Aspergillus spinal epidural abscess masquerading as Pott's disease in a person with HIV: A case presentation and review of the literature

Rule R[a,b,#], Mitton B[a,b], Govender N P[c,d], Hoffmann D[a,b], Said M[a,b]

a. Department of Medical Microbiology, University of Pretoria, Pretoria, South Africa
b. Tshwane Academic Division, National Health Laboratory Service, Pretoria, South Africa
c. Centre for Healthcare-Associated Infections, Antimicrobial Resistance and Mycoses, National Institute for Communicable Diseases, a Division of the National Health Laboratory Service, Johannesburg, South Africa
d. School of Pathology, University of Witwatersrand, Johannesburg, South Africa

Corresponding author: Roxanne Rule (MBChB)

roxyrule@gmail.com

Address: 2-27 Pathology Building, University of Pretoria
Corner of Steve Biko & Dr Savage Roads
Pretoria
South Africa
0002

Tel: +2712 319 2338

Other contributing authors:

Barend Mitton (MBChB)

Address: 2-27 Pathology Building, University of Pretoria

Corner of Steve Biko & Dr Savage Roads

Pretoria

South Africa

0002

Nelesh Govender (FC Path (Micro))

Address: Centre for Healthcare-Associated Infections,

Antimicrobial Resistance & Mycoses

National Institute of Communicable Diseases

1 Modderfontein Road

Sandringham

Johannesburg

2192

Daniel Hoffmann (MBChB)

Address: 2-27 Pathology Building, University of Pretoria

Corner of Steve Biko & Dr Savage Roads

Pretoria

South Africa

0002

Mohamed Said (MBChB)

Address: Pathology Building, University of Pretoria

Corner of Steve Biko & Dr Savage Roads

Pretoria

South Africa

0002

Abstract

Aspergillus spinal epidural abscess is a debilitating form of invasive aspergillosis that may easily be misdiagnosed as Pott's disease due to shared risk factors and clinical features. To optimise outcomes in infected patients, it is essential to make an early accurate diagnosis and immediately initiate antifungal therapy. We describe a case of thoracic *Aspergillus* spinal epidural abscess in a patient with underlying HIV infection. The initial diagnostic consideration was that of spinal tuberculosis manifesting as Pott's disease. Consequently, despite positive microbiological cultures of *Aspergillus fumigatus*, antifungal therapy was delayed until histopathological evaluation of the affected tissue confirmed the presence of fungal hyphae. The patient showed an initial favourable response after surgical removal of the infected focus, but unfortunately never returned to premorbid functioning. This case highlights the importance of early diagnosis and treatment of *Aspergillus* spinal epidural abscesses. Morbidity and mortality associated with this condition may be significantly increased if physicians fail to institute antifungal therapy timeously.

Keywords

Invasive aspergillosis, *Aspergillus* spinal epidural abscess, Pott's disease, *Aspergillus fumigatus*, HIV

Introduction

Aspergillus spinal epidural abscess is an infrequently described syndrome of invasive aspergillosis (IA), that is associated with a high morbidity and mortality for affected patients.[1] It is most common among patients with advanced immunosuppression.[1] The diagnosis and treatment of this invasive disease remains a challenge, especially in tuberculosis-endemic areas, where it is often misdiagnosed as spinal tuberculosis (TB) due to the overlap of non-specific clinical features.[2-4] The lack of sensitive and specific non-invasive diagnostic modalities is an additional challenge.[5]

Case Presentation

We describe a case of a 42-year-old HIV positive woman who presented to the neurology department of a tertiary hospital in Pretoria, South Africa. She was being treated with a fixed-dose combination of tenofovir, emtricitabine and efavirenz. Her CD4+ T-cell count was 233 cells/μL on admission. No recent HIV viral load was available. She had also reported a previous history of drug-susceptible pulmonary TB seven years previously for which she had completed six months of first line anti-tuberculous therapy. Her presenting signs and symptoms were consistent with a T4 myelopathy, comprising lower back pain for the preceding three months accompanied by progressive sensory fallout below the level of the T5 dermatome, poor central stability, no motor power of the lower limbs as well as faecal and urinary incontinence. She also had a concomitant lower respiratory tract infection. A magnetic resonance imaging (MRI) thoracic scan was performed on admission and revealed left upper lobe fibro-cavitatory lung changes with ipsilateral tracheal and mediastinal displacement. In addition, thickened left pleura and paravertebral soft tissue with multiple small rim enhancing abscesses was seen. Intraspinal extension of this infectious process with

