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Current strategies against invasive fungal infections in patients with aplastic anemia, strong power and weak weapon, a case report and review of literature

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ABSTRACT

We report an 18 year old boy with Aplastic anemia complicated by serious fungal rhinosinusitis. Despite prompt treatment and early repeated surgical debridements, he died after about more than 6 weeks of hard challenges with fungal infections. Current strategies against invasive fungal infections (IFIs) in patients with Aplastic anemia may be inadequate for the management of serious complications. Antifungal prophylaxis is highly recommended in pre-transplant period for severe form of Aplastic anemia.
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1. Introduction

Aplastic anemia is characterized by bone marrow failure and marked decrease in all marrow elements. In severe form of *Aplastic anemia*, rapid bone marrow transplantation after primary workup is life saving; however, protected environment and prevention of opportunistic infections may be difficult in these cases [1].

Most patients with *Aplastic anemia* experience repeated episodes of infection during their life. Gram positive (predominantly gram-positive cocci) and gram negative organisms (especially Multi-Drug Resistance (MDR) negative bacilli) are the most common causes of infections, but IFIs remain the main cause of death and increase the mortality among respective patients [2,3]. *Aspergillosis* and *Mucormycosis* are the most common mold infections in patients with *Aplastic anemia* [2].

In reported case series by Valera (2011), in 32 patients with acute invasive fungal rhinosinusitis, all deaths were reported among patients with *Aplastic anemia* despite surgical debridement and systemic antifungal therapy [4].

Severe neutropenia predisposes these patients to more severe forms of IFIs with a wide range of clinical manifestations. Gastrointestinal [5], upper air way [6], musculoskeletal [7] cardiac [8], renal [9], disseminated infection [10] and Rhinocerebral/sino-orbital/rhinosinusitis [11] among the most common reported manifestations of IFIs in patients with *Aplastic anemia*. Of these conditions, the last one is the most serious and fatal [4]. We report on a serious fungal infection in a case of *Aplastic anemia* and offer an appropriate strategy for the treatment and prevention in such patients.

2. Case

An 18 year old boy, known case of *Aplastic anemia* since 7 years ago, admitted with severe headache and fever in emergency ward. He was a candidate for bone marrow transplantation because of standard treatment failure that included corticosteroid, anti-thymocyte globulin (ATG) and cyclosporine, and put on in transplant waiting list. He had frequently received blood and platelet transfusion due to low hemoglobin (Hb) level and often nose bleeding.

On admission, he had severe leukopenia [white blood cell count (WBC): 100 (without cell differentiation)], anemia (Hb: 6.5) and severe thrombocytopenia (platelet count: 6000). Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were 130 and 48, respectively.

After initial assessment, broad spectrum antibiotics (Piperacillin-Tazobactam) were started for him. Two days later, he developed pain, swelling and redness of the right side of face.

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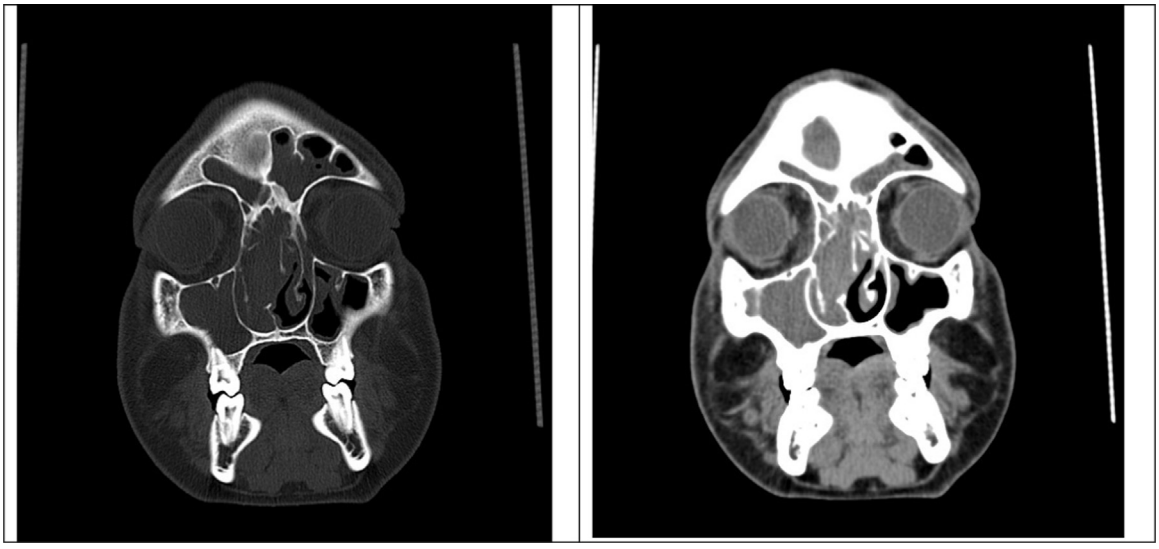

