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Full Length Research Paper

When and which fungal pathogens should come to mind in patients with hematological malignancies?

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We retrospectively evaluated the febrile neutropenia episodes of hematological patients and their outcomes with respect to fungal pathogens and antifungal therapy. The study covers all consecutive patients older than 14 years of age and who developed febrile neutropenia episodes from September 2011 and September 2012. 68 consecutive patients with neutropenia and their 129 febrile episodes were analyzed. Mean age was 59.36±15.22 years (range: 17-80 years) and 41 cases were male. MASCC score was 19.56 ± 9.04 in the patients with hematological malignancies. VOR, CAS and AM-B were used to treat in 40 episodes of 40 patients, 34 episodes of 27 patients and 12 episodes of 12 patients as first-line therapy, respectively. *Candida albicans* is still dominant in hematological patients followed by other non-albican *Candida* species that leads to fatal outcome in patients with history of azole exposure. Design and equipments (HEPA filter, single-room design, etc.) of hematology ward are very important for environmental contamination by *Aspergillus spp.* that leads to mortal and common invasive fungal infections in patient with hematological malignancies.

Key words: Hematological patients, febrile neutropenia,fungal pathogen, antifungal treatment, invasive fungal infection, candidemia.

INTRODUCTİON

Febrile neutropenia (FN) is generally a complication of cancer chemotherapy (Freifeld et al., 2011). Invasive fungal infections are important leading to poor outcome in patients with prolonged neutropenia (Ellis, 2008). Early diagnosis of fungal infection and antifungal treatment improves outcome (Aisner et al., 1977). Diagnosis of invasive fungal infections has been a challenge. Mortality related with FN has steadily decreased with guidelines, new laboratory tests and serial computed tomography (CT) scanning, between 5% in patients with solid tumors (1% in low-risk patients) and 11% in some hematological malignancies (De Naurois et al., 2010). Patients who have prolonged and profound neutropenia due to hematologic malignancy or allogeneic hematopoietic stem cell transplantation are at risk of invasive aspergillosis due to (De Naurois et al., 2010). Patients who have persistent neutropenic fever under antibacterial therapy should be undergone to serial CT scanning for early detection of pulmonary infiltrates related with invasive aspergillosis and then empirical antifungal therapy is recommended (De Naurois et al., 2010). However, invasive fungal infection was demonstrated among only 4% of patients who comprised 22-34% of the neutropenic patients who had cancer and received an antifungal drug according to established criteria (Walsh et al., 1999). CT scanning and galactomannan, with a reported sensitivity rate of 58-65% and specificity of 65-95% are recommended for preemptive antifungal therapy (Freifeld et al., 2011). Here, we retrospectively evaluated the febrile neutropenia episodes of hematological patients and their outcomes with respect

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Table1. Distribution of hematologic malignancies in the patients with febrile neutropenia (n:124).

Hematologic Malignancies	n (%)
Acute myeloblastic leukemia	37 (57)
Acute lymphocytic leukemia	17 (25)
Non-Hodgkin lymphoma	4 (6)
Multiple myeloma	1 (1)
Hairy cell leukemia	2 (3)
Mantle-cell lymphoma	1 (1)
Aplastic anemia	2 (3)
Plasma cell leukemia	2 (3)
Chronic myeloid leukemia	2 (3)
Total	68 (100)

to fungal pathogens, primary antifungal prophylaxis antifungal therapy.

MATERİALS AND METHODS

All consecutive patients between September 2011 and September 2012, who were older than 14 years of age and who developed febrile neutropenia episodes during chemotherapy due to hematological cancers in the hematology department at the Ministry of Health, Okmeydanı Training and Research Hospital, Istanbul, Turkey were included in the study. The hematology department is equipped with 23 beds. Patients rooms were designed as single, double and four-person without HEPA filters. Febrile neutropenia (FN) was defined as an oral temperature >38.5°C or two consecutive readings of >38.0°C for 2 h and an absolute neutrophil count $<0.5 \times 10^9$/L, or expected count to fall below 0.5×10^9/L (Freifeld et al., 2011). Collected data included patient demographics and diagnosis, episode data, clinical presentation and laboratory findings, clinical therapy, microbiological data and outcome. The febril neutropenia treatment protocol was based on clinical practice guidelines for the use of antimicrobial agents in neutropenic patients with cancer from 2002 and the update in 2010 by the Infectious Diseases Society of America (Freifeld et al., 2011; Hughes et al., 2002).

Empirical antifungal treatment was considered for patients with persistent or recurrent fever after 4-7 days of antibiotics and whose overall duration of neutropenia was expected to be more than 7 days. If CT showed changes associated with fungal infection, amphotericin B (conventional or liposomal, AM-B) or voriconazole (VOR) were initiated. Cavitation, air-crescent sign and halo sign were classified as the major changes. Nodules and new infiltrates, including consolidation and effusions, were classified as minor changes (Caillot et al., 2001). Possible invasive pulmonary aspergillosis (IPA) was defined as clinical and radiologic findings highly suggestive of without histopathologic and microbiologic evidence of infection. Probable IPA was defined as positive culture for Aspergillus species from a respiratory specimen or two consecutive galactomannan assays with an index of 0.5 and greater with clinical and radiologic findings suggestive of IPA. Proven IPA was defined as histopathologic evidence of tissue invasion and damage by Aspergillus species with clinical and radiologic findings (Ascioglu et al. 2002).

Caspofungin (CAS) was chosen as the empirical treatment for patients without the above-mentioned pulmonary findings. Galactomannan assay could not be implemented for all patients due to the unavailability of the test in the laboratory. Blood samples drawn from vein or catheter were inoculated into BactAlert 3D bottles (bioMérieux Diagnostics, Lyon, France). In addition, samples from urine, sputum, wound, conjunctive, abscess, blood and catheter were inoculated onto 5% sheep-blood agar (Salubris Inc., Istanbul, Turkey), or chocolate agar (Salubris Inc., Istanbul, Turkey) and Mac Conkey agar (Salubris Inc., Istanbul, Turkey). Isolated yeasts from blood cultures were identified by morphologic examination on Sabouraud Dextrose Agar (Unipath Ltd., Basingstoke, England) plates, germ-tube formation and API ID 32C (bioMérieux Diagnostics, Lyon, France) at the species level. The ATB Fungus 2 microdilution kit was used (bioMérieux Diagnostics, Lyon, France) for susceptibility testing according to the CLSI (formerly NCCLS) broth microdilution M27-A2 protocol. Aspergillus galactomannan antigen test was performed using the Platelia commercial enzyme immunoassay kit (Platelia Aspergillus EIA; Bio-Rad Laboratories, Marnes-la-Coquette, France). Blood samples of patients were analyzed twice weekly and results were computed as an index, which 0.5 and greater was considered as positive relative to optical density of the control sample measured with a semiautomatic analyzer (Behring ELISA processor III; Dade Behring, Marburg, Germany) (Maertens et al., 2004).

Possible causes of false positive and negative results were eliminated. The test was considered significant if patient had two consecutive galactomannan assays with an index of 0.5 and greater under persistent or recurrent fever after 4-7 days of antibiotics with or without microbiologic or radiological findings associated with fungal infections as mentioned above and whose overall duration of neutropenia was expected to be more than 7 days (Freifeld et al., 2011). Catheters were removed if patient was no responder to antifungal therapy with deteriorating clinical, radiological and laboratory findings. Response to treatment was defined as defervescence in 48-72 h subsequent to initiation antifungal therapy, recovery of increased C reactive protein (CRP) level, leukocytosis or leukopenia, recovery of vital signs and clinical symptoms associated with infection (improvement in arterial blood-gas values, radiological improvement, negative urine culture for urinary system infection and recovery of signs and symptoms related to other infections). In-hospital mortality during the neutropenic phase and the clinical outcomes of febrile neutropenic episodes were the primary consequences that were investigated in this study.

Statistical analysis

Continuous variables were described as mean± standard deviation and range. Percentile values were described without decimals. Overall mortality associated with febrile neutropenia was defined as death within 30 days after development of neutropenia.

RESULTS

We retrospectively analyzed 68 consecutive patients with neutropenia and their 129 febrile episodes. Mean age was 59.36±15.22 years (range: 17-80 years) and 41 cases were male. MASCC score was 19.56 ± 9.04 in the patients with hematological malignancies (Table 1). Systemic antifungal drugs was initiated to eight patients with 10 culture-proven fungal infections, 20 patients with probable pulmonary aspergillosis infection who had GM positivity and thorax CT findings in 23 attacks, and 15 patients with possible pulmonary aspergillosis infection who had clinical findings with thorax CT findings in 17 attacks, and in 21 attacks of 20 patients with suspected invasive fungal infection (Table. 2). VOR, CAS and AM-B

Table 2. Isolated fungal pathogens and antifungal treatment response in the patients with febrile neutropenia.

Patient	Age	Gender	Hematologic malignancy	Sample	Isolated fungal pathogen	Antifungal Resistance	Empirical antifungal treatment before identification	Treatment modification	Outcome
1	50	Male	AML+Lung cancer	Blood	*Candida parapsilosis*		VOR	From VOR to AM-B, central line removed	Survived
				Blood	*Candida albicans*	-	VOR	-	Survived
2	61	Male	AML	Blood, urine	*Candida albicans*				Died
3	73	Female	NHL	Blood, urine	*Candida albicans*				Survived
				Blood, Central Line	*Candida albicans*	-	AM-B	Central line removed	Survived
4	35	Male	AML	Blood	*Candida albicans*		CAS		Survived
5	44	Female	ALL	Blood	*Candida albicans*		AM-B		Survived
6	63	Female	NHL	Blood	*Candida krusei*		VOR	Catheter removed	Survived
7	63	Female	AML	Blood	*Trichosporon asahii*		AM-B	From AM-B to VOR, central line removed	Died
8	52	Female	ALL	Blood	*Geotrichum capitatum*	FLC, ITR	CAS	Central line removed	Survived

