

Concomitant invasive pulmonary aspergillosis and *Candida zeylanoides* bloodstream infection in an acute myeloblastic leukemia patient

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Abstract

Current management of acute myeloblastic leukemia (AML) involves induction chemotherapy followed by risk stratified consolidation approaches, consisting of high dose chemotherapy or allogeneic stem cell transplantation. AML patients are at high risk of invasive fungal disease, particularly mold infections, due to the leukemia and chemotherapy related immune deficit. We present an AML patient with prolonged fever and neutropenia after induction therapy, during which she developed concomitant *Candida zeylanoides* bloodstream infection and invasive pulmonary aspergillosis. The response to Caspofungin and Voriconazole therapy was delayed, and this dictated the surgical management of the remnant pulmonary lesions. A histological confirmation of aspergillosis was thus obtained. Evaluation of host risk factors for invasive fungal disease, prompt scale-up of the diagnosis scheme and initiation of antifungal therapy are mandatory in order to ensure patient survival.

Keywords: Pulmonary Aspergillosis, Acute Myeloid Leukemia

Introduction

Acute myeloblastic leukemia (AML) is a highly aggressive proliferation of the immature myeloid precursor cells, whose optimal management depends upon timely administration of chemotherapy regimens and allogeneic stem cell transplantation availability. Disease and therapy related immune suppression result in a high risk of opportunistic infection. Since the introduction of *Candida* prophylaxis with Fluconazole, we have witnessed a shift in the spectrum of

fungal infections in these patients, with mold infections and particularly *Aspergillus fumigatus* accounting for the majority of cases [1]. Invasive pulmonary aspergillosis causes important morbidity and mortality in AML patients and favors disease relapse by delaying therapeutic sequences and thus altering the dose intensity of the intended treatment regimen [2].

Diagnosing in due time an invasive pulmonary aspergillosis requires, besides a high clinical suspicion, the preemptive use of imagistic and serologic diagnostic tools and prompt initiation of the antifungal therapy. Voriconazole is considered the backbone treatment of invasive pulmonary aspergillosis [3].

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Case report

We present the case of a 31 year-old pregnant female patient, admitted to the Hematology Clinic of the Regional Oncology Institute, Iasi in February 2014 for marked fatigue and productive cough, experienced during the previous month. The blood count revealed pancytopenia with grade IV neutropenia and 35% blast cells on the peripheral blood smear. Bone marrow examination revealed extensive infiltration with myeloid blasts displaying an M5 immunophenotype (CD45-/+, low, CD117+, CD34+, CD13+, CD33+ low, HLA/DR+, CD38+ high, CD36-, CD64-/+, low (6%), CD56+, CD123+, MPO-/+, (2%), NG 2+). Molecular testing did not identify any specific gene fusion products. Karyotype examination was normal. The patient had no compatible HLA sibling.

A diagnosis of intermediate risk Acute Monoblastic Leukemia- not otherwise specified (NOS) was established according to the 2008 World Health Organization Classification of Tumors of Hematopoietic and Lymphoid Tissues and the patient was considered eligible for allogeneic stem cell transplantation from unrelated donor after achieving complete remission (CR). There is no evidence regarding the influence of pregnancy upon the evolution of acute leukemias [4]. On the other hand, as the patient was in the first trimester of pregnancy, a therapeutic abortion had to be performed, considering the potential adverse effects of chemotherapy upon the fetus.

A standard "7+3" induction therapy (Cytarabine 100-200 mg/m² continuous IV infusion for 7 days and Idarubicin 12 mg/m²/day for 3 days) was initiated, with immediate onset of profound leukopenia and neutropenia, with a total duration of 30 days. Taking into account the high risk of developing invasive fungal disease, we considered the patient eligible for Posaconazole prophylaxis. However, as Posaconazole was not available in our center at that moment, Fluconazole prophylaxis was performed instead.

On day 6 post induction the patient became febrile and presented X-ray features of left inferior lobe pneumonia. She immediately defervesced after empirical

Piperacilin plus Tazobactam and Gentamicin therapy (providing broad spectrum coverage against Gram positive and Gram negative bacteria, including *Pseudomonas aeruginosa*) was initiated, but on day 19 post induction the fever relapsed, without any other further clinical evidence of infection.

The antibiotic regimen was switched to Meropenem (targeting Gram negative bacteria, especially extended spectrum beta-lactamase producing microorganisms) and Teicoplanin (targeting Gram positive bacteria and especially methicillin resistant staphylococci), but without resolution of the febrile syndrome over the next 48 hours. On day 21 the blood cultures became positive for *Candida zeylanoides*, so that broad-spectrum antibiotherapy was stopped, Fluconazole prophylaxis was discontinued and intravenous Caspofungin was added. Despite that, the fever persisted and we noted the onset of respiratory symptoms (severe cough, anterior thoracic pain, and dyspnea) on day 30 post induction.