enhancing soft tissue and an extradural abscess (T2-T4) with marked cord compression and oedema were also noted. The radiology report concluded that the features were most likely related to tuberculosis. The differential diagnosis included bacterial or fungal infection. Figures 1a, b and c depict the MRI findings described above. A working diagnosis of spinal TB was made. This diagnosis was supported by the history of immunosuppression, previous pulmonary TB, a current concomitant lower respiratory tract infection, an elevated erythrocyte sedimentation rate (ESR) of 90 mm/hr and the imaging findings. Subsequent respiratory specimens failed to detect *Mycobacterium tuberculosis* DNA on the Gene Xpert Ultra platform (Cepheid, Sunnyvale, CA). Liquid TB culture of these respiratory specimens yielded no growth following 42 days of incubation. Consequently, she was referred to neurosurgery for a decompressing laminectomy. Intra-operative tissue specimens were sent for microscopy, culture and sensitivity tests as well as histopathology. Microbiological testing revealed acute angle branching, septate fungal hyphae on direct microscopy with potassium hydroxide (KOH) preparation of the tissue. Culture subsequently yielded a pure growth of *Aspergillus fumigatus* on all primary microbiological media as well as Sabouraud agar (Diagnostic Media Products (DMP), Johannesburg, South Africa). The mould identification was determined phenotypically with macroscopic and microscopic analysis of growth on Sabouraud agar (DMP) and the use of a lactophenol cotton blue stain (DMP), respectively. Figure 2 demonstrates the typical macroscopic morphology of *A. fumigatus*, while figure 3 demonstrates the microscopic characteristics observed. The identification was confirmed by means of ITS gene sequencing.[6] Antifungal susceptibility testing was performed on the isolate at the national mycology reference lab. The isolate was susceptible to amphotericin B (MIC of 0·50 µg/mL), voriconazole (MIC of 0·064 µg/mL) and itraconazole (MIC of 0·38 µg/mL), as determined by the Etest method (bioMérieux Diagnostics, Marcy l'Etoile, France) and interpreted using EUCAST breakpoints version

10.0.[7] At this point, the treating clinicians were doubtful of the significance of the culture, given the ubiquity of *Aspergillus* species in the environment and the possibility of specimen contamination. They considered the diagnosis of spinal TB as more probable and opted to wait for the histopathology results before initiating antifungal therapy. Anti-tuberculous therapy was also withheld while awaiting the biopsy results. A serum (1,3) beta-D-glucan test revealed a markedly elevated level of >500 pg/mL. Fifteen days after surgery, the histology results were made available to the treating clinicians. Granulomatous inflammation was noted (Figure 4) and invasive mycosis was confirmed with branching, septate hyphae visualised on Groccot's methenamine silver stain (DMP) (Figure 5). A real-time PCR (Genesig® Advanced Kit, Primerdesign Ltd, Southampton, United Kingdom) done on the tissue for TB was negative. The patient was initiated on amphotericin B at a dose of 50 mg intravenously daily while awaiting stock of voriconazole. After eight days of intravenous amphotericin B, her therapy was altered to oral voriconazole 200 mg twice daily, to complete a total duration of 6 – 12 weeks, based on clinical response. She was subsequently down-referred to a rehabilitation centre for continued physiotherapy. While in the rehabilitation centre, she completed four weeks of voriconazole. Due to further stock shortages, her therapy was amended to oral itraconazole 100 mg twice daily to complete the total duration of therapy. Although an initial improvement in symptoms was noted soon after surgery with a mild improvement in motor power of her lower limbs, this improvement was not sustained. In the rehabilitation facility, despite daily physiotherapy and occupational therapy visits, she remained wheelchair bound with no regain of bladder or bowel function. No follow up scans were performed during her stay in the rehabilitation facility. She has since been discharged home and has unfortunately been lost to follow up.

Discussion

Mycology

Aspergillus species were first described by Micheli in 1729 in his famous book titled the *Nova Geneva Plantarum*.[8] The name *Aspergillus* is derived from the striking resemblance of the conidial head to an aspergillum, a perforated metal globe on the end of a short rod-like handle used to sprinkle holy water during religious practices.[8]

Aspergillus species are ubiquitous, saprophytic moulds found throughout the world.[5,8] They are found in large numbers in the environment, with their ecological niche being decaying vegetable matter and soil.[8,9] As a result, exposure to these organisms is inevitable. The genus comprises more than 250 species with approximately 33 associated with human disease.[9] *Aspergillus* section *Fumigati* accounts for most cases of human disease, followed by the other species in the genus, including, but not limited to, *A. flavus, A. terreus, A. niger and A. nidulans*.[9]

Pathogenesis and immune response

Disease caused by *Aspergillus* species occurs primarily as a result of inhalation of aerial fungal conidia.[5] Despite the abundance of spores in the environment and the almost constant exposure to them, disease only occurs in a select subgroup of patients.[5] In most patients, the immune system is able to overcome the fungal onslaught through both innate and adaptive immune mechanisms.[5] In healthy individuals, mucociliary clearance eliminates most inhaled conidia.[5] Conidia that are able to reach the alveoli then encounter alveolar epithelial cells.[5] It has been demonstrated that these epithelial cells are able to kill conidia by means of actin-dependent phagocytosis and acidified phagolysosomes.[5] Likely the most critical immune components in eradication of fungal conidia are the alveolar macrophages and neutrophils.[5]

These cells are capable of engulfing and destroying germinating conidia through various mechanisms.[5] They are capable of killing fungal cells by means of acidified phagolysosomes within 24 hours as well as through NADPH oxidase-dependent reactive oxidant species (ROS) generation.[5] A more detailed explanation of the immune mechanisms involved in protection against *Aspergillus* species can be found in the article recently published by Latge et al (2019).[5] In susceptible individuals, inhaled conidia are able to reach the alveoli and evade the immune response, leading to germination and proliferation of fungal cells within the lungs.[5] Fungal hyphae may then invade pulmonary arterioles and lung parenchyma leading to pulmonary necrosis.[5] In addition, haematogenous dissemination may occur leading to invasion of distant organs.[5]

The increased susceptibility of HIV-seropositive individuals to invasive fungal infections (IFI) is multifactorial and may occur at all stages of HIV disease.[10] These patients have defects in both innate and adaptive immune responses contributing to their predisposition to IFI.[11] HIV infected macrophages exhibit weakened fungal phagocytosis and processing as well as impaired cytokine production.[11] In addition, dendritic cells have dysregulated responses and no longer adequately present fungal antigens to T-lymphocytes.[11]

