Invasive pulmonary co-infection caused by *Aspergillus* sp. and *Pneumocystis jirovecii*, complicated by *Cytomegalovirus* reactivation in a patient following second allogeneic hematopoietic stem cell transplantation – Case report

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ABSTRACT

Invasive fungal infections are common complication in hematopoietic stem cell transplant recipients, often leading to high morbidity and mortality rates. Furthermore, when invasive fungal co-infections are diagnosed the prognosis is rarely favorable. Here, we present a rare case of a 47-year-old HIV-negative male with invasive pulmonary co-infection caused by *Aspergillus* sp. and *Pneumocystis jirovecii*, complicated by *Cytomegalovirus* reactivation following second allogeneic hematopoietic stem cell transplantation with a fatal outcome. 2012 Elsevier Ltd. All rights reserved.

1. Introduction

With an incidence between 10 and 20% the fungal infections are a frequent companion of hematopoietic stem cell transplantation (HSCT), and the mortality associated with these complications can reach 90% [1]. The type of transplantation (allogeneic and HLA mismatched), the presence of acute or chronic graft-versus-host disease (GVHD), prolonged neutropenia ($<0.5 \times 10^9$/L) corticosteroid therapy (≥ 2 mg/kg) for extended period of time, *Cytomegalovirus* (CMV) reactivation, and antimicrobial prophylaxis are among the major risk factors for the development of invasive fungal infections (IFIs) after HSCT [1,2].

Herein, we present a clinical case of invasive pulmonary co-infection caused by *Aspergillus* sp. and *Pneumocystis jirovecii*, complicated by *Cytomegalovirus* reactivation in a patient following second allogeneic hematopoietic stem cell transplantation.

2. .Case presentation

A 47-year-old male was admitted to the transplantation ward of our hospital for a second allogeneic HSCT. The patient was diagnosed with acute myeloid leukemia in June 2018 and chemotherapy was initiated

according to established protocols. The first allo-HSCT was performed in November 2020. After 6 months a relapse of the underlying disease was registered and in October 2021 (Day 0) a second salvage allogeneic transplantation of peripheral stem cells from a fully matched unrelated donor (10/10 HLA) was performed. Both the recipient and the donor were CMV IgG positive and the patient's HIV status was negative. Antimicrobial prophylaxis with amikacin (1g/d), piperacillin/tazobactam (3 × 4.5g/d), fluconazole (400 mg po), trimethoprim/sulfamethoxazole (480 mg po twice a day 3 times a week), acyclovir (3 × 400 mg/d) was started, and cyclosporine A (5 mg/kg/d) and mycophenolate (15 mg/kg/d) were added against GVHD. Neutrophil recovery ($>0.5 \times 10^9$/L) occurred on D+13, and for platelets ($>20 \times 10^9$/L) on D+14. Immediately after transplantation an increase in body temperature up to 40 °C was registered. On D+5, the high-resolution lung computed tomography (CT) scan revealed multiple nodules in both lungs with ground-glass opacity and halo sign, suspicious for a multifocal IFI (Fig. 1).

The positive serum galactomannan (GM) antigen test (Platelia *Aspergillus* Ag, Bio-Rad, USA) (D+5) resulted in initiation of voriconazole treatment (loading dose 12 mg/kg day 1 and maintenance dose 8 mg/kg thereafter). Later, on D+20, CMV reactivation was

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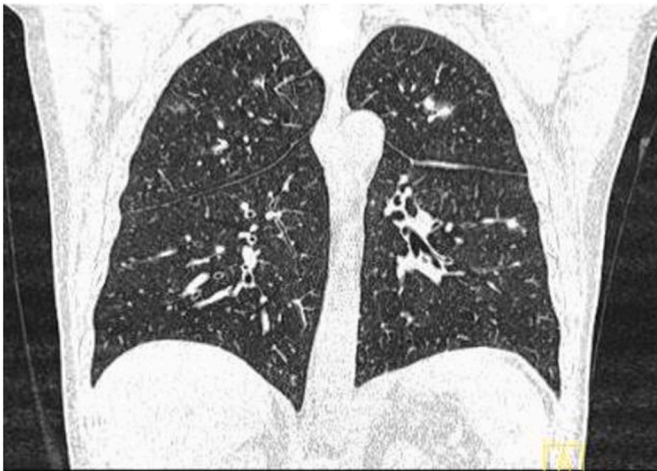
Fig. 1. Lung CT scan performed on D+5 after second allogeneic HSCT.

Fig. 3. Giemsa staining on BAL demonstrating blue dot-like intracystic bodies of *Pneumocystis jirovecii* on a pale eosinophilic background (arro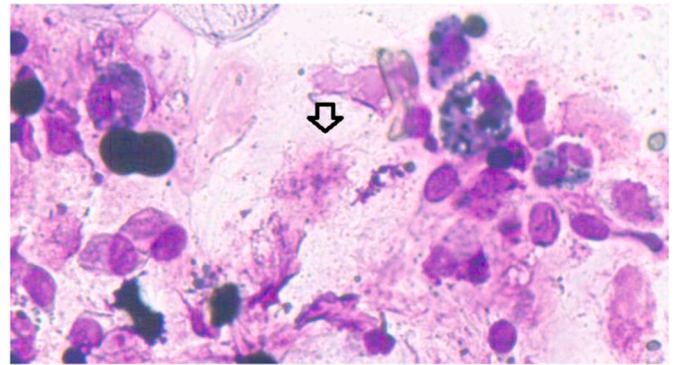w). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

diagnosed by qPCR (734 copies/mL) and treatment with ganciclovir (2 × 5 mg/kg/day) was initiated. The CT scans revealed cavitations in the lungs with persistence of ground-glass opacity and halo sign changes (Fig. 2). Due to high body temperature (up to 39 °C) and severe fatigue, a bronchoscopy was performed on D+29 and a bronchoalveolar lavage fluid (BAL) was subjected to a variety of molecular-genetic, serological, cultural and cytological tests.

The cytological examination of the BAL sample (Giemsa, Diff-quick, Grocott's methenamine silver stain and hematoxylin and eosin staining) was positive for *P. jirovecii* (Fig. 3). A positive *Aspergillus* GM Ag (OD index 2.9) in the same sample was also found. The standard microbiological cultural examination was negative for *Aspergillus* spp. and other opportunistic pathogens. Additionally, PCR for *Mycobacterium tuberculosis* (Xpert® MTB/RIF, Cepheid, USA) and Multiplex PCR (QIAstat-Dx® Respiratory SARS-CoV-2 Panel, QIAGEN, The Netherlands) for a wide spectrum of respiratory pathogens were also performed but were negative.

Based on the positive laboratory results for *P. jirovecii* and *Aspergillus*

the trimethoprim/sulfamethoxazole dosing regimen was adjusted to 15 mg/kg/d and the voriconazole treatment was extended. Because an intestinal form of GVHD was diagnosed, methylprednisolone (2 mg/kg) was added to the therapy.

During the course of hospitalization and as a part of the routine screening for colonization of gastrointestinal and respiratory system with multidrug-resistant (MDR) bacteria, *Enterococcus faecalis* and MDR *Acinetobacter baumannii* were isolated from multiple throat swabs and urine samples, and the antibacterial therapy was changed to vancomycin (2 × 1 g/d) and meropenem (3 × 2 g/d).

Following the revised and updated European organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium guidelines the patient was diagnosed with probable invasive pulmonary aspergillosis (IPA) and proven *Pneumocystis jirovecii* pneumonia (PJP) [3].

