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Research Article

Diagnosis and Treatment of Central Nervous System Aspergillosis: A Report of 3 Cases and Literature Review

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Abstract

Background: Central nervous system (CNS) aspergillosis is a rare but devastating invasive fungal infection, with mortality exceeding 80% even with antifungal therapy. Patients with hematologic malignancies and those undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT) are at particularly high risk, especially when receiving prolonged glucocorticoids or T-cell immunosuppressants. The diagnosis is notoriously difficult because clinical manifestations are nonspecific, conventional cerebrospinal fluid (CSF) cultures have very low sensitivity, and brain biopsy is often contraindicated due to thrombocytopenia. Next-generation sequencing (NGS) of CSF has emerged as a promising unbiased diagnostic tool, but its clinical utility in this setting requires further illustration. **Methods:** We retrospectively collected three cases of CNS aspergillosis in patients with underlying hematologic diseases treated at our institution. All patients had received prolonged immunosuppressive therapy, including glucocorticoids for >3 weeks; two also received T-cell inhibitors such as ruxolitinib or basiliximab. CSF samples were obtained by lumbar puncture and subjected to metagenomic NGS for pathogen identification. Antifungal regimens, imaging findings, and clinical outcomes were reviewed. **Results:** The three patients (one with pancytopenia complicated by hemophagocytic syndrome, two post-allo-HSCT with severe graft-versus-host disease) all presented with fever and neurological symptoms (headache, aphasia, confusion, or seizure). Two had documented pulmonary aspergillosis prior to CNS involvement. CSF NGS detected Aspergillus flavus in two cases and A. fumigatus in one, whereas conventional cultures and serum biomarkers remained negative. Brain MRI revealed characteristic lesions including choroid plexus enhancement, multifocal infarcts, and hemorrhagic changes. Two patients developed CNS disease while already on liposomal amphotericin B for pulmonary infection (one also had isavuconazole added), indicating breakthrough infections. Despite antifungal therapy, all patients showed rapid neurological deterioration; one died of cerebral hemorrhage, and the other two were discharged against medical advice with

irreversible neurological damage and likely fatal outcomes. **Conclusions:** CSF NGS is a highly sensitive and specific diagnostic tool for CNS aspergillosis in hematologic patients, offering a major advantage over conventional methods, particularly when prior antifungal therapy has been given. The occurrence of breakthrough infections despite combination therapy with agents that penetrate the blood-brain barrier strongly suggests the presence of azole resistance. Our cases underscore the urgent need for early CSF NGS, routine antifungal susceptibility testing, and therapeutic drug monitoring to improve the currently grim prognosis of this lethal complication.

Key words: Central nervous system (CNS); aspergillosis; hematologic diseases; next-generation sequencing (NGS); breakthrough.

1. Introduction

Central nervous system (CNS) aspergillosis is a rare but devastating invasive fungal infection, predominantly affecting profoundly immunosuppressed patients (1). Among all forms of invasive aspergillosis (IA), CNS involvement carries the worst prognosis, with mortality rates often exceeding 80–90% despite modern antifungal (2). This dismal outcome stems from late diagnosis, poor blood–brain barrier (BBB) penetration, and the inherent angioinvasive nature of Aspergillus. In patients with hematologic malignancies and allogeneic hematopoietic stem cell transplantation (allo-HSCT) recipients, IA incidence has risen steadily over the past two decades, driven by intensified chemotherapy, broad-spectrum antibiotics, and expanded immunosuppressants used for graft-versus-host disease (GVHD) (3). While these advances improve survival from underlying diseases, they concurrently create a vulnerable niche for opportunistic moulds, with Aspergillus remaining the most common and lethal (4).

Epidemiology has shifted (5). Global incidence varies by region and prophylaxis policy, but a clear upward trend is seen in high-risk populations—acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and all-HSCT recipients (6). In Europe and North America, IA cumulative incidence ranges from 5% to 15% in AML patients and exceeds 20% in allo-HSCT recipients with grade II-IV acute GVHD. Asian data, though historically limited, show comparable or even higher burdens, with A. fumigatus and A. flavus dominant (7). Notably, non-fumigatus species (A. flavus, A. terreus, A. niger), and other moulds (Fusarium, Scedosporium, Mucorales) are increasing, posing additional diagnostic and therapeutic challenges.

