The Use of Respiratory-Tract Cultures in the Diagnosis of Invasive Pulmonary Aspergillosis

Joseph A. Horvath, MD, *Memphis*, Stephen Dummer, MD, *Nashville, Tennessee*

PURPOSE: To define the role of lower-respiratory-tract cultures in the diagnosis of invasive pulmonary aspergillosis (IPA) in immunocompromised hosts.

METHODS: Immunocompromised patients with a positive, nonbiopsy, lower-respiratory-tract culture for *Aspergillus* species were classified as having definite, probable, indeterminate, or no IPA. Culture data, positive predictive values (PPVs), correlation with clinical and radiographic findings, and the relationship between the number of specimens submitted and the likelihood of recovering *Aspergillus* were assessed.

RESULTS: Definite or probable IPA was diagnosed in 72% of episodes from patients with hematologic malignancy, granulocytopenia, or bone-marrow transplant; in 58% of those with solid-organ transplant or using corticosteroids; and in 14% of those with human immunodeficiency virus infection. The PPV of cultures ranged from 14% in the latter group to 72% in the first group (bone-marrow-transplantation subgroup, 82%). Fungal cultures were more often positive than were routine cultures ($P < 0.001$). Clinical and radiographic findings suggestive of IPA were present more frequently in infected than uninfected patients (59% versus 24%, $P < 0.025$); and 73% versus 6%, ($P < 0.0001$, respectively). Infected patients with ≥1 positive node had more cultures submitted than a control group of patients with no positive cultures (5.8 ± 4.7 versus 2.1 ± 2.2 cultures, $P < 0.001$).

CONCLUSION: Recovery of *Aspergillus* species from high-risk patients is associated with invasive infection. Clinical and radiographic correlations help to separate true- from false-positive cultures. At least 3 sputum specimens should be submitted for fungal culture whenever fungal infection is suspected.

Acute, invasive pulmonary aspergillosis (IPA) is a serious infection of immunocompromised hosts. Patients at the highest risk for invasive aspergillosis are those with hematologic malignancies or granulocytopenia,[1-5] bone-marrow transplantation (BMT),[6-8] solid-organ transplantation,[9-12] or corticosteroid use.[13] More recently, persons infected with the human immunodeficiency virus (HIV) also have been shown to be at increased risk.[14-16] The mortality associated with *Aspergillus* infections in patients with these risk factors remains high, and can approach 90% in neutropenic patients or BMT recipients.[8,13,17-19] Much of this high mortality may reflect the difficulty in establishing an early, definitive diagnosis. Some authors have observed improved survival from invasive aspergillosis when high-dose amphotericin is initiated early, that is, before or within a few days of the appearance of pulmonary infiltrates.[7,12,18,20-25] Invasive procedures to establish a definitive diagnosis are associated with a high risk of complications. Noninvasive methods such as antibody or antigen detection remain investigational. Thus, therapeutic decisions are frequently based solely on presumptive diagnoses.[13,26-28]

Sputum cultures have been reported to be insensitive for the diagnosis of IPA in immunocompromised hosts. In studies that predominately involved patients with acute leukemia or BMT, 15% to 69% of patients with IPA had positive cultures before death, with the higher figures coming from the most recent reports.[1-3,17,24,25,29,30] A slightly greater sensitivity of respiratory cultures (78%) was reported a large series of IPA in recipients of liver transplants,[12] but Weiland et al[10] found that only 40% of 21 kidney transplant recipients with IPA had positive sputum cultures. Cultures of nonbiopsy materials obtained at bronchoscopy, such as lavage fluid, washings, or brushings, also have been found to be insensitive; yielding positive results in ≤50% of cases of IPA.[31-35] A number of investigators have shown that positive cultures are usually specific for aspergillosis when taken from patients at high risk, such as leukemic patients who are neutropenic after receiving chemotherapy.[29,36,37] Yet the positive predictive value (PPV) of respiratory-tract cultures for *Aspergillus* in patients with other IPA risk factors remains imprecise. Furthermore, earlier studies have not included BMT or HIV-infected patients. The present investigation was undertaken to further define the predictive value of positive respiratory cultures, evaluate the role of clinical variables in interpreting culture results, and identify ways to improve the use of respiratory-tract cultures in the diagnosis of IPA in immunocompromised hosts.