The role of T-cell depletion and dysfunction is well documented and includes the following factors: The absolute reduction in CD4+ T cells as a result of HIV infection leads to failure of organism-specific effector populations.[11] Evidence of impairment of antigen-specific cytokine responses in alveolar T-cells may allow for respiratory fungal pathogens, such as *Aspergillus* species, to evade the initial pulmonary immune responses.[11] In addition, impaired T-lymphocyte production of interferon-γ, which plays a crucial role in the defence against

invasive aspergillosis by enhancing oxidative responses and hyphal damage in phagocytes, has also been demonstrated in HIV patients.[11,12]

Risk factors for IA

Patients with underlying immunosuppressive conditions, such as HIV, haematopoietic stem cell transplantation, neutropenia, corticosteroid use, chronic granulomatous disease and neutrophil dysfunction disorders, among others, are susceptible to the more severe forms of disease known as invasive aspergillosis.[13] With regard to HIV, IA is seen most frequently in those with advanced disease, whose CD4+ T cell counts are typically <100 cells/mm^3, those with significant neutropenia (traditionally in the setting of AZT use) and those with concomitant corticosteroid use.[11,14] In keeping with a previous South African case reported by Rossouw et al (2011), our patient did not have a remarkably low CD4+ T cell count as one would expect. [15]

A change in the epidemiology of invasive aspergillosis is being observed, and less classical, emerging risk factors have now been identified and should also be remembered when considering a diagnosis of IA.[16,17] These conditions include solid organ transplantation, chronic obstructive pulmonary disease (COPD), cytomegalovirus (CMV) infection and reactivation, liver disease, diabetes mellitus and *Aspergillus* infection in critically ill patients.[17]

Epidemiology

Globally, the incidence of IA is increasing.[18] A recent review analysing data from 40 countries, accounting for approximately 29% of the world's total population, found the average global incidence of IA to be 4·10 cases per 100 000.[18] Unfortunately, none of the countries included in the review had estimates of IA in HIV patients.[18] In keeping with international data, South Africa's estimated IA incidence is at 4·8 cases per 100 00 population, with approximately 3 885 cases of IA occurring per year.[19] Due to challenges with diagnosing IA, this incidence rate may represent an underestimation.

Clinical Presentation

Aspergillus fumigatus is known to cause a spectrum of diseases, determined by host immune status as well as underlying pulmonary integrity.[13] Invasive aspergillosis is the most severe form and may manifest as various syndromes, including invasive pulmonary aspergillosis as well as the many extrapulmonary invasive aspergillosis syndromes, involving the central nervous system, eyes, heart, abdominal cavity and bones.[20] Among these extrapulmonary aspergillosis syndromes, is invasive spinal aspergillosis, a less frequent but debilitating condition, as seen in our patient.

Invasive spinal aspergillosis may occur as an osteomyelitis involving one or more vertebral bodies or as *Aspergillus* spinal epidural abscess.[1,15,21] Various mechanisms of acquisition of *Aspergillus* spinal epidural abscess have been described. *Aspergillus* spinal epidural abscess may arise as a result of contiguous spread from vertebral osteomyelitis, haematogenous dissemination from a distant focus of infection, direct inoculation of *Aspergillus* into the epidural space during iatrogenic procedures or, in the case of our patient, contiguous spread

from a pulmonary focus.[1,15,22] Most commonly, *Aspergillus* spinal epidural abscess manifests in the thoracic or lumbar spine.[1]

Shweikeh et al (2018) performed a systematic review and found 26 previously reported cases of *Aspergillus* spinal epidural abscess.[1] They found that these infections most commonly occur in the third to sixth decades of life, with a higher incidence in males (66.7%) and that the thoracic spine was most often implicated.[1] Patients commonly presented with symptoms of back pain, focal neurological deficits and fever.[1] Common comorbid conditions were renal impairment, malignancy, diabetes mellitus, immunosuppression and tuberculosis.[1] Management commonly included a combination of antifungal therapy and surgery.[1] Clinical outcomes were poor with a 52% case-fatality ratio.[1] Only 26% of patients studied made a full recovery.[1] To our knowledge, *Aspergillus* spinal epidural abscess has only been described once previously in South Africa in an HIV infected patient who succumbed to his illness within three weeks of antifungal therapy.[15]

Invasive aspergillosis syndromes, notably *Aspergillus* spinal epidural abscess, are commonly misdiagnosed as tuberculosis.[2-4] The risk factors and clinical features of both diseases often overlap making the clinical distinction especially challenging.[2] A review of the literature has revealed four cases of *Aspergillus* spinal epidural abscess initially diagnosed as Pott's disease. These patients were all started on empiric anti-tuberculous therapy before the diagnosis of IA was confirmed.[2-4] Two of the four patients demised shortly after starting antifungal treatment.[3,4] These cases emphasise the importance of maintaining a high index of suspicion of IA in order to optimise outcomes of patients with *Aspergillus* infection.