Pathogenesis links host immunity and fungal virulence. In immunocompetent hosts, inhaled conidia are cleared by alveolar macrophages and neutrophils. In hematologic patients, this first-line defence is compromised. Prolonged severe neutropenia is a classic risk factortor, but CNS aspergillosis also occurs in non-neutropenic patients, receiving high-dose glucocorticoids or T-cell inhibitors (calcineurin inhibitors, mTOR inhibitors, ATG). Glucocorticoids impair the phagocytosis and cytokine production; T cell inhibitors disrupt the adaptive immunity. Their combination-often used in GVHD-creates an "immunoparalysis" that facilitates hematogenous dissemination from pulmonary foci to the CNS. Central venous catheters, prior pulmonary aspergillosis, and viral reactivations are additional independent risk factors (8).

Aspergillus invades the CNS via three routes: (i) hematogenous spread from a distant focus (most commonly lung), causing microabscesses, hemorrhagic infarctions, and mycotic aneurysms; (ii) direct extension from sinuses, mastoid, or the orbit; and (iii) iatrogenic introduction (rare). The angioinvasive property is central: hyphae invade endothelium, causing thrombosis, ischaemia, and necrosis, mediated by fungal proteases,

phospholipases, and gliotoxin. Cerebral vessel occlusion manifests, as stroke-like syndromes, hemorrhage, or rapidly progressive encephalopathy.

Clinically, CNS aspergillosis is notoriously nonspecific and insidious (9). Common symptoms—fever (often refractory to antibiotics), headache, altered mental status, focal neurological deficits, seizures, and raised intracranial pressure—are not pathognomonic and may be attributed to other complications (chemotherapy encephalopathy, thrombocytopenic bleeding, viral encephalitis, toxoplasmosis, cryptococcosis). Absence of fever or meningeal signs does not exclude the diagnosis, especially in blunted inflammatory response. Some cases present with sudden stroke-like events. The insidious onset and lack of early clues often delay diagnosis until an advanced, irreversible stage (10).

Neuroimaging is pivotal. Magnetic resonance imaging (MRI) is superior to computed tomography (CT). Typical findings include single or multiple ring-enhancing lesions, hemorrhagic transformation, and vasogenic edema; diffusion-weighted imaging (DWI) may show restricted diffusion. The "target sign" is inconsistent. Fungal vasculitis can cause mycotic aneurysms. However, these features overlap with bacterial abscesses, tuberculosis, toxoplasmosis, neoplasms, and demyelinating diseases, so imaging alone is insufficient—microbiological or molecular confirmation is mandatory (11).

Microbiological diagnosis remains formidable. The gold standard—histopathology of brain tissue with positive culture—is rarely feasible due to thrombocytopenia or coagulopathy, and biopsy yield is low. Cerebrospinal fluid (CSF) analysis is often unrevealing: routine parameters are normal or mildly abnormal, culture sensitivity is extremely low (<10–20%), and results take days. CSF galactomannan (GM) and (1→3)-β D-glucan (BDG) have variable sensitivity and false-positive issues (12). Serum biomarkers have limited sensitivity for CNS disease.

Over recent years, CSF next-generation sequencing (NGS) has emerged as a transformative unbiased, culture-independent tool that detects a broad range of pathogens in a single assay (13). It is particularly valuable when conventional tests are negative or inconclusive, and when the differential is broad (14). Several studies demonstrate high sensitivity and specificity for Aspergillus, even with low fungal burden or prior antifungal therapy (15–16). Turnaround time has shortened to 48–72 hours (17). In our three cases, CSF NGS was pivotal in establishing the final diagnosis, underscoring its clinical utility.

Treatment is hampered by pharmacological and microbiological hurdles. Voriconazole is first-line due to superior CNS penetration (CSF level 50–80% of plasma) and proven efficacy. Isavuconazole has comparable penetration, better safety, and fewer drug interactions, but less CNS experience. Posaconazole has unpredictable CNS penetration and is reserved for salvage. Liposomal amphotericin B (L AmB) poorly penetrates the BBB and is mainly empirical or combination. Echinocandins do not achieve therapeutic CSF levels and are ineffective as monotherapy.

A growing concern is azole resistance in Aspergillus, particularly A. fumigatus, but increasingly also in A. flavus and A. terreus (18). Resistance can be environmental (agriculture azoles) or acquired through prolonged patient azole exposure (19-20). Common mechanisms involve cyp51A mutation (e.g., TR34/L98H, TR46/Y121F/T289A) (21-22). In Chinese multicenter surveillance, resistance prevalence among clinical A. fumigatus isolates is 6–10%, with higher rates in some regions (23-24). Resistance is associated with significantly increased treatment failure and mortality-about 20% higher in hematological patients infected with resistant strains (25). “Breakthrough” IA (developing during antifungal prophylaxis or therapy) is increasingly recognized26, often caused by resistant strains or intrinsically resistant species, and is particularly challenging (27-28).