On D+50, a control CT scan was performed, revealing a reduction of the cavitating nodules. Based on the regression of the laesions in the lung as shown on the CT-chest and a favorable clinical response (no fever, normotensive, normal breathing), the treatment was considered to be
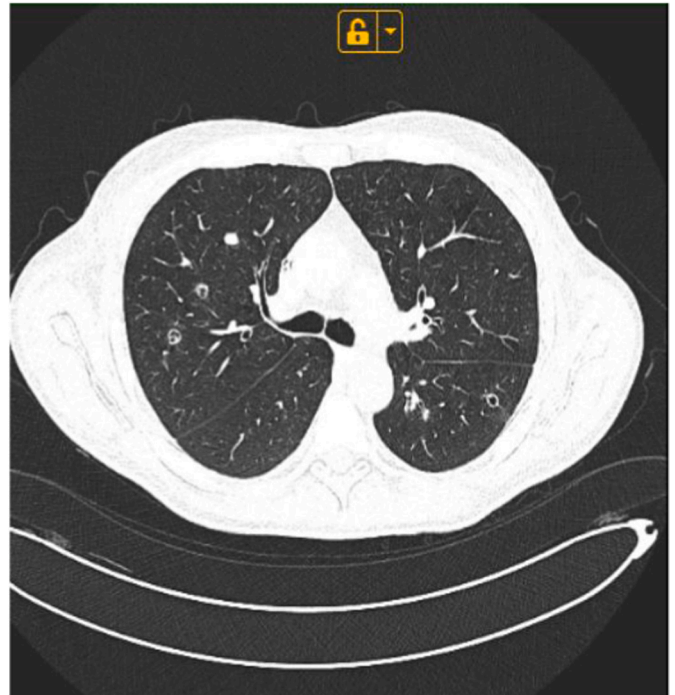

Fig. 2. Lung CT scan performed on D+20 after second allogeneic HSCT.

Fig. 4. Lung CT scan performed on D+50 after second allogeneic HSCT.

effective (Fig. 4). On D+55 the clinical status of the patient changed and rapidly deteriorated. He developed profuse hemoptysis and progressive shortness of breath, which necessitated oxygen therapy. On D+56, the patient was in a critical condition with severe shortness of breath and high-grade fever, and despite resuscitation measures, a fatal outcome occurred.

3. Discussion

The presented case is one of the few described cases presenting co-infection with IPA and PJP in HIV-negative patients. Currently, the separately described cases are less than 15 [4–6]. The largest study on co-infections with IPA and PJP and the risk factors for occurrence of these IFIs, following a 10-year period (2011–2021), was conducted by Zhong Y et al. [7]. The low incidence of such co-infections is thought to be associated with the appropriate anti-PJP prophylaxis in the risk groups and with the low resistance rate to the first line agent for prophylaxis (trimethoprim/sulfamethoxazole) [4].

In the presented clinical case, multiple risk factors for the development of IFI were identified - the type of the underlying hematological disease (acute leukemia), subsequent stem cell transplantation, myeloablative regimen with subsequent profound neutropenia, immunosuppressive prophylaxis against GVHD and CMV reactivation.

The IPA/PJP co-infection is characterized with a complex pathogenesis. It has been reported that the co-infection is more likely to be diagnosed in HIV-negative patients [7]. The reason might be the adequate antifungal prophylaxis or the anti-retroviral therapy in HIV-positive individuals. It is speculated that HIV-negative people need lower fungal burden to develop PJP. Also, compared to the HIV-negative, the HIV-positive patients are less likely to take corticosteroids, especially high dose steroids for prolonged periods of time, which is known as a main risk factor for PJP and IPA [7].

Post-HSCT patients usually suffer from severe inflammation caused by the myeloablative conditioning regimen and the chemotherapy (GVHD and mucositis), which are considered as risk factors for opportunistic fungal infections. In addition, the immune reactivity plays a significant role in the occurrence and outcome of the IPA/PJP infection. It is known that the immune response in HIV-infected individuals is altered, and the mortality rates are higher in the hematology non-HIV group compared to the HIV-positive group. Even more, it is reported that the co-infection of IPA and PJP further destroys the lungs' functions, enhances inflammatory reactions, and predisposes to fatal outcome [7].

The rapid diagnosis of the infectious complications in HSCT patients plays an important role in preventing the fatal outcome. A variety of methods (microbiological, histological, imaging) have been introduced in the laboratory practice for IFIs diagnosis. Circulating components of the fungal cell (GM, (1–3)-β-D-glucan and DNA) can be detected by ELISA or molecular-genetic methods [3]. The non-invasive serological tests for GM Ag and beta-D-glucan can be very useful when performed regularly for IFIs screening. An advantage of the beta-D-glucan test is the potential to detect both IPA and PJP as a mono- or co-infection and some other important fungal pathogens. Although widely used, these diagnostic methods have their drawbacks. Fungi other than *Aspergillus* spp. also produce GM antigen (*Penicillium* spp., *Fusarium* spp., *Histoplasma* spp.) [1]. Similarly, (1–3)-b-D-glucan is a component of the wall of fungi, representatives of different genera (*Candida* spp., *Aspergillus* spp., *Scedosporium* spp.) [9]. In addition, the use of certain antibiotics (amoxicillin/clavulanate, piperacillin/tazobactam), as well as the presence of GVHD can lead to false-positive results when using a GM test, especially in serum samples [10].

Histological and cytological examination of BAL, sputum or tissue samples for mycotic hyphae or cysts detection, is also an important component in the arsenal for the diagnosis of IFIs [3,9]. There are some PCR protocols that use universal pan-fungal oligonucleotides able to detect the DNA of wide range fungal pathogens [11]. The positive result

requires further investigations. Although highly sensitive, the molecular-genetic methods do not always distinguish between infection and colonization when BAL fluid or sputum are used [12]. This disadvantage can be resolved by using quantitative tests, as a higher DNA copy number would favor infection. However, the gold standard for diagnosing IFIs remains the BAL examination, which is not always available due to possible complications (bleeding, pneumothorax) [12].

The specific elements visualized on the imaging studies are very helpful in establishing the diagnosis. The consolidation, cavitation, halo sign and air crescent sign on CT scan are characteristic for IPA [3], while the bilateral involvement of the lungs with ground-glass opacity, as observed in the presented case, are typical for PJP [9]. Very often in fungal co-infection these lung changes can overlap [13]. Fungal co-infections should always be suspected in high-risk patients even if antifungal prophylaxis is initiated. Because of the wide fungal spectrum and some diagnostic challenges, a combined diagnostic approach is recommended when cough, high fever, fatigue, elevated inflammatory blood markers or no response to the antimicrobial therapy are documented. The diagnosis of fungal co-infections should be based on clinical symptoms, microbiological (classical, serological, PCR) and histological studies, and radiological examinations.

It should be taken into consideration that the lung changes, detected by imaging methods, could be non-infectious in nature and should be differentiated from those with infectious origin. Bronchiolitis obliterans, alveolar hemorrhage, fibroelastosis and interstitial lung disease are among the pulmonary complications associated with the post-transplant chemotherapy, drug toxicity or GVHD, often presented with respiratory symptoms (cough, dyspnea) and lung changes (halo sign, ground-glass opacities and consolidation), typical also for certain infectious agents [14].

The prevention of IFIs is also an important aspect in the management of the infectious complication in this severely immunocompromised patient group. Different regimens are used to protect patients at high risk. Posaconazole (300 mg/d) or voriconazole (2 × 200 mg/d) is recommended for prophylaxis against IPA [15]. For PJP, trimethoprim/sulfamethoxazole (160/800 mg/d) is still the first-line option for prophylaxis [12]. The presented clinical case is important as it demonstrates that patients under prophylactic regimen against PJP can still develop the complication. A possible reason for such breakthrough infections could be an acquired resistance of *P. jirovecii* to trimethoprim/sulfamethoxazole [8].

The recommended therapy of IPA and PJP includes the administration of the same antimicrobial agents, but in higher doses: voriconazole 12 mg/kg/d and then 8 mg/kg/d for IPA and trimethoprim/sulfamethoxazole 15–20 mg/kg/d p.o. divided in 3 doses [12]. Because of the possible toxic side effects of both voriconazole and trimethoprim/sulfamethoxazole (bone marrow suppression, liver and kidney damage), routine monitoring of blood parameters for organ damage is recommended.

In the presented clinical case, simultaneously with the fungal co-infection, CMV reactivation was diagnosed. The CMV reactivation in patients after allogeneic HSCT is recognized as a major risk factor for IFIs complications, especially IPA and PJP [16]. In this case, the development of IFI preceded the CMV reactivation. Regardless of timing, co-infection with fungi and CMV is considered an indicator of poor prognosis [4]. Even without the intervention of CMV and the presence of only IPA and PJP, the probability of a fatal outcome is high [5].

In conclusion, the presented clinical case demonstrates that even with an adequate diagnostic, therapeutic and resuscitation measures, mortality associated with invasive infections can hardly be avoided. IFIs must always be taken into account when severely immunocompromised patients present with high fever, fatigue and pulmonary changes on CT scan and prompt therapy must be initiated without delay.

Ethical form

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

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

Declaration of competing intrest

There are none.

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