciences, Health Services Laboratories, 1 Mabledon Pl, London, WC1H 9AX, United Kingdom*

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ABSTRACT

Invasive fungal infection is a life-threatening complication of chemotherapy and neutropaenia in the haematology population. *Trichoderma* species rarely cause human disease but have been reported to cause invasive infection in the immunosuppressed. We present a case of invasive *Trichoderma longibrachiatum* pulmonary infection with fatal outcome in a neutropaenic patient with acute myeloid leukaemia. 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Trichoderma species are saprophytic filamentous fungi found in soil and decaying plant matter. They are a rare cause of human infection but can cause life-threatening disease in immunosuppressed patients with approximately 50 % mortality [1]. The few reports of *Trichoderma* infection in the literature are in patients with haematological malignancies and those requiring peritoneal dialysis with common sites of infection being lung and peritoneum [1]. In the absence of clinical breakpoints to interpret antifungal susceptibility testing or established clinical treatment guidelines, systemic antifungals such as amphotericin B or voriconazole are commonly used, in some cases combined with surgery. We present the case of invasive pulmonary infection with *Trichoderma longibrachiatum* in a neutropaenic patient being treated for acute myeloid leukaemia.

2. Case presentation

A 51-year-old UK born female was electively admitted to a tertiary hospital in October 2023 (Day 0) for her first cycle of (Fla-Ida) (fludarabine, cytarabine (Ara-C), and idarubicin) chemotherapy for measurable-residual disease (MRD) positive acute myeloid leukaemia (AML). She had contracted SARS-CoV-2 1 month prior to admission and

continued to test positive for SARS-CoV-2 during admission. She became neutropaenic on day 6 and on day 9 she developed neutropenic sepsis requiring a 4-day intensive care unit (ICU) admission for blood pressure support due to *Enterobacter cloacae* bacteraemia. Meropenem 1g TDS was started and continued throughout admission.

On day 25 of her admission, she again developed neutropenic fevers [Fig. 1] while on meropenem and prophylactic itraconazole with temperatures up to 39.7 °C and an associated cough productive of brown sputum. On day 29 a high-resolution computed tomography (HRCT) of her chest was performed. This demonstrated multifocal consolidation and nodularity, many nodules being bronchocentric [Fig. 2]. There was also mediastinal stranding in keeping with mediastinitis and the tracheal wall was thickened. The following day serum β-D-glucan was positive at 360.4 pg/mL (Fungitell® assay, internally verified positive threshold of 110 pg/mL). Galactomannan was negative on days 30, 43 and 44. On day 29 prophylactic itraconazole was stopped and intravenous caspofungin 70mg once daily was started as per the protocol at our centre for suspected invasive fungal infection (IFI). Itraconazole drug trough levels had been satisfactory on day 30 at 0.84 mg/L. Intravenous catheter-related *Enterococcus faecium* and *Staphyloccoccus epidermidis* bacteraemias were identified on day 30 and lines were removed.

Caspofungin was changed to intravenous liposomal amphotericin B (AmBisome®) 3mg/kg once daily on day 34 due to persistent fevers

>39 °C. Her fever and cough persisted despite this, and she developed intermittent chest pains, so a further HRCT was performed on day 41. This showed progressive consolidation within the lower lung lobe as well as scattered lung nodules and diffuse tracheal wall thickening [Fig. 3].

On the same day a mould was identified on Sabouraud agar from a sputum sample taken 9 days earlier. This was identified morphologically and by matrix-assisted laser desorption/ionisation time-of-flight (MALDI-ToF) as *Trichoderma longibrachiatum* [Fig. 4]. *T. longibrachiatum* was isolated on a total of 4 sputum samples taken on days 32, 38, 48, and 55. Repeat serum β-D-glucans were considered equivocal (between 50 and 110 pg/mL) on day 42 (104 pg/mL), 43 (68 pg/mL) and 46 (93 pg/mL). The initial isolate was sent to the UK Health Security Agency National Mycology Reference Laboratory, where isolate identification was confirmed and antifungal susceptibility testing was performed using CLSI broth microdilution methodologies [2]. Minimum inhibitory concentrations (MICs), available on day 50, were as follows: amphotericin B 1.0 mg/L, caspofungin 0.03 mg/L, itraconazole 2 mg/L, posaconazole 0.5 mg/L and voriconazole 0.25 mg. In the absence of species-specific interpretive breakpoints for *Trichoderma* spp., MICs were interpreted against breakpoints developed for *Aspergillus fumigatus*, indicating likely susceptibility to amphotericin B, caspofungin and voriconazole, resistance to itraconazole and intermediate susceptibility to posaconazole.

