Journal of Infection and Public Health (2017) 10, 49—58

http://www.elsevier.com/locate/jiph

A retrospective study of the epidemiology and clinical manifestation of invasive aspergillosis in a major tertiary care hospital in Bahrain

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Received 16 July 2015; received in revised form 23 December 2015; accepted 20 February 2016

KEYWORDS
Aspergillus;
Epidemiology;
Treatment;
Mortality;
Infection

Summary Limited data are available on the epidemiology, clinical manifestations and outcomes of patients with invasive aspergillosis in Bahrain. This study was conducted retrospectively to determine the epidemiology of invasive aspergillosis and its risk factors, clinical presentation, underlying conditions, and outcomes over the past five years in a major hospital.

The medical records of patients with positive Aspergillus cultures admitted to a major tertiary care hospital in Bahrain during 2009—2013 were reviewed. Cases were classified according to (1) the European Organization for the Research and Treatment of Cancer/Mycoses Study Group (MSG) criteria (proven, probable, possible IA or not classifiable) and (2) ''validated'' criteria to distinguish Aspergillus colonization from IA (putative or proven IA). Demographic, microbiologic and diagnostic data were collected, and outcomes were recorded.

A total of 60 patients were included, of whom 44 were colonized (73.3%), and 16 had probable IA (26.7%); no proven or possible IA cases were identified according to the EORTC/Mycoses Study Group (MSG) criteria. In comparison, with the alternative ''validated'' criteria, 32 were colonized (53.3%), 28 had putative IA (46.7%), and none had proven IA (0%). The lung was the most common site of infection,

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http://dx.doi.org/10.1016/j.jiph.2016.02.015

and *Aspergillus fumigatus* was the most commonly isolated species (53%). Mortality was 25% among colonized patients, 44% in probable cases and 32% in those with putative IA. All patients were immunocompromised or had one or more predisposing factors. Independent risk factors for death among patients with IA included older age, history of mechanical ventilation, renal replacement therapy and higher sequential organ failure assessment scores at diagnosis.

Introduction

Aspergillosis refers to a wide variety of diseases caused by members of the genus Aspergillus [1]. *Aspergillus* species are ubiquitous organisms commonly found in soil, water and decaying vegetation [2]. There are approximately 200 species of Aspergillus [3]. However, *A. fumigatus* is the most frequently encountered species in human disease, followed by *A. flavus*, *A. terreus* and *A. niger* [4,5].

There are several types of aspergillosis: the most common are allergic aspergillosis, aspergilloma and invasive aspergillosis [6].

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity reaction to the fungus, mostly due to *A. fumigatus*. It is commonly seen in patients with long-standing asthma or cystic fibrosis [7].

Aspergilloma is the most common form of pulmonary involvement by *Aspergillus* species. The aspergilloma (fungal ball) consists of a mass of fungal hyphae, inflammatory cells, fibrin, mucus and tissue debris and usually develops in a pre-existing lung cavity [8].

Invasive aspergillosis (IA) is a serious opportunistic infection that commonly affects immunocompromised patients, such as those with prolonged neutropenia and cancer [9]. Patients with immune suppression as a result of solid organ or bone marrow transplantation and patients with AIDS are also at high risk [10,11].

In clinical practice, a diagnosis of IA is often suspected when Aspergillus is isolated from non-sterile body sites, particularly tracheal and bronchial aspirates [12]. However, because *Aspergillus* spp. are ubiquitous, one must be cautious in attributing a pathogenic role to fungus isolated from these samples [13].

This study was therefore conducted to collect data from patients with either Aspergillus colonization or invasive disease in order to investigate the epidemiology of invasive aspergillosis and determine the risk factors for Aspergillus infections along with the associated clinical presentation, underlying conditions and outcomes.

Materials and methods

Patients and settings

The Salmaniya Medical Complex is the largest public hospital located in the Salmaniya district of Manama, in the Kingdom of Bahrain. Established in 1979, the hospital has approximately 1200 beds. The hospital receives an average of 900—1000 patients a day and employs more than 2000 physicians, nurses and workers. It is a multi-specialty tertiary hospital. It includes specialties in cardiology, dermatology, diabetes, endocrine glands, the digestive system, hematology, infectious diseases, internal diseases, kidney and lung diseases, rheumatic diseases and oncology; it also provides outpatient services.

In this retrospective study, the medical records of all patients with a positive Aspergillus culture admitted to the Salmaniya Medical Complex, a major tertiary care hospital in Bahrain, between January 2009 and December 2013 were reviewed. All consecutive adult (>14 years) patients with a culture, direct examination and/or histopathologic sample positive for *Aspergillus* spp. at any site were eligible for inclusion. The study was approved by the local ethics and research committees.

Data collection and outcomes

Outpatients were excluded from the study, which includes only hospitalized patients. Unfortunately, it was not possible to report the number of outpatients with a positive Aspergillus culture.

Collected patient data included demographics (i.e., age, sex, and nationality), risk factors, underlying diseases and clinical presentation, including signs and symptoms compatible with invasive fungal disease (that is, refractory or recrudescent fever, pleuritic chest pain or rub, dyspnea, hemoptysis or worsening lung function). The organs which were affected by Aspergillus and the isolated species were recorded. The date of the first positive Aspergillus culture was consider the date of diagnosis of IA.

Although *Aspergillus* species are well recognized as common environmental airborne contaminants, the detection of *Aspergillus*, especially *A. fumigatus* and *A. flavus* in sputum cultures from patients with appropriate predisposing conditions, is likely to be of diagnostic importance, and empiric antifungal therapy should be considered.

In our study, the microbiological diagnosis of aspergillosis from sputum depends on the Gram stain. To evaluate whether the sample is acceptable, the presence or absence of squamous epithelial cells, polymorphonuclear leukocytes and microorganisms was evaluated. Filamentous hyphae are usually seen in aspergillosis. A direct sputum smear using a wet preparation of 10% potassium hydroxide was performed in suspected cases or if hyphae were seen in the Gram stain. The presence of hyaline, branching, septate hyphae consistent

with Aspergillus in any specimen from a patient with supporting clinical symptoms was considered significant. A semi-quantitative, culture-based method involving ordinary media containing blood agar, in which Aspergillus can grow easily, and Sabouraud dextrose agar was used in suspected cases and in cases in which the Gram stain and/or wet preparation revealed hyphae. Aspergillus usually grows rapidly; incubation lasted for 7 days at 25 °C and 37 °C. Identification of most species of Aspergillus in culture is generally performed by examining the colony-level and microscopic morphological features of conidia and conidiophores.

Radiologic data from chest X-rays or computed tomography (CT) scans of involved organs were also collected. Findings suggestive of invasive pulmonary aspergillosis on chest CT scans were defined as ''typical'' if at least one of the following three signs was present: wedge-shaped lesion, halo or air-crescent sign, lung cavitation or nodule [14].

We also collected data on patients admitted to the ICU and acute illness severity scores, including the acute physiology and chronic health evaluation (APACHE) II score at admission [15] and the sequential organ failure assessment (SOFA) score [16] on the day of the positive Aspergillus culture. Our collected clinical data also included the use of mechanical ventilation, vasopressor agents and/or renal replacement therapy (RRT) both at the time of the first Aspergillus-positive culture and during the ICU stay.

Data regarding antifungal therapy and its duration as well as the survival rate were also recorded.

In this study, we classify patients using both the validated criteria and the old EORTC/MSG criteria (without GM) when the validated criteria were not available in our hospital, and we compare the results.

Cases were classified according to (1) The EORTC/Mycoses Study Group (MSG) criteria (proven, probable, and possible IA or not classifiable) [14] and (2) ''validated'' criteria to distinguish Aspergillus colonization from IA (i.e., putative or proven IA) [17].

Statistical analysis

Descriptive statistical analyses. Discrete variables are reported as counts (percentage) and continuous variables as the mean ± standard deviation or median (first to third interquartile range).

Results

Clinical characteristics of the data

Analysis of our data revealed that there were a total of 60 patients with a positive Aspergillus culture from 2009 through 2013, of which 40 were Bahrainis and 20 were non-Bahrainis. The male:female ratio for Bahrainis was 16:24, and that for non-Bahrainis was 15:5. The majority of patients were in the age group of 45—65 years, with a mean age of 52.85 years.

The clinical characteristics of the data are presented in Tables 1 and 2. Most patients were admitted for a medical condition ($n = 42$, 70%), and most were admitted for respiratory care ($n = 34$, 57%). As noted in Tables 1 and 2, all patients had one or more predisposing factors to Aspergillus infection. The most common underlying diseases were diabetes ($n = 14$, 23%) and chronic obstructive pulmonary disease (COPD) ($n = 10$, 17%). Malignancy was the underlying condition in nine (15%) patients; one patient (2%) had undergone solid organ transplants, and one patient had HIV. Fourteen (23%) patients were receiving corticosteroid therapy, seven (12%) patients were receiving radiotherapy/chemotherapy, and four (7%) were on immunosuppressive therapy. Neutropenia was present in four patients (7%); however, it was prolonged (>10 days) in only one patient.

Of all cases, 19 patients were admitted to the ICU, and the most common reason for admission was respiratory disease ($n = 18$, 30%). Sepsis and ARDS were diagnosed on the day of admission in 11 (18%) and four (7%) patients, respectively.

Of the 60 total patients, 16 had probable IA (26.7%), and 44 were colonized (73.3%); no proven or possible IA cases were identified (0%) according to the EORTC/Mycoses Study Group (MSG) criteria. In contrast, according to the alternative ''validated'' criteria, 32 patients were colonized (53.3%), and 28 had putative IA (46.7%); again, no proven IA cases were identified (0%) (Tables 1 and 2).

Aspergillus fumigatus was the most commonly isolated species ($n = 32$, 53%), followed by *Aspergillus niger* in 17 cases (28%), *Aspergillus flavus* in seven (12%), and *Aspergillus* spp. in four (7%) specimens (Table 3).

Clinical signs and radiological imaging

Clinical and radiologic findings are described in Tables 4 and 5. In the current study, 60% of patients presented with dyspnea, 33% had refractory fever, and 18% suffered from worsening lung function. Chest pain and hemoptysis were observed in 5% of patients. On the day of diagnosis of IA, patients with IA had more compatible clinical signs than those with colonization.

The radiologic findings were abnormal in all patients with IA. Chest CT was performed in 36 (60%) patients, and it revealed that there were significantly more radiologic findings typical of IA in patients with probable and putative IA than in those with colonization.

Site of infection and diagnostic classification

The lung and/or trachea were the most common sites of infection. Most species were isolated from sputum/ETA. Bronchoalveolar lavage (BAL) was performed in 12 (20%) patients (Table 6).

Our data revealed that COPD was observed more frequently in patients with IA than in patients with colonization. Furthermore, patients with IA were more likely to have hematologic malignancy than were those with Aspergillus colonization, which likely explains the greater proportion of patients receiving chemotherapy, radiotherapy and immunosuppressive drugs in these groups. Among the patients with IA, 26 (40%) stayed in an immunosuppressed state according to the EORTC criteria (Tables 4 and 5).

In addition, compared with colonized patients, patients with probable and putative IA also had higher SOFA scores on the day of the first positive Aspergillus culture and were more likely to have a diagnosis of sepsis or ARDS at ICU admission.

Table 1 EORTC/Mycosis Study Group (MSG) criteria classification: clinical characteristics of the data.

	All patients ($n = 60$)	Probable IA ($n = 16$)	Colonization ($n = 44$)
Male, *n* (%)	31 (52)	5 (31)	26 (59)
Underlying conditions, *n* (%)			
COPD	10 (17)	4 (25)	6 (14)
Chronic heart failure	3 (5)	0	3 (7)
Diabetes	14 (23)	6 (38)	8 (18)
Solid tumor	3 (5)	2 (13)	1 (2)
Hematologic cancers	6 (10)	6 (38)	0
Neutropenia	4 (7)	0	4 (9)
Radiotherapy/chemotherapy	7 (12)	7 (44)	0
Solid organ transplant	1 (2)	1 (6)	0
Immunosuppressive drugs	4 (7)	3 (19)	1 (2)
HIV	1 (2)	0	1 (2)
Liver disease	2 (3)	0	2 (5)
Chronic hemodialysis	1 (2)	0	1 (2)
Smoking	18 (30)	6 (38)	12 (27)
Diagnostic categories, *n* (%)			
Medical admission	42 (70)	9 (56)	33 (75)
Cardiovascular	4 (7)	0	4 (9)
Respiratory	34 (57)	9 (56)	25 (57)
Gastrointestinal	1 (2)	0	1 (2)
ID	2 (3)	0	2 (5)
Rheumatology	1 (2)	0	1 (2)
Hematology/oncology	6 (10)	5 (31)	1 (2)
Nephrology	2 (3)	1 (6)	1 (2)
Post-operative	9 (15)	1 (6)	8 (18)
Trauma	2 (3)	0	2 (5)
Others	1 (2)	0	1 (2)
ICU admission	19 (32)	11 (69)	8 (18)
Severity scores and main diagnoses			
APACHE II score on admission	15 (17—28)	11 (18—30)	4 (17—28)
Sepsis on ICU admission	11 (18)	6 (38)	5 (11)
ARDS on ICU admission	4 (7)	3 (19)	1 (2)
Septic shock, *n* (%)	8 (13)	5 (31)	3 (7)
Pneumonia, *n* (%)	18 (30)	12 (75)	6 (14)
Antifungal therapy, *n* (%)	15 (25)	6 (38)	9 (20)

APACHE, acute physiology and chronic health evaluation; ICU, intensive care unit; ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; IA, invasive aspergillosis.

These patients additionally had a greater requirement for mechanical ventilation, vasopressors or RRT during their ICU stay.

The length of ICU stay was similar in patients with probable IA, putative IA and Aspergillus colonization (median, 10 days [1st—3rd quartile, 3—27.5 days] for both probable and putative IA vs. 13 days [1st—3rd quartile, 4—20 days] for colonization).

Antifungal therapy and outcomes

Antifungal therapy was started in 19 (32%) patients. In 41 additional cases, therapy was not administered on the basis of a clinical judgment call. The median time from positive Aspergillus culture to initiation of treatment was 2 (0—5) days for patients with Aspergillus colonization, 1 (0—5) day for probable IA and 1 (0—4) day for putative IA.

Regarding types of antifungal therapy, voriconazole was the treatment of choice for 15 patients; the remaining four patients received fluconazole, which is also an antifungal triazole but is not active against invasive aspergillosis, so we regarded those patients as having no therapy. Regarding the mortality rate, it was higher among those who received fluconazole (75%) than those who received voriconazole (36%).

Mortality was higher in patients with probable IA (seven of 16, 44%) and putative IA (nine of 28, 32%) than in those with colonization (eight of 32, 25%). Voriconazole was given to five of 16 total patients with probable IA and to 10 of 28 total patients with putative IA; among those who received treatment, the mortality rate was 40% in probable IA and 30% in putative IA cases. There were 18 untreated patients with probable IA and putative IA; among them, the mortality rate was 45% and 38%, respectively. As a result, no changes in overall survival were observed when patients who received voriconazole were compared with untreated patients (Fig. 1).

Table 2 Alternative ''validated'' criteria classification: clinical characteristics of the data.

	All patients ($n = 60$)	Putative IA ($n = 58$)	Colonization ($n = 32$)
Male, *n* (%)	31 (52)	14 (50)	17 (53)
Underlying conditions, *n* (%)			
COPD	10 (17)	9 (32)	1 (3)
Chronic heart failure	3 (5)	1 (4)	2 (6)
Diabetes	14 (23)	6 (21)	8 (25)
Solid tumor	3 (5)	2 (7)	1 (3)
Hematologic cancers	6 (10)	6 (21)	0
Neutropenia	4 (7)	1 (4)	3 (9)
Radiotherapy/chemotherapy	7 (12)	7 (25)	0
Solid organ transplant	1 (2)	1 (4)	0
Immunosuppressive drugs	4 (7)	3 (11)	1 (3)
HIV	1 (2)	0	1 (3)
Liver disease	2 (3)	0	2 (6)
Chronic hemodialysis	1 (2)	0	1 (3)
Smoking	18 (30)	11 (39)	7 (22)
Diagnostic categories, *n* (%)			
Medical admission	42 (70)	19 (68)	23 (72)
Cardiovascular	4 (7)	1 (4)	3 (9)
Respiratory	34 (57)	18 (64)	16 (50)
Gastrointestinal	1 (2)	0	1 (3)
ID	2 (3)	0	2 (6)
Rheumatology	1 (2)	0	1 (3)
Hematology/oncology	6 (10)	5 (18)	1 (3)
Nephrology	2 (3)	1 (4)	1 (3)
Post-operative	9 (15)	2 (7)	7 (22)
Trauma	2 (3)	0	2 (6)
Others	1 (2)	0	1 (3)
ICU Admission	19 (32)	11 (39)	8 (25)
Severity scores and main diagnoses			
APACHE II score on admission	15 (17—28)	11 (18—30)	4 (17—28)
Sepsis on ICU admission	11 (18)	6 (21)	5 (16)
ARDS on ICU admission	4 (7)	3 (11)	1 (3)
Septic shock, *n* (%)	8 (13)	5 (18)	3 (9)
Pneumonia, *n* (%)	18 (30)	12 (43)	6 (19)
Antifungal therapy, *n* (%)	15 (25)	10 (36)	5 (16)