Diagnosis

Making the diagnosis of IA can be challenging, but a timely diagnosis is of paramount importance due to the high mortality rate associated with this condition. A high index of suspicion should be maintained at all times. Latge et al (2019), have proposed an algorithm to assist in making an accurate diagnosis of IA in haematologic malignancy and haematopoietic stem cell transplant patients.[5] This algorithm may also prove to be useful for patients with other immune compromising conditions, such as HIV. The described approach includes assessing host, clinical/radiological, microbiological factors and histopathological factors and classifies patients as either proven IA, probable IA or possible IA.[5]

A diagnosis of proven IA can be made if histopathological results confirm the presence of fungal hyphal elements and angioinvasion on tissue specimens examined with various fungal stains.[5] In our patient hyphal elements were seen in tissue on the Grocott's methenamine silver stain. A patient has probable IA if host, clinical/radiological and microbiological criteria are met. All three criteria were met in the patient described in this case. This is in contrast to possible IA, where only host and clinical/radiological criteria are met.[5]

Histopathological diagnosis in tissue sections is made by identifying the fungal hyphae.[20] *Aspergillus* hyphae are typically septate, of narrow width (3 to 12 µm) and acutely or dichotomously branched at 45° angles.[23] Invasive aspergillosis is confirmed by the presence of these characteristic hyphae within a vessel wall with accompanying haemorrhage.[23] Granuloma formation and necrosis may also occur.[23]

Clinical and radiological features of invasive pulmonary aspergillosis (IPA), suggested by Latge et al (2019) are non-specific and often subtle and lack sensitivity to make an informed diagnosis.[5] Patients may present with a low-grade fever and mild non-productive cough.[5] Once angioinvasion is established, pleuritic chest pain and haemoptysis may occur.[5] In disseminated cases, clinical features are usually related to the site of infection.[5] Radiological changes in IPA are best appreciated with high resolution CT scans and comprise cavitation formation, pulmonary nodules and the highly suggestive but infrequent halo sign indicative of thrombotic pulmonary infarction with surrounding haemorrhage and oedema.[5]

Microbiological diagnosis of IA is challenging. Respiratory cultures in patients with suspected IPA yield a low sensitivity (<30%) and therefore cannot be solely relied upon.[5] In addition to this, due to the ubiquity of the organism in the environment and high likelihood of airway colonisation in patients with underlying pulmonary disease, a positive respiratory culture cannot conclusively make the diagnosis and can only be used as a surrogate in patients with host and clinical features to suggest the diagnosis of IPA.[5,16,24] Obtaining sterile deep tissue specimens in patients with IA may not always be feasible and requires invasive and difficult sampling from the patient. When *Aspergillus* species is cultured from a sterile site, however, it should be considered clinically significant.[16]

Due to the limitations associated with microbiological culture, many non-culture-based methods have been developed. These include biomarkers such as cell wall polysaccharide antigens (galactomannan and β-1,3-glucan) and fungal nucleic acid.[5] Galactomannan is the preferred antigen for diagnosing IA, demonstrating excellent sensitivity (67% - 100%) and specificity (86% - 99%) in numerous studies.[5] The galactomannan assay also has the

advantage in that it can be performed on both serum and bronchoalveolar lavage fluid.[5] β-1,3-glucan can be used as a non-specific marker of invasive fungal infection, including IA, with sensitivities ranging from 67% to 100%.[5] Lastly, detection of fungal DNA with PCR assays can be used, however, widespread utility of this technique has been limited by the lack of standardisation of the technical components of these PCR assays.[16] Progress in standardising PCR techniques for *Aspergillus* detection has been made possible by the by the European Aspergillus PCR Initiative (EAPCRI) Working Group of the International Society of Human and Animal Mycoses (ISHAM).[25] This has allowed for a number of commercially available assays to be approved for diagnostic use, such as AsperGenius (PathoNostics, Maastricht, Netherlands) and MycAssay *Aspergillus* (Myconostica Ltd., Cambridge, United Kingdom), to name a few.[25]

Treatment and prognosis

A number of antifungals exhibit *in vitro* activity against *Aspergillus* species, including the triazoles, polyenes and echinocandins.[20] The preferred drug for IA is voriconazole, as recommended in the 2016 Infectious Disease Society of America (IDSA) practice guidelines for the diagnosis and management of aspergillosis.[20] Early initiation of therapy is critical for improved outcomes, and treatment should not be delayed while awaiting diagnostic evaluation in suspected IA cases.[20] The treatment duration is typically protracted in nature, ranging from 6 – 12 weeks, depending on the host immune status and duration of immunosuppression, location of the infection and response to therapy.[20] Where feasible, surgical source control is also advised.[20] Follow up imaging (high resolution CT) is advised after a minimum of two weeks of antifungal therapy to monitor response to treatment.[20]

In this case, voriconazole was changed to oral itraconazole due to stock outages of voriconazole. The choice of standard dosing of oral itraconazole may have been suboptimal due to the co-administration of efavirenz.[26] Many drug-drug interactions are known to occur with the non-nucleotide reverse transcriptase inhibitor drugs and itraconazole.[26] As a result, itraconazole concentrations are significantly reduced in the presence of efavirenz.[26] In addition, the oral bioavailability of itraconazole is poor and largely influenced by gastric acidity and the presence of food.[26] For these reasons, therapeutic drug monitoring of itraconazole serum concentrations would be advised, but unfortunately, this practice is unavailable in South Africa.

Invasive aspergillosis syndromes are associated with high mortality rate and are almost always fatal in the absence of treatment.[27] The attributable mortality is 40% for invasive pulmonary disease but is much higher for disseminated disease and central nervous system involvement, with reported mortality rates exceeding 90%.[27] For this reason, it is crucial to promptly and accurately diagnose these invasive syndromes so that appropriate antifungal treatment can be instituted early, ensuring the best possible chance of survival for each infected patient.