Fig. 1. Para-nasal CT scan (PNS CT) of patient before second surgical debridement which revealed complete right maxillary sinus opacity and involvement of bilateral ethmoid sinuses without any obvious bone destruction.

Gradually, the patient exhibited high grade fever, chills, intolerable headache and periodic disorientation, so due to poor clinical response, an antifungal agent (Amphotericin-B deoxycholate) was added to his antibiotic regimen on fifth day of admission.

In serial physical examination (after primary unilateral face swelling and cellulitis), he developed necrotic lesions in soft and hard palate followed by nasal septum, right alar groove and right nasolabial fold necrosis.

Tomography scan (CT scan) was requested, which revealed right maxillary and ethmoidal sinus involvement (Fig. 1).

Surgical consult has been also requested for diagnostic aspiration and evaluation for surgical debridement. Despite low platelet count, after receiving single donor platelet transfusion, right maxillary sinus debridement was performed and samples were sent for pathology. Fungal elements similar to mucoral hyphae were reported by pathologist.

After primary aspiration, right maxillary sinus debridement was performed during single donor platelet transfusion and samples were sent for pathology. Upon early surgical debridement and short time clinical improvement, all signs and symptoms exacerbated again after few days. Caspofungin was added and second surgical debridement planned 10 days after the first one, and has been organized for short interval surgical sinus debridements during platelet transfusion, till it becomes completely clear.

In the next surgical debridement tissue samples were cultured on Sabouraud dextrose agar (Merck, Darmstadt, Germany) and also examined for Aspergillus and Candida DNA by real time polymerase chain reaction (PCR) and Mucoral by nested PCR [12,13]. Bacterial culture and specimen were sent for microbiology and pathology, respectively.

Other successful debridements were done twice in short intervals. Finally, all involved sinuses, nasal cavity and overlying soft tissue were completely removed by anterior and posterior ethmoidectomy and sphenoidectomy. Also, posterior part of septum was removed. Detailed information about time and results of clinical samples were included in Table 1.

Adjuvant therapy with gamma interferon 100 μgr/day in combination with Granulocyte-colony stimulating factor (G-CSF) [300 μgr/day primarily and then with full dose of 600 μgr/day in two divided doses] was added to the broad antibacterial and antifungal treatments.

Other assessments including blood culture and urine culture were negative and chest x-ray, abdominal ultrasonography and echocardiography were normal in primary evaluations.

However in serial chest x-rays, early possible signs of pulmonary involvement detected in about 3 weeks after his admission (Fig. 2).

Bilateral well-circumscribed ground-glass gray opacities were detected in these chest x-rays confirmed by spiral chest CT scan (Fig. 3).

Our further investigation into fungal infection revealed positive *Mucormycosis*, *Aspergellosis* and *Candidiasis* by PCR and positive fungal culture for *Aspergillus flavus* and *Candida albicans* in repeated debridement (Fig. 4C).

No positive culture was obtained from *Mucormycosis*. During admission, the patient had several positive blood cultures (Table 2). His antibiotic was changed based on antibacterial susceptibility test. Sinus debridement during antifungal treatment was done in four times, but the patient's condition gradually worse and eventually expired.

Table 1
Summary information of repeated debridement and direct mycology test results.

	Date*			pathology	Tissue PCR *Aspergillosis*	Tissue PCR *Mucormycosis*	Tissue PCR *Candida*	Culture
First surgical debridement	20	July	2015	*Mucormycosis*	Not sent	Not sent	Not sent	
Second surgical debridement	30	July	2015	*Mucormycosis*	+	Neg.	Neg.	
3th surgical debridement	7	August	2015	*Mucormycosis*	+	Neg.	+	+**
4th surgical debridement	21	August	2015	*Mucormycosis*	+	+	+	

* Admission date: 13 July 2015.
** Aspergillosis Flavus.