Despite advances, the overall prognosis of CNS aspergillosis remains grim. A systematic review (2000–2020) reported a pooled mortality of 86% in hematological patients (29). Poor prognostic factors include severe persistent immunosuppression, prior azole exposure, delayed diagnosis, multiple/large lesions, cerebral hemorrhage or herniation, and uncontrolled GVHD or relapse. Survivors often have long-term neurological sequelae. Lack of effective salvage options for azole-resistant disease compounds the problem.

Given the rarity, heterogeneity, and diagnostic difficulty of CNS aspergillosis in hematological patients, reporting individual cases and small series remains valuable to raise awareness and illustrate current challenges (30). Each case offers insights into varied presentations, the utility of CSF NGS, pitfalls of antifungal therapy, including breakthrough infections, and the importance of susceptibility testing. Here, we present three cases from our institution, describing their clinical courses, imaging, microbiological data (including CSF NGS), antifungal regimens, and outcomes. Through literature review, we aim to highlight host risk factors, the imperative of early CSF NGS, the reality of breakthrough infection during azole prophylaxis/therapy, and the urgent need for susceptibility testing in failing patients—ultimately to improve recognition and management of this lethal complication in a highly vulnerable population.

2. Materials and Methods

Case 1

A 59-year-old male presented with a 17-year history of pancytopenia and complaints of general fatigue for more than 20 days. He had previously been suspected of having aplastic anemia (AA) or myelodysplastic syndrome (MDS), but showed poor response to azacitidine and hematopoietic supportive therapy. Upon admission to our department, bone marrow examination revealed hemophagocytes (Figure 1A), elevated soluble CD25, decreased natural killer (NK) cell activity, and elevated ferritin levels, leading to a diagnosis of hemophagocytic syndrome. He received glucocorticoids, hematopoietic supportive therapy, and platelet transfusion.

Subsequently, he developed fever accompanied by knee joint pain after two months of treatment with glucocorticoids. A test for influenza A was positive, and he received antiviral therapy for one week. His body temperature returned to normal, and he was discharged after four days. However, on the day of discharge, fever recurred with a mild headache. Further diagnostic workup did not reveal a clear cause for about one month. Finally, a lumbar puncture was eventually performed, and NGS of CSF detected Aspergillus flavus. Brain MRI (May 12, 2023) showed right lateral ventricle choroid plexus enlargement with heterogeneous enhancement, suggestive of granulomatous lesions. After treatment with voriconazole for three days, his fever and headache improved, and he was discharged.

Later, he stopped voriconazole by himself after one month's treatment because of weakness in legs. He then developed high fever, headache, dizziness, and fatigue, follow-up cranial MRI (June 24, 2023) revealed progressive lesions. He was admitted to the neurology department about 2 weeks later, where imaging showed subfalcine herniation. The patient was discharged against medical advice and died shortly thereafter at home.

Case 2

A 24-year-old male had a history of AML who relapsed nine months after haploidentical hematopoietic stem cell transplantation (Haplo-HSCT). He received donor lymphocyte infusion (DLI) three times and subsequently developed acute severe graft-versus-host disease (GVHD) involving the skin, liver, and gastrointestinal tract. He was treated sequentially with first-line glucocorticoid therapy, and second-line regimens included ruxolitinib, basiliximab, anti-thymocyte globulin (ATG), mesenchymal stem cells (MSC), and so on

for GVHD control. Concurrently, the patient presented with low-grade fever accompanied by diarrhea and abdominal pain. Perianal swab culture yielded Candida glabrata, Enterococcus faecium, and Stenotrophomonas maltophilia. So, he received meropenem, vancomycin, and caspofungin for anti-infective therapy. During this period, the patient's diarrhea showed no significant improvement, accompanied by markedly elevated IL-6 and increased EB viral DNA levels. Tocilizumab combined with rituximab was administered for the uncontrolled GVHD. Subsequently, his fever, diarrhea, and abdominal pain were gradually brought under control. Given his cough and expectoration, a chest CT was performed, which showed newly developed multiple pulmonary inflammatory infiltrates, and the antifungal agent was therefore switched to liposomal amphotericin B. And his sputum culture grew Aspergillus fumigatus complex. One week later, repeat imaging showed progression of the pulmonary infection, and isavuconazole was added.

One day later, the patient suddenly developed slurred speech, apathy, slowed response, and decreased muscle strength in the right limb. CT suggested cerebral infarction involving multiple hypodense lesions. MRI revealed multiple abnormal signals in bilateral cerebral hemispheres, and lesions demonstrated hypointense or isointense signal on T1-weighted imaging and hyperintense signal on T2-weighted imaging. Slightly hyperintense or hyperintense signals were observed within partial lesions on DWI, accompanied by mild reduction of ADC values in the corresponding focal areas; concurrent elevation of peripheral blood schistocytes was also observed (Figure 1B). Early the next morning, he experienced seizures and subsequently progressed to a somnolent state by morning. A lumbar puncture was performed, and CSF was sent for next-generation sequencing (NGS), which detected a high load of Aspergillus fumigatus and herpesvirus. On the third day, he developed hypotension and was transferred to the intensive care unit (ICU), and eventually died.

Case 3

A 41-year-old female underwent umbilical cord blood transplantation for myelodysplastic syndrome (MDS, Figure 1C). On day +4 post-transplantation, the patient developed fever and sepsis. After initiation of anti-infective therapy with carbapenems combined with vancomycin, persistent fever remained, accompanied by elevated IL-6 levels. Pre-engraftment syndrome could not be excluded as the cause of fever, and glucocorticoidsicoids were therefore added at a dose of 1 mg/kg for anti-inflammatory management. However, fever persisted. The dosage of glucocorticoids was increased to 2 mg/kg, and the prophylactic antifungal agent was switched to voriconazole.

On day +15 post-transplantation, she developed abdominal pain, hematochezia, elevated creatinine, and hypertension. Elevated factor Ba levels and schistocytes led to a diagnosis of thrombotic microangiopathy (TMA) with steroid-refractory acute gastrointestinal GVHD (Figure 1D). Ruxolitinib, iptacopan, and eculizumab were added. Repeated blood NGS detected Ralstonia mannitolilytica, Enterococcus faecium, Aspergillus flavus, Candida glabrata, and human herpesvirus 6B (HHV-6B). Aspergillus flavus was isolated from sputum culture. Then her anti-infective regimen was therefore adjusted to cefoperazone-sulbactam, levofloxacin, caspofungin, and liposomal amphotericin B. During this period, the patient's fever and infection improved, but TMA recurred repeatedly. After treatment with basiliximab, the patient achieved short remission of TMA.

On day +52, she developed confusion and incoherent speech without unilateral limb weakness. Cranial CT (April 22, 2026) revealed hypodense lesions involving the left parieto-occipital lobe. And multiple patchy signal abnormalities were identified in the left cerebral region, which were iso- to slightly hypointense on T1-weighted images and

hyperintense on T2-weighted images. Scattered punctate mild hyperintensities were seen on DWI, with iso- to slightly reduced ADC signals. On day +54, a lumbar puncture was performed following platelet transfusion, and CSF NGS detected Aspergillus flavus with 536 sequence reads. Voriconazole and liposomal amphotericin B were combined for antifungal therapy. Thereafter, her neurological status did not improve, and the family signed for discharge against medical advice.

For studies that generate large-scale datasets, the authors should indicate the public repository where the data have been archived and supply the corresponding accession identifiers. If these identifiers are not yet available upon first submission, the authors shall confirm that they will be furnished later.

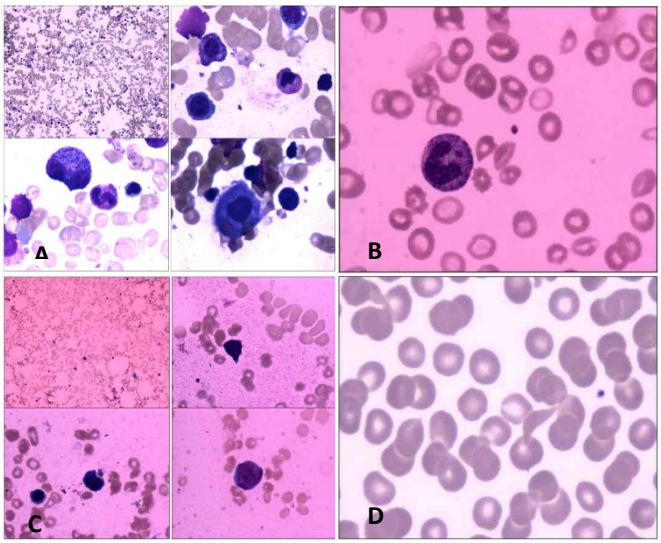


Figure 1. Bone marrow and peripheral blood examination.

A: Bone marrow smears demonstrated hemophagocytes (case 1). B: Peripheral blood smears showed 0.7% schistocytes (case 2). C: Initial diagnostic bone marrow aspirate smears showing hypocellular marrow with 5% blasts (case 3). D: Peripheral blood smears showed 0.2% schistocytes (case 3).

3. Results

3.1 Clinical Diagnosis

The clinical data of the three cases are summarized in the following Table 1.

A total of three patients with CNS aspergillosis were included in this study. All cases presented with well-defined host risk factors for invasive fungal infection. One patient had pancytopenia without a definitive underlying diagnosis; the other two patients developed serious complications following allo-HSCT, including GVHD and TMA. Regarding host susceptibilities, all three patients had received glucocorticoid therapy for more than three weeks, two had neutropenia, two had an indwelling central venous catheter, and two had been treated with T-cell immunosuppressants.ppressants (ciclosporine and basiliximab). Collectively, these factors created a high-risk background for fungal infection in this cohort.

In terms of clinical presentations, fever was the initial symptom in all patients. One patient experienced unexplained persistent fever lasting over one month, which eventually prompted CSF examination. The other two patients had complex post-transplant courses, with overlapping infectious complications and active GVHD, making differential diagnosis challenging. Neurological manifestations included seizures in one patient and

neuropsychiatric symptoms (e.g., aphasia) in two patients.

3.2 Microbiological and imaging diagnosis

Two patients had a history of pulmonary aspergillosis. Microbiological evidence was positive for Aspergillus in both: Aspergillus flavus was detected in two cases and Aspergillus fumigatus in one. All patients underwent cranial MRI examinations. Contrast-enhanced brain MRI performed in Case 1 revealed lesions involving the right choroid plexus and lateral ventricles; Figure 2 shows the dynamic changes on two follow-up scans during treatment. Case 2 demonstrated multiple lesions in the bilateral cerebral hemispheres and cerebellum (Figure 3). Case 3 showed patchy opacities in the left frontal, parietal, and occipital lobes, as well as the corpus callosum and basal ganglia; Figure 4 presents comparative MRI scans before and after the onset of neurological symptoms.

Brain imaging confirmed intracranial lesions in all patients, and CSF next-generation sequencing (NGS) was positive for Aspergillus in every case. In addition, two patients had positive sputum cultures (detailed in Table 2). The rapid neurological deterioration observed in two patients, despite prior antifungal exposure, suggests that established pulmonary foci may serve as silent cerebral embolic sources. Moreover, the single case of voluntary voriconazole discontinuation exemplifies the critical vulnerability of long-term oral antifungal maintenance, where drug-drug interactions, intolerances, or non-adherence can precipitate fatal recrudescence.

Table 1. Clinical summary of the three cases.

No	Age/sex	Underlying disease	Neutropenia	Allo-HSCT	Catheter	Glucocorticoid	T-cell inhibitors
1	59/M	Pancytopenia	+	-	-	+	-
2	24/M	AML, allo-HSCT, DLI	+	+	+	+	+
3	41/F	MDS, allo-HSCT, TMA, GVHD	-	+	+	+	+

Table 1. Clinical summary of the three cases (continued).

No	Fever	Neurological symptoms	Chest CT	Brain CT/MRI	Blood/sputum culture	CSF NGS	Treatments	Outcome
1	+	+	-	+	-/-	+	Voriconazole	death
2	+	+	+	+	-/+	+	L-AmB+ isavu	death
3	+	+	+	+	-/+	+	L-AmB + Vori	death

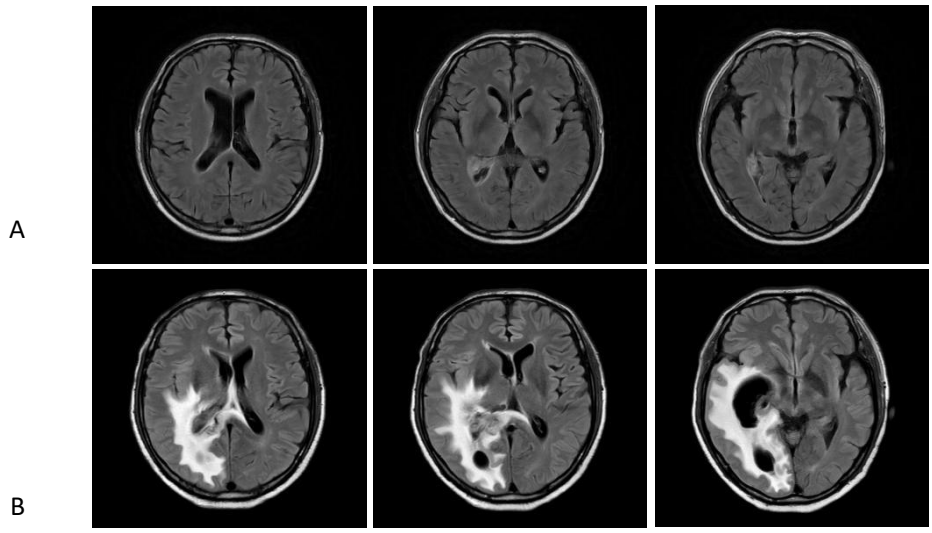


Figure 2. Contrast-enhanced brain MRI (T2WI) of Case 1: (A) right lateral ventricle choroid plexus enlargement with heterogeneous enhancement (May 12, 2023); and (B) progressive enlargement of lesions (June 24, 2023).

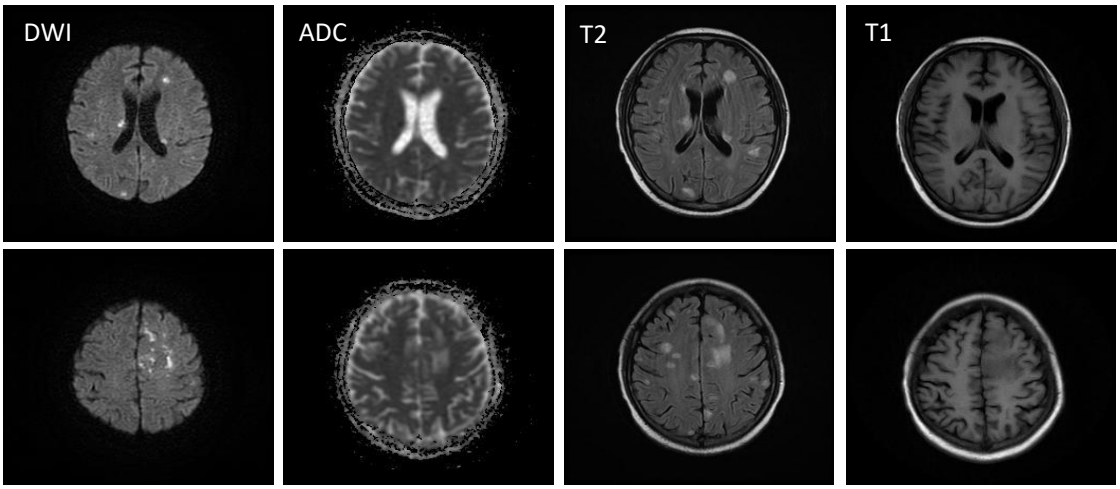


Figure 3. Brain MRI of Case 2 revealing multiple abnormal signal foci in bilateral cerebral hemispheres and cerebellum (T1, T2 FLAIR, DWI, and ADC sequences).

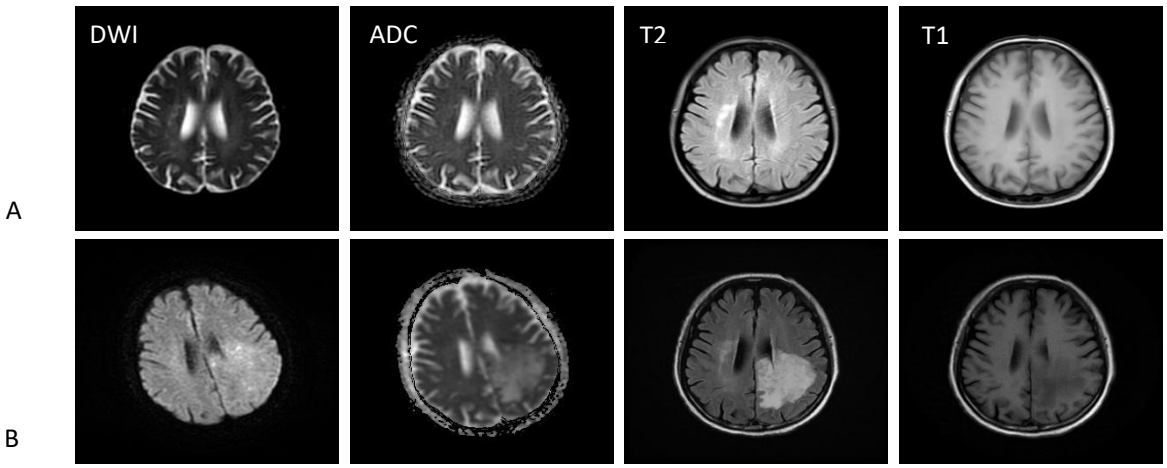


Figure 4. Serial brain MRI of Case 3: (A) before neurological symptom onset (April 6, 2026); (B) after onset (April 23, 2026), showing progressive patchy abnormalities in the left cerebral hemisphere.