Conclusion

Aspergillus spinal epidural abscess is an infrequent but important cause of compressive myelopathy. Immunocompromised patients, including those that are HIV infected, are at risk of this invasive form of aspergillosis. Despite challenges associated with the diagnosis of *Aspergillus* spinal epidural abscess, especially in TB endemic regions, it is critical to

maintain a high index of suspicion, in order to improve patient outcomes through timeous administration of antifungal therapy.

Ethical Approval and Informed Consent

Ethical approval was obtained from the Research Ethics Committee, University of Pretoria, Faculty of Health Sciences, ethics reference number 296/2020. Written informed consent for publication was obtained from the patient.

Contributors

RR, BM and MS conceptualised the case report, collected and analysed case data, performed the literature search, obtained images, and drafted, revised and approved the final manuscript. NPG analysed the case data and drafted, revised and approved the final manuscript. DH drafted, revised and approved the final manuscript.

Declaration of interests

Dr Govender reports grants from US NIH, grants from US CDC, grants from Bill and Melinda Gates Foundation, grants from UKRI Medical Research Council, grants from NHLS Research Trust and non-financial support from Gilead, outside the submitted work.

The other authors have no conflicts of interest to declare.

Acknowledgements

The authors would like to acknowledge the department of Anatomical Pathology, University of Pretoria, for providing the photomicrographs of the patient's tissue sections.

Funding Source

No funding was obtained for this study.

I, Roxanne Rule, the corresponding author, acknowledge that I had full access to all the data in the study and had the final responsibility for the decision to submit for publication.

References

1. Shweikeh F, Zyck S, Sweiss F, Sangtani A, Shweikeh M, Issa H, et al. *Aspergillus* spinal epidural abscess: case presentation and review of the literature. Spinal Cord Ser Cases. 2018;4:19.

2. Sethi S, Siraj F, Kalra K, Chopra P. *Aspergillus* vertebral osteomyelitis in immunocompetent patients. Indian J Orthop. 2012;46(2):246-50.

3. Vaishya S, Sharma MS. Spinal *Aspergillus* vertebral osteomyelitis with extradural abscess: case report and review of literature. Surg Neurol. 2004;61(6):551-5.

4. Tew CW, Han FC, Jureen R, Tey BH. *Aspergillus* vertebral osteomyelitis and epidural abscess. Singapore Med J. 2009;50(4):e151 - 4.

5. Latge JP, Chamilos G. *Aspergillus fumigatus* and Aspergillosis in 2019. Clin Microbiol Rev. 2019;33(1).

6. Maphanga TG, Birkhead M, Muñoz JF, Allam M, Zulu TG, Cuomo CA, et al. Human Blastomycosis in South Africa Caused by *Blastomyces percursus* and *Blastomyces emzantsi* sp. nov., 1967 to 2014. J Clin Microbiol. 2020;58(3).

7. EUCAST. Breakpoint tables for interpretation of MICs for antifungal agents, version 10.0. 2020. Available from http://www.eucast.org/astoffungi/clincalbreakpointsforantifungals/.

8. Denning DW. Invasive Aspergillosis. Clin Infect Dis. 1998;26:781-805.

9. Cadena J, Thompson GR, 3rd, Patterson TF. Invasive Aspergillosis: Current Strategies for Diagnosis and Management. Infect Dis Clin N Am. 2016;30(1):125-42.

10. Kuritzkes DR. Neutropenia, Neutrophil Dysfunction, and Bacterial Infection in Patients with Human Immunodeficiency Virus Disease: The Role of Granulocyte Colony-Stimulating Factor. Clin Infect Dis. 2000;30:256-60.

11. Armstrong-James D, Meintjes G, Brown GD. A neglected epidemic: fungal infections in HIV/AIDS. Trends Microbiol. 2014;22(3):120-7.

12. Warris A, Netea MG, Verweij PE, Gaustad P, Kullberg BJ, Weemaes CMR, et al. Cytokine responses and regulation of interferon-gamma release by human mononuclear cells to *Aspergillus* fumigatus and other filamentous fungi. Med Mycol. 2005;43(7):613-21.

13. Kosmidis C, Denning DW. The clinical spectrum of pulmonary aspergillosis. Thorax. 2015;70(3):270-7.

14. Danion F, Rouzaud C, Duréault A, Poirée S, Bougnoux ME, Alanio A, et al. Why are so many cases of invasive aspergillosis missed? Med Mycol. 2019;57(Supplement_2):S94-S103.

15. Rossouw I, Goedhals D, van der Merwe J, Stallenberg V, Govender N. A rare, fatal case of invasive spinal aspergillosis in an antiretroviral-naive, HIV-infected man with pre-existing lung colonization. J Med Microbiol. 2011;60:1534-8.

16. Hope WW, Walsh TJ, Denning DW. Laboratory diagnosis of invasive aspergillosis. The Lancet Infect Dis. 2005;5:609-22.

17. Kanj A, Abdallah N, Soubani AO. The spectrum of pulmonary aspergillosis. Respir Med. 2018;141:121-31.

18. Bongomin F, Gago S, Oladele RO, Denning DW. Global and Multi-National Prevalence of Fungal Diseases-Estimate Precision. J Fungi. 2017;3(4):57.

19. Schwartz IS, Boyles TH, Kenyon CR, Hoving JC, Brown GD, Denning DW. The estimated burden of fungal disease in South Africa. S Afr Med J. 2019;109(11).

20. Patterson TF, Thompson GR, Denning DW, Fishman JA, Hadley S, Herbrecht R, et al. Practice Guidelines for the Diagnosis and Management of Aspergillosis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016;63(4):e1-e60.