METHODS

Patients. Records from the microbiology laboratory of Vanderbilt University Hospital were searched for all

From the Departments of Medicine and Surgery (SD), Vanderbilt University Medical Center, Vanderbilt University Medical Center, Nashville, and the Department of Medicine (JAH), University of Tennessee, Memphis, Tennessee.

Requests for reprints should be addressed to Stephen Dummer, MD, 911 Oxford House, Vanderbilt University, School of Medicine, Nashville, Tennessee 37232-4751.

Manuscript submitted November 8, 1994 and accepted in revised form August 1, 1995.

reports of *Aspergillus* species isolation from January 1980 through November 1993. Patients >10 years old who had a positive culture of a lower-respiratory-tract specimen for *Aspergillus* were identified. These specimens included sputa, endotracheal aspirates, bronchial washings or brushings, and bronchoalveolar lavage fluid, but excluded biopsy samples. Upper-respiratory-tract specimens, such as nasal or throat swabs, were excluded because there were too few to analyze. Charts of patients were then reviewed to determine the presence or absence of one or more of the following conditions: (1) active leukemia or lymphoma; (2) granulocytopenia, defined as <1,000/mm^3 circulating granulocytes for ≥7 days immediately prior to the first positive culture; (3) solid-organ or bone-marrow transplantation; (4) corticosteroid usage, equivalent to ≥10 mg per day for each of the 30 days immediately prior to the first positive culture; or (5) HIV infection. Patients with risk factors for chronic invasive aspergillosis, such as chronic granulomatous disease or chronic obstructive lung disease, were not included unless one of the above five conditions also existed. Patients with a positive respiratory-tract culture were then grouped as follows: group I—leukemia, lymphoma, BMT recipients, or those with granulocytopenia; group II—solid-organ transplant recipients; group III—those using corticosteroids; or group IV—those with HIV infection.

Diagnosis of IPA. Patient records were reviewed to establish the presence of the following clinical or radiologic features previously shown to be suggestive of acute IPA in immunocompromised hosts.[22,26,27,38] These included (1) any degree of hemoptysis; (2) pleuritic chest pain; (3) pleural rub; and (4) new pulmonary infiltrates described as alveolar, nodular, cavitary, or wedge shaped. Data were collected regarding the onset of these features, as well as the use of and initiation of amphotericin at doses above and below 0.8 mg/kg per day, in relation to the submission day of the first culture positive for *Aspergillus*.

Each patient was assigned to one of four diagnostic categories as follows: (1) Definite IPA—This necessitated a histologically proven invasive pulmonary infection; patients with nonpulmonary sites (eg, skin) demonstrating forms consistent with *Aspergillus* species on histologic examination and having pulmonary infiltrates were not considered definite unless IPA was also demonstrated in lung tissue. (2) Probable IPA—these patients had suggestive pulmonary infiltrates without another apparent cause and no response to broad spectrum antibacterial agents (patients meeting these criteria, who experienced recovery without receiving ≥500 mg of amphotericin B, were considered indeterminate). (3) Indeterminate—these patients had interstitial or lobar infiltrate(s) or pleural effusions, but without a pattern suggestive of aspergillosis, as defined above, and with no apparent cause (patients with a potential pathogen [besides *Aspergillus*] isolated from sputum or blood were also placed in this category, regardless of type of infiltrate). Patients were also placed in the indeterminate category if their pulmonary process resolved completely with the administration of <500 mg of amphotericin B, or if they had absence of *Aspergillus* species at autopsy, if death occurred after >2 months of therapy with amphotericin B or itraconazole. (4) Uninfected—these patients did not demonstrate IPA at autopsy or after lung biopsy, or had a full recovery from the pulmonary process without any amphotericin B therapy.

Respiratory-tract culture. Specimens for routine bacterial pathