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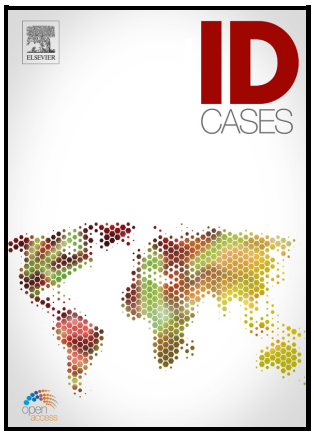

Severe Dual Fungal Infection After Bispecific Antibody Therapy: A Case of Invasive Aspergillosis and Mucormycosis in Immunocompromised Patient

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Title: Severe Dual Fungal Infection After Bispecific Antibody Therapy: A Case of Invasive Aspergillosis and Mucormycosis in Immunocompromised Patient

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KEYWORDS: case report, mycotic bi-therapy, aerosol, immunocompromised patient

Abstract

Bispecific antibody is a new treatment for hematological disease, especially for lymphoma, myeloma and acute lymphoblastic leukemia. This class of treatment presents the same kind of side effect as CAR-T cell which are immune-mediated. Nevertheless, infectious complication

remains a major concerns with related mortality. Fungal infection are rarely reported in clinical trial but remains a major concern. We report a case of a co-infection of *Aspergillus* and *Mucorales* in a patient with diffuse large B-cell lymphoma (DLBCL) following treatment with the bispecific antibody epcoritamab. The patient developed severe cytokine release syndrome (CRS) and subsequent fungal infections, which were challenging to diagnose and treat due to the complexities of managing immunocompromised patients and co-infection. Advanced diagnostics, including PET-CT, and a combination of antifungal therapies were crucial in achieving remission. The case underscores the need for early diagnosis, multidisciplinary management, and innovative treatment strategies in similar high-risk patients.

Introduction

CAR-T cell therapy and bispecific antibodies are new treatment options for patients with lymphomas, myeloma, and B-acute lymphoblastic leukemia. They induce immune response mediated by T-cell lymphocytes. However, these therapies are associated with significant complications, particularly cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity (ICAN). Additionally, infectious complications remain a leading cause of non-relapse mortality (1). Clinicians managing these patients are aware of the risk of infections with unusual pathogens in this specific context.

We report a patient with a severe co-infection of Aspergillus and Mucorales following treatment of CRS complications from the bispecific antibody epcoritamab.

Case report

A 54-year-old man experienced a relapse of diffuse large B-cell lymphoma (DLBCL) after CAR-T cell therapy and was subsequently treated with epcoritamab, an anti-CD3 and anti-CD20 bispecific antibody. Shortly after completing his ramp-up doses, he developed fever and hypotension, leading to a diagnosis of severe grade 4 CRS. He was treated with high-dose corticosteroids (up to 1 gram per day due to severe organ failure), noradrenaline support, and broad-spectrum antibiotics. During his hospitalization inside the Intensive Care Unit (ICU), he presented severe neutropenia and hyperglycemia (Fig.1). One month after recovering from CRS, the patient developed acute respiratory distress due to a complete obstruction of his trachea by a pseudo-tumor mass. Incomplete endoscopic removal revealed a dual infection with proven invasive mucormycosis and *Aspergillus-like* infection. Pan-*Aspergillus* and Mucorales PCRs were positive for this mass, and *Aspergillus flavus* (*A.Flavus*) was isolated on Sabouraud agar, confirming the positive histology and PCR results. *A. flavus* was also cultured from the sinuses, and the bronchoalveolar lavage (BAL), with positive PCR for both *Aspergillus* and Mucorales in the BAL. No Mucorales were cultured from any sample. A full work-up with 2-deoxy-2-[18F]fluoro-d-glucose positron emission tomography/computed tomography scan

(FDG PET-CT) was made to evaluate the localization and spread of the fungal infection and revealed multiple lesions (infiltration of the trachea, lungs and sinuses) (Fig.2). As tracheal and lung resection were not feasible, we initiated combination therapy with Liposomal amphotericin B (10 mg/kg, reduced to 5 mg/kg daily due to renal failure) and isavuconazole (200 mg/day after a loading dose). By day 10, a partial response was observed in the lungs and trachea, but the sinus lesions progressed. Suspecting inadequate drug penetration into the sinus due to complete necrosis, we switched from isavuconazole to voriconazole (as *A. flavus* was resistant to isavuconazole (MIC 2mg/L) but susceptible to Liposomal amphotericin B (MIC 1mg/L) and voriconazole (MIC 0.5mg/L)). Voriconazole was administered both orally (with therapeutic monitoring between 1-6 µg/mL, targeting 4 µg/mL) and via aerosol (40 mg of nebulized voriconazole from a 10 mg/mL solution three times per day). We also performed multiple surgical removals of necrotic sinus material. After 6 weeks of this treatment, the patient showed a partial response with clinical improvement, so, we continued the combination therapy for 6 months. A follow-up FDG PET-CT scan confirmed complete remission of both DLBCL and the fungal infection. Given the patient's ongoing immunosuppression, we instituted prophylaxis with posaconazole (300 mg once daily).

Discussion

This case illustrates the challenge in diagnosing, treating, and monitoring fungal infections, particularly mucormycosis, in immunocompromised patients. Early diagnosis is crucial, as studies have shown that delayed treatment correlates with higher mortality (delays of more than five days can double the mortality rate) and the mortality associated with this infection remains high (50 to 60%) (2–4). A recent meta-analyse described some improvement in the overall survival over time (72% vs 58% vs 49% for studies before 2000, 2000-2009, and 2010-2020)(4).

Diagnosing fungal infections remains complex, despite the availability of various techniques. The diagnostic process relies on direct examination, histopathology, culture, and more recently molecular analysis. The sensitivity and specificity of these tests vary significantly and depend on factors such as sample type, preparation, and the expertise of the analyst (5). The European Confederation of Medical Mycology (ECMM) has summarized procedures for diagnosing fungi, but diagnosing mucormycosis is challenging due to the difficulty in culturing the fungus (over 50% of histologically positive tissues fail to grow in culture) (6). Furthermore, poor-quality staining or fungal damage can lead to misidentification, such as mistaking Mucorales for *Aspergillus*, resulting in incorrect diagnoses and treatment (7). Guidelines for mucormycosis do not consider PCR as a microbiology criterion which may reduce diagnostic accuracy (5,8). In this case, both *Aspergillus* and Mucorales PCRs were systematically applied to deep respiratory samples in our laboratory, leading to the diagnosis of the co-infection, confirmed by dual positive histology. Histologically, *Aspergillosis* is characterized by the presence of thin (3- to 12-µm) hyphae that are septate and branched at acute angles (45°) or dichotomously. In contrast, mucormycosis is identified by nonpigmented, wide (5- to 20-µm) hyphae that are thin-walled, ribbon-like, with few septations and branched at right angles. These characteristics can be highlighted by using special stains such as Gomori-Grocott or Periodic-Acid-Schiff. Immunohistochemistry can also enhance specificity (5) but it was not the case for our patient. PCR may play a role even if it is not yet strongly endorsed by guidelines. Some studies have demonstrated active infection with positive PCR despite negative microscopy for Mucorales (9) or when other infections, like aspergillosis, mask the presence of mucormycosis (8)).

Monotherapy with liposomal amphotericin B remains the gold standard for the treatment of mucormycosis combined with surgery. However, mortality remains up to 60% with some improvements this last decade(4,10). One preliminary study suggested that higher doses of Liposomal amphotericin B (10 mg/kg vs. 5 mg/kg) may be beneficial, though clinical application is challenging due to nephrotoxicity (11). While dual therapy with Liposomal amphotericin B and another antifungal is not fully recommended in current guidelines (5), some in vitro and animal studies have shown synergistic effects when combining Liposomal amphotericin B with azoles or either echinocandins (12). A retrospective series showed some efficacity inside a very difficult population of patients with hematological malignancies (13).In aggressive mucormycosis cases, dual therapy may be considered (14). Given our patient's ineligibility for tracheal surgery with complete removal of trachea and lung resection and the severity of his respiratory failure due to tracheal infiltration, we opted for combination therapy with Liposomal amphotericin B and isavuconazole. Interestingly, the mucor infiltration

responded well to treatment, but the sinus lesions, infected only by *A. flavus*, did not. Antifungal susceptibility testing revealed that *A. flavus* was resistant to isavuconazole but susceptible to amphotericin B and voriconazole. We hypothesized that the fungi grew in necrotic tissue with poor drug diffusion, leading us to add aerosolized voriconazole. Aerosolized voriconazole may be an option for sinus aspergillosis (15) even if it is not part of recent guideline (5). It is less described for nebulized posaconazole and rarely for isavuconazole (15). We chose voriconazole because our main issue was sinus aspergillosis with good sensibility to voriconazole and some report of the dosage(16), meanwhile poor data regarding the other azoles were found. Based of some report in the literature, we opted for a reconstitution of voriconazole from a vial used for intravenous purpose (10mg/ml, 4 ml three time per day). We used a commercial nebuliser and the patient had to benefit of nebulization until end of emanation (16,17). During this combination of therapy, we controlled weekly the blood level of voriconazole to avoid to high blood concentration and possible toxicity. New therapeutic option with nebulized formulation , opelconazole, is currently under investigation combined with systemic therapy in a phase 3 study for the indication of difficult case to threat (for patients with refractory invasive pulmonary aspergillosis (NCT05238116). We combined this therapy with multiple surgical debridement of necrotic material in the sinus of the patient. Such surgical procedures remained invasive and aggressive but it is of importance to enhance the local control of the infection and give opportunity to obtain sample for culture, identification and antifungal susceptibility testing (18).

Another critical aspect of treatment is to control the risk factors for Mucorales or *Aspergillus*, infection. It is well-established that diabetes mellitus, prolonged neutropenia, corticosteroid use, and hematologic malignancies are major risk factors (5,19). Strict blood sugar control is essential, though correcting neutropenia is more challenging. In such cases, even with low quality of data (20), granulocyte transfusions may be considered (19) but it is not recommended in recent guideline (5).

New therapeutic options like CAR-T cell and bispecific antibodies offered new curative options in patients with acute leukemia, aggressive lymphoma and multiple myeloma. Several reports highlighted the minor risk of fungal infection (both aspergillosis, pneumocystis, candida and mucormycosis) with CAR-T cell (around 3%) (21) and do not support the use of routine primary prophylaxis (22) . It is supposed that these therapies are associated with a risk of invasive fungal infection due to the use of high doses of corticosteroids for the management of specific complications like CRS and ICAN (21,22) but this association could not be well demonstrated in other report (23)

Bispecific antibodies, which bind both the tumor and the patient's T-lymphocytes, share similar complications of CRS and ICAN. This treatment is also associated with an increasing risk of developing bacterial and viral infection which dominate the infectious risk (24). To our knowledge, there is no case of invasive co-infection of aspergillosis and mucor described with the use of this bispecific treatment. Indeed, other bispecific used for myeloma showed an other pattern of infectious complications (25).

Despite current guidelines and the limited studies on mucormycosis, determining the optimal timing for treatment evaluation remains challenging. European guidelines recommend initiating treatment with the highest feasible dose of Liposomal amphotericin B combined with surgical debridement of infected tissue. Once a positive response is achieved, switching to an oral azole (posaconazole or isavuconazole) is advised (5)(posaconazole or

isavuconazole), However, the precise timing for this switch is not specified. Some experts suggest continuing Liposomal amphotericin B for at least three weeks (19). For our patient, we decided to continue as long as the PCR remained positive. After obtaining three negative samples, a follow-up FDG PET-CT showed a good response in both the mucor respiratory tract lesions and the sinus aspergillosis. We continued dual therapy with Liposomal amphotericin B and aerosolized voriconazole. After six months, a new FDG PET-CT confirmed complete remission of both the lymphoma and the infectious complications. Given the patient's ongoing immunosuppression, we decided to continue secondary prophylaxis with oral posaconazole (targeted serum level of 4 µg/mL), as *A. flavus* was resistant to isavuconazole but susceptible to posaconazole.

While high-resolution CT scans remain the gold standard for diagnosing and monitoring fungal infections, FDG PET-CT has recently been introduced into guidelines (26). FDG PET-CT is valuable for detecting sites of dissemination that may not be visible on conventional CT scans and can guide invasive diagnostic procedures (27). Additionally, because FDG PET-CT evaluates metabolic activity, a negative FDG PET-CT result can be associated with complete remission, providing insight into the optimal timing for discontinuing or switching treatment. Studies have shown that FDG PET-CT can influence therapeutic strategies in at least 50% of patients (28).

Conclusion

This case highlights several challenges in treating immunocompromised patients with severe fungal infections. Firstly, the potential for co-infection with other fungi must be carefully considered due to shared risk factors. Secondly, while surgery is a critical component of treatment and should always be performed, some patients may not be candidates. Thirdly, if infection control is not achieved within a few days, alternative treatment strategies (e.g., dual therapy, nebulized drugs) should be considered. Fourth, FDG PET-CT can be invaluable in guiding treatment duration, with negative results serving as a strong indicator of remission. Finally, new immunosuppressive therapies can lead to severe complications, and clinicians must remain vigilant.

Informed Consent

The patient provided written consent to use his medical information and images for a clinical case report.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used GRAMMARLY to review the English level. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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Figure 1. Biological evolution of the parameter of glycemia and absolute neutrophile count (/mm³) during the first cycle of epcoritamab with the admission of ICU for grade 4 CRS with use of high dose of corticosteroid and associated hyperglycaemia and a long period of neutropenia. The second admission for respiratory distress leading to the diagnosis of co-infection is also shown. CRS: Cytokine Release Syndrome, ICU: Intensive Care Unit.

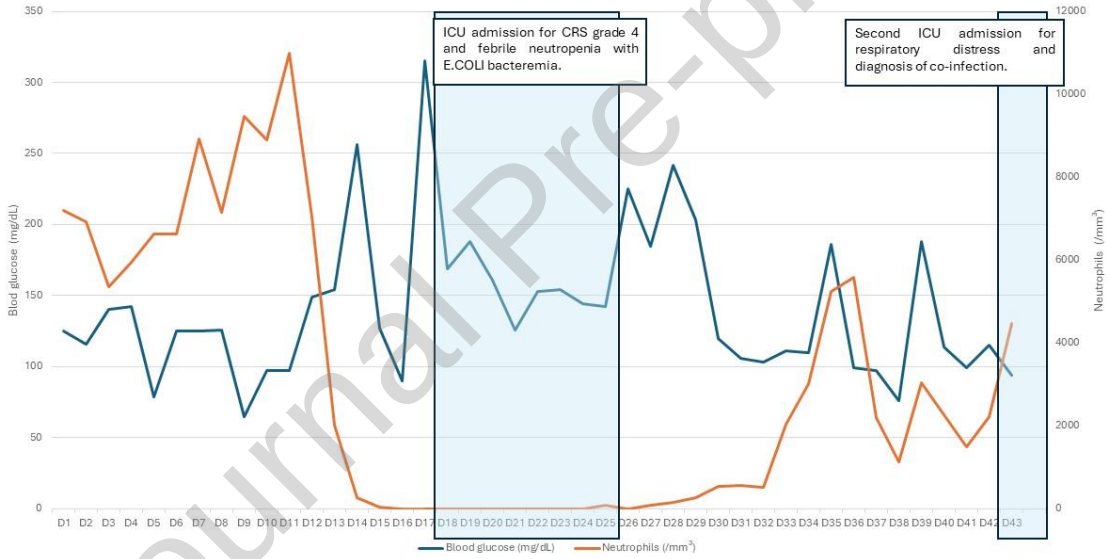
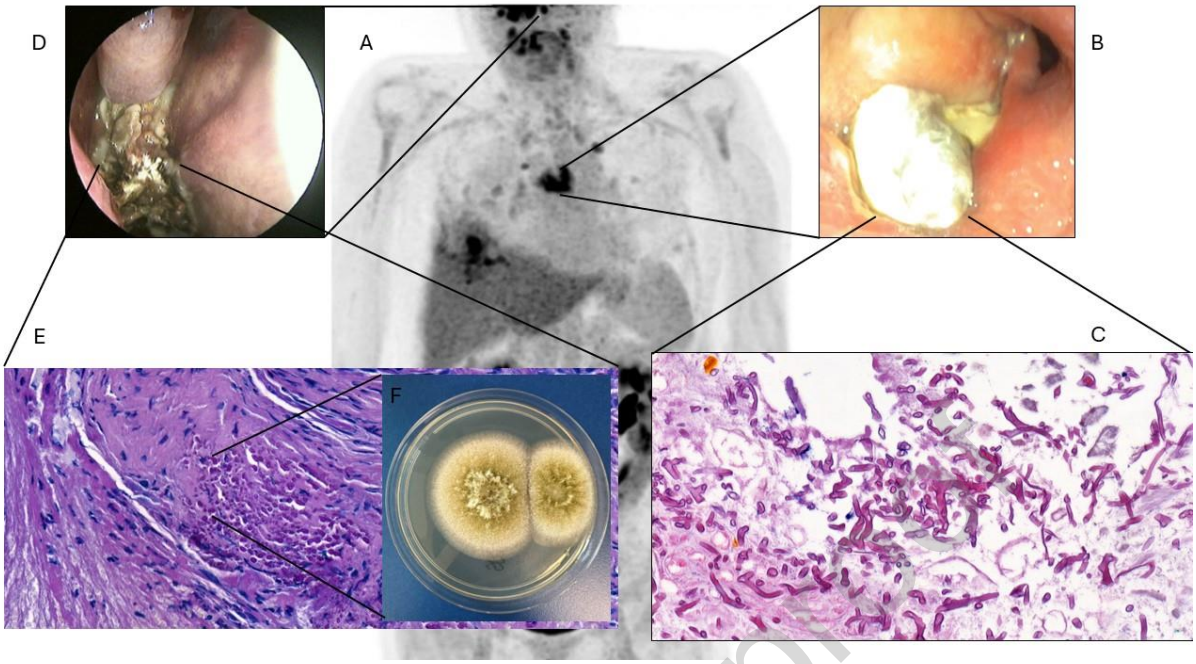


Figure 2. Clinical Presentation at Diagnosis.

A. FDG PET-CT scan performed at diagnosis showing involvement of the trachea, lungs, and sinuses. **B.** Endoscopic image from bronchoalveolar lavage revealing a mobile fibrin aggregate with a stalk attached to the medial side of the trachea, intermittently obstructing the right main bronchus. **C.** Microscopy (x40) of the mass showing a large-caliber, septate filament at 90° (Mucor) in the center of the image, with smaller septate filaments at 45° surrounding it (Aspergillus). **D.** Nasal endoscopy showing mycotic material in the right nasal cavity, adjacent to the sphenopalatine foramen. **E.** Microscopy of sinus biopsies showing degenerated mycelial filaments (PAS staining) with a filamentous appearance suggestive of Aspergillus. F. Culture of *A.Flavus*. PAS: Periodic-Acid-Schiff, FDG PET-CT: 2-deoxy-2-[18F]fluoro-d-glucose positron emission tomography/computed tomography



Author Statement

We warrant that this manuscript represents our original work and has not been previously published in any form. Furthermore, it is not under consideration for publication elsewhere.

All authors have contributed substantially to the conception, drafting, or revision of the work and take full responsibility for its content.

Disclosure

For the submission of this article, I report my disclosure. I received a travel and research grant from Gilead. Gilead is not aware of this work and so, I do feel fully impartial in this submission.

Other authors reported no disclosure related to this work.

Conflicts of interest

Adrien DE VOEGHT received a travel grant and research grant from Gilead.

Other authors reported no other conflict of interest.

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Ethical approval:

As the article is a case report without new drug use, no ethical approval was asked.

Consent:

The patient gave his consent (oral and writing).

Author contribution

Sarah SAKALIHASAN : conceptualisation, data collection, writing original draft & Frédéric LIFRANGE : conceptualisation, data collection, writing original draft

Mathieu CZAJKOWSKI : data interpretation, writing, review Veronique GONCETTE: data interpretation, writing, review Bernard DUYSINX : data interpretation, writing, review Pierre LOVINFOSSE : data interpretation, writing, review Damla CAN : data interpretation, writing, review Raphael SCHILS : data interpretation, writing, review

Marie-Pierre HAYETTE : conceptualisation, data collection, formal analysis, methodology, supervision, writing original draft and editing & Adrien DE VOEGHT : conceptualisation, data collection , formal analysis, methodology, supervision, writing original draft and editing