Medical Mycology Case Reports 44 (2024) 100637

Contents lists available at ScienceDirect

Medical Mycology Case Reports

journal homepage: www.elsevier.com/locate/mmcr

A case of intracranial infection caused by *Aspergillus flavus* originating from chronic otitis media

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ARTICLE INFO

Handling Editor: Dr Adilia Warris

Keywords:
Central nervous system aspergillosis
Next-generation sequencing
Voriconazole concentration

ABSTRACT

Central nervous system (CNS) aspergillosis is uncommon in immunocompetent patients. We present a 64-year-old man with chronic otitis media and uncontrolled diabetes. *Aspergillus flavus* was identified in cerebrospinal fluid via metagenomics next-generation sequencing technology. Initial voriconazole treatment offered limited relief, but personalized dosage adjustments, guided by drug concentration, led to remission. This case underscores the importance of diverse diagnostic approaches and tailored therapy for CNS *Aspergillus* infections.

1. Introduction

Although central nervous system (CNS) aspergillosis is a rare disease, its prevalence has been growing due to the extensive use of immunosuppressants and antifungal medications [1]. The majority of instances of CNS aspergillosis arise when the fungus enters the body via the respiratory system and travels to the brain via the bloodstream. In some cases, the infection spreads from neighboring tissues such as the paranasal sinuses, middle ear, and mastoid, with the fungus infiltrating the intracranial space by destroying the thin bony walls that separate it [2–4]. In rare situations, nasal, ocular, and cerebral spread, affecting the base of the skull and intracranial regions, might induce rhino-brain-eye syndrome [1].

The gold standard for diagnosing CNS aspergillosis is histopathological evidence or a positive culture result for a biopsy or cerebrospinal fluid (CSF) [5]. However, these methods are time-consuming and laborious. Metagenomic next-generation sequencing (mNGS) is a high-throughput sequencing approach that has been used effectively to identify causal agents in a variety of clinical cases [6]. Aspergillosis may now be discovered via NGS, which shows potential for detecting CNS infection.

Voriconazole or isavuconazole is recommended as initial treatment for invasive aspergillosis [7]. Clinical trials have shown that voriconazole is more effective than amphotericin B formulations in various populations with invasive aspergillosis [8,9].

2. Case presentation

A 64-year-old male was admitted to the hospital with a complaint of recurrent chronic left ear pain, purulent discharge, and recurring headaches over the past 3 months (day 0). Three months before presentation (day −90), he was diagnosed with a left mastoiditis after having suffered from chronic left ear pain with discharge and headaches for 8 months, and underwent a left tympanotomy and tympanoplasty. Culture from the ear secretions revealed *Pseudomonas aeruginosa,* and he was treated with intravenous ceftazidime (2g every 8 hours) and intravenous amikacin (0.4g once daily) for 2 weeks. The patient had a well-documented history of diabetes spanning over two decades, with documented poor blood sugar control.

Relevant laboratory findings on the first day of hospitalization included: White Blood Cell Count: 5.43 × 10^9/L, Neutrophil Percentage: 75.0%, CRP: 17.69 mg/L, ESR: 68 mm/h, Procalcitonin: 0.05 ng/mL. Random Blood Sugar: 24.53 mmol/L. Blood galactomannan (GM) index was 0.8 (positive) with the (1–3)-β-D glucan being negative. Culture of ear secretions revealed the presence of *Staphylococcus lugdunensis*. The radiological findings disclosed abnormalities. The CT scan images of the head revealed an increased density in the left mastoid air chamber and signs of bone destruction, indicative of left middle ear mastoiditis (Fig. 1) (day 2). Additionally, the head MRI scan images depicted a substantial area of abnormal enhancement in the left middle skull base (Fig. 2) (day 2).