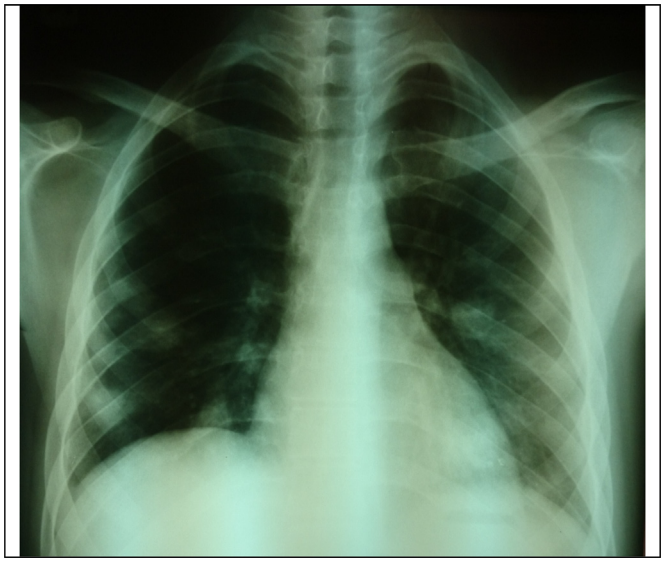

Fig. 2. Multiple bilateral ground glass opacities on chest x-ray.

3. Discussion

Frequent episodes of profound and prolonged neutropenia, aggressive chemotherapy, exposure to fungal spores in non protected environment and consequently preadmission colonization with fungal agents and high risk condition such as *Aplastic anemia* and *Acute Myeloid Leukemia* (AML) are some of typical examples that increase the risk of IFIs [14].

Along with high clinical suspicions, early diagnosis and treatment; multidisciplinary approach against IFIs significantly reduced mortality [15]. Constantly low absolute neutrophil count (ANC), lack of standard and secure control measures such as high-efficiency-particulate-air (HEPA) in many centers and progressive nature of certain fungal infections such as *Mucormycosis*; all are important obstacles to our attempts toward controlling fungal infections in these patients.

Despite very low platelet count (less than 6×10^3/microliter), our patient underwent four times of successful surgical debridement, during continuous single donor platelet transfusions, without any hemostatic complications. In each times he was referred to operation room after preparation of donor platelet for infusion during surgical debridement (Fig. 5).

Along with severe neutropenia, prolonged hospital stay and broad spectrum antibiotic therapy with damaged skin and mucosal barriers and presence of indwelling catheters predisposed our patient to colonization and infection with hospital acquired multidrug resistant organisms. Such patients are at greater risk for acquisition of MDR gram positive and gram negative organisms such as *Acinetobacters*, *Methicillin-resistant Staphylococcus aureus (MRSAs)* and *vancomycin-resistant Enterococcus (VREs)* [2]. This is an important point in management of these patients that should also take into consideration such as our patient which finally complicated by VRE Bacteremia.

Diagnostic misconception based on primary clinical presentation is one of the other important challenges in the management of IFIs in severe neutropenic patients.

Although rhinocerebral/sino-orbital/rhinosinusitis IFIs are frequently considered as clinical manifestation of *Mucormycosis*, it is critical to obtain proper tissue specimens for culture and biopsy, whenever possible; given the similar clinical presentations of *Aspergillosis*. Mucoral family is difficult to cultivate and sensitivity of culture for this family was reported around 50% [16] and thus other diagnostic modalities (histopathological carachtristics and molecular test) should be considered, if needed.

Co-detection of multiple fungi also is not an uncommon event in severe neutropenic patients and accurate diagnosis can help correct decision making in choosing proper antifungal regimen.

Although fever-driven approach for empirical antifungal therapy; based on existing guidelines is currently applied in many hematology oncology centers worldwide, but this issue continues to be a challenge and IFIs still is one of the leading causes of mortality and morbidity in neutropenic patients with persistent fever [17].

Other different treatment approaches either pre-emptive (diagnostic-driven approach) or targeted therapy also may be used, according to radiological findings, clinical symptoms and mycology test results. These approaches have been tested by researchers in different populations and in various settings in neutropenic patients.

