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ARTICLE

Culture-positive invasive aspergillosis in a medical center in Taiwan, 2000–2009

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Abstract We reviewed 776 patients who were culture positive for *Aspergillus* species at the hospital from 2000 to 2009. The isolates were collected for species identification by oligonucleotide hybridization and sequence analysis. A total of 96 cases of proven or probable IA were identified according to published criteria. The incidence of IA has increased significantly during the study period. *Aspergillus fumigatus* and *A. flavus* (41.7% each) were equally prevalent causative species. IA due to unusual species including *A. nidulans* (n=2), *A. versicolor* (n=2), and *A. tubingensis* (n=1) were also found. Among patients with IA, 55.2% had hematological disorder, 19.8% had underlying lung disorder, and 10.4% had autoimmune disease. The isolates species (P<0.001) and underlying disease (P<0.001) significantly affect the association of a positive culture with invasive disease. The overall mortality at three months was 62.5%, which remained stable throughout the study period. Multivariate analysis identified prior steroid use (P=0.007) as a significant risk factor for death, while surgery (P=0.030) and voriconazole (P=0.012) had protective effects. In conclusion, autoimmune disorders and underlying pulmonary diseases should also be considered as important predisposing factors of IA. Further emphasis on surgery and voriconazole in the management of IA might be beneficial.

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Introduction

Invasive aspergillosis (IA) is a significant cause of infectious mortality in severely immunocompromised patients [1–3]. Patients with hematological malignancies and recipients of haematopoietic stem cell (HSCT) or solid organ transplant (SOT) are high risk groups [4–9]. The majority of IA is caused by *Aspergillus fumigatus*, accounting for 60–90% of cases [4, 5, 7, 8]. IA is associated with poor prognosis, and reported mortality at three months ranged from 20% to 60% [1, 4–6, 9].

The European Organization for the Research and Treatment of Cancer/Mycosis Study Group (EORTC/MSG) definition of invasive fungal infection [10] has been widely adopted. The definition focused on highly immunocompromised populations (e.g. HSCT, SOT recipients). However, recent literature has described IA in various patient groups, including patients with chronic obstructive pulmonary disease (COPD) [7, 11], autoimmune diseases [12, 13], and intensive care unit patients [14]. Definitions of invasive pulmonary aspergillosis (IPA) for COPD patients have also been proposed [11].

While the spectrum of patients at risk of IA is expanding, most published studies target selected patient groups [2, 3, 7, 15, 16], and few surveys of the general patient

population that develops IA exist. The objective of this study was to investigate the clinical and microbiological features of culture-confirmed IA at a tertiary care center in Taiwan.

Materials and methods

Patients and setting

The study was carried out at the National Taiwan University Hospital, a 2,900-bed tertiary care hospital. Patients with cultures yielding *Aspergillus* species during the period of January 2000 to December 2009 were identified. A patient with multiple positive cultures during one infection episode was considered as one case. The medical records of these patients were reviewed to collect the following data: age, gender, underlying medical conditions, receipt of corticosteroid therapy, sites of infection, diagnostic procedures, results of galactomannan (GM) antigen detection by enzyme-linked immunosorbent assay (Platelia, Bio-Rad, Hercules, Calif.) if available, antifungal treatment, surgical procedures, and outcome. Patients with more than one risk factor were included in each risk group. GM antigen detection was introduced to our institute in January 2005. Patients with at least one GM index of 0.5 were recorded as having positive GM detection.

Definitions

Invasive mold infections were defined in accordance with the EORTC/MSG criteria [10], and in patients with COPD, the Bulpa criteria [11]. The use of steroids was defined as usage ≥0.2 mg/kg/day within three months prior to obtaining the positive culture. Infection with the involvement of two or more noncontiguous organ sites was considered as a disseminated disease. The day of diagnosis of IA was the day the first culture positive for *Aspergillus* species was obtained. The outcome of the patients was defined as their status at 3 months after diagnosis. Attributable mortality was defined as progressive organ failure involving the organ(s) in which IA was diagnosed and death due to the toxicity of antifungal therapy.