A lumbar puncture procedure was conducted, and cerebrospinal

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https://doi.org/10.1016/j.mmcr.2024.100637
Received 13 November 2023; Received in revised form 18 February 2024; Accepted 22 February 2024
Available online 28 February 2024

fluid (CSF) specimens were collected following standard aseptic protocols. Subsequently, the CSF samples were subjected to mNGS within a 24-h timeframe. Glass beads were introduced into the CSF samples, followed by rigorous agitation, DNA extraction, and the construction of DNA libraries. These quality-controlled libraries were sequenced using a BGISEQ-500/50 platform (BGI-Tianjin, Tianjin, China), resulting in an average of 20 million reads per sample. After eliminating human sequences, the remaining data were aligned against bacterial, viral, fungal, and protozoan databases [6].

Following admission, the patient was empirically treated with ceftazidime at a dosage of 2 g every 8 hours, and intravenous amikacin at a dose of 0.4g once daily for 7 days (from day 0).

A lumbar puncture procedure was performed, revealing abnormal CSF characteristics. The CSF pressure was measured at 215 mmH_2O, and CSF biochemistry and routine tests yielded the following results: nucleated cell count of 48 × 10ˆ6/L, with all these cells being mononuclear, protein level of 0.80 g/L, glucose level of 4.64 mmol/L (with simultaneous fingertip blood glucose at 9.77 mmol/L), and chloride levels at 120 mmol/L. CSF bacterial and fungal cultures were negative. The cerebrospinal fluid (1–3)-β-D glucan test and GM test were negative. However, mNGS in CSF identified the presence of *Aspergillus flavus* with a sequence count of 28, with no other bacteria or viruses detected.

As a result of these findings, the patient's treatment was adjusted (day +7). The revised treatment regimen included intravenous voriconazole at a dosage of 200 mg every 12 hours (on the first day 400 mg q12h), combined with antimicrobial agents against *Pseudomonas aeruginosa* for another 7 days.

On day +15, the patient's headache symptoms did not get better. Subsequent follow-up CSF results displayed no significant improvement. A follow-up CSF examination showed the results below (day +15): The pressure of CSF pressure was 216 mmH_2O, and CSF nucleated cell count of 20 × 10ˆ6/L, with 80% of mononuclear cells, protein level of 1.304 g/L, glucose level of 4.2mmol/L (with simultaneous fingertip blood glucose at 9.0 mmol/L), and chloride levels at 122 mmol/L. Repeat mNGS in CSF continued to detect *Aspergillus flavus* with a sequence count of 42.

Monitoring of voriconazole trough concentration was performed by high performance liquid chromatography (HPLC), and was below 0.45 μg/mL (day +10), which prompted additional investigation of voriconazole metabolism gene testing. Contrary to expectations, the patient exhibited intermediate metabolizer phenotype of cytochrome (CYP) 2C19. Compared to normal metabolism, voriconazole concentration tends to increase in intermediate metabolizers. Recognizing the limitations of genotype-based dosing, adjustments were made according to the measured concentration.

Considering the patient's poor response, liposomal amphotericin B was initiated (day +16). However, due to adverse gastrointestinal effects, liposomal amphotericin B was used for only three days and discontinued (liposomal amphotericin B 50mg on the 1st day, 300mg on the 2nd day, 300mg on the 3rd day). Dose adjustments were made based on the voriconazole trough concentration by 50% following the "Voriconazole Personalized Medication Guidelines" [10]. As a result, the dose was adjusted to 300 mg q12h intravenous infusion (from day +18). After two weeks of the adjusted treatment plan (day +32), the patient experienced significant relief from headaches, a marked reduction in purulent discharge and the voriconazole concentration reached 1.92 μg/mL (day +35), indicating therapeutic levels. The patient was discharged two days later (day +37). Treatment with voriconazole 300mg q12h and sitafloxacin were prescribed after hospital discharge. The patient was re-admitted to the hospital for a follow-up examination following nearly two months of voriconazole treatment with 300mg q12h (day +80). The CSF pressure was measured at 125 mmH_2O, and CSF biochemistry and routine tests yielded the following results: nucleated cell count of 13 × 10ˆ6/L, Neutrophil Percentage: 92.3%, protein levels of 1.423 g/L (The normal value is less than 0.45 g/L), glucose levels of 3.9 mmol/L (with simultaneous fingertip blood glucose at 11.6 mmol/L), and chloride levels at 121 mmol/L. It is noteworthy that the cell count, although still elevated, exhibited a decrease from 48 × 10ˆ6/L (day 0) to 13 × 10ˆ6/L (day +80).

Blood GM test was <0.5 (negative) on day +80. Due to the high cost, NGS was not repeated. Voriconazole trough concentration was 0.93μg/mL. Upon re-examination by MRI of the head, improvement was noted in the nasopharyngeal lesions. Otorhinolaryngology consultation confirmed the absence of pus or exudation in both ears and the absence of evident signs of acute infection. Inflammatory markers, including WBC, CRP, and ESR, exhibited a decrease. Consequently, the antibiotics were ceased, while the antifungal therapy was sustained. The case was lost to follow-up since then.

3. Discussion

Aspergillus infection is difficult to diagnose in patients with normal immunity compared with those with low immunity. The diagnosis of cerebral aspergillosis can be challenging due to two reasons: (1) the clinical and radiographic features of intracranial aspergillosis are not highly specific, and (2) the positive culture rate for Aspergillus in blood

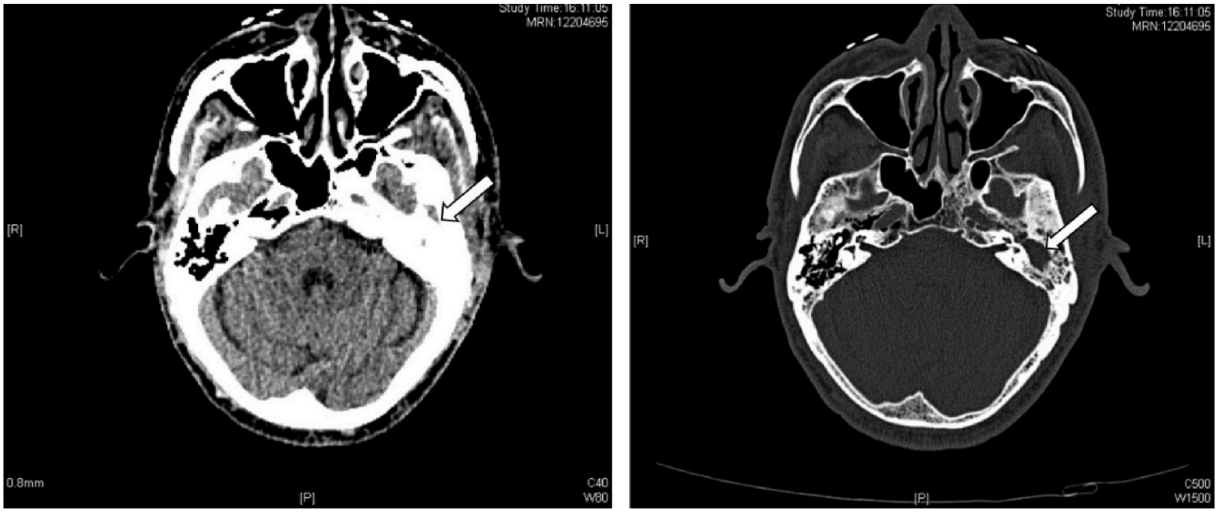

Fig. 1. Brain CT scan Images. The above CT images of the head showed increased density of the left mastoid air chamber and bone destruction (marked by the white arrow) (day +2).

and CSF is extremely low. However, CNS aspergillosis has a high mortality rate in immunocompetent individuals if not diagnosed and treated early. Especially for cases of nasal, ocular, and cerebral disseminated aspergillosis, the mortality rate can be as high as 88%–90% [11,12]. The preceding reports indicate that a significant proportion of patients treated with Voriconazole as their initial therapeutic intervention exhibited both survival and improvement [13,14]. The mortality rate associated with Aspergillus infection in the immunocompetent population ranges between 10% and 20% [8,15].