A CT examination of the thorax revealed a consolidation syndrome in the right superior lobe, a paracardiac abscess in the right middle lobe and an infiltrative nodule in the right inferior lobe (Figure 1, Figure 2). Serum Galactomannan antigen (a component of the *Aspergillus* cell wall), routinely investigated in neutropenic patients with high suspicion of invasive fungal disease, was negative at this moment.

Even though specific diagnostic methods (such as PCR for the ITS2 (internal transcribed spacer 2) sequences [5, 6] were not available, taking into account the patient's risk factors and the imaging data according to the 2008 EORTC criteria (see supplementary material), we considered the possibility of invasive pulmonary aspergillosis and we empirically initiated the therapy with Voriconazole. The patient defervesced within 24 hours. Neutrophil and platelet recovery occurred on day 32 post induction, allowing us to perform a broncho-alveolar lavage (BAL). Galactomannan testing and *Aspergillus* spp. cultures from the BAL fluid were positive, allowing us to consider the diagnosis of probable invasive pulmonary aspergillosis. Progressive improvement of the clinical status

permitted the discharge of the patient on day 41 post induction. Complete remission was confirmed at this moment by morphological and flow cytometrical bone marrow

examination. Intravenous Caspofungin was discontinued and the patient remained on oral Voriconazole.

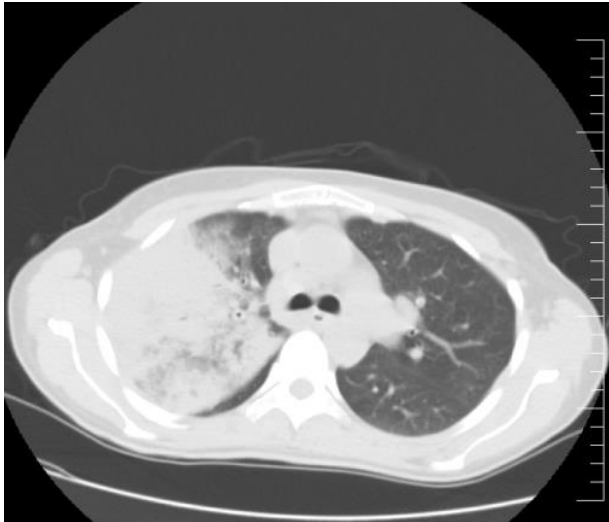


Fig. 1. Extensive infiltrates in the right superior lobe (Day 30 post induction).

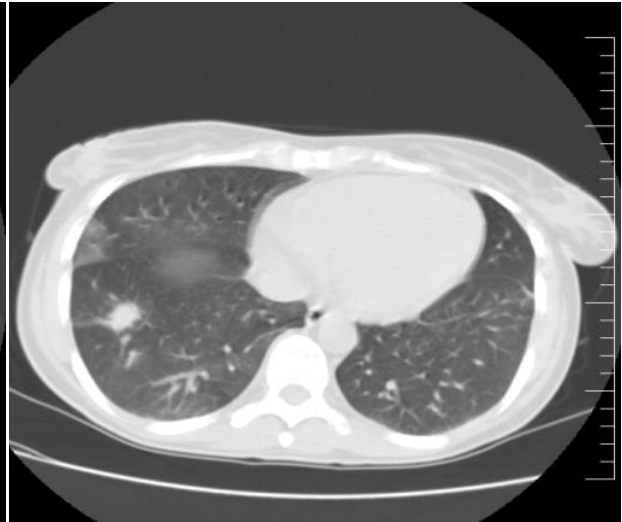


Fig. 2. Infiltrative nodule with discrete halo sign in the lateral segment of the right inferior lobe (Day 30 post induction).

The patient was readmitted on day 69 post induction with residual persistent cough. A thoracic CT scan after 38 days of Voriconazole therapy showed marked regression of the upper lobe infiltrates, but progression of the right inferior lobe lesion. Under these circumstances, the patient required a surgical approach, so that an atypical inferior lobe

resection was performed on day 88 post induction.

The pathology examination revealed the presence of narrow tubular septate *hyphae*, branching at angles of 45° in all the examined sections, supporting the diagnosis of invasive pulmonary aspergillosis (Figure 3). No angioinvasive features were present.