Microbiology

All isolates from the study cases had been identified by the conventional method of morphological identification. A total of 697 isolates were preserved for further species identification and confirmation by oligonucleotide array hybridization and sequence analysis as previously described [17]. Fungus-specific universal primers ITS1 and ITS4 were used to amplify the region comprising the ITS 1 and ITS 2 regions of the rRNA genes [17]. The determined sequence was then compared with sequences available in the GenBank using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

The association between isolated species and invasive disease was tested by the χ^2 analysis. The incidence of IA was compared between two year groups: 2000–2004 and 2005–2009 using the Student's *t*-test. Stepwise logistic regression model was used to examine the association of underlying conditions with invasive disease. The survival distribution was estimated by the Kaplan and Meier method, and the log-rank test was used to compare the survival curve of different groups. Multivariate analysis of risk factors associated with death was carried out by the Cox regression proportional hazards model. All parameters with a P value<0.05 in the univariate analysis were included in the multivariate analysis. P values <0.05 were considered as statistically significant in all analyses. The statistical software used was SigmaPlot (version 11, Systat Software Inc., San Jose, Calif.).

Results

A total of 776 patients with culture positive for *Aspergillus* species were identified. *A. niger* (26.8%) was the species most frequently isolated, followed by *A. flavus* (23.6%), and *A. terreus* (17.4%) (Table 1). When diagnostic criteria were applied, 96 patients were recognized as having either proven (n=35) or probable (n=61) IA. Nineteen patients had positive cultures obtained from usually sterile sites (blood cultures in 2, lung tissue in 7, pleural effusion in 6, and other tissues in 4).

A. fumigatus (40 patients [41.7%]) and *A. flavus* (40 [41.7%]) were the leading causative pathogens of IA. Several unusual species, including *A. nidulans* (2 [2.1%]), *A. versicolar* (2 [2.1%]), and *A. tubingenesis* (1 [1.0%]), were also found responsible for IA. The *A. nidulans* isolates were identified only to the genus level by conventional morphological method, and species level identification was achieved by oligonucleotide hybridization. The *A. tubingensis* isolate was initially reported as *A. niger*, but sequence analysis confirmed it as the former (GeneBank accession no. EU440769.1).

The *Aspergillus* species differ significantly in their association with invasive diseases (P <0.001). Although *A. niger* was the most frequently isolated species, it was rarely associated with IA (3 in 206 [1.5%]). On the contrary, 50% (2 in 4) of *A. nidulans*, 31.7% (40 in 126)

Table 1 Distribution of *Aspergillus* species in all cases with positive cultures and those with invasive aspergillosis (IA)

Aspergillus species	No. (%) of cases with positive culture results	
	All isolates	IA
A. flavus	183 (23.6)	40 (41.7)
A. fumigatus	126 (16.2)	40 (41.7)
A. nidulans	4 (0.5)	2 (2.1)
A. niger	206 (26.5)	3 (3.1)
A. sydowii	(1.9)	0 (0)
A. terreus	136 (17.5)	5 (5.2)
A. versicolor	22 (2.8)	2 (2.1)
A. tubingensis	1 (0.1)	1 (1.0)
Others[a]	4 (0.5)	0 (0)
Unidentified species[b]	79 (10.2)	2 (2.1)
Total	776 (100)	96 (100)

[a] Includes *A. clavatus* (n=3) and *A. glaucus* (n=1)

[b] These isolates were not preserved for further species identification

of *A. fumigatus* and 21.9% (40 in 183) of *A. flavus* isolates were associated with an invasive disease.

The annual hospital-wide incidence of IA has increased significantly during the study period, from an average of 0.099 cases per 1,000 admissions in 2000–2004 to 0.166 cases in 2005–2009 ($P<0.05$) (Fig. 1). This occurred in the context of a relatively stable incidence of *Aspergillus* isolation and a rising admission rate of cancer (including hematologic and solid organ) patients.

Predisposing factors were identified in 93 patients (Table 2). Patients with more than one risk factor were included in each risk group. Underlying hematological disorder, including malignancy ($n=41$) and non-malignant disease ($n=12$), was present in 53 (55.2%) patients. Sixteen (12.5%) patients were transplant recipients, 11 received HSCT and five received SOT. Autoimmune disease was diagnosed in 11(11.5%) patients, and underlying lung disease in 19 (19.8%). Worthy of note, four patients with underlying lung disease had concomitant autoimmune or hematological disorder, and were included only in the latter two groups in mortality analysis.

When patients with positive culture but no IA were compared with those with IA, underlying disease was found to have a significant impact on the association of a positive culture with invasive disease ($P<0.001$) (Table 3). A positive culture was associated with IA in >40% of patients with hematological malignancies, non-malignant hematological disorders, autoimmune diseases, HSCT, SOT, and neutropenia; 10–30% with underlying lung disease and HIV infection; and <10% in patients with solid organ cancer and diabetes mellitus.

The majority of IA was localized to the lungs (61.5%) and sinuses (15.6%). Disseminated disease was diagnosed in 5 (5.2%) cases (lung and CNS in 3, lung and skin in 2) (Table 4), and multisite involvement was found in 8 (rhinocerebral in 4, sinus and lung in 3, lung and ribs in 1). Fifty-one IA patients received GM antigen test and the result was positive in 33 (64.7%) patients.

Overall, 90 (93.8%) patients received antifungal therapy. Amphotericin B (including lipid formulation) was the most

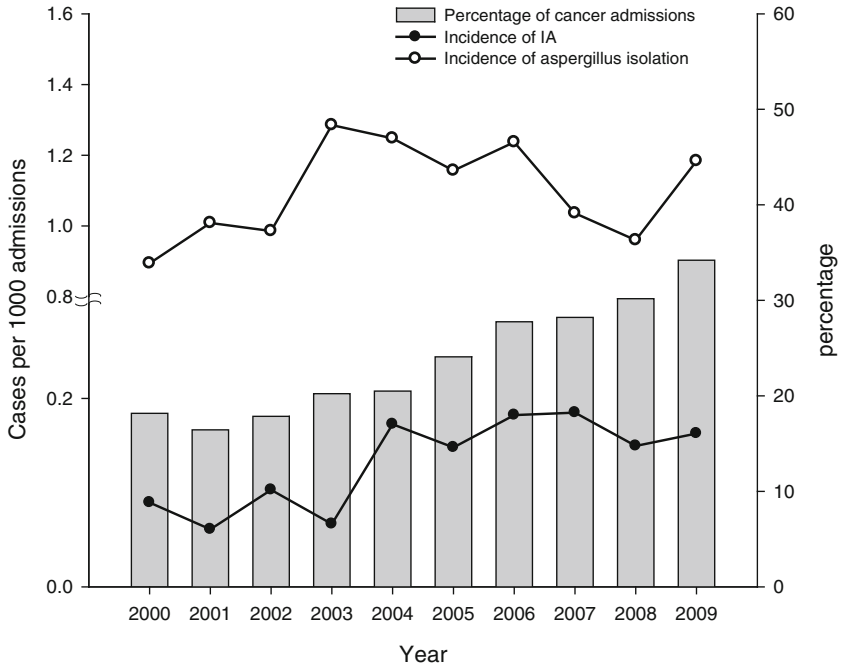

Fig. 1 Incidence of *Aspergillus* isolation, invasive aspergillosis, and number of hospitalized cancer patients, 2000–2009

Table 2 Clinical characteristics of 96 patients with proven or probable invasive aspergillosis, 2000–2009

Characteristic	Value / no. (%) of patients
Age in years, median (range)	47 (1–86)
Male	58 (60.4)
Underlying disease	
Acute leukemia	33 (34.4)
Non-Hodgkin lymphoma	7 (7.3)
Multiple myeloma	1 (1.0)
Aplastic anemia	7 (7.3)
Myelodysplastic syndromes	4 (4.2)
Myelofibrosis	1 (1.0)
Solid organ tumor	5 (5.2)
Autoimmune disease[a]	11 (11.5)
HIV infection	2 (2.1)
Chronic granulomatous disease	1 (1.0)
Underlying lung disease[b]	19 (19.8)
Diabetes Mellitus	3 (3.1)
HSCT	11 (11.5)
Solid organ transplant	5 (5.2)
Heart	1 (1.0)
Liver	1 (1.0)
Lung	3 (3.1)
Immunologic risk factors	
Steroid use	29 (30.2)
Neutropenia <500cells/μL	41 (42.7)
Site of infection	
Lung alone	59 (61.5)
Sinus alone	15 (15.6)
Disseminated	5 (5.2)
GM antigen positive	33 (34.3)
Surgical intervention	21 (21.9)
Antifungal therapy	
Amphotericin-B-containing	62 (64.6%)
Voriconazole-containing	38 (39.6)
Voriconazole monotherapy	10 (10.4)