APACHE, acute physiology and chronic health evaluation; ICU, intensive care unit; ARDS, acute respiratory distress syndrome; COPD, chronic obstructive pulmonary disease; IA, invasive aspergillosis.

Table 3 Isolated *Aspergillus* species.

Aspergillus species	NO	%
A. fumigatus	32	53
A. niger	17	28
Aspergillus flavus	7	12
Aspergillus spp.	4	7
Total	60	100

Furthermore, among patients with IA, independent risk factors for mortality were older age, higher SOFA scores and the need for mechanical ventilation or RRT.

Discussion

Aspergillus spp. are ubiquitous filamentous fungi that are widespread in the environment and are responsible for a broad spectrum of illnesses, from saprophytic colonization to rapidly disseminated and invasive disease.

The true epidemiology of IA remains uncertain and may be influenced by many environmental factors and diagnostic strategies.

In our retrospective study of 60 patients with Aspergillus-positive cultures, 26.7% were diagnosed with probable IA and 46.7% were diagnosed with putative IA according to the EORTC/Mycoses Study Group (MSG) criteria [14] and the alternative ''validated'' criteria [17]. Compared with patients with Aspergillus colonization, more patients with IA had COPD, hematologic malignancies and/or were receiving radiotherapy/chemotherapy; additionally, most patients were on immunosuppressive drug or corticosteroid therapy. In reviewing the surveillance of IA in Europe, the USA and Japan, we found that over 10 million patients are at high risk of invasive aspergillosis (IA) each year because of corticosteroid treatments or other immunosuppressive therapies. Among those cases, more than 200,000

Table 4 EORTC/Mycosis Study Group (MSG) criteria classification: clinical, radiologic and microbiologic findings correlated to Aspergillus diagnosis.

	All patients ($n = 60$)	Probable IA ($n = 16$)	Colonization ($n = 44$)
SOFA II score at diagnosis	26 (4—14)	11 (9—14)	15 (4—10)
EORTC host factors, *n* (%)			
Neutropenia	4 (7)	1 (6)	3 (7)
Allogeneic stem cell transplant	1 (2)	1 (6)	0
Prolonged use of corticosteroids	1	1 (6)	0
T-cell immunosuppressant	11	11 (67)	0
Inherited severe immunodeficiency	3	2 (13)	1 (2)
Abnormal radiologic findings			
Chest X-ray/CT scan, *n* (%)	48 (80)	16 (100)	32 (73)
Non-specific chest CT scan	32 (53)	0	32 (73)
''Typical'' chest CT scan findings, *n*	16 (27)	16 (100)	0
Mycological criteria			
Direct test	60	16 (100)	44 (100)
Indirect test	NA	NA	NA
Microbiologic findings			
BAL[a]/ETA/Sputum, *n*	60	16	44
Sputum, *n* (%)	30 (50)	10 (63)	20 (45)
ETA, *n* (%)	18 (30)	4 (25)	14 (32)
BAL[a] performed, *n* (%)	12 (20)	2 (13)	10 (23)
Isolated species, *n* (%)			
Aspergillus fumigatus	32 (53)	6 (38)	26 (59)
Aspergillus flavus	7 (12)	3 (19)	4 (9)
Aspergillus niger	17 (28)	3 (19)	14 (32)
Others	4 (7)	4 (25)	0
Organ support at time of diagnosis, *n* (%)			
Vasopressor therapy	8 (13)	5 (31)	3 (7)
RRT	3 (5)	1 (6)	2 (5)
Mechanical ventilation	11 (18)	5 (31)	3 (7)

[a] BAL, bronchoalveolar lavage; CT, computed tomography; EORTC, European Organization for Research and Treatment of Cancer; NA, not available; ETA, endotracheal aspirate; IA, invasive aspergillosis; RRT, renal replacement therapy; SOFA, Sepsis Organ Failure Assessment.

patients develop IA annually; the key groups that develop IA include acute leukemia patients, among whom approximately 10% develop IA (300,000 new acute leukemia cases and 30,000 IA cases, annually) and patients receiving stem cell and other transplants (>75,000 cases annually in the USA, Europe and Japan, approximately 10% develop IA, leading to 7500 IA cases). Additionally, 1.3% of COPD patients admitted to the hospital develop IA (7% of the global total of 65M moderate and severe COPD cases (WHO), leading to 60,000 confirmed IA cases) [18—20].

In patients with COPD, the frequency of Aspergillus isolates from lower respiratory tract samples has progressively increased [21]. In our study, 10 (17%) positive Aspergillus cultures were isolated from COPD patients; four patients were diagnosed with probable IA, and nine patients were diagnosed with putative IA. The patients with IA frequently presented with severe disease, were recurrently admitted to the hospital with exacerbation of symptoms and had been receiving prolonged courses of corticosteroid therapy.

In one study, of 118 patients with COPD, 40% had IA and 60% were colonized [22]. The patients with IA in that study were categorized as having advanced respiratory disease according to the Global Initiative for Obstructive Lung Disease classification. The study showed that the patients with IA had higher severity scores and worse prognoses than the patients with Aspergillus colonization. The emergence and occurrence of IA in patients with COPD is mainly attributed to prolonged administration of corticosteroid therapy [23]. It appears that corticosteroids predispose patients to opportunistic infections through quantitative and qualitative functional impairment of neutrophil and macrophage function [23].

Invasive aspergillosis is a life-threatening complication as well in immunocompromised patients, particularly in those undergoing bone marrow transplantation, receiving chemotherapy for hematological malignancy and/or on immunosuppressive drugs [24]. Prolonged neutropenia and solid organ transplant have also been recognized as strong risk factors for IA [25—28].

In our study, six patients with IA had a hematologic malignancy and were receiving chemotherapy. The estimated mortality among those patients was 57%. In the present study, we reported on only one patient who had undergone renal transplantation, had a positive Aspergillus culture and was diagnosed with probable/putative IA. Neutropenia was noted in four patients with a positive Aspergillus culture, but it was prolonged in only one patient who was diagnosed with

Table 5 Alternative ''validated'' criteria classification: clinical, radiologic and microbiologic findings correlated to Aspergillus diagnosis.

	All patients ($n = 60$)	Putative IA ($n = 28$)	Colonization ($n = 32$)
SOFA II score at diagnosis	26 (4—14)	14 (9—14)	12 (4—10)
Clinical signs, *n* (%)			
At least one of the following signs			
Refractory fever	20 (33)	13 (46)	7 (22)
Pleuritic chest pain	5 (8)	3 (11)	2 (6)
Pleuritic rub	2 (3)	2 (7)	0
Dyspnea	36 (60)	25 (89)	11 (34)
Hemoptysis	5 (8)	4 (14)	1 (3)
Worsening lung function	11 (18)	5 (18)	6 (19)
Abnormal radiologic findings			
Chest X-ray/CT scan, *n* (%)	48 (80)	26 (93)	22 (69)
Non-specific chest CT scan	32 (53)	12 (43)	20 (63)
''Typical'' chest CT scan findings, *n*	16 (27)	16 (57)	0
Microbiologic findings			
BAL[a]/ETA/Sputum, *n*	60	28	32
Sputum, *n* (%)	30 (50)	16 (57)	14 (44)
ETA, *n* (%)	18 (30)	6 (21)	12 (37)
BAL[a] performed, *n* (%)	12 (20)	6 (21)	6 (19)
Isolated species, *n* (%)			
Aspergillus fumigatus	32 (53)	14 (50)	18 (56)
Aspergillus flavus	7 (12)	4 (14)	3 (9)
Aspergillus niger	17 (28)	6 (22)	11 (35)
Others	4 (7)	4 (14)	0
EORTC host factors, *n* (%)			
EORTC host factor present on diagnosis	26 (43)	21 (75)	5 (16)
Neutropenia	4 (7)	1 (4)	3 (9)
Malignancy under cytotoxic therapy	7 (12)	7 (25)	0
Glucocorticoid treatment	14 (23)	12 (43)	2 (6)
Inherited severe immunodeficiency	1 (2)	1 (4)	0
Organ support at time of diagnosis, *n* (%)			
Vasopressor therapy	8 (13)	5 (18)	3 (9)
RRT	3 (5)	2 (7)	1 (3)
Mechanical ventilation	11 (18)	5 (18)	3 (9)

[a] BAL, bronchoalveolar lavage; CT, computed tomography; EORTC, European Organization for Research and Treatment of Cancer; ETA, endotracheal aspirate; IA, invasive aspergillosis; RRT, renal replacement therapy; SOFA, Sepsis Organ Failure Assessment.

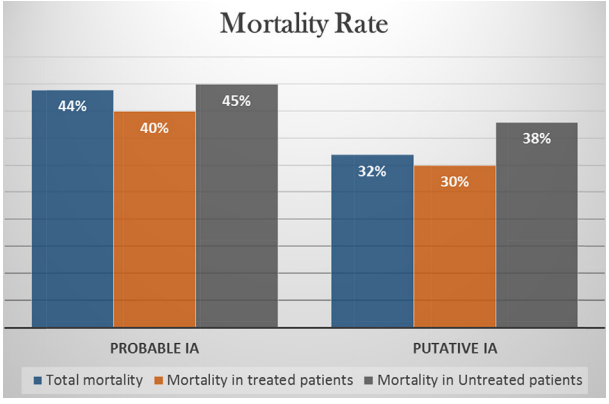

Figure 1 Mortality rate in patients with probable and putative IA.

probable/putative IA. Fungal infections continue to be a major problem for these patients [10]. As described by Gerson et al. [29], the key risk factor of IPA occurrence was the intensity and duration of neutropenia.

Another significant risk factor in this study was diabetes mellitus, which occurred in six patients with IA; five of them had more than one risk factor. Diabetes is a common metabolic disorder with significant morbidity and mortality. Diabetes is commonly considered as a risk factor of mycosis [10,30,31]. The most common fungal infections are isolated from the respiratory tract, skin and urinary tract [30]. However, the reason for high susceptibility in diabetic patients remains unclear.

Fever is a common presentation in neutropenic patients and should not be considered an index of aspergillosis [32]. However, in our study, 46% of patients who developed invasive pulmonary aspergillosis (IPA) had refractory fever in the days before IPA diagnosis. The other distinguishing clinical signs of IPA are chest pain

Table 6 Specimens with positive Aspergillus culture.

Sample	NO	%
Sputum	30	50
ETA[a]	18	30
BAL[b]	12	20
Total	60	100

[a] ETA, endotracheal aspirate.
[b] BAL, bronchoalveolar lavage.

and hemoptysis [33]. We observed these signs with a frequency of 11% and 14%, respectively.

In our study, the radiologic manifestations were nonspecific and may have been masked by an underlying acute process. Our study found only 16 patients with probable/putative IA who had a halo sign or cavitation visualized on chest CT scans. Although Barberan et al. revealed that radiologic worsening or cavitation visualized on chest X-rays and/or CT scans was associated with IA [34], in another study, up to 60% of cases of IA were exclusively diagnosed by autopsy because of the absence of reliable clinical and radiologic signs [35]. The use of a clinical algorithm to discriminate colonization from IA [17] remains a valid option for recognizing patients who require a more extensive diagnostic workup.

