



Successful treatment of severe aplastic anemia with syngeneic stem cell transplantation in the setting of active disseminated mucormycosis



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ABSTRACT

Severe aplastic anemia (SAA) is a hematological disease resulting in pancytopenia due to bone marrow failure. Treatment consists of immunosuppressive therapy, or allo-SCT. Patients with aplastic anemia are predisposed to invasive fungal infections due to mucormycosis. Till now, syngeneic SCT in the context of active mucormycosis infection for patients with severe aplastic anemia is lacking in the literature.

Here, we report a case of severe aplastic anemia with disseminated mucormycosis infection undergoing syngeneic transplant.

1. Introduction

Severe aplastic anemia (SAA) is a life-threatening hematological disease characterized by suppression of the bone marrow. Diagnosis is made in the context of pancytopenia associated with a persistent hypocellular marrow without major dysplastic signs or marrow fibrosis [1]. Treatment consists of immunosuppressive therapy with anti-thymocyte globulins (ATG) and cyclosporine (CSA), or allogeneic stem cell transplantation (allo-SCT) especially for patients who have a matched related donor. Allo-SCT as front-line therapy has comparable overall survival (OS) (82%) as immunosuppressive therapy (86%), but a high rate of relapse is observed after immunosuppressive therapy and hence the event free survival (EFS) is by far lower after this kind of treatment as compared to transplanted patients [2].

Patients with SAA are predisposed to recurrent bacterial infections and invasive fungal infections (IFI) due to profound and persistent neutropenia. Infections are the leading cause of death in patients with SAA [3]. Mucorales are the second most common cause of IFI encountered in SAA and disseminated mucormycosis has a dismal prognosis with death occurring within few weeks. There are no previously reported cases of allo-SCT for SAA in patients with active mucormycosis. We herein report a case of SAA with active disseminated mucormycosis infection of the lungs and sinuses who was successfully treated with aggressive surgical debridement, antifungal therapy and underwent syngeneic SCT while neutropenic and with active infection.

2. Case

The patient is a 36-year-old Iraqi man patient with diabetes mellitus type I treated with insulin diagnosed with SAA in Iraq and had received immunosuppression therapy with CSA and ATG and was referred to the American University of Beirut (AUBMC) for further management. At presentation on February 14th, 2017 (day 0) CBC showed a hemoglobin of 9.3 g/dl, hematocrit of 27%, White blood cell count of 500 /cu.mm and platelets count of 120 000 cu.mm. He gave a history of recurrent epistaxis and relapsing fever.

On presentation, patient had low-grade fever and altered level of consciousness but was hemodynamically stable. Computerized tomography scan (CT-scan) of chest and brain on day 0 showed scattered right lung nodules with surrounding ground-glass halo, suspicious for an atypical infectious process, and findings of chronic sinusitis in the right maxillary, frontal, sphenoid and ethmoid air cells. Patient was started on piperacillin-tazobactam, and clarithromycin. Antifungal therapy with voriconazole was added for primary prophylaxis because of neutropenia [4]. On day + 1 bone marrow (BM) evaluation showed aplastic appearance with less than 5% cellularity and no blasts. Flow cytometry of the BM sample showed no evidence of paroxysmal nocturnal hemoglobinuria clone (PNH) or increased blasts. Cytogenetic studies showed normal karyotype 46, XY. Serology for hepatitis B and C and autoimmune antibodies (ANA, c-ANCA and p-ANCA) were all negative. Folate level was normal, and vitamin B12 level was low at 148 pg/ml (lower limit of normal: 240 pg/ml). Blood film inspection

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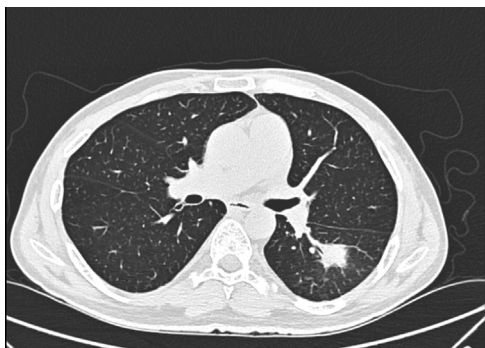


Fig. 1. Axial cut of CT chest without contrast showing left lower lobe nodule. Before transplant.

showed marked neutropenia, slight hypochromia with ovalocytes and few platelets. Other laboratory studies including liver function tests were normal.

