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Invasive Aspergillosis

Disease Spectrum, Treatment Practices, and Outcomes

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Introduction

Invasive aspergillosis remains a major cause of morbidity and mortality in immunosuppressed patients (8, 12, 29, 36, 38, 40). Prognosis of invasive aspergillosis has in general relied on making a prompt diagnosis of infection, and in administration of high doses of amphotericin B (3, 8, 29). The utility of an early diagnosis and the institution of aggressive antifungal therapy have been suggested as a means for reducing the high mortality of disseminated aspergillosis (3, 46). Unfortunately, the rapid diagnosis of invasive aspergillosis is difficult, as no rapid methods to establish definitely the diagnosis of infection are available in most clinical settings (15, 44, 57, 60, 61, 64). An ELISA for detecting *Aspergillus galactomannan* is used to establish an early diagnosis in Western Europe, but it is not currently available for use in the United States (63, 65). Others have advocated use of radiologic techniques to establish an early diagnosis of infection, but clinical detection remains a central means of establishing a diagnosis of infection (2, 3, 30, 31, 35), as cultures may not be positive early in the course of infection (26, 32, 42).

The outcome of therapy for invasive aspergillosis is dependent not only on intensive antifungal therapy but also on recovery of underlying host defense defects such as resolution of neutropenia (21, 38, 50, 54, 66). Therapy of invasive aspergillosis has been difficult due to dose-limiting toxicity associated with amphotericin B deoxycholate (18) and due to the fact that other agents such as the azoles have limited utility in critically ill patients with invasive infection (9, 10, 11, 13, 58). Itraconazole is the only noninvestigational triazole antifungal with activity against *Aspergillus* species, but its use has been limited due to the fact that its oral absorption is erratic in critically ill patients. A solution offering better absorption than capsules and an intravenous formulation have only recently received approval for clinical use (1, 4, 27, 45, 48, 59).

In patients who have been treated with an initial course of amphotericin B, the sequential use of long-term itraconazole is attractive as a means to provide an extended course of *Aspergillus* therapy with reduced toxicity and ease of administration. However, extensive clinical data supporting this approach in invasive aspergillosis are not available and concerns regarding potential antagonism between amphotericin B and the azoles have muted clinical enthusiasm for this approach (52, 53).

In this study, we examined a large cohort of patients treated for invasive aspergillosis to establish the current epidemiology and clinical outcomes of infection in these patients. In addition, we also aimed to examine the utility of specific treatment regimens including sequential therapy with amphotericin B followed by itraconazole in this disease.

Methods

Investigators

In 1994–1995, investigators from the United States, Canada and Western Europe were selected by us (TFP and JRG) for inclusion in this study based on their likelihood of treating patients with invasive aspergillosis. Investigators were asked to provide case record data, which would be used to establish a database to assess current treatment practices and outcomes for patients with invasive aspergillosis. Case records were received from 89 physicians representing a diverse geographic area of the United States and Canada and also included 3 participants from Western Europe (see Appendix for full list of study investigators).

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Case record

We developed a case report form with limited and simple questions focusing on demographics, presentation of disease, management, and outcome, and asked each participant to describe on these forms the most recent cases treated for invasive aspergillosis, with a maximum of 10 per investigator to limit site case bias. Cases selected for review were to have been treated after 1990 with instructions for inclusion of most recent cases. The majority of cases reviewed were diagnosed and treated between 1994 and 1995. Participating physicians included infectious disease physicians, hematologists, oncologists, general internists, family physicians, physicians specializing in care of patients with HIV/AIDS, and pulmonary or intensive care unit specialists. Approximately half of the patients came from academic centers and half from private practices.

Data analysis

The case form included specific questions related to risk factors for infection, extent of infection, radiographic lesions, serology, cultures, histopathology, and therapy. Because lipid formulations of amphotericin B were investigational at the time of the study, few case records were received with patients on those therapies. In addition, because most of the patients receiving lipid therapies were participating in other clinical trials, outcome data from the 34 patients receiving liposomal amphotericin were excluded from analysis. The case record form was modified to request additional demographic and epidemiologic data in the last 280 patients reviewed. Data from case forms were extracted, entered into a central database, and analyzed. Statistical analysis included chi-square and Fisher exact test comparisons where appropriate (EpiInfo, Centers for Disease Control, Atlanta, GA).

Case definition

Cases were defined using modifications of the Mycoses Study Group Criteria (6, 58). Case forms requested submission of *definite* and *probable* cases of invasive aspergillosis. A definite case was defined as a compatible clinical illness accompanied by a tissue biopsy with isolation of *Aspergillus* species on culture and/or histopathology showing hyphae compatible with the organism. A probable case was defined as a compatible clinical illness including pneumonia, cavitary infiltrates or nodules, signs of sinusitis, hemoptysis, or pleuritic chest pain in a patient at risk for invasive aspergillosis who also had a bronchoalveolar lavage culture positive for *Aspergillus* species, 2 positive sputum cultures, or cytology smears. Radiographic signs such as the "halo sign" or "air crescent sign" on computed tomography (CT) scans of the chest (2, 30, 31) and the detection of *Aspergillus* antigen in serum or bronchoalveolar lavage were also considered criteria for a compatible clinical illness (63, 65). *Possible* cases were based on clinical characteristics including new pulmonary infiltrates in a high-risk patient but without documentation of mycologic evidence of invasive *Aspergillus* infection. Patients classified as having *central nervous system* (CNS) involvement included those patients with disseminated infection who were specifically noted to have infection at that site as well as patients with isolated CNS disease.

Treatment regimens and response

In this retrospective case study, each investigator independently selected treatment regimens including antifungal drugs and duration of therapy. Response rates were defined as *complete* if there was resolution of all signs and symptoms attributed to invasive aspergillosis and negative follow-up cultures if any were done. A *partial* response was defined as resolution of most but not all signs and symptoms attributed at baseline to aspergillosis. Patients were considered *stable* with a significant but less than major resolution in baseline signs or symptoms. *Antifungal failure* was defined as minimal or no improvement or progression of disease at the time of the last assessment, and *other failures* included toxicity or a concurrent independent process, as assessed by each clinical investigator, which caused progression of disease or death. There was no minimum duration of treatment for a patient to be evaluated.

Results

Demographics

Five hundred ninety-five cases were submitted for review. Of these, 439 patients (74%) had definite aspergillosis, and 135 patients (22%) had probable aspergillosis. Twenty-one patients (4%) were considered possible cases of invasive aspergillosis. The basis for the diagnoses was by cultures (511 positive cultures in 440 patients) and histopathology. Three hundred four patients (51%) had a positive biopsy, while 124 (21%) had invasive aspergillosis detected on autopsy. Overall, 222 (37%) patients were female and 373 (63%) were male. The mean age of the patients reported was 42.3 ± 18.4 years (range, 0–86 yr).

Predisposing risk factors for invasive aspergillosis are shown in Table 1. As expected the most common predisposing conditions were hematologic diseases including patients undergoing bone marrow transplantation (32%) and those with other hematologic conditions including leukemia or lymphoma (29%), which comprised 61% of the study population. Both autologous transplantation in 43 patients (7%) and allogeneic transplantation in 151 (25%) were significant underlying risk factors for invasive aspergillosis. Notably, a wide range of conditions was associated with the diagnosis although all of the other conditions

TABLE 1. Underlying diagnosis in 595 patients with invasive aspergillosis

Diagnosis	Number (%)
Bone marrow transplant	
Autologous	43 (7)
Allogeneic	151 (25)
Hematologic disease	
Leukemia/lymphoma/other	169 (29)
Solid organ transplant	52 (9)
AIDS	48 (8)
Other immune deficiencies	
Solid organ tumor	25 (4)
Chronic granulomatous disease	9 (2)
Other pulmonary diseases	56 (9)
Miscellaneous	33 (5)
None	9 (2)
Total	595 (100)

contributed less than 10% each to the study population. Only 9 patents (2%) had no apparent underlying condition before the diagnosis of invasive aspergillosis.

The absolute neutrophil count at time of diagnosis was requested from the last 280 patients studied. Of these 280 patients absolute neutrophil data were provided from 268: 110 (34%) had an absolute neutrophil count $<1{,}000/mm^3$, (90 [32%] $<500/mm^3$, and 34 [12%] $<100/mm^3$).

Identification of the *Aspergillus* species was provided in 261 cases. The most common *Aspergillus* species identified was *A. fumigatus* in 173 (66%), followed by *A. flavus* in 36 (14%). However, a wide variety of *Aspergillus* species were associated with invasive infection including *A. niger*, 17 (7%); *A. terreus*, 11 (4%); and other species in 5 patients (2%) (*A. nidulans*, 1; *A. versicolor*, 2; *A. oryzae*, 1; and *A. glaucus* group, 1.) The species was not identified or not reported in 19 (7%).

Radiographic findings

Of the 595 patients, 96% were reported to have had chest radiographs. Of these, 70% were abnormal and compatible with aspergillosis (that is, cavitating infiltrates, nodules, and focal infiltrates), 16% were abnormal but not thought to be suggestive of aspergillosis, and 10% were normal. One hundred fifty-six patients had pulmonary CT scans, and of these 85% were considered suggestive of aspergillosis, which included, but was not limited to, nodules with or without a "halo" or "air crescent" sign; 12% were abnormal but not suggestive of aspergillosis, and 3% were normal. Of 66 patients who had head CT scans, 53% were abnormal and suggestive of aspergillosis, while 24% were abnormal but not suggestive of aspergillosis, and 23% were normal.

Site of infection

Sites of involvement with invasive aspergillosis as determined by each investigator based on histopathology, radiology, and signs and symptoms are shown in Table 2. Patients were considered to have CNS involvement whenever specifically noted by the investigator. The majority of patients, 330 (56%), had disease restricted to the lungs, but disseminated infection was present in 114 (19%). An additional 34 patients (6%) had CNS infection. Isolated skin and sinus infections were not common in this series and were detected in 28 (5%) and 27 (5%) patients, respectively.

TABLE 2. Site of infection in 595 patients

Organ or Tissue	Number	(%)
Pulmonary	330	(56)
Multiorgan dissemination	114	(19)
Skin	28	(5)
Paranasal sinuses	27	(5)
Central nervous system	34	(6)
Tracheobronchitis	9	(1)
Other	53	(9)

Therapy

The treatment regimens used in these patients are shown in Table 3. Of the multiple regimens used, amphotericin B alone (187 patients, 31%), amphotericin B followed sequentially by itraconazole (93 patients, 16%), and itraconazole alone (58 patients, 10%) comprised the largest treatment groups, and about 57% of patients overall. The only formulation of itraconazole used in this study was the capsules. A variety of other antifungal combinations and mixed regimens were used, although the numbers in all of those groups were small with each comprising less than 5% of the study population. Thirty-four patients receiving lipid formulations of amphotericin B all received those drugs on an investigational basis so that outcomes of those patients were not considered as outlined above. Thus, further analysis of outcome focused on patients receiving amphotericin B alone, itraconazole alone and sequential therapy with amphotericin B followed by itraconazole.

Information on the duration of therapy with 1 of those 3 regimens was provided from 168 patients. In those patients receiving amphotericin B alone, amphotericin B was administered for a median duration of 15 days, but with a range from 1 to 116 days. In contrast those patients receiving itraconazole received therapy for a median duration of 90 days with the range from 1 to 380 days. Those patients receiving sequential amphotericin B to itraconazole received a median of 28 days of amphotericin B followed by another 35 days of itraconazole therapy.

Outcome

The outcome of invasive aspergillosis according to underlying primary diagnosis is presented in Table 4.

TABLE 3. Treatment regimens for 595 patients with invasive aspergillosis

	Number	(%)
Single drug regimens		
Amphotericin B	187	(31)
Itraconazole	58	(10)
Sequential regimens		
Amphotericin B to itraconazole	93	(16)
Amphotericin B to lipid formulation amphotericin B	21	(4)
Amphotericin B lipid formulation to itraconazole	13	(2)
Itraconazole to amphotericin B	10	(2)
Combination regimens		
Amphotericin B and flucytosine	10	(2)
Amphotericin B and rifampin	10	(2)
Amphotericin B and itraconazole	19	(3)
Miscellaneous other regimens	174	(28)

TABLE 4. Outcome according to primary diagnosis

	Complete Response No. (%)	Partial Response No. (%)	Stable No. (%)	Treatment Failure No. (%)	Other Failure No. (%)	Other No. (%)
Severely immunosuppressed	80 (22)	23 (6)	9 (2)	174 (48)	77 (21)	0
Auto BMT	5 (12)	4 (9)	0	24 (56)	10 (23)	0
Allo BMT	15 (10)	5 (3)	4 (2)	93 (62)	34 (23)	0
Hematologic malignancy	60 (36)	14 (8)	5 (3)	57 (34)	33 (19)	0
Not severely immunosuppressed	81 (35)	37 (16)	12 (5)	42 (18)	47 (20)	13 (6)
Solid organ transplant	25 (48)	6 (12)	0	14 (26)	6 (12)	1 (2)
AIDS	8 (17)	8 (17)	2 (3)	10 (21)	20 (42)	0
Solid tumor	6 (24)	7 (28)	3 (12)	4 (16)	2 (8)	3 (12)
Pulmonary diseases	20 (36)	11 (20)	6 (10)	2 (4)	12 (21)	5 (9)
Other	22 (43)	5 (10)	1 (2)	12 (23)	7 (14)	4 (8)
All patients	161 (27)	60 (10)	21 (4)	216 (36)	124 (21)	13 (2)

Abbreviations: auto BMT = autologous bone marrow transplant; allo BMT = allogeneic bone marrow transplant.

Those patients with most severe immunosuppression—those undergoing bone marrow transplantation or with a primary diagnosis of a hematologic malignancy including leukemia or lymphoma—accounted for 363 (61%) of the total 595 patients. Complete or partial responses were noted in 103/363 (28%) of patients with severe immunosuppression as compared with complete or partial responses in 118/232 (51%) patients with less severe immunosuppression ($p < 0.0001$). Of those severely immunosuppressed patients, complete responses were seen in 80/363 (22%) with partial responses occurring in 23/363 (6%). In the patients with less severe immunosuppression, complete and partial responses occurred in 81/232 (35%) and 37/232 (16%) patients, respectively.