Despite the fact that implementation of these strategies substantially has reduced the burden of IFIs in high risk patients, antifungal prophylaxis still is an important strategy for the prevention of IFIs in certain circumstances.

Currently, there is no recommendation about starting antifungal prophylaxis for patients with *Aplastic anemia* (as a one of

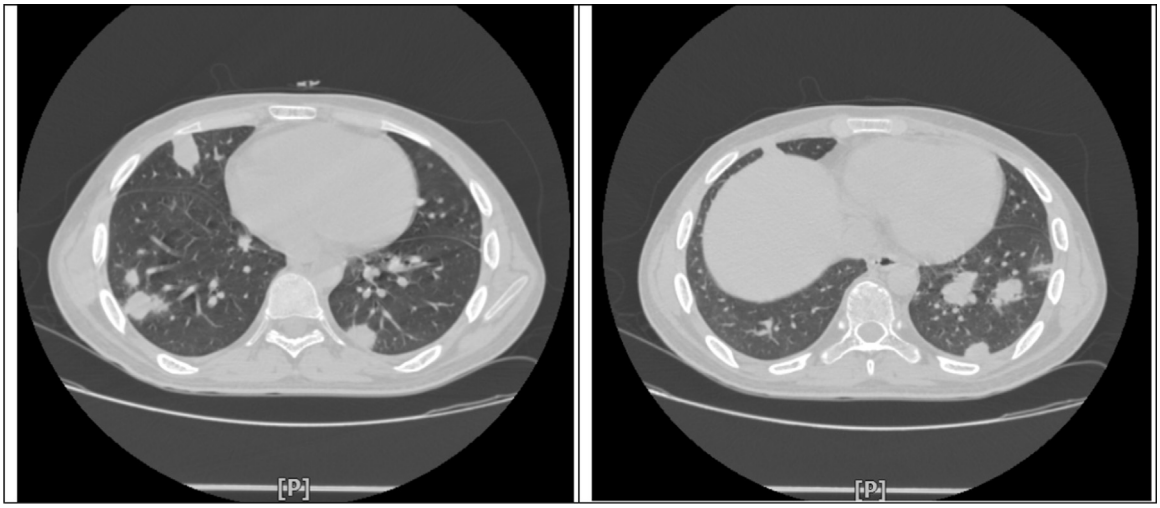

Fig. 3. Multiple bilateral round dense ground glass opacities on spiral chest CT scan.