BAL, Bronchoalveolar lavage; VOR, Voriconazole; AM-B, Liposomal Amphotericin B; CAS, Caspofungin; FLC, Fluconazole; ITR, Itraconazole.

were used to treat in 40 episodes of 40 patients, 34 episodes of 27 patients and 12 episodes of 12 patients as first-line therapy, respectively. Two patients were treated with CAS due to hepatosplenic candidiasis depending on clinical and radiological findings. Central venous catheter could be removed from five of 10 patients whose blood cultures yielded yeast and two of those (40%) died (Table 2). There was no proven infection associated with *Zygomycetes* or *Fusarium* species. Two patients with acute lymphocytic leukemia were treated with a combination of antifungal drugs as salvage therapy with AM-B combined with CAS and VOR combined with CAS due to persistent fever and negative culture, progression in thorax CT findings and deterioration. Both responded to combination therapy. Overall one-year crude mortality rates were 25% (17/68). The number of patients who died of infections was 12 (17%). Of 12 fatal cases associated with infection, four died of culture-proven or probable invasive fungal infections (Table 2).

DISCUSSION

Invasive fungal infections are a complication of hematologic maligancies due to immunosuppresive conditions. *Aspergillus* and *Candida* species are the most common pathogens of invasive fungal infections in patients with hematological malignancies (Viscoli et al., 2005). A*spergillus* spp. is ubiquitous in the environment and is commonly isolated from environment including hospitals. It is more likely that our hematology ward has no single-rooms with HEPA filter and private toilet, probable and possible pulmonary aspergillosis infection predominated among invasive fungal infections in our study. In relation to that inconvenience, VOR was the most initiated anti-fungal drug with excessive number of patients. And also antifungal drugs were initiated in 71 of 129 episodes. That number indicates that frequencies of fungal infection attacks and initiations of antifungal drugs are related with setout and conditions of ward where patients with hematological malignancies are followed-up (Martino and Subirà, 2002). However, empirical antifungal therapy was reported to be initiated to 67% of patients followed-up at ward with low environmental contamination in microbiological surveillance (Montagna et al., 2012) Sputum and BAL cultures of three patients' yielded *Aspergillus fumigatus* in favor of probable pulmonary aspergillosis infection. Galactomannan which has positive predictive value with 10-94%

and negative predictive value with 95-98% depending on incidence of invasive aspergillosis between 0, 5 and 20%, was more used to diagnose aspergillus infection in our patients (Martino and Subirà, 2002).

Frequencies of invasive aspergillosis and candidiasis were reported as 5-24% that is lower than our numbers especially for aspergillosis among patients with hematological malignancies (Martino and Subirà, 2002). However, mortality rates were reported higher than those in our study. *Candida albicans* (n=6) predominated among fungal pathogens caused to blood stream infection in our study as reported in other studies (Zirkel et al., 2012). Only one patient had *Candida parapsilosis* related blood stream infection. Colonization of the mucosal membranes by *Candida* species preceded invasive diseases, while *Aspergillus* and other molds invade the respiratory tract (De Pauw and Donnelly, 2007). *C. parapsilosis* was also reported to be related with vascular catheters and parenteral nutrition (Clark et al., 2004). *C. tropicalis* was reported to be related with cancer and *neutropenia. C. krusei* and *C. glabrata* fungemias were reported to be associated with previous exposure to azoles (Bassetti et al., 2009). *Trichosporon asahii* and *Geotrichum capitatum* have emerged leading to death among our patients with blood stream infections. *Trichosporon* was reported as the second most common cause of disseminated yeast infections and also can cause to breakthrough under CAS treatment as found in our study (Miceli et al., 2011). Triazole (fluconazole, voriconazole) therapies are recommended with removing of central line (Colombo et al., 2011). *Geotrichum capitatum* (formerly *Trichosporon capitatum*) is a part of the normal flora of the human digestive and respiratory tracts and skin and may be a pathogen in case of disruption of physiological barriers in the digestive tract in patients receiving chemotherapy (Martino et al., 1990).

Voriconazole and micafungin were reported successful treatment regimens (Sagrahouni et al., 2012).

Although, catheter removal is recommended after identifying the candidemia, catheter removal by 24 or 48 h after treatment initiation was reported to have no effect on overall treatment response, mortality and mycological eradication in 842 patients with candidemia (Morgan et al., 2005; Nucci et al., 2010; Mermel et al., 2009). Catheter was removed from our patients if patients' conditions were convenient to remove it. Catheter should be taken into consideration especially in cases with either *Trichosporon asahii* and *Geotrichum capitatum* owing to the fact that they lead to fatal outcomes.

Conclusion

Candida albicans is still dominant in hematological patients followed by other non-albican *Candida* species that leads to fatal outcome in patients with history of azole exposure. Design and equipments (HEPA filter, single-room design, etc.) of hematology ward are very important for environmental contamination by *Aspergillus spp.* that leads to mortal and common invasive fungal infections in patient with hematological malignancies. Catheter should be removed in cases with fungal blood stream infection due to the fact that treatment response, colonization and outcomes are likely to be related with it.

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