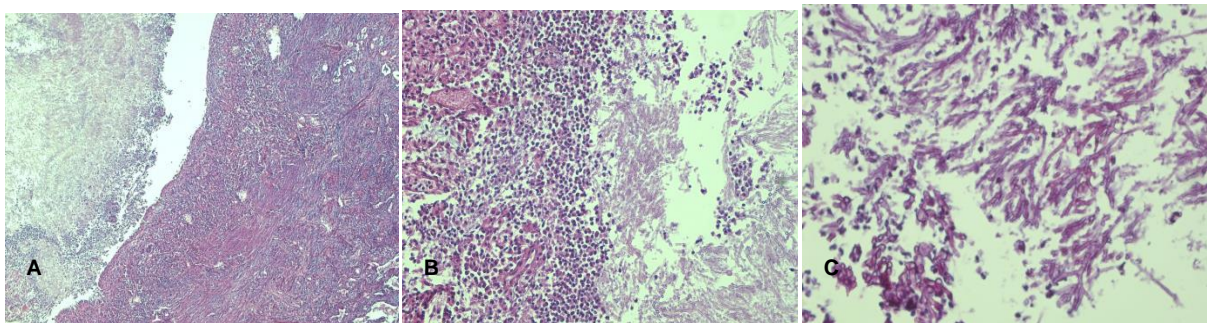


Fig. 3. Pathology examination reveals presence of *Aspergillus hyphae* in all examined sections. **A:** Fragment of the pulmonary tissue displaying a central cavity encircled by a granulation tissue with chronic inflammatory infiltrate. In the cavity lumen multiple colonies of *hyphae* are present ("fungus ball" appearance). (HE, x5) **B:** granulation tissue surrounding hyphal elements. (HE, x20) **C:** tubular narrow (4-6 μ m) monomorphic *hyphae*, regularly septate, branching at angles of 45° . Both viable (basophilic) and necrotic (eosinophil) *hyphae* were present. (PAS, x40)

The evolution after surgery was favorable and the patient continued the oral Voriconazole therapy for a total of 5 months.

Repeated chest CT examinations revealed a complete resolution of the pulmonary infiltrates (Figure 4).

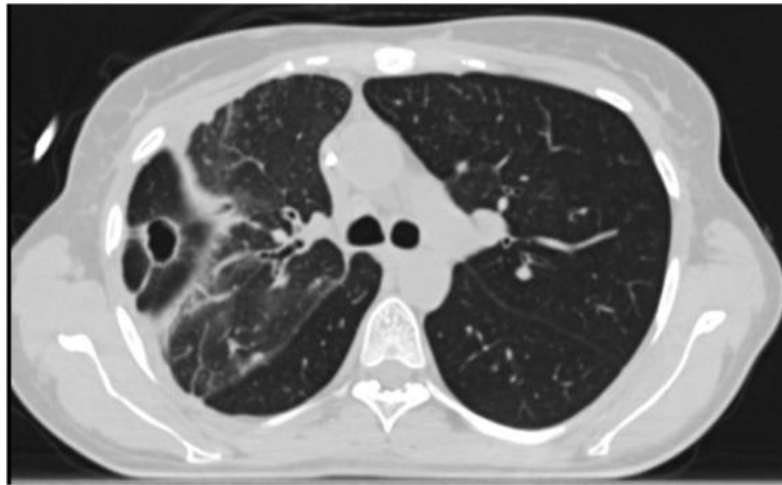


Fig. 4. Resolution of right upper lobe infiltrates (Day 90 post induction). Minimal post surgery encapsulated pneumothorax is present in the lateral segment of the middle lobe

Consolidation chemotherapy, consisting of two cycles of high dose Cytarabine, was administered starting day 94 post induction. Taking into account the preexisting fungal infection, secondary Voriconazole prophylaxis was employed.

The patient suffered leukemia relapse 10 months after diagnosis. We have opted for Ida-FLAG (Idarubicine, Fludarabine, high dose Cytarabine, followed by Filgrastim administration) as a reinduction regimen, obtaining a second complete remission. Secondary Posaconazole prophylaxis was used during this period, as the patient developed a severe sun-burn-like skin reaction after oral Voriconazole. No other fungal related manifestations could be noticed. The patient is currently in complete remission, awaiting an allogeneic stem cell transplantation from an unrelated donor.

Discussions

We present the case of a young female patient with intermediate risk AML, eligible for allogeneic stem cell transplantation from an unrelated donor, for which severe infectious complications prevented the administration of consolidation chemotherapy for more than 90

days, thus favoring leukemia relapse. Despite combined Caspofungin and Voriconazole therapy, the delayed response (> 30 days) of the fungal pulmonary lesions imposed surgical management.