21. Ono R, Sato S, Okada S, Kanbe E, Tanaka E, Tamai Y. Invasive Pulmonary Aspergillosis in the Epidural Space in a Patient with Acute Myelogenous Leukemia with Myelodysplasia-related Changes: A Case Study and Literature Review of Vertebral Aspergillosis in Japan. Intern Med. 2018;57(21):3205-12.

22. Kim CW, Perry A, Currier B, Yaszemski M, Garfin SR. Fungal infections of the spine. Clin Orthop Relat Res. 2006;444:92-9.

23. Guarner J, Brandt ME. Histopathologic diagnosis of fungal infections in the 21st century. Clin Microbiol Rev. 2011;24(2):247-80.

24. Barton RC. Laboratory diagnosis of invasive aspergillosis: from diagnosis to prediction of outcome. Scientifica. 2013;2013:459405.

25. Rath PM, Steinmann J. Overview of Commercially Available PCR Assays for the Detection of *Aspergillus* spp. DNA in Patient Samples. Front Microbiol. 2018;9:740.

26. Schwartz IS, Wasserman S. Itraconazole and antiretroviral therapy: strategies for empirical dosing. The Lancet Infect Dis. 2017;17(11):1122-3.

27. Muñoz P, Guinea J, Bouza E. Update on invasive aspergillosis: clinical and diagnostic aspects. Clin Microbiol Infect. 2006;12 (suppl 7):24-39.

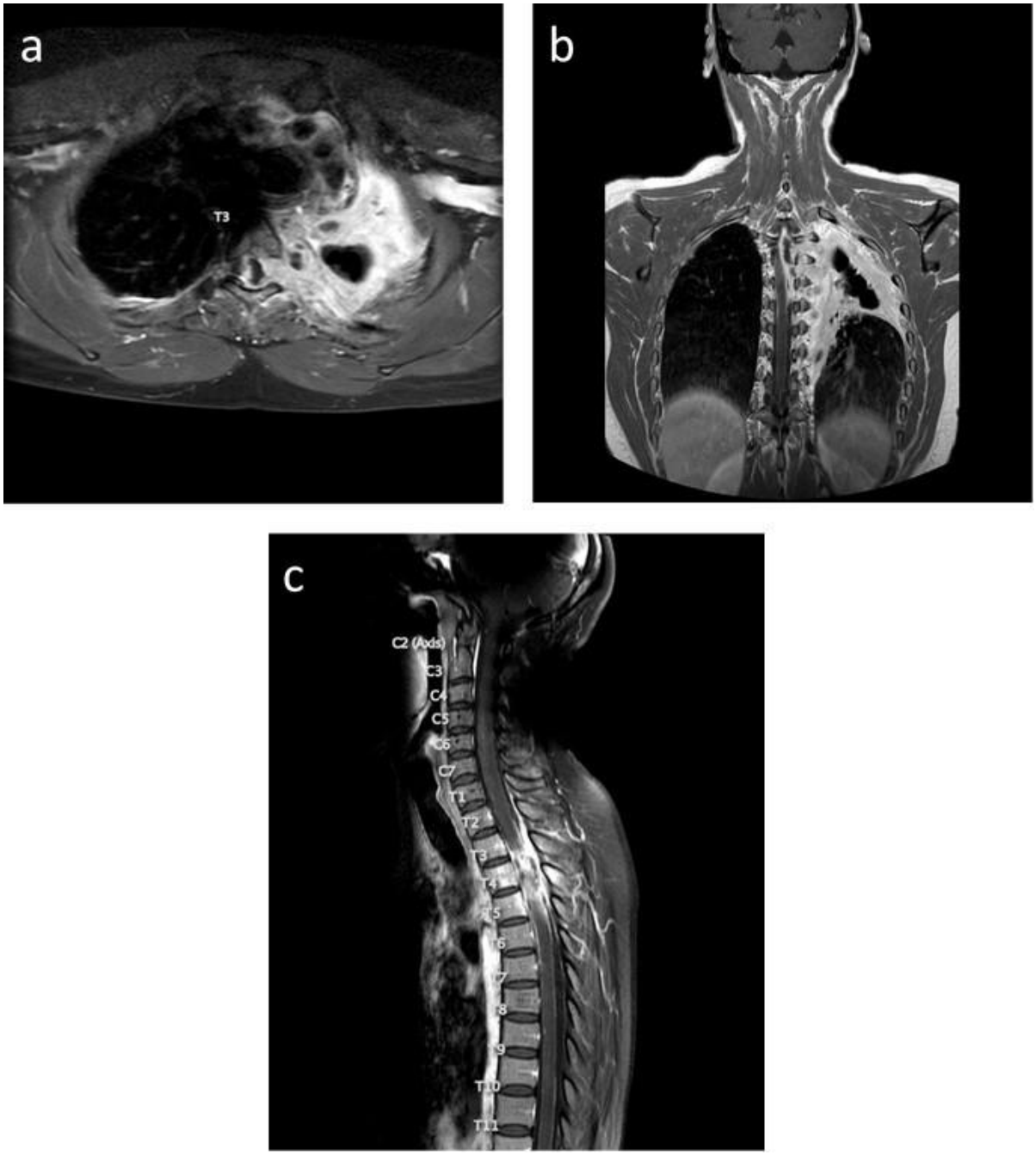

Figure 1: MRI images of a) transverse plane, b) coronal plane and c) sagittal plane showing left upper lobe cavitation, thickened and enhancing medial pleura and left para-spinal soft-tissue, with multiple small pockets of non-enhancing fluid collections. This can be seen extending into the spinal canal via the left T3/T4 and T4/T5 neural foraminae encasing the exiting nerves. An irregularly rim enhancing intraspinal, extradural collection can be seen

between the lower end of T2 and T4, compressing and displacing the adjacent thoracic cord to the right.