With the recent development of mNGS, it has demonstrated significant advantages in detecting difficult-to-culture pathogens and rare pathogens. In this case, mNGS technology identified the presence of *Aspergillus* in the CSF, providing evidence for early diagnosis and treatment. In a prospective study of 213 patients with CNS infection, five cases were diagnosed with CNS aspergillosis. Using species-specific read numbers (SSRN) ≥ 2 as a threshold, the sensitivity of mNGS for diagnosing CNS aspergillosis was 80% (4/5), and the specificity was 79.3% (165/208) [6]. Though mNGS emerges as a superior diagnostic tool compared to conventional methodologies when identifying infectious encephalitis and/or meningitis, it is crucial to employ this innovative approach in tandem with established microbiological testing protocols. In another retrospective study of 10 patients with central aspergillosis, the combined use of mNGS and GM testing resulted in diagnostic sensitivities and specificities of 61.9% and 82.6%, respectively [16]. Therefore, clinicians complemented mNGS with traditional methods such as GM testing and *Aspergillus* serology to provide more evidence for clinical diagnosis [17–19].

Voriconazole is a broad-spectrum triazole antifungal drug. Voriconazole should be used with caution since it is primarily metabolized by CYP2C19, with minor contributions from CYP3A4 and CYP2C9. Voriconazole is also a potent inhibitor of CYP3A4, which lead to drug interactions with various medications, including calcineurin inhibitors, sirolimus, vincamine, cyclophosphamide, and HMG-CoA reductase inhibitors [20]. Medication interactions resulted in suboptimal medication concentrations, reducing voriconazole effectiveness and necessitating therapeutic drug monitoring (TDM). Based on pharmacokinetic principles, steady-state blood concentrations is obtained by the second day following delivery with a first-day loading dosage. Blood samples should be collected on the third day following administration for the initial voriconazole concentration measurement. The recommended target trough concentration range for voriconazole is 0.5 mg/L to 5 mg/L. For patients with cerebral aspergillosis, it is preferable to achieve voriconazole trough concentrations above 1 mg/L.

In this case, voriconazole medication did not improve the patient's headache symptoms or CSF examination, and the monitored voriconazole concentrations were persistently low, indicating the need for additional research and adjustment of the treatment plan. After medication interactions were ruled out in this patient, hereditary variables should be considered.

According to the Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline "CYP2C19 and Voriconazole Therapy" [20], individuals with ultra-rapid metabolism of CYP2C19 have reduced voriconazole trough concentrations, leading to delayed achievement of target blood levels. In contrast, individuals with slow metabolism have increased voriconazole trough concentrations and an increased risk of adverse drug events. This patient had an intermediate metabolizer phenotype of the CYP2C19 gene. Compared to normal metabolism, voriconazole concentration tends to increase in intermediate metabolizers.

However, why did this patient have a lower concentration of voriconazole? In some cases, rare CYP2C19 mutations were usually not included in routine genotyping tests. These patients were typically classified as wild type (CYP2C19 $\times$ 1*1) by default. Therefore, in very few cases, the defined wild type allele might have decreased or increased functional variants. In addition, the individual prediction of CYP2C19 metabolic status also depended on other factors, including epigenetic phenomena, diet, comorbidities, or concomitant medications. Therefore, CYP2C19 genotype testing cannot replace therapeutic drug concentration monitoring, and voriconazole concentration monitoring is more meaningful for dose adjustment [10,21]. The dosage was increased to 300 mg every 12 hours in this case until therapeutic voriconazole level was attained.

In summary, *aspergillus* infection is difficult to diagnose in patients with normal immunity compared with those with low immunity. NGS provided a more convenient approach for the early diagnosis of CNS aspergillosis. Once *Aspergillus* infection is confirmed, voriconazole should be administered in time and the prognosis is favorable in patients with normal immunity. Voriconazole concentration monitoring is more meaningful for dose adjustment. The case underscores the critical role of personalized therapeutic adjustments in instances where standard treatment approaches fail to yield the anticipated clinical response.

CRediT authorship contribution statement

Ling Yang: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Jiacun Su:** Resources, Investigation.

Fig. 2. Brain magnetic resonance imaging (MRI) scan Images. Head scan images showed a large area of left middle skull base abnormally enhanced (marked by the white arrow) (day +2).

Chao Zhuo: Conceptualization, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

There are none.

Acknowledgements

We appreciated the patient allowing us to use his case to improve the scientific medical literature.

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