Patient had persistent fever, along with worsening level of consciousness on day + 5. He was alert, somnolent, oriented to time, place and persons with normal cranial nerves, motor and reflexes exam. Repeat CT scan of the chest without contrast on February 19 showed worsening pulmonary nodules with interval appearance of surrounding ground-glass abnormalities (Fig. 1).

CT scan of the abdomen and pelvis with intravenous contrast showed new edematous changes in the distal left psoas muscle and surrounding tissue, most pronounced in the pelvis and upper thigh. Investigations included a broncho-alveolar lavage (BAL) on day + 6 which grew methicillin resistant *Staphylococcus aureus* (MRSA) and *Haemophilus parainfluenza*. BAL PCR for RSV and *Mycobacterium tuberculosis* were negative. Aspergillus galactomannan was negative in the blood and the BAL. The patient received targeted antibacterial therapy and because of persistent fever, he was switched from voriconazole to liposomal Amphotericin B at 5 mg/kg/day (L-Amb) starting day + 9. However, patient remained persistently febrile with declined level of consciousness, lethargy and decreased PO intake. Magnetic resonance venography (MRV) of the brain was negative for dural venous thrombosis or meningitis. Repeat CT scan of the chest and sinuses on day + 12 showed worsening disease and increased mucosal disease in the right aspect of the paranasal sinuses with obstruction of the osteomeatal complex, associated with bony erosion with adjacent soft tissue stranding and thickening highly suspicious for an aggressive fungal sinusitis suspicious for mucormycosis (Fig. 2). The patient underwent urgent surgical debridement with pathology showing numerous septate fungal hyphae in vessels and invading surrounding tissues necrosis.

CT guided biopsy of a left lung nodule showed aseptate fungal



Fig. 2. Axial cuts of CT sinuses with intravenous contrast showing right acute sinusitis with right laminal and septal erosions. Before transplant.

hyphae suggestive of mucormycosis [5]. The slides were reviewed by international experts and the general morphology was suggestive of mucormycosis Fig. 5. Treatment consisted of L-Amb at 7 mg/kg, piperacillin-tazobactam and linezolid. Because of slow improvement posaconazole at 200 mg every 6 h was added. He was persistently pancytopenic and required multiple red blood cells and platelets transfusions. The patient underwent three surgical debridements in one week on day + 12, + 18, and + 30.

Despite the high risk of SCT, we decided to proceed with it after consulting with international experts in the field as it was his only chance for survival. The donor was a syngeneic twin brother. Conditioning regimen consisted of Fludarabine 30 mg/m² starting day – 4 till day – 2, along with total body irradiation (TBI) of 4 Gy on day – 1. A total CD34 + cell dose of 7.9×10^6 /kg collected from peripheral blood was infused on day + 28 of admission, Day 0 Post transplant (PT). No immunosuppression was given after the transplant as graft versus host disease (GVHD) prophylaxis. G-CSF was started on day + 5 PT along with eltrombopag 50 mg daily and erythropoietin 40,000 units/week starting day + 2 PT.

On day + 5 PT, the patient had a left wrist skin indurated and erythematous lesion that was urgently debrided with pathology showing fungal pseudohyphae and culture was positive for *Candida tropicalis*. The patient had multiple episodes of fever after transplant for which antibiotics were changed. On day + 9 PT, he had engraftment of his BM with recovery of his hemoglobin and platelets count. Repeat imaging on day + 12 PT showed decrease in the mucosal inflammatory changes in the paranasal sinuses, mild decrease in size with interval development of an air-crescent in the necrotic left lower lobe lung consolidation (Fig. 3), a new hypodense splenic lesion, probably related to splenic abscesses and an interval increase in the intramuscular ring enhancing collections (Fig. 4). CT drainage of left thigh collection yielded *Enterococcus faecalis*.

A second drainage of a residual collection was also done on day + 24 PT. The patient became afebrile and antibiotics were deescalated then stopped. On day + 32 PT, he was discharged home with full recovery from pancytopenia, afebrile. Last imaging done on day + 34 PT showed clearing of fluid within the left lower lobe cavity and decrease in the surrounding post obstructive changes and air space abnormality and minimal residual intramuscular abscesses in the left inguinal region. Repeat BM evaluation prior to discharge showed 70–80% cellular marrow with mild dysmyelopoiesis. The patient received a total of 50 days of L-Amb and 35 days of posaconazole.

He returned to Iraq and his last follow up on 7 months post-transplant showed hemoglobin of 12 g/dl, WBC of 4600/mm³ and platelet count of 140,000/mm³. CMV and EBV PCR were negative.

3. Discussion

SAA is a hematological disease defined as pancytopenia with



Fig. 3. Axial cut of CT chest without intravenous contrast showing clearing of fluid within the left lower lobe cavity and decrease in the surrounding post obstructive changes. After transplant.



Fig. 4. Axial cut of CT pelvis with intravenous contrast showing ring-enhanced left psoas collection. Day + 12 PT.

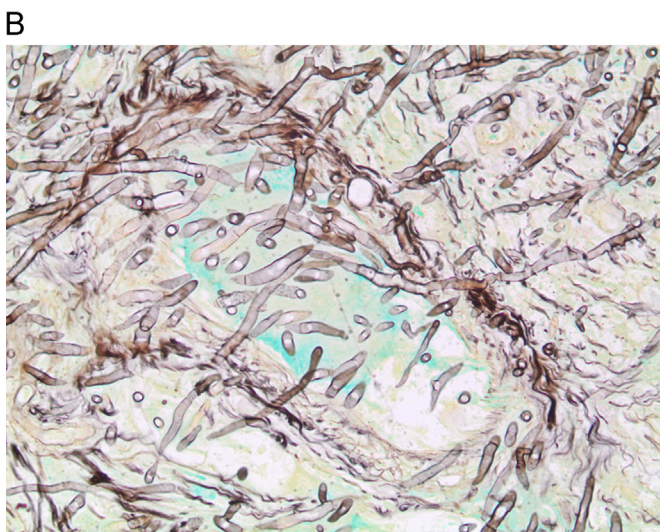
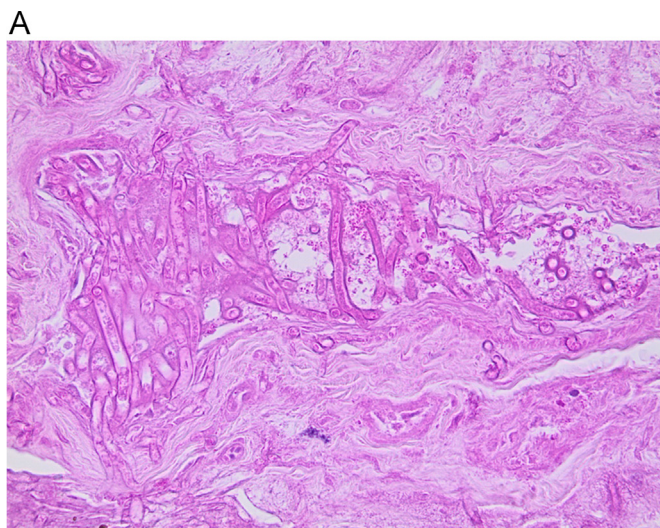


Fig. 5. (A) (Hematoxylin and eosin stain, 400x magnification). (B) Gomori Methenamine Silver stain highlights the septate fungal hyphae (400x magnification). Histologic examination of multiple specimens from the nasal septum, paranasal sinus, and nasal turbinates shows widespread areas of necrosis and foci of chronic inflammation. There are numerous septate fungal hyphae showing acute and right angle branching and demonstrating extensive angioinvasion (Fig. 5A and B).

hypocellular bone marrow in the absence of abnormal infiltrates or fibrosis. SAA is defined in the presence of two of the following: hemoglobin less than 10 mg/dl, platelet count less than $50 \times 10^9/l$ and neutrophil count less than $1.5 \times 10^9/l$ [1,6]. The severity of the disease

is defined according to the blood count parameters and bone marrow cellularity. The standard treatment for patients with SAA is either allo-SCT from an HLA-identical donor or immunosuppressive therapy with a combination of ATG and CSA. Currently allo-SCT is the first choice treatment in patients younger than 40 who have a matched sibling donor. Patients with SAA have many complications related to the disease such as bleeding caused by thrombocytopenia and infections related to the profound and persistent neutropenia. From these, fungal pathogens contribute to high morbidity and mortality and IFI are the leading cause of death in these patients. *Aspergillus species* are the most common infection reported in the literature, while mucorales are the second most common IFI encountered in SAA. Mucorales have been reported to cause pulmonary, rhinocerebral and cutaneous and disseminated infections [7]. Prolonged and severe neutropenia (ANC less than 200 for more than 3 weeks), prolonged high dose systemic corticosteroids (prednisone or equivalent of more than 1 mg/kg/day for more than 3 weeks), iron overload, diabetes mellitus and ketoacidosis are the main risk factors for the development of mucormycosis infection in hematologic malignancies [8]. Other risk factors have been proposed such as use of voriconazole prophylaxis, and the increasing use of intense immunosuppression [9]. The diagnosis is suspected by the clinical manifestations in susceptible patients with continuous fever despite antibacterial therapy and is confirmed by the detection of the fungus by histological analysis or culture of a tissue specimen taken from a site of disease [5,10]. There is no serological test currently available for mucormycosis detection. Cultures typically show organisms with broad, ribbon-like, irregularly shaped non-septate or sparsely septate hyphae while histopathological findings reveal neutrophilic infiltration and angioinvasive pattern.

Morphologic overlap can occur between mucorales and *Aspergillus* making accurate distinction between the two, based on histopathologic examination alone, sometimes difficult. Even though *Aspergillus* are characterized by having septate hyphae with dichotomous branching, mucorales can be pauciseptate, exhibiting occasional septae [11,12]. In addition, the angle of hyphal branching in Mucorales can vary from 45 to 90 degrees.^a Angioinvasion is seen in both fungal species. In the absence of culture results, accurate identification of the involved fungal species may not be possible.

Treatment of post-transplant mucormycosis include early surgical debridement if possible especially in rhino cerebral infection along with antifungal therapy including polyene (Amphotericin B), triazoles (posaconazole and isavuconazole), and echinocandins with combination of previous agents [13].

The transplant in our case was preceded by non-myeloablative conditioning and with PBSC. In an analysis of all syngeneic transplants performed in SAA in the EBMT registry, 88 patients undergoing 113 syngeneic SCT were reported between 1976 and 2009. Results showed that engraftment was similar in patients with or without conditioning but it was more rapid with PBSC compared to BM (median 12 days vs. 17 days; $P = 0.001$). With a median follow up of 7.28 years, survival showed a 10-year overall survival (OS) of 93%. As for graft failure (GF), a multivariate analysis of all transplants showed that the lack of conditioning has a statistically significant influence on the risk of GF. Another significant risk factor for GF was graft source with higher 3-year incidence for BM compared to PBSC [14].

In our case, the patient underwent syngeneic SCT from his twin brother despite invasive mucormycosis infection. A multicenter prospective study looking at the risks and outcomes of IFI in SCT recipients after non myeloablative conditioning showed that acute GVHD grades III to IV, chronic extensive GVHD, corticosteroid therapy (2 mg/kg/d prednisolone equivalents), and positive CMV antigenemia were all associated with increased risk of invasive mold infections [15]. Once IFI diagnosis is made, outcomes were dependent on GVHD treatment requirements. The 1-year OS was higher (44%) in patients receiving lower doses corticosteroids (2 mg/kg or less) compared to those receiving higher doses or immunosuppressive therapy such as sirolimus.

This data reinforces the feasibility of the syngeneic transplant in our case taking into consideration that our patient had negative CMV antigenemia, and will not require any corticosteroids or immunosuppressive therapy to prevent GVHD in this setting. Outcomes, thus, will not be affected by these factors.

In conclusion, our case confirms that syngeneic transplant is a reasonable option in SAA with active mucormycosis, taking into consideration the absence of need for corticosteroids and immunosuppressive therapy post-transplant.

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Conflict of interest

The authors have no conflicts of interest to declare.

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