Responses according to site of infection are shown in Table 5. Complete and partial responses for patients with invasive pulmonary aspergillosis were seen in 132/330 (40%), which was significantly higher than complete or partial responses in patients with more widespread infection, such as those with disseminated infection or CNS infection, where complete and partial responses were seen in 23/148 (16%) patients ($p < 0.007$). Complete or partial responses occurred in 20/114 (18%) patients with disseminated disease, while the poorest responses occurred in patients with CNS involvement, with complete or partial responses reported in 3/34 (9%) patients ($p < 0.0017$ compared with pulmonary infection and $p < 0.027$ compared with disseminated infection).

Utilization of each the major therapeutic regimens is shown for each of the underlying risk groups (Table 6). In comparing the severity of immunosuppression between each of the 3 major treatment groups, 130/187 (70%) patients receiving amphotericin B alone were severely immunosuppressed—autologous or allogeneic bone marrow transplant recipients or with a hematologic malignancy—compared with 48/93 (52%) patients receiving sequential amphotericin B to itraconazole and only 10/58 (17%) patients receiving itraconazole alone ($p < 0.005$ and $p < 0.00001$, respectively). Patients receiving sequential amphotericin B and itraconazole were also significantly more likely to be severely immunosuppressed (52%) as compared with itraconazole (10%) ($p < 0.0001$). Of the patients receiving amphotericin B as the sole antifungal, 68 (36% of the amphotericin B recipients) received an autologous or allogeneic bone marrow transplant and an additional 62 patients (34% of those receiving amphotericin B) had a hematologic malignancy. Notably, only 1 patient (2% of the itraconazole recipients) with a bone marrow transplant (an autologous marrow recipient) received itraconazole as the sole antifungal therapy, and an additional 9 patients (15% of those receiving itraconazole alone) had a diagnosis of a hematologic malignancy. Patients receiving sequential amphotericin B followed by itraconazole reflected an immune status between that of those receiving amphotericin B alone or itraconazole: 48/93 (52%) patients receiving sequential amphotericin B to itraconazole were severely immunosuppressed and 21 (23%) of those had received a bone marrow transplant.

TABLE 5. Response to therapy by site of infection in 595 patients

Response	Site of Infection			
	Pulmonary No. (%)	Disseminated No. (%)	Cerebral No. (%)	Other No. (%)
Complete response	89 (27)	17 (15)	2 (6)	53 (45)
Partial response	43 (13)	3 (3)	1 (3)	13 (11)
Stable	10 (3)	3 (3)	2 (6)	6 (5)
Treatment failure	106 (32)	69 (60)	22 (65)	19 (16)
Other failure*	82 (25)	22 (19)	7 (20)	26 (23)

*Clinical failure from drug toxicity, progression of primary disease, or other factors independent of aspergillosis.

TABLE 6. Treatment given according to underlying disease

Primary Diagnosis	Treatment Regimen		
	Amphotericin B (n = 187)	Amphotericin B to Itraconazole (n = 93)	Itraconazole (n = 58)
Severely immunosuppressed	130 (70)	48 (52)	10 (17)
Autologous BMT	15 (8)	2 (2)	1 (2)
Allogeneic BMT	53 (28)	19 (21)	0
Hematologic malignancy	62 (34)	27 (29)	9 (15)
Not severely immunosuppressed	57 (30)	45 (48)	48 (83)
Solid organ transplant	14 (7)	12 (13)	4 (7)
AIDS	11 (6)	9 (10)	12 (20)
Solid tumor	8 (4)	3 (3)	7 (12)
Other immune deficiency	3 (2)	3 (3)	0
Pulmonary diseases	9 (5)	13 (13)	15 (26)
Miscellaneous	10 (5)	4 (4)	6 (10)
No underlying disease	2 (1)	1 (2)	4 (7)

The clinical responses to antifungal therapy for each of the 3 major drug regimens are shown in Table 7. Notably, complete or partial responses were significantly lower to amphotericin B alone—which was the group comprising the highest percentage of severely immunosuppressed patients—than to either sequential therapy with amphotericin B and itraconazole or itraconazole alone. Complete or partial responses occurred in 60/187 (32%) of patients receiving amphotericin B alone as compared with 50/93 (54%) of patients receiving combination therapy ($p < 0.001$) and 33/58 (57%) of patients receiving itraconazole therapy alone ($p < 0.001$). In the most severely immunosuppressed patients complete and partial responses to amphotericin B were seen in 31/130 (24%) patients as compared with responses in 29/57 (51%) of patients with less immunosuppression. Responses occurred in 20/48 (42%) patients with severe immunosuppression who received sequential amphotericin B followed by itraconazole. Only 10 severely immunosuppressed patients received itraconazole alone.