On day 48, due to persistent fevers despite liposomal amphotericin B, voriconazole 200mg twice daily was added as a second agent. Her fevers began to improve 48 hours following this addition, and she began to feel brighter and sit out of bed more. This was accompanied by a fall in *C*-reactive protein from 143 mg/L on day 47–88 mg/L on day 55. The patient's renal function deteriorated therefore amphotericin B was changed back to caspofungin and voriconazole was continued. Voriconazole trough levels were supratherapeutic at 14.4 mg/L and 5.5 mg/L on day 52 and 55 (target trough level 1 to <5.5mg/L).

On day 54, the patient deteriorated clinically. She felt unable to expectorate and had an audible chest rattle. A repeat HRCT the following day showed stable appearances of the pulmonary nodules, however there was ulceration and perforation of the posterior wall of the trachea with surrounding emphysema. That evening the patient sustained a cardiac arrest and cardiopulmonary resuscitation was initiated. During intubation the trachea was found to be very oedematous, with blood-stained frothy secretions. After return of spontaneous circulation, she was transferred to the ICU. Bronchoscopy in the ICU demonstrated a lesion on the posterior wall of the trachea which was almost obstructing
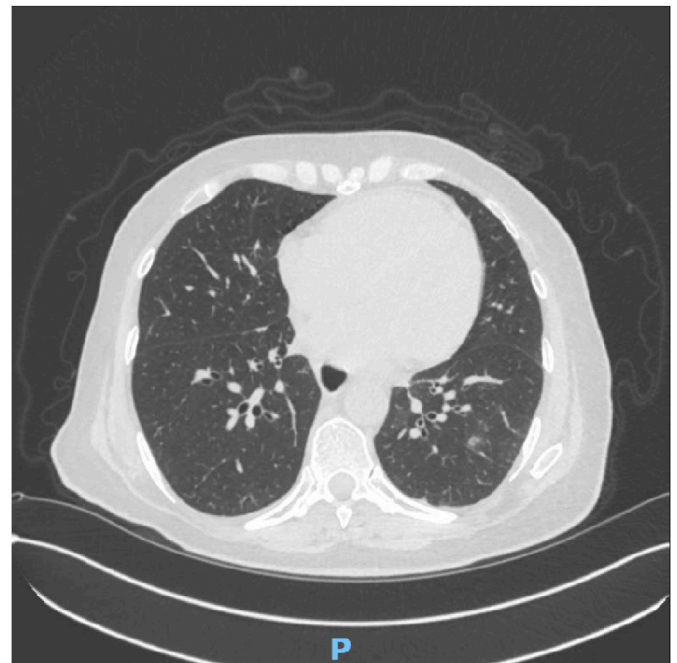

Fig. 2. HRCT scan on day 29. This demonstrates several areas of subtle abnormality. The distribution is not centrifugal, as would be expected for *Aspergillus* infection. There is a poorly defined lesion in evolution in the lateral segment of the left lower lobe. There is tracheal thickening and mediastinal stranding.

the entire lumen and thought to be an eroding fungal lesion. She later desaturated further, with heavily blood-stained secretions, suffering a further hypoxic cardiac arrest from which she did not recover.

3. Discussion

To our knowledge, we report the first United Kingdom case of invasive fungal infection (IFI) due to *T. longibrachiatum*. This case fits the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium (EORTC/MSGERC) consensus definition of "probable" IFI [3]. Major radiological criteria were fulfilled and while deep microbiological sampling was not felt to be clinically feasible, *T. longibrachiatum* was isolated from sputum
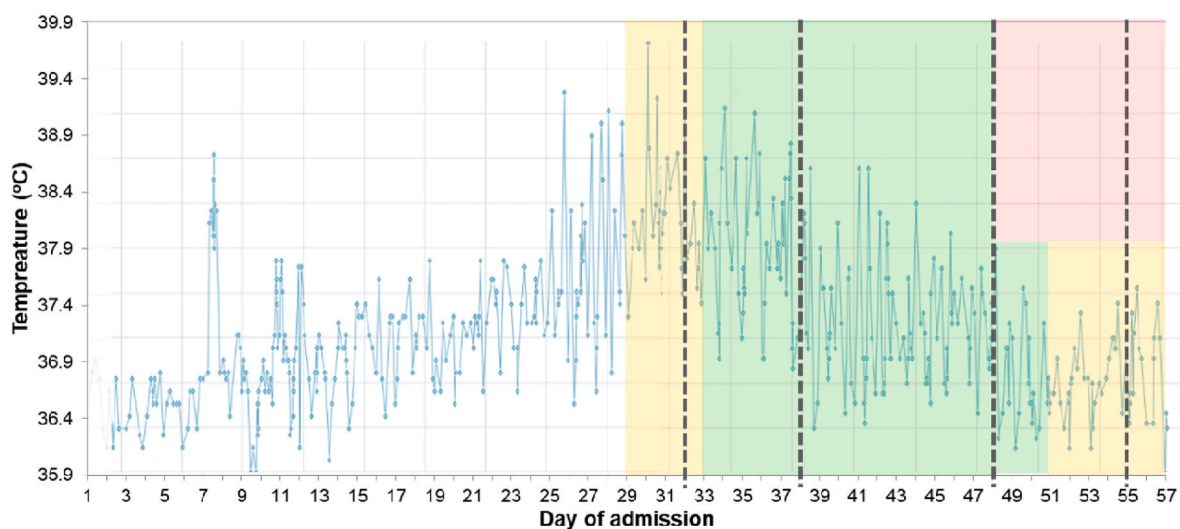

Fig. 1. Temperature, antifungal use and positive sputum cultures for *T. longibrachiatum* over time. Black dashed line denotes day of positive sputum culture for *T. longibrachiatum*. Yellow area denotes use of caspofungin, green area denotes use of amphotericin B, and pink area denotes use of voriconazole.
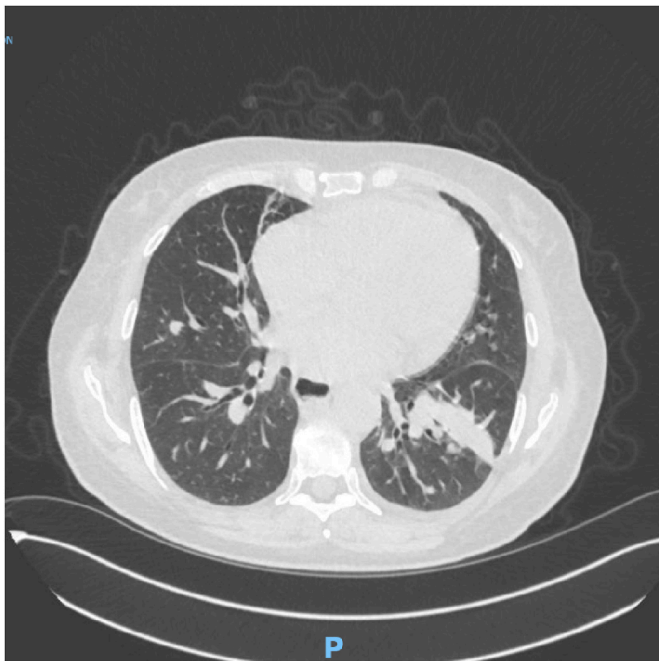
Fig. 3. HRCT scan on day 41. This demonstrates considerable evolution of the lesion in the lateral segment of the left lower lobe, which now suggests haemorrhage. There are several other clearly demarcated nodular lesions with no halo sign, again suggesting local haemorrhage/necrosis. There is an usual appearance in the posterior trachea, suggesting the presence of a mass, some of which seems indistinguishable from the trachea itself.

on four occasions during the patient's illness.

Trichoderma is a genus of saprophytic filamentous fungi found in humid soil and decaying plant matter. It is commonly found in agricultural environments and certain isolates are reported to biologically control plant pathogens [4]. There are several species of *Trichoderma* thought to cause disease in humans, with *T. longibrachiatum* being the most frequently reported.

Little is known about the natural history, epidemiology and optimal management of this infection because the literature base consists of a small number of case reports and one case series. Diagnosis is limited to areas where there is access to laboratory mycology methods and expertise. Although not noted in our case, soil exposure has previously been suggested as risk factor. For example, cases have been linked to

farming and exposure to close contact with an indoor potted plant, the planting soil in one case found to be culture positive for *T. longibrachiatum*. [5,6] Our patient's closest exposure to soil was sitting next to a wood burner at home between cycles of chemotherapy.

In a study of 50 cases of *Trichoderma* spp. Infection, 38 % had haematological or oncological disease and just over a quarter were neutropenic and/or receiving systemic anti-cancer therapy [1]. The lung was the most common organ involved, affected in 42 % of cases. Pulmonary, peritoneal, and central nervous system disease have been reported as well as endocarditis, fungaemia or disseminated disease [1]. Nodular infiltration is commonly seen on cross-section imaging of patients with lung infection, which may be accompanied by the halo sign, air crescent sign or cavitation [5]. Focal dense consolidation, pleural effusions or pericardial effusions may also be seen. In our case, radiological features were haemorrhagic on later imaging, and it is likely that *T. longibrachiatum* hyphae colonized the bronchi and arteries, leading to arterial perforation and pulmonary haemorrhage. The predominantly centripetal and tracheobronchial distribution of fungal lesions seen in this case might in part be explained by the size of *T. longibrachiatum* conidia (2.5–3 x 3-5microns) which are significantly larger than those of *Aspergillus fumigatus* (diameter 2–3 μm), potentially limiting penetration into deeper lung tissues. However, these radiological features are not specific for *Trichoderma* infection, making mycological evidence essential for its diagnosis and treatment.

Laboratory investigation involves culture, microscopy and molecular techniques. *Trichoderma* is a filamentous fungus which causes hyalohyphomycosis; with hyaline septate hyphae seen in tissue sections that are difficult to differentiate from *Aspergillus* species. Flat green colonies rapidly grow on Sabouraud dextrose agar. Potato dextrose agar can also be helpful, on which they produce a yellow pigment [7]. Lactophenol blue stain demonstrates hyaline septate hyphae and branching divergent whorls of stout, flask-shaped conidiophores [8].

Our case had a positive serum β-D-glucan (BDG) which fell with treatment. Since *Trichoderma* possesses BDG, and published cases of invasive *Trichoderma* infection where BDG was measured reported strong BDG antigen positivity, it is likely that the BDG trend observed in our patient represented a response to treatment [1,9]. Although helpful as a screening test for IFI in high-risk patients, positive serum BDG may indicate infection by a wide range of fungal pathogens and has a considerable false positive rate [10]. The BDG was not repeated within a short time frame of the first positive test, which would have been desirable from a stewardship perspective in the absence of convincing radiological evidence of IFI.

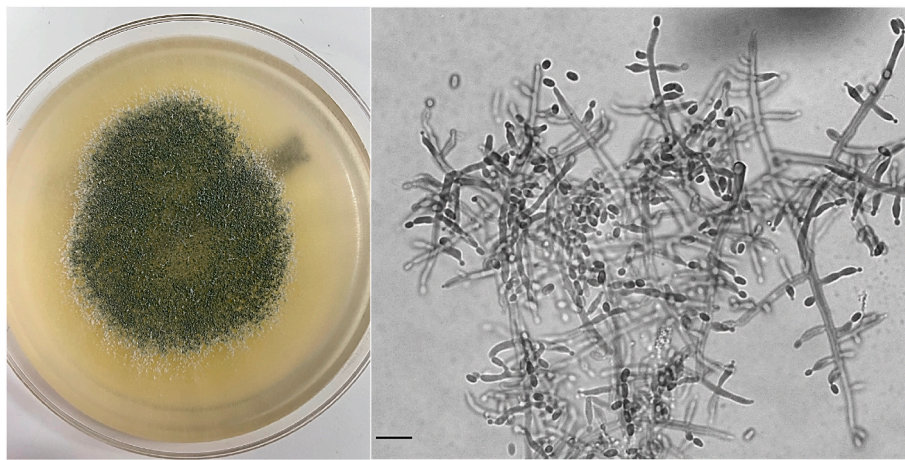Invasive *Trichoderma* infections occur less commonly in patients

Fig. 4. Colonial appearance of *T. longibrachiatum* on Sabouraud dextrose agar after 5 days incubation at 30°C (left-hand side) and microscopic appearance of tease mounts showing flask-shaped phialides arising both singly along conidiophore axes and in loosely arranged whorls, producing ellipsoidal to oblong conidia (right-hand side; scale bar = 10 μm).

receiving antifungal prophylaxis. Only 20 % of *Trichoderma* infections in one study of febrile neutropenic patients had received prophylaxis when diagnosed [11]. Our patient was taking itraconazole prophylaxis, however the isolate had a high in vitro MIC (2 mg/L) to itraconazole. This might explain the apparent breakthrough infection despite adequate serum itraconazole concentrations at the onset of her illness. This is consistent with MICs in the literature of 8 mg/L using the microdilution method and 32 mg/L using the E-test method [1].

Caspofungin was started as pre-emptive therapy for prolonged neutropenic fever and continued when invasive pulmonary aspergillosis was suspected based on radiological findings. Caspofungin is the preferred first line agent at our centre, primarily due to tolerability and toxicity concerns. In a large systematic review and meta-analysis by Chen et al. involving 4583 patients in 17 randomised control trials, empirical caspofungin appeared to be the most effective agent for all-cause and fungal-specific mortality [11]. However, it should be noted that several international guidelines recommend voriconazole, isavuconazole, or amphotericin B based on intrinsic resistance of the species [12,13]. Recommendations for use of echinocandins in these guidelines is as third line salvage therapy, often in combination with another agent. Of note, the MIC of our isolate for caspofungin was 0.03 mg/L, suggesting it was more susceptible to caspofungin than other reported *T. longibrachiatum* clinical isolates with MICs of 4 isolates reported as 0.5 mg/L [1].

Trichoderma species in general have high MICs to most antifungals except for echinocandins and voriconazole [1,14]. The antifungal regimens used for our case are consistent with those reported in the literature. Amphotericin B is the most commonly used antifungal agent, followed by voriconazole and caspofungin [1]. 24 % of cases in the literature were treated with combination therapy [1]. There are no guidelines for management of *Trichoderma* infections, however American guidelines for rare mould infections recommend a triazole or amphotericin-based therapy [15]. Clinical and biochemical parameters appeared to improve following institution of voriconazole. This is consistent with another case in which the patient became afebrile shortly after starting IV voriconazole for *T. longibrachiatum* pulmonary infection [5]. It has been suggested that caspofungin and voriconazole may have synergistic antifungal effects [8]. A meta-analysis by Panackal et al. suggested some benefit in salvage therapy, however a study on patients with haematological malignancies, specifically, did not demonstrate any benefit from the combination of caspofungin and voriconazole [16,17]. These studies were based upon in vitro testing in aspergillosis, so their application to *Trichoderma* infection must be inferred. Clinical data is lacking to support routine use of dual antifungal therapy in IFI. We utilized dual therapy due to the severity of illness, lack of clinical response to liposomal amphotericin and after considering the sparse evidence. It should generally be reserved for salvage therapy in cases where there is a lack of clinical response to a single agent.

Tracheal invasion may have contributed to the poor clinical response to caspofungin observed despite apparently satisfactory MICs for liquid phase tissues. This is because caspofungin is a highly hydrophilic molecule which would not be expected to penetrate tracheal cartilage. In contrast, voriconazole is moderately lipophilic and has a much higher volume of distribution and therefore would be preferred therapy in IFI with suspected tracheal invasion [18]. Direct instillation of tracheal amphotericin B or nebulised amphotericin may also have been considered, as described in some cases in the literature Cases demonstrating similar lung infiltration, infarction and necrosis have been reported [19]. On autopsy of a bone marrow transplant patient with *T. longibrachiatum* infection there were multiple areas of infarction with infiltration by branching septate hyphae in both lungs [20]. On bronchoscopy of an infected diabetic farmer, a distal main bronchus was found to be partially obstructed by whitish necrosis, and biopsy could not be performed due to risk of massive haemorrhage [5]. Imaging of immunosuppressed patients should be actively inspected for abnormal large airways.

In summary, we report a case of invasive *T. longibrachiatum* infection in an adult with acute myeloid leukaemia. Risk factors for infection were immunosuppression with chemotherapy, prolonged neutropaenia and antibiotic use. The patient died secondary to tracheal perforation and possible mediastinitis despite aggressive antifungal therapy with caspofungin, amphotericin B and voriconazole. This outcome, alongside radiologic features suggest a predilection for large airways and risk of broncho-tracheal invasion. Early mycological diagnosis and susceptibility testing were important in guiding antifungal choice. Reporting of additional cases will be required to help develop evidence on appropriate management of patients infected with this rare and life-threatening fungus.

Conflict of interest

Author AJ Wilson receives speakers fees and research funding from Gilead.

Ethical Form

Please note that this journal requires full disclosure of all sources of funding and potential conflicts of interest. The journal also requires a declaration that the author(s) have obtained written and signed consent to publish the case report/case series from the patient(s) or legal guardian(s).

The statements on funding, conflict of interest and consent need to be submitted via our Ethical Form that can be downloaded from the submission site www.ees.elsevier.com/mmcr. **Please note that your manuscript will not be considered for publication until the signed Ethical Form has been received.**

Funding source

Consent

Written informed consent was obtained from the patient or legal guardian(s) for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

Consent has been obtained from next of kin.

CRediT authorship contribution statement

Penelope J. Teoh: Writing – original draft, Conceptualization. **Emma McGuire:** Writing – review & editing, Conceptualization. **Andrew M. Borman:** Writing – review & editing. **Andrew J. Wilson:** Writing – review & editing. **Chloe Merrion:** Writing – review & editing. **Vanya Gant:** Writing – review & editing, Conceptualization.

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References

[1] E. Sal, J. Stemler, J. Salmanton-Garcia, I. Falces-Romero, L. Kredics, E. Meyer, et al., Invasive *Trichoderma* spp. infections: clinical presentation and outcome of cases from the literature and the FungiScope® registry, J. Antimicrob. Chemother. 77 (10) (October 2022) 2850–2858.

[2] Clinical and Laboratory Standards Institute, Reference method for broth dilution antifungal susceptibility testing of filamentous fungi, in: Approved Standard. 3rd Ed. Document M38-Ed3, CLSI, Wayne, PA, 2017.

[3] S. Ascioglu, J.H. Rex, B. de Pauw, J.E. Bennett, J. Bille, F. Crokaert, et al., Invasive fungal infections cooperative Group of the European organization for research and treatment of cancer; mycoses study Group of the national institute of allergy and infectious diseases. Defining opportunistic invasive fungal infections in immunocompromised patients with cancer and hematopoietic stem cell transplants: an international consensus, Clin. Infect. Dis. 34 (1) (2002 Jan 1) 7–14, https://doi.org/10.1086/323335. Epub 2001 Nov 26. PMID: 11731939.

[4] L. Hatvani, M. Homa, K. Chenthamara, F. Cai, S. Kocsubé, L. Atanasova, et al., Agricultural systems as potential sources of emerging human mycoses caused by Trichoderma: a successful, common phylotype of Trichoderma longibrachiatum in the frontline, FEMS Microbiol. Lett. 366 (21) (2019 Nov 1) fnz246, https://doi.org/10.1093/femsle/fnz246. PMID: 31816013.

[5] Q. Yu, Q. Zhang, J. Yu, Y. Song, W. Zhang, C. Que, A 66-year-old woman with progressive dyspnea and obstructive pneumonia, Chest Journal 160 (2) (August 2021) E177–E180, https://doi.org/10.1016/j.chest.2021.02.078.

[6] S. Wang, J. Tu, Invasive pulmonary infection caused by *Trichoderma longibrachiatum*, Thorax 78 (6) (2023 Jun) 632–633, https://doi.org/10.1136/thorax-2022-219855. Epub 2023 Feb 8. PMID: 36754640.

[7] G.J. Samuels, A. Ismaiel, T.B. Mulaw, G.M. Szakacs, I.S. Druzhinina, C.P. Kubicek, et al., The Longibrachiatum Clade of *Trichoderma*: a revision with new species, Fungal Divers. 55 (2012) 77–108, https://doi.org/10.1007/s13225-012-0152-2.

[8] A. Alanio, B. Brethon, M.F. de Chauvin, E. de Kerviler, T. Leblanc, C. Lacroix, et al., Invasive pulmonary infection due to *Trichoderma longibrachiatum* mimicking invasive aspergillosis in a neutropenic patient successfully treated with voriconazole combined with caspofungin, Clin. Infect. Dis. 46 (10) (2008) e116–e118, https://doi.org/10.1086/587750.

[9] A.M. Markantonatou, K. Samaras, E. Yannaki, E. Zachrou, T.A. Vyzantiadis, *Aspergillus* galactomannan detection: *Trichoderma* as a cause of positive results, Curr. Microbiol. 76 (2019) 48–51, https://doi.org/10.1007/s00284-018-1582-1.

[10] M.A. Finkelman, Specificity influences in $(1\rightarrow3)$-β-d-glucan-supported diagnosis of invasive fungal disease, J Fungi (Basel) 7 (1) (2020 Dec 29) 14, https://doi.org/10.3390/jof7010014. PMID: 33383818; PMCID: PMC7824349.

[11] K. Chen, Q. Wang, R.A. Pleasants, L. Ge, W. Liu, K. Peng, et al., Empiric treatment against invasive fungal diseases in febrile neutropenic patients: a systematic review and network meta-analysis, BMC Infect. Dis. 17 (1) (2017 Feb 20) 159, https://doi.org/10.1186/s12879-017-2263-6. PMID: 28219330; PMCID: PMC5319086.

[12] R.F. Patterson, G.R. Thompson, D.W. Denning, J.A. Fishman, S. Hadley, R. Herbrecht, et al., Clinical practice guideline for the diagnosis and management of aspergillosis: 2016 update by IDSA, Published: 29 June 2016, Clin. Infect. Dis. 63 (4) (2016) e1–e60, https://doi.org/10.1093/cid/ciw326.

[13] A.J. Ullmann, J.M. Aguado, S. Arikan-Akdagli, D.W. Denning, A.H. Groli, K. Lagrou, et al., Diagnosis and management of *Aspergillus* diseases: executive summary of 2017 ESCMID-ECMM-ERS guideline, Clin. Microbiol. Infection 24 (Supplement 1) (May 2018) E1–E38, https://doi.org/10.1016/j.cmi.2018.01.002.

[14] C. Kratzer, S. Tobudic, M. Schmoll, W. Graninger, A. Gerogopoulos, et al., In vitro activity and synergism of amphotericin B, azoles and cationic antimicrobials against the emerging pathogen Trichoderma spp, J. Antimicrob. Chemother. 58 (2006) 1058–1061.

[15] M. Hoenigl, J. Salmanton-García, T.J. Walsh, M. Nucci, C.F. Neoh, J.D. Jenks, et al., Global guideline for the diagnosis and management of rare mould infections: an initiative of the European confederation of medical mycology in cooperation with the international society for human and Animal mycology and the American society for microbiology, Lancet Infect. Dis. 21 (2021) e246–e257.

[16] A.A. Panackal, E. Parisini, M. Proschan, Salvage combination antifungal therapy for acute invasive aspergillosis may improve outcomes: a systematic review and meta-analysis, Int. J. Infect. Dis. 28 (2014) 80–94, https://doi.org/10.1016/j.ijid.2014.07.007.

[17] I.I. Raad, A.E. Zakhem, G.E. Helou, Y. Jiang, D.P. Kontoyiannis, R. Hachem, Clinical experience of the use of voriconazole, caspofungin or the combination in primary and salvage therapy of invasive aspergillosis in haematological malignancies, Int. J. Antimicrob. Agents 45 (3) (2015) 283–288, https://doi.org/10.1016/j.ijantimicag.2014.08.012.

[18] T.J. Walsh, E.J. Anaissie, D.W. Denning, R. Herbrecht, D.P. Kontoyiannis, K.A. Marr, V.A. Morrison, B.H. Segal, W.J. Steinbach, D.A. Stevens, J.A. van Burik, J.R. Wingard, T.F. Patterson, Treatment of aspergillosis: clinical practice guidelines of the infectious diseases society of America, Clin. Infect. Dis. 46 (3) (2008 Feb 1) 327–360.

[19] H. Boettcher, B. Bewig, S.W. Hirt, F. Möller, J. Cremer, Topical amphotericin B application in severe bronchial aspergillosis after lung transplantation: report of experiences in 3 cases, J. Heart Lung Transplant. 19 (12) (2000 Dec) 1224–1227.

[20] S. Richter, M.G. Cormican, M.A. Pfaller, C.K. Lee, R. Gingrich, M.G. Rinaldi, et al., Fatal disseminated Trichoderma longibrachiatum infection in an adult bone marrow transplant patient: species identification and review of the literature, J. Clin. Microbiol. 37 (4) (1999 Apr) 1154–1160, https://doi.org/10.1128/JCM.37.4.1154-1160.1999. PMID: 10074541; PMCID: PMC88664.