Figure 2: Typical macroscopic appearance of *Aspergillus fumigatus* grown from this patient's tissue on Sabouraud agar.

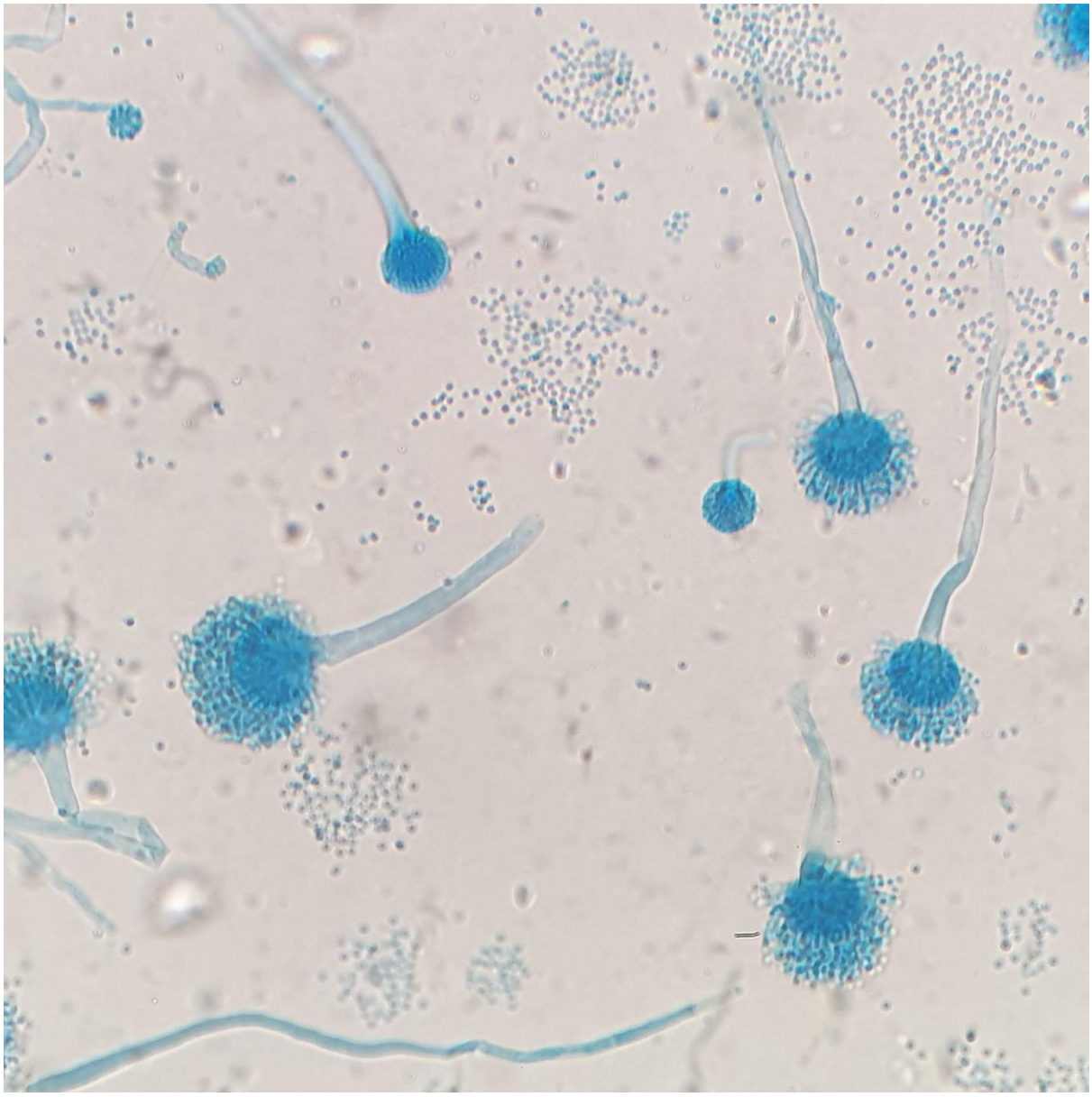

Figure 3: Microscopic morphology of *Aspergillus fumigatus* as seen with lactophenol cotton blue stain under 400X magnification.