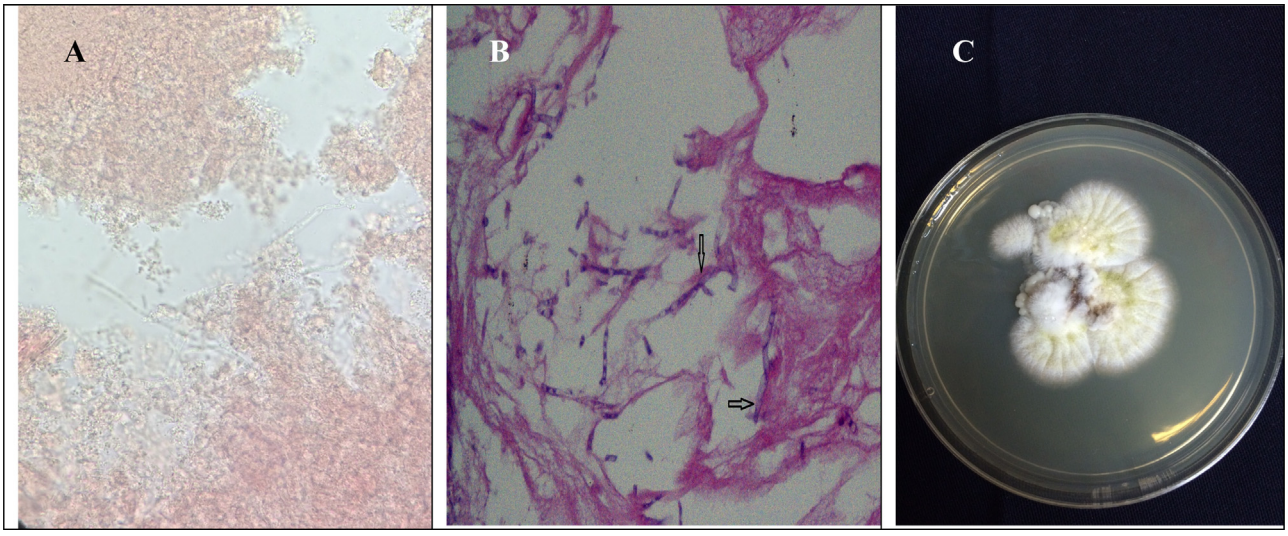

Fig. 4. A, KOH preparation reveals hyphea, B: Histology show broad septated hyphea mixed with non-septated hyphea (H&E X 250); C: Positive culture for *Aspergillus flavus* and *Candida albicans* on Sabouraud dextrose agar.

Table 2
Summary of indirect mycology test, bacterial cultures, antifungal agents and antibiotics in the course of treatment.

Date	Blood PCR *Aspergillosis*	Blood PCR *Mucormycosis*	Blood PCR candida	Culture	Antibiotic regimen	Antifungal and adjuvant therapy
18 July 2015	*	*	*	Neg.	Clindamycin + Tazocin	Amp-B deoxycholate[a,b]
20 July 2015	Neg.	Neg.	Neg.	Neg.		IVIG[c]
2 August 2015	Neg.	Neg.	Neg.	Neg.	Vancomycin + Imipenem	Caspofungin[d]+gamma-INF added[e]
16 August 2015	Neg.	Neg.	Neg.	Non hemolytic streptococcus group D	Vancomycin switched to Targocid	**
22 August 2015	Neg.	Neg.	Neg.	Non hemolytic streptococcus group D	Linezolid start and B/C (BACTE) send to CMRC[f]	**
26 August 2015	*	*	*	VRE[g]		**

[a] Amphotericin B (Amp-B) added to his antibacterial regimen because of poor clinical response before any clinical evidence of fungal infection in ENT.
[b] Change to liposomal Amphotericin B because of hypokalemia in 22 July 2015.
[c] Intravenous immunoglobulin added in 31 July 2015 as a single dose.
[d] Caspofungin added in 20 July 2015 because of poor clinical response and ocular involvement.
[e] Gamma-INF 100 μgr plus G-CSF 300 μgr were added for 14 doses/every other day.
[f] Professor Alborzi Clinical Microbiology Research Center.
[g] Vancomycin resistant entroccocus.
[*] Not check.
[**] The patient maintained on combination antifungal treatment till end of admission.

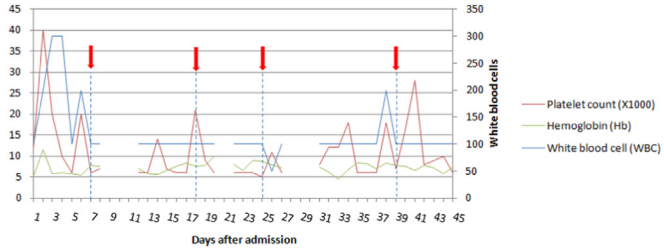

Fig. 5. Serial complete blood counts (CBC) with differential were recorded since admission. Red arrows demonstrate time of debridements. Note to very low platelet counts in the days in which patient candidate to surgical debridement. He received multiple donor platelet infusions during repeated operation.

the high risk groups) in the latest guideline of Infectious Diseases Society of America (IDSA, 2011) and 4th European Conference on Infections in Leukemia (ECIL-4, 2011) [18,19].

Although prompt diagnosis and early transplantation seem to be the only reliable modality in the protection of such patients against serious infectious complications (mainly IFIs), but based on our experience and also other similar reports we recommend that patients with *Aplastic anemia* receive anti-mold prophylaxis during the period of profound and prolonged neutropenia (ANC less than 500/ microliter) instead of other proposed strategies for the management of patients within pre-transplantation period. It should be noted that in the case of *Aspergillosis* indirect tests such as galactomannan and moleculer tests have been improved our diagnostic power for early detection of IFIs due to *Aspergillosis*. Yet, there is no standard diagnostic test for early detection of *Mucormycosis* except histopathology and culture [20].

Also, in centers with high incidence of *Mucormycosis*, it seems better to better that use an agent which is active against both *Mucormycosis* and *Aspegillosis* for prophylaxsis.

Conflict of interest

The authors did not have any financial or other relationships, which could be regarded as a conflict of interest.

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