Death due to or with *Aspergillus* was reported in 122/187 (65%) patients receiving amphotericin B, while death due to or with *Aspergillus* occurred in 34/93 (36%) patients receiving sequential amphotericin B following by itraconazole ($p < 0.00001$) and 15/58 (26%) patients receiving itraconazole alone ($p < 0.00001$).

TABLE 7. Clinical response by treatment regimen and immunosuppressive status

Response	Response by Regimen for All Patients		
	Amphotericin B (n = 187) No. (%)	Amphotericin B to Itraconazole (n = 93) No. (%)	Itraconazole (n = 58) No. (%)
Complete response	47 (25)	36 (39)	23 (40)
Partial response	13 (7)	14 (15)	10 (17)
Treatment failure	81 (43)	20 (22)	4 (7)
Stable	2 (1)	4 (4)	5 (9)
Other failure	44 (24)	19 (20)	16 (27)
	Response by Regimen for Severely Immunosuppressed Patients		
	Amphotericin B (n = 130)	Amphotericin B to Itraconazole (n = 48)	Itraconazole (n = 10)
Complete response	26 (20)	17 (35)	3 (30)
Partial response	5 (4)	3 (6)	1 (10)
Treatment failure	70 (54)	13 (27)	2 (20)
Stable	2 (1)	4 (9)	0
Other failure	27 (21)	11 (23)	4 (40)
	Response by Regimen for Patients Not Severely Immunosuppressed		
	Amphotericin B (n = 57)	Amphotericin B to Itraconazole (n = 45)	Itraconazole (n = 48)
Complete response	21 (37)	19 (42)	20 (42)
Partial response	8 (14)	11 (24)	9 (19)
Treatment failure	11 (19)	7 (16)	2 (4)
Stable	0	0	5 (10)
Other failure	17 (30)	8 (18)	12 (25)

Discussion

The present study is a retrospective survey of cases obtained from 89 physicians experienced in treating patients with invasive aspergillosis. The study was designed to capture current treatment practices from a wide variety of practitioners in order to establish outcomes of infection with antifungal regimens in current use and to reflect actual clinical outcomes achieved in patients in clinical settings. The retrospective nature of the case report design limits the data to those that were collected—the case record form was intentionally designed for simple outcome and risk factor measures for ease of participation and collection. The cases submitted may also be influenced by selection of particular cases by each practitioner. However, in order to limit site and/or investigator bias, investigators were asked to submit a maximum of 10 cases, with the cases to be the most recent cases treated. The large number of participating physicians and the extensive collection of cases were also aimed at producing a cross-sectional view of treatment outcomes in a variety of practices. Thus, this extensive collection—which is 1 of the largest collected for invasive aspergillosis from participating investigators rather than from a literature review—is aimed at providing a broad assessment of therapeutic regimens and outcomes.

Criteria for diagnosis and for therapeutic responses were adapted from those developed by the Mycoses Study Group to evaluate invasive aspergillosis. These criteria have been used successfully to evaluate therapeutic outcomes in other recent studies and provide a standard for comparison of diagnosis as well as outcomes between studies (6, 58). Favorable responses in our study required either a complete or partial response, which was defined in this study as major improvement in attributable signs and symptoms, although specific criteria for a partial response were not assigned. Treatment failures were those patients who had a stable disease or progression. However, the participating physician determined assessment of responses and causes of failure. There were no required therapeutic regimens in terms of antifungal choices, duration of therapy, or for assessing response. Nevertheless, these were all cases treated by individual study investigators who also determined clinical outcome. Notably, the antifungal treatment regimens evaluated for this report—amphotericin B, itraconazole, and sequential amphotericin B followed by itraconazole—are currently major therapeutic options. Results from this study clearly demonstrate the need for new therapeutic compounds including the need to evaluate lipid formulations of polyenes, which may improve efficacy as well (22, 23, 24, 25).

The largest series of treatment outcomes in invasive aspergillosis reported to date, including cases collected from the literature, is by Denning and Stevens in 1990 (8). In that review, 2,121 published cases were assessed, and from these, 440 evaluable courses of infection in 379 patients were analyzed. Treatment regimens during the period of their observations were primarily amphotericin B, amphotericin B combined with flucytosine, and surgical resection of operable lesions. Of 15 bone marrow transplant recipients with pulmonary aspergillosis, none responded to therapy. Responses were much higher in other groups, including patients with neutropenia (68% of 75 patients), renal or heart disease (76% of 34 patients), chronic granulomatous disease (83% of 12 patients), and patients with other predisposing conditions (86% of 21 patients). Of 33 patients with cerebral aspergillosis, 24% responded. The overall response rate to amphotericin B was 55% in 320 sites of infection. Sixty-three patients were treated with amphotericin B and flucytosine, and 68% of these responded, including 11 of 19 with acute leukemia. In that review, it was suggested that flucytosine or other agents such as rifampin be considered in addition to amphotericin B based on those data, provided antagonism can be excluded in vitro. In our series, few patients (only 2% of the series studied) received a combination of amphotericin B and flucytosine, so no firm conclusions as to the utility of that regimen can be made. However, flucytosine can be associated with significant toxicity, particularly in the setting of renal insufficiency, which has blunted enthusiasm for its use. In addition, its availability only as an oral agent in the United States makes administration in critically ill patients with invasive aspergillosis more difficult, which has further limited its use in that combination.