Table 2. CSF NGS results confirming Aspergillus detection in all three patients.

	Genus			Species	
Case	**Species Name**	**Sequence Count**	**Relative Abundance**	**Name**	**Sequence Count**
Case 1	Aspergillus	758	87.63%	Aspergillus flavus	388
Case 2	Aspergillus	317648	90.22%	Aspergillus fumigatus	199328
Case 3	Aspergillus	563	54.50%	Aspergillus flavus	536

3.3 Treatment and outcomes

Two patients had already received liposomal amphotericin B for pulmonary aspergillosis but still developed CNS dissemination, and their clinical condition deteriorated rapidly after neurological symptom onset. The third patient voluntarily discontinued voriconazole after two months of antifungal therapy, subsequently developed subfalcine herniation, and ultimately died. Regarding treatment and outcomes, two patients had already received liposomal amphotericin B for pulmonary aspergillosis but still developed CNS dissemination, and their clinical condition deteriorated rapidly after neurological symptom onset. The third patient voluntarily discontinued voriconazole after two months of antifungal therapy, subsequently developed subfalcine herniation, and ultimately died. Overall, one of the three patients had a fatal outcome, underscoring that CNS aspergillosis carries an exceedingly high mortality and rapid progression even in the setting of prior antifungal treatment.

4. Discussion

In this report, we describe three cases of CNS aspergillosis in immunocompromised patients with hematologic diseases, each illustrating distinct yet interconnected challenges in the modern management of this fatal infection. Our cases underscore the pivotal role of CSF NGS in achieving a timely diagnosis, the reality of breakthrough infections during antifungal prophylaxis, and the poor prognosis that often follows.

The diagnostic value of CSF NGS is paramount. In all three patients, conventional workups, including blood and sputum cultures, were unrevealing or non-specific. A definitive diagnosis was established only through CSF NGS, which identified the Aspergillus species—A. flavus in two cases and A. fumigatus in one. This aligns with a growing body of evidence supporting CSF NGS as a transformative tool for CNS infections, particularly when the fungal burden is low or when prior antifungal therapy has been administered, as was the case for our patients. In Case 2 and Case 3, who were already on liposomal amphotericin B or voriconazole for pulmonary disease, CSF NGS remained positive, demonstrating its superior sensitivity over culture and its ability to detect pathogens despite ongoing treatment. Given the prohibitive risks of brain biopsy in thrombocytopenic patients, we advocate for the early use of CSF NGS in any hematologic patient with unexplained neurological symptoms and risk factors for invasive fungal disease (31).

The phenomenon of breakthrough CNS aspergillosis is a critical clinical concern. Two of our patients (Case 2 and Case 3) developed CNS disease while receiving systemic antifungal therapy (liposomal amphotericin B) for prior pulmonary aspergillosis. Although in Case 2, isavuconazole—an agent with good central nervous system penetration—was added to liposomal amphotericin B, the CNS lesions still progressed rapidly (32). This is a stark illustration of treatment failure, which can be attributed to several factors. First, the poor penetration of certain antifungals, such as echinocandins and even liposomal amphotericin B, across the blood-brain barrier limits their efficacy in the CNS. Second, and more importantly, prolonged azole exposure may select for resistant strains. In case 3, voriconazole was administered at an early stage. While we were unable to perform antifungal susceptibility testing due to the lack of cultured isolates, the clinical trajectory of these cases—rapid neurological decline despite therapy—is highly suggestive of either azole resistance or inadequate CNS drug concentrations. These findings echo recent multicenter studies reporting a rising prevalence of azole resistance in Aspergillus spp. and the associated increase in mortality. Despite the availability of antifungals (like voriconazole, isavuconazole) with favorable CNS penetration (33), breakthrough fungal infections still occurred in patients, suggestive of azole-resistant isolates. This strongly indicates that drug resistance—rather than suboptimal CNS drug exposure—was the primary driver of breakthrough infection in these cases. Our cases serve as a potent reminder of the urgent need for routine antifungal susceptibility testing and therapeutic drug monitoring in patients failing therapy, especially in the era of increasing azole usage.

The clinical presentation and rapid progression are sobering. The neurological symptoms in our patients—ranging from headache and fever (Case 1) to acute aphasia and hemiparesis (Case 2) and confusion (Case 3)—highlight the protean and non-specific nature of CNS aspergillosis. In Case 2, the presentation with a stroke-like syndrome due to cerebral infarction is a classic manifestation of fungal angioinvasion, leading to rapid deterioration and death from cerebral hemorrhage. This aggressive clinical course underscores the narrow window for therapeutic intervention. In our study, intracranial aspergillosis may have already developed in these two patients prior to the onset of neurological symptoms, concurrently with pulmonary fungal infection. Severe and recurrent GVHD and TMA occurred in the patients, causing extensive vascular endothelial injury and severe disruption of the blood-brain barrier, which rendered these patients highly susceptible to intracranial infection. So, for patients with hematological malignancies receiving long-term immunosuppressive therapy after allo-HSCT, active screening for intracranial fungal infection should be performed in the event of recurrent polymicrobial infections. The poor outcomes in our series, including one death and two instances of discharge against medical advice due to irreversible neurological damage, are consistent with the dismal prognosis reported in the literature, where mortality rates often exceed 80%.

Finally, our cases highlight the complex interplay between host immunity and infection. All three patients had profound and multifaceted immunosuppression. Case 2 and Case 3, both post-allo-HSCT, were battling severe GVHD and its treatment with a cocktail of glucocorticoids and T-cell inhibitors (ruxolitinib, basiliximab, ATG). This "perfect storm" of immunosuppression not only predisposes to invasive fungal infections but also hinders the host’s ability to clear the pathogen, rendering even optimal antifungal therapy less effective.

Our case series reinforces that CNS aspergillosis remains a devastating complication in hematologic patients. The key takeaways are: (1) a high index of suspicion must be maintained for any neurological symptom; (2) CSF NGS should be performed early, as it is a highly sensitive and

specific diagnostic tool that can circumvent the limitations of conventional methods; (3) the development of neurological symptoms in a patient on azole therapy should prompt immediate investigation for a breakthrough CNS infection, and antifungal susceptibility testing should be prioritized. Larger, prospective studies are urgently needed to define optimal management strategies, particularly for patients with azole-resistant disease or those with breakthrough infections, to improve the currently grim prognosis of this condition.

Several limitations of this study should be acknowledged. First, microbial testing of CSF relied solely on NGS, with no corresponding antimicrobial susceptibility test results available. Second, the small sample size of cases prevents us from drawing generalizable conclusions. In addition, tissue pathological biopsy specimens could not be obtained from any patients due to their critical clinical conditions. Furthermore, patients who underwent allo-HSCT experienced multiple complications besides infection, meaning the intracranial imaging findings may not be solely attributable to fungal infection.

5. Conclusions

CNS aspergillosis remains one of the most devastating complications in immunocompromised patients with hematologic diseases, carrying a mortality rate that has seen little improvement over the past decades. Our three-case series reinforce several critical messages for clinical practice. First, CSF NGS is a highly sensitive and specific diagnostic tool that should be employed early in any hematologic patient presenting with unexplained neurological symptoms and risk factors for invasive fungal disease, particularly when conventional tests remain negative. Second, the occurrence of breakthrough CNS aspergillosis despite combination therapy with agents that penetrate the blood–brain barrier—such as liposomal amphotericin B and isavuconazole—strongly suggests that azole resistance, rather than inadequate drug exposure, is the primary driver of treatment failure in this setting. Third, the rapid neurological deterioration and uniformly poor outcomes in our patients underscore the narrow window for therapeutic intervention and the urgent need for routine antifungal susceptibility testing and therapeutic drug monitoring. Larger, prospective studies are urgently needed to define optimal management strategies, particularly for patients with azole-resistant disease or breakthrough infections, to improve the currently grim prognosis of this lethal complication

Declarations

1. ***Consent to publication***
   We declare that all authors agreed to publish the manuscript in this journal based on the signed Copyright Transfer Agreement and followed publication ethics.
2. ***Ethical approval and consent of participants***
   This case series study was reviewed and approved by the Institutional Ethics Committee of the Seventh Hospital of Sun Yat-sen University. Written informed consent has been obtained from all three patients for the publication of their clinical data and relevant imaging findings in this article. All personally identifiable information has been fully anonymized to protect patient privacy.
3. ***Disclosure of conflict of interests***
   We declare that no conflict of interest exists in this work.
4. ***Funding***
   This work was supported by the Guangdong Provincial Medical Science and Technology Research Fund (A2026225).
5. ***Availability of data and material***
   Raw clinical data and imaging materials cannot be shared publicly to protect the confidentiality of the enrolled patients. Researchers who meet the criteria for access to confidential data may

contact the corresponding author to request the data.

6. ***Authors' Contributions***

LFJ and WXB conceived and designed the study. HYL and DHM collected clinical data and drafted the manuscript. LB performed image analysis and data sorting. XXJ revised the manuscript critically for important intellectual content. All authors read and approved the final version of the manuscript.

7. **Acknowledgement**

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8. ***Authors' biography***

None.

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