Compared with patients with Aspergillus colonization, patients with probable/putative IA are more critically ill and require ICU care. Patients with IA had a higher incidence of medically necessary ICU admissions and higher SOFA scores than patients with colonization. Furthermore, patients with IA more frequently presented with sepsis and/or ARDS upon admission, needed supportive therapy, mechanical ventilation or RRT and were associated with poor outcomes.

In the present study, 11 patients with probable/putative IA were admitted to the ICU with high APACHE II scores on admission. Six patients were diagnosed with sepsis and three patients with ARDS upon ICU admission. Five patients developed septic shock, and 5 patients required mechanical ventilation. Five patients received vasopressor therapy and 2 needed RRT.

Limited studies have been carried out on the epidemiology of IA in ICUs. In a large US cohort, ICU patients with aspergillosis were found to have several comorbidities, prolonged hospitalization, high mortality rates and increased costs [36]. More specifically, over 70% of patients required ventilation and received high-dose corticosteroids, and more than 35% had acute renal failure, COPD or septic shock.

In a multicenter Italian study, aspergillosis represented 35 of 384 invasive mycoses in ICU patients [37].

Antifungal therapy was started in 19 (32%) patients. No significant differences in the median time from positive Aspergillus culture to the initiation of treatment were reported between the groups (i.e., 2 days for patients with Aspergillus colonization, 1 day for patients with probable IA and 1 day for patients with putative IA).

Mortality was higher in patients with probable IA (7 of 16, 44%) and putative IA (9 of 28, 32%) than in those with colonization (8 of 32, 25%). Among patients with IA, no changes in overall survival were observed when patients who received antifungal treatment were compared with untreated patients (Fig. 1).

In our study, voriconazole was the treatment of choice for 15 of 19 total patients who received antifungal therapy. The mortality rate was higher in those who received other types of antifungal therapy (75%) than those who received voriconazole (36%).

Studies show that voriconazole has become the new standard of care in the treatment of patients with invasive aspergillosis, which may occur in immunocompromised patients, including patients experiencing hematologic cancers, allogeneic BMT and solid organ transplants. This has been established based on the results of a large, randomized study in which voriconazole proved superior to amphotericin B; 53% of subjects had complete or partial response to voriconazole, whereas only 32% had a similar response to amphotericin B [38].

Other independent risk factors were associated with mortality in patients with IA, including older age, higher SOFA scores and the need for mechanical ventilation or RRT.

Our study has some limitations. First, we could not use a specific multimodal diagnostic approach for our analysis because not all patients underwent CT scans. Furthermore, GM measurements are not available in our hospital. Additionally, we included only patients with Aspergillus-positive cultures, thereby eliminating patients with suspected disease based on radiologic imaging or biomarkers.

Second, we did not assess causes of death and cannot exclude the possibility that some patients died because of concomitant illnesses.

Third, we did not gather data on daily corticosteroid doses and could not fully assess the influence of dose or duration on mortality.

Fourth, autopsy would likely have increased the number of cases of IA diagnosis. Autopsy studies have shown that IA is the most commonly missed infectious diagnosis in patients needing ICU admission [39—41].

Conclusion

This study implies that clinicians should be aware of the potential fungal etiology of cases of refractory fever that are unresponsive to conventional medical therapy. Therefore, immediate testing, including sampling of respiratory fluids, biopsy specimens and other specimens that can be processed for fungal culture, must be part of a patient's diagnostic workup. There is a clear delay in the diagnosis and treatment of these cases.

Invasive aspergillosis is a life-threatening disease, so an aggressive approach for high risk patients is necessary; the approach should aim to recognize patients as soon as possible and initiate antifungal therapy promptly. Additionally, early treatment may improve the prognosis and outcome. However, this aim remains challenging to achieve. When diagnosis is confirmed, it is often already too late.

Authors' contribution

Jameela Alsalman is the main editor and involved in writing and revising the research. Thuraya Zaid involved in data collection, data entry, statistical analysis, writing and revising the research. Mohamed Makhlooq involved in

data collection, data entry and statistical analysis. Maysa Madan, Zahra Mohamed, Amani Alarayedh, Aysha Ghareeb involved in data collection and data entry. Nermin Kamal involved in data collection.

Funding

No funding sources.

Competing interests

None declared.

Ethical approval

Not required.

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