In the series by Denning and Stevens (8), a favorable response rate of 78% was found with itraconazole therapy in which capsules were also used. Notably, in invasive pulmonary aspergillosis, with 11 of 14 (79%) cases responding, and in bone and joint disease, with 4 of 5 responders, efficacy of itraconazole appeared good. However, few highly immunosuppressed patients received itraconazole therapy alone (8). In a larger series of patients treated with itraconazole by the Mycoses Study Group (6), more than 50% of patients responded to itraconazole therapy. However, again the patient population was notable for the small numbers of patients with leukemia or lymphoma and bone marrow transplant recipients, which likely reflected the avoidance of an oral triazole with erratic absorption and slower onset of antifungal activity than amphotericin B in patients who were less critically ill.

More recently, an extensive review by Stevens and Lee (58) used Mycoses Study Group criteria to assess outcomes for 125 patients with invasive aspergillosis who received compassionate use itraconazole be-

fore its United States Food and Drug Administration (FDA) approval. In that review, 34 (27%) patients receiving itraconazole had a complete response and another 45 (36%) improved. Patients receiving less than 2 weeks of itraconazole had a worse outcome, as did those with CNS, sinus, or disseminated infection. As was seen in our results, *Aspergillus* species did not impact results, but in contrast to our report, underlying disease also was not a factor contributing to a worse outcome. In contrast to other series, including ours, 57 of 125 (46%) patients receiving itraconazole reported by Stevens and Lee were from groups with more immunosuppression including hematologic malignancy and bone marrow transplantation. Notably, response rates in that group were not different from other patient groups with complete or partial responses in 66%. Responses were also seen in 8 of 11 (73%) patients undergoing bone marrow transplantation. As these patients were all receiving open-labeled itraconazole, they comprise a highly select population; the investigator may well have chosen this oral compound for use in less severely ill patients with the likelihood of a favorable response.

In our series, as expected, more than 41% of patients came from highly immunosuppressed groups, although the list of underlying conditions was extensive including only 9 patients without known underlying immunodeficiency. Most infections were pulmonary, but disseminated disease was also common. As reported in other series, outcomes of infection were highly dependent on the underlying diagnosis and immune status of the patient (8, 11, 32, 38, 41, 50). Responses of only 28% were seen in severely immunosuppressed patients (those undergoing bone marrow transplantation or with a hematologic malignancy), which were significantly less than the 51% responses in less immunosuppressed patients. Site of infection had a less major impact, although patients with pulmonary infection had responses (40%) better than those with disseminated disease (16%) (with or without CNS involvement). Immune reconstitution may improve outcome, but serial assessment of neutrophil counts was not obtained. However, only one-third of the patients was reported to have neutrophil counts less than 500/mm^3 at time of diagnosis.

Several less common *Aspergillus* species were documented to be associated with infection, confirming reports of the pathogenicity of these species. *A. fumigatus* and *A. flavus* were the most common, comprising 66% and 14% of positive culture results, respectively. The number of patients with other species, such as *A. terreus* (11 patients, 4%), was too small to draw meaningful conclusions on outcome, which has been reported to be poor in some series (28).

Although the focus of this report was current treatment practices and outcome, it is important to note that by far the most common clinical presentation in this series was pulmonary infection (56%). Primary sinus and skin infections were uncommon, making up only 5% of patients each. Disseminated infection (which could also include the lungs) was present at time of diagnosis in 19%, suggesting that the diagnosis was not established early in the course of infection in many of these patients.

Serologic methods may facilitate the diagnosis of invasive aspergillosis and could improve the outcome of infection with the prompt recognition of infection and aggressive initiation of antifungal therapy (64). In these patients, serologic methods were not used to establish the diagnosis, as antibody testing is not predictive in the diagnosis of invasive infection, and commercial tests for detecting *Aspergillus* antigen were not available at the time of this study. Recently, a sandwich ELISA technique that utilizes a monoclonal antibody to galactomannan has been developed (63, 65). This assay (Sonofi Diagnostics Pasteur, Marnes-la-Coquette, France) has been used most extensively in Europe, where it is approved for clinical use (63–65). Recent prospective studies in hematology patients have demonstrated a sensitivity and specificity of >90% with this method (64). However, there have been false-positive reactions, so that more than 1 positive sample is required for a positive result. In addition, other false-positive results have been seen in pediatric patients, perhaps related to dietary intake, as well as cross-reactions with fungi other than *Aspergillus* (64).

Our series confirms that up until the mid-1990s, amphotericin B was used widely for treatment of aspergillosis, being used alone in 187 patients, in combinations in 39 patients, and as initial therapy with a change to another agent in 127 patients, for a total of 353 patients. The patients who received amphotericin B as sole therapy represented the most seriously ill patients studied, with 70% of those patients being highly immunosuppressed including hematologic malignancy or bone marrow transplantation. Those who received amphotericin B alone appear to do the worst of the 3 major regimens studied, with a 32% response rate and death due to or with *Aspergillus* in 65%. This emphasizes the importance of immunosuppression in determining outcome, and also illustrates the need for alternative approaches to this infection in the highly immunosuppressed population.

The length of time between diagnosis and initiation of antifungal therapy was not collected, and a delayed initiation of amphotericin B potentially could have impacted outcomes adversely. However, few highly immunosuppressed patients received itraconazole alone, and few received initial therapy with itraconazole followed by amphotericin B, so that delayed initiation of amphotericin B as compared to the other regimens because of toxicity concerns or other factors seems unlikely.

More favorable responses with sequential amphotericin B followed by itraconazole were seen, with responses of 54% in a population who had underlying immunosuppression similar to those receiving amphotericin B (50% of those receiving sequential amphotericin B and itraconazole were highly immunosuppressed). Death due to or with *Aspergillus* occurred in 36% of the 93 patients receiving sequential amphotericin B followed by itraconazole, which likely reflected the selection of patients surviving long enough to receive an oral regimen following initial amphotericin B, which was administered for a mean duration of 4 weeks. However, in the absence of randomized, controlled clinical trial data to support the approach of azole therapy following initial polyene regimens, these data support the use of such an approach, as poorer outcomes did not occur, which might have occurred if antagonism had been seen.

The response rate for patients receiving itraconazole alone in this series is very favorable, with responses of 57% and death due to or with *Aspergillus* in only 26%. However, it should be cautioned that a very select group of patients was likely chosen by individual providers to receive itraconazole alone: only 58 patients from the entire group received itraconazole alone and of those patients, only 10 were highly immunosuppressed, including 1 undergoing autologous bone marrow transplantation and 9 with a hematologic malignancy. Notably, none of the patients receiving itraconazole alone underwent allogeneic bone marrow transplantation, a group with a particularly poor prognosis. Thus, from these data it could be reasoned that oral itraconazole should be reserved for use as a sole antifungal agent for primary *Aspergillus* therapy in patients from whom oral absorption is more likely and in those with less extensive immunosuppression and/or infection (46, 58). The availability of itraconazole in oral cyclodextrin and intravenous formulations opens the possibility of early intravenous therapy with this compound, which may further the use of itraconazole in more immunosuppressed patients groups (1, 27, 48, 59). Nevertheless, the present study reinforces results in earlier series that suggest patients with milder predisposing conditions may do very well with oral azole therapy for invasive aspergillosis (8, 13, 14). An advantage of such an approach is ease of prolonged therapy and improved tolerance of drug.

One anatomic site associated with a particularly poor outcome, which might be improved with azole therapy, is that of CNS disease (7, 19, 39, 51, 55). While 3 responses occurred in CNS infection in our series, none of those patients received itraconazole alone. However, Sanchez and colleagues (51) reported efficacy of itraconazole at 800 mg per day in 3 of 4 patients with CNS *Aspergillus* infection. In addition, using the investigational triazole voriconazole, Denning and colleagues (7) reported 25% response rates in CNS disease.

Another major use for the azoles with activity against *Aspergillus* is likely to be in long-term oral sequential therapy and perhaps in combinations with amphotericin B and/or other drugs such as the echinocandins that have *Aspergillus* activity. In the past, lack of an intravenous formulation for azoles (and other drug classes) with *Aspergillus* activity has severely limited the potential for combination therapy for this lethal disease. Now with intravenous availability of less toxic lipid formulations of amphotericin B (25, 33, 68, 69), intravenous azoles (itraconazole as well as investigational compounds voriconazole and Sch 59884, the intravenous prodrug of the antifungal triazole Sch 56592) (7, 14, 34, 43) and new drug classes, such as echinocandins (caspofungin [MK-0991], FK463, and others) (5, 23, 47, 49, 56), early combination(s) may be a means for improving the outcome of this lethal disease. In this report the success of sequential therapy with amphotericin B followed by itraconazole provides clinical support for this approach, which has been used successfully in treating cryptococcal meningitis and other mycoses (46, 62). Antagonism has been reported rarely and occurs when the triazole is begun before the amphotericin B (53). However, in our series the number of patients given combined amphotericin B and itraconazole or itraconazole followed by amphotericin B is too small for meaningful conclusions. Recently, susceptibility testing for molds has been standardized, which may help guide antifungal compounds and regimens (17, 37). The clinical impact of other combinations is also not established and should be investigated in animal models as well as in clinical disease (20).

The overall poor outcomes in this study, particularly in highly immunosuppressed patients, emphasize the continued need for new therapies against this disease and the development of new therapeutic strategies for the prevention and early therapy of this infection (16, 33, 67).

Summary

A review of representative cases of invasive aspergillosis was conducted to describe current treatment practices and outcomes. Eighty-nine physicians experienced with aspergillosis completed case forms on 595 patients with proven or probable invasive aspergillosis diagnosed using modifications of the Mycoses Study Group criteria. Pulmonary disease was present in 56%, with disseminated infection in 19%. The major risk factors for aspergillosis were bone marrow transplantation (32%) and hematologic ma-

lignancy (29%), but patients had a variety of underlying conditions including solid organ transplants (9%), AIDS (8%), and pulmonary diseases (9%). Overall, high antifungal failure rates occurred (36%), and complete antifungal responses were noted in only 27%. Treatment practices revealed that amphotericin B alone (187 patients) was used in most severely immunosuppressed patients while itraconazole alone (58 patients) or sequential amphotericin B followed by itraconazole (93 patients) was used in patients who were less immunosuppressed than patients receiving amphotericin B alone. Response rate for patients receiving amphotericin B alone was poor, with complete responses noted in only 25% and death due to or with aspergillosis in 65%. In contrast, patients receiving itraconazole alone or following amphotericin B had death due to or with *Aspergillus* in 26% and 36%, respectively. These results confirm that mortality from invasive aspergillosis in severely immunosuppressed patients remains high even with standard amphotericin B. Improved responses were seen in the less immunosuppressed patients receiving sequential amphotericin B followed by itraconazole and those receiving itraconazole alone. New approaches and new therapies are needed to improve the outcome of invasive aspergillosis in high-risk patients.

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Appendix

Investigators in the I[3] *Aspergillus* Study Group included the following: Atlanta, GA: Devine S; Baltimore, MD: Lee E, Miller C; Bay Shore, NY: Samuels S; Boston, MA: Antin J, Miller K, Sugar A; Bradenton, FL: Bach M; Brooklyn, NY: Berkowitz L; Brussels, Belgium: Meunier F; Buffalo, NY: Campbell L; Chapel Hill, NC: Wiley J; Charleston, MA: Fishman J; Charleston, SC: Frei-Lahn D; Chicago, IL: Bernales R, Noskin G, Pottage J; Columbia, SC: Pati A; Columbus, OH: Copelan E; Dallas, TX: Fay J; Detroit, MI: Chandrasekar P, Vasquez J; Duarte, CA: Ito J, O'Donnell M; Durham, NC: Laughlin M, Perfect J; Edina, MN: Belani K; Freiberg, Germany: Fetscher S; Gainesville, FL: Wingard J; Hartford, CT: Lyons R; Hermitage, TN: Bodner S; Hershey, PA: Zurlo J; Houston, TX: Fainstein V; Kansas City, MO: Bamberger D, Dall L, Klotz S, McKinsey D; Lacrosse, WI: Agger W; Little Rock, AR: Anaissie E, Bradsher R; Los Angeles, CA: Kapoor N; Louisville, KY: Stevens D; Manhasset, NY: Schuster M; Maywood, IL: Garrity E Jr, Yeldandi V; Memphis, TN: Flynn P, Santana V; Milwaukee, WI: Bernstein B; Mineola, NY: Cunha B; Minneapolis, MN: Hertz M, Morrison V; Nashville, TN: Dummer S; New York, NY: Klapholz A, Bonvino S, White M; Norfolk, VA: Green S; Oakland, CA: Feusner J; Oklahoma City, OK: Greenfield R; Omaha, NE: Penn R; Palm Springs, CA: Kerkar S; Paris, France: Dupont B; Philadelphia, PA: Blumberg E, Schuster M, Styler M; Pittsburgh, PA: Colodny S, Mirro J; Portland, ME: Hurwitz C; Richmond, VA: Yanovich S; Rochester, MN: Keating M; Salt Lake City, UT: Petersen F; San Antonio, TX: Patterson T, Graybill J; San Diego, CA: Catanzaro A; San Diego, CA: Kirkland T; San Francisco, CA: Gordon S; San Jose, CA: Stevens D; Sarasota, FL: Lipman M; Shaker Heights, OH: Lazarus H; Springfield, IL: Tewari R; Stanford, CA: Long G, Negrin R; Syracuse, NY: Grethlein S; Tampa, FL: Hiemenz J; Temple, TX: Winn R; Toledo, OH: Jauregui L; Tucson, AZ: Graham M; Washington, DC: Cahill R, Mazumder A; Worcester, MA: Esposito A.

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