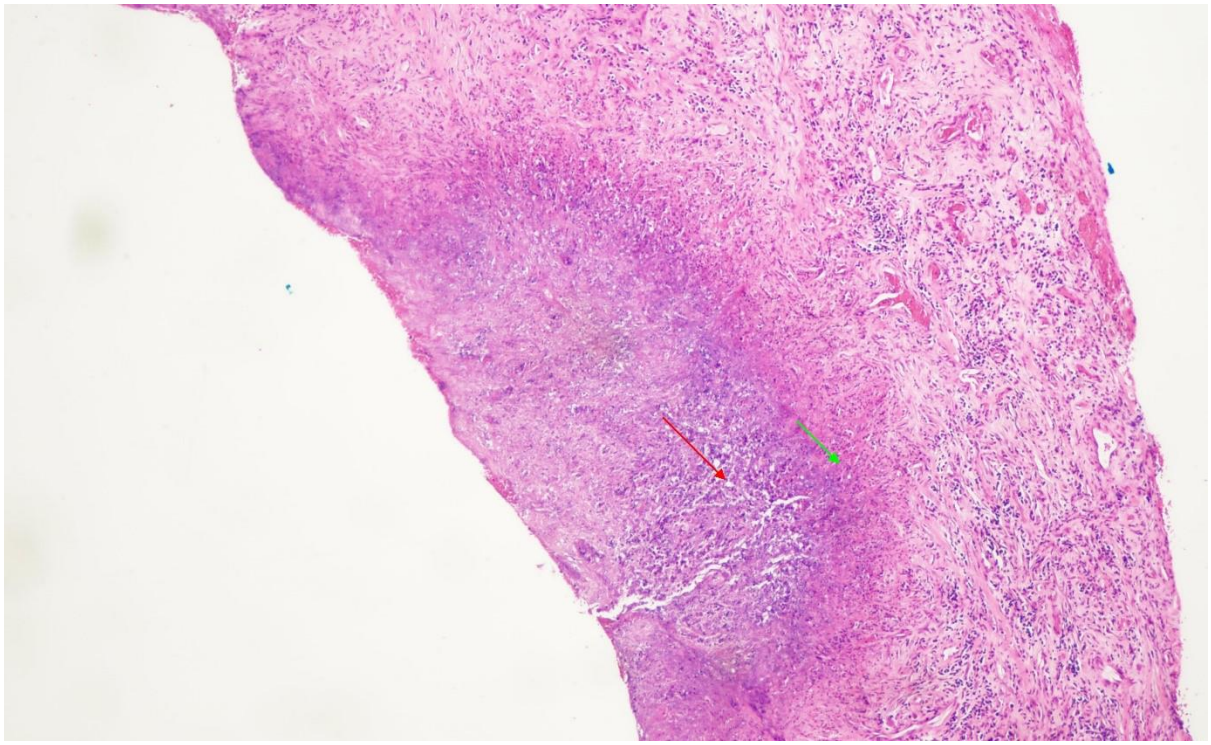

Figure 4: A photomicrograph of an H&E section from the patient, 20X magnification, showing necrotizing microabscess (indicated by the red arrow) with palisading single lying epithelioid cells (indicated by the green arrow).

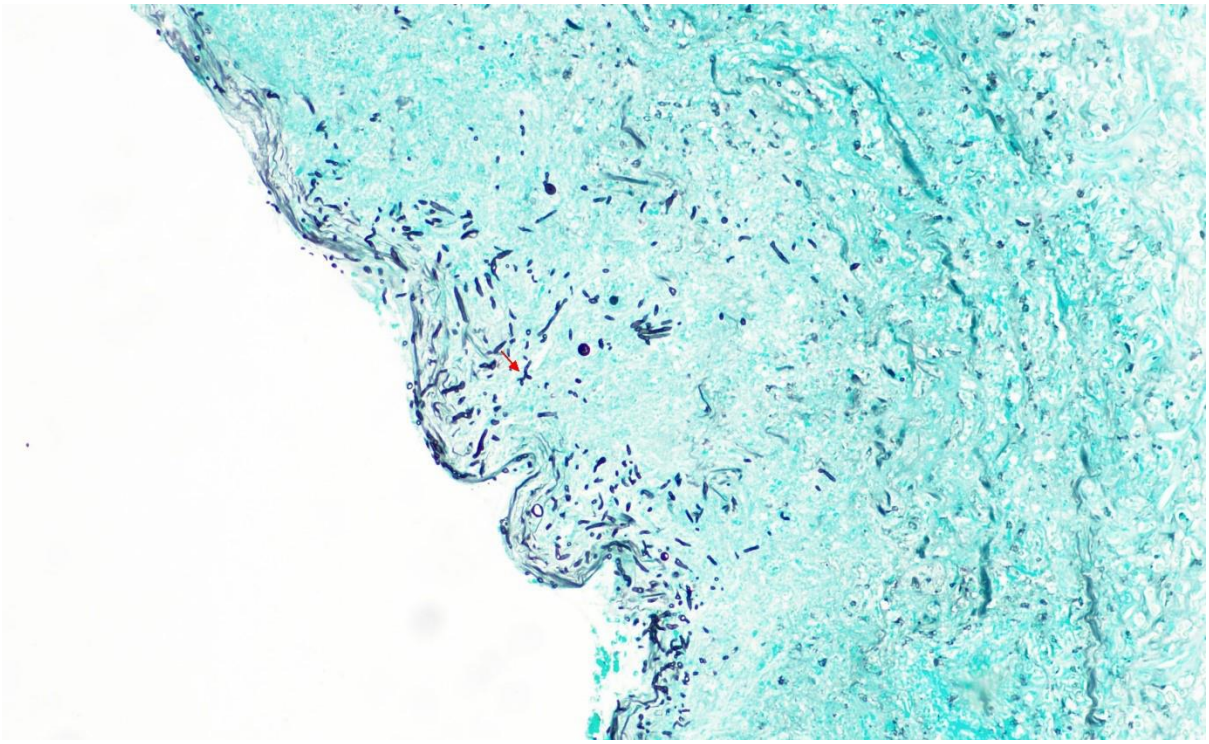

Figure 5: A photomicrograph of the patient's tissue section stained with Grocott's methenamine silver stain, 20X magnification, showing septate hyphae branching at acute angles (indicated by the red arrow).