HSCT haematopoietic stem cell

[a] Includes systemic lupus erythematosus (SLE) in 5 patients, rheumatoid arthritis in 2, Sjogren's syndrome in 1, coexisting SLE and Sjogren's syndrome in 1, dermatomyositis in 1, and pemphigus vulgaris in 1

[b] Includes sequelae of tuberculosis in 8 patients, COPD in 7, bronchiectasis in 3, pulmonary fibrosis in 1, bronchiolitis obliterans in 1, alveolar proteinosis in 1, bronchogenic cyst in 1, and sarcoidosis in 1

frequently administered antifungal agent, being used either alone or in combination in 62 patients (64.6%). Voriconazole was used in 38 (39.6%) patients, was the first-line therapy in 14 cases (14.6%) and monotherapy in 10 cases (10.4%). Other administered agents included itraconazole and echinocandins. Surgical intervention was carried out in 21 (21.9%) patients, including endoscopic sinus surgery in 7 patients, lung lobectomy in 6, wedge resection in 3, decortication in 2, finger amputation in 1, odontectomy in 1, and removal of Tenckhoff catheter in 1. Two patients were treated only surgically, both by endoscopic sinus surgery.

Table 3 Risk of invasive aspergillosis (IA) in patients who had an *Aspergillus* species isolated according to underlying conditions

Underlying conditions	Risk of IA / No. of cases (%)[a]
Non-malignant hematological disease	12/16 (75.0)
Neutropenia	41 /60 (68.3)
Solid-organ transplantation	5/10 (50.0)
Autoimmune diseases	11/23 (47.8)
HSCT	11/25 (44.0)
Hematological cancer	41/102 (40.1)
HIV infection	2/7 (28.6)
Underlying pulmonary disease	19/100 (19.0)
Solid-organ cancer	5/110 (4.5)
Diabetes mellitus	3/72 (4.2)

[a] Expressed as no. of cases of IA / no. of cases with *Aspergillus* species isolated

A total of 59 (61.5%) patients died within 3 months of receiving a diagnosis of IA. Of these deaths, 43 (72.9%) were attributed to IA. Mortality rate of different patient subgroups is as follows: autoimmune disease 81.8% (9 in 11 patients died), SOT 80% (4 in 5), HSCT 72.7% (8 in 11), haematological disorder 64.2% (34 in 53), and underlying lung disease 53.3% (8 in 15). Mortality rate was 58% (36 in 62) in patients treated with amphotericin-B-containing regimen, 52% (20 in 36) with voriconazole-containing regimen, 81% (13 in 16) with caspofungin monotherapy, and 85% (6 in 7) with fluconazole monotherapy. Mortality rate remained constant during the study period, being 60.6% in the years 2000–2004 and 61.9% in 2005–2009. However, in subgroup analysis, a trend of improved outcome was observed in patients with hematological disorder and SOT; the mortality rate was 71.4% in 2000–2004 and 58.3% in 2005–2009. The reasons for the improvement of outcome remained unclear.

Results of univariate and multivariate analysis are listed in Table 5. Univariate analysis demonstrated prior steroid use ($P<0.001$), positive GM antigen ($P<0.001$), and a disseminated disease ($P=0.006$) as poor prognostic factors, while surgery ($P=0.002$) and voriconazole ($P=0.02$) were protective against death from IA. Results of multivariate analysis were similar to those by univariate analysis except that disseminated disease did not reach prognostic significance. GM detection was not included in the multivariate analysis since only 51 patients received the test. In our analysis, neutropenia, receipt of HSCT and

Table 4 Summary of clinical characteristics and outcome of patients with disseminated invasive aspergillosis (IA) and IA caused by unusual species

No. (year)	Age/ sex	Underlying disease or conditions	Site(s) of infection	*Aspergillus* species	Specimen of isolation/ histology proof	Specimen with positive galactomannan	Antifungal therapy	Outcome
Disseminated IA								
1. (2005)	11/F	Sever aplastic anemia	Lung, skin	*A. flavus*	Sputum, skin / skin	Serum	CAS, L-AMB	Expired
2. (2006)	74/M	COPD, CKD	Lung, CNS	*A. fumigatus*	Sputum	Serum, cerebrospinal fluid	L-AMB, VOR	Expired
3. (2007)	27/M	NHL	Lung, skin	*A. flavus*	Sputum, skin	Serum	AMB	Expired
4. (2007)	47/F	SLE	Lung, central nervous system	*A. fumigatus*	Sputum	Serum, cerebrospinal fluid	AMB	Expired
5. (2009)	78/M	MDS with leukemic change	Lung, central nervous system[a]	*A. fumigatus*	Sputum	Serum	AMB, VOR	Expired
IA due to unusual species								
1. (2002)	26/F	ALL, HSCT	Lung	*A. versicolor*	Pleural effusion	Not available	AMB	Expired
2. (2002)	21/F	NHL, HSCT	Lung	*A. versicolor*	Sputum	Not available	L-AMB, AMB, ITR	Survived
3. (2006)	84/F	DM	Sinus, central nervous system	*A. nidulans*	Nasal swab/ sphenoid sinus	Not available	AMB, VOR	Expired
4. (2007)	55/M	ALL	Lung	*A. tubingensis*	Sputum	Serum	FLU	Expired
5. (2008)	22/F	Myelofibrosis, HSCT	Lung	*A. nidulans*	Sputum	Serum	VOR, AMB, CAS	Expired

[a] Head MRI revealed multiple nodular lesions with surrounding hemorrhage and halo zone edema, compatible with cerebral aspergillosis

SOT, underlying lung disease, and infecting species did not significantly affect mortality.

Discussion

Our understanding of the epidemiology and outcome of IAs has derived mainly from studies on selected patient subgroups. In this study, we collected cases of IA in the general patient population from 2000 to 2009 at a large Taiwanese institute. Major changes regarding the diagnosis and management of aspergillosis occurred during this period, including the introduction of voriconazole in 2004 and GM antigen detection in 2005.

An increase in the incidence of IA was observed over the past decade. The abrupt rise in incidence during 2003–2004 might be attributed to the severe acute respiratory syndrome outbreak in 2003, which resulted in much fewer hospital visits. Furthermore, the proportion of cancer admissions has increased steadily during the study period. This might contribute to increased cases of IA, since 47.9% (n=46) of IA in our series developed in patients with malignancies. Finally, it is also possible that IA was under-diagnosed in the early study period due to inadequate clinical suspicion. Increased IA incidence in the past two decades have been reported [2, 3]. A previous study on IPA conducted at our hospital during 1999 to 2005 also revealed a rise in incidence [18].

Overall, IA was diagnosed in 12.4% of patients from whom an *Aspergillus* species was isolated. This rate is similar to a previously published hospital-based survey [8]. Underlying conditions should be considered when evaluating the significance of a positive *Aspergillus* culture. Hematological disease and neutropenia are well-known risk factors for IA. However, in our series, autoimmune disease was also an important predisposing factor. Among cases with IA, 11.5% had autoimmune disease, and 47.8% of autoimmune patients with positive *Aspergillus* culture developed IA. Although infection is a major cause of mortality in autoimmune patients, invasive fungal infection occurring in this patient group has seldom been described [12, 13]. High SLE disease activity, *Aspergillus* infection, and receipt of high dose steroid following infection have been associated with high mortality [12, 13]. In our series, the mortality of autoimmune patients with IA was extremely high (9 in 11 patients, 81.8%), despite that most of them received appropriate therapy (amphotericin B in 6 patients, voriconazole in 2, and caspofungin in 1). The majority of patients (10 out of 11) received steroid therapy prior to acquiring infection, and both of the 2 patients who had pulse steroid therapy after being diagnosed with IA died within 1 month. Our finding indicates that autoimmune patients undergoing steroid therapy should be considered at

Table 5 Results of multivariate analysis of factors of prognostic significance of overall and attributable mortality at 3 months among patients with invasive aspergillosis

Variable	Reference category	Univariate	Multivariate	
		P	HR (95% CI)	*P*
ANC count				
<500 cells/μL	>500	0.200	–	–
HSCT				
Yes	No	0.893	–	–
SOT				
Yes	No	0.167	–	–
Underlying lung disease				
Yes	No	0.167		
Steroid use				
No	Yes	<0.001	0.5 (0.3–0.8)	0.007
Site of infection				
Disseminated	Single/multisite	0.006	1.9 (0.8–5.3)	0.167
Voriconazole use				
Yes	No	0.02	0.5 (0.3–0.9)	0.012
Underwent surgery				
Yes	No	0.002	0.4 (0.2–0.9)	0.030
GM antigen detection				
Positive	Negative	<0.001	–	–
Causative pathogen				
A. fumigatus	*A. flavus*	0.079	–	–

high risk of acquiring IA. More studies are needed to elucidate the burden of fungal infection in this patient group.

A. fumigatus is the predominant etiological agent of aspergillosis reported in the United States and Europe [8]. In the present study, however, *A. flavus* and *A. fumigatus* contributed the same proportion of IAs (41.7% respectively). Predominance of *A. flavus* has been reported in studies from India and Africa [19], which focused on sinusitis and keratitis, as well as in studies of the pediatric group [20]. This reflects the geographic and institution-specific difference in the relative distribution of *Aspergillus* species. More importantly, a large scale study in Taiwan disclosed *A. flavus* as the *Aspergillus* species least susceptible to amphotericin B in this region [21]. In the present series, however, the survival rates of IA caused by *A. flavus* and *A. fumigatus* were not significantly different (P=0.079).

In the present case series, we also identified several cases of IA caused by less common *Aspergillus* species. *A. nidulans* is strongly associated with osteomyelitis in patients with chronic granulomatous disease (CGD) [22, 23], but otherwise remains an infrequent cause of cerebral [24] and pulmonary [25] aspergillosis. Here we report invasive *A. nidulans* infection in two non-CGD patients. *A. tubingensis* is closely related to and morphologically indistinguishable from *A. niger* [26], and was first recognized as a cause of IA only recently [27]. In our series, it was responsible for IPA in a leukemic patient. As molecular methods become increasingly applied in species identification, more IAs caused by previously unidentifiable or erroneously identified species are expected to appear.

In our series, 61.5% of patients died within 3 months of diagnosis, and mortality rate remained unchanged throughout the study period. A trend toward improved survival was observed in hematology and SOT patients, a finding that was in accordance with previous reports [6, 9, 15]. However, there was a substantial increase in IA that occurred in autoimmune patients (9 out of the 11 cases occurred in the second half of the study period), whose mortality rate was over 80%. Our finding emphasized that while outcome of IA in patients with well-established predisposing factors has improved, more efforts should be directed to IAs that develop in less-studied patient groups.

Several important prognostic factors of IA were identified in this Taiwanese series. Receipt of steroid therapy was consistently associated with poor outcome of IA [6, 9]. Our study demonstrated high dose steroid therapy as an independent risk factor for mortality in patients with various medical conditions. While the sensitivity of GM detection for IA was only 64.7% in this study, a positive GM detection was correlated with significantly poorer survival. GM index has been proposed as a surrogate end point outcome of aspergillosis [28, 29], and our result further supports its role as an outcome indicator. Both

medical treatment with voriconazole and surgical intervention were associated with decreased risk of death. It is noteworthy that surgery was successfully undertaken in seven hematology patients in our study. One explanation for the protective effect of surgery was the intrinsically better prognosis of infections with operable lesions [16, 30].

This retrospective study had three noteworthy limitations. First, this study takes as its starting point patients who were culture positive for *Aspergillus* spp. and then seeks to identify from this small subset of cases thought to have proven or probable IA. The findings of this study do not represent the whole spectrum of IA in the hospital. Second, although a high overall mortality of IA was noted in this study, it is difficult to conclude that patients with IA and a positive culture have a more serious progression of disease. Third, this study was conducted in a tertiary care center and, as such, its findings might not reflect the overall situation in Taiwan.

In conclusion, the present series from Taiwan suggests that despite recent advances in the diagnosis and management of aspergillosis, the incidence of IA is rising and the outcome remains suboptimal. Autoimmune disorder and underlying pulmonary diseases are important predisposing factors of IA, aside from malignancies and receipt of transplantation. Physicians should maintain high suspicion of IA in caring for predisposed patients, and prompt administration of highly effective antifungal agents and surgical intervention may be of benefit.

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