We have considered this patient to be at high risk for developing invasive fungal disease according to the following criteria: the induction treatment for AML, history of use of broad spectrum antibiotics and history of prolonged neutropenia (> 10 days).

On day 6 post induction therapy a broad spectrum antibiotic therapy was initiated and while the initial response was favorable, the fever relapsed and persisted despite the *Candida zeylanoides* targeted Caspofungin treatment.

Furthermore, multiple other diagnosis issues had to be considered. We could not rule out completely the possibility of a bacterial infection, even though the fever had not responded to the Meropenem and Teicoplanin combination. Given the normal transthoracic echocardiography, a bacterial or fungal endocarditis was considered less probable, even if its negative predictive value is not 100%. The co-existence of an alternative invasive fungal infection, particularly pulmonary aspergillosis, was also taken into account and we have thus performed a chest

CT that raised indeed a high suspicion of invasive pulmonary aspergillosis in this case. Under circumstances such as these it is highly recommended to enhance the diagnostic work-up by including imaging tests, serial serum Galactomannan assessment and, whenever possible, Galactomannan testing and cultures from BAL fluid, rather than relying solely on empirical broad spectrum antifungal therapy. Histological confirmation following lung biopsy is considered ideal; however, severe thrombocytopenia often prevents this approach.

Scaling-up the diagnostic approach in due time for febrile neutropenic patients is essential for the successful management, especially in patients suspected of invasive pulmonary aspergillosis [7]. It is known that the transition from the bronchoalveolar to the angioinvasive phase of the infection evolves in a very narrow timeframe of just several hours in neutropenic patients [8]. Prompt preemptive initiation of antifungal treatment is thus vital.

When facing high risk neutropenic patients with persistent fever, for which invasive fungal disease is ruled out, it is a common approach in our center to test for pulmonary tuberculosis and even administrate empiric tuberculostatic therapy. Finally, in patients with persistent febrile syndrome, bone marrow examination should be considered to exclude refractory disease. It is questionable whether primary Posaconazole prophylaxis would have been able to prevent the *Aspergillus* infection, allowing for timely consolidation

chemotherapy. However, it is known that Posaconazole prophylaxis reduces the incidence of invasive fungal disease from 8% to 2% in high risk AML and myelodysplastic syndrome patients [9-11]. At least part of its superiority in prophylaxis over other antifungal azoles, like Fluconazole, appears to be due to its hydrophobic nature and due to the preferential concentration (up to 400 fold) in the membrane compartments of alveolar macrophages and epithelial cells [12].

Conclusions

We present a rare case of concomitant pulmonary invasive aspergillosis and *Candida zeylanoides* bloodstream infection. The diagnosis and management of invasive pulmonary aspergillosis in neutropenic patients is difficult and challenging, requiring careful consideration of the patients' risk factors, dynamical clinical assessment and early preemptive use of paraclinical tools and antifungals.

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Supplimentary Materials

TABLE 1. EORTC criteria for proven lower respiratory tract invasive mold infection

Analysis/specimen	
Microscopic analysis	Histopathologic, cytopathologic, or direct microscopic examination of a specimen obtained from a normally sterile site
Culture: sterile material	Recovery of a mold by culture of a specimen obtained by a sterile procedure from a normally sterile and clinically or radiologically abnormal site consistent with an infectious disease process (excluding bronchoalveolar lavage fluid)
Culture: blood	Blood culture that yields a mold

TABLE 2. EORTC criteria for probable lower respiratory tract invasive fungal disease

Host criteria	Recent history of neutropenia ($<0.5 \times 10^9$ neutrophils/L or <500 neutrophils/mm ³] for >10 days) Allogeneic stem cell transplant recipient Prolonged corticosteroid therapy Treatment with nucleoside analogues and T cell immunosuppressants during the past 90 days Inherited severe immunodeficiency
Clinical criteria	Presence of 1 of the following signs on CT: - Dense, well circumscribed lesion(s), with or without a halo sign; - Air crescent sign; - Cavity; - Tracheobronchitis; Tracheobronchial ulceration, nodule, pseudomembrane, plaque, or eschar seen on bronchoscopic analysis
Mycological criteria	Direct tests (direct microscopy, cytology or culture): - Presence of mold elements in sputum, bronchoalveolar (BAL) lavage fluid, bronchial brush; - Recovery by culture of a mold; Indirect tests: detection of a mold antigen or cell wall component: - Galactomannan in serum or BAL fluid; - Beta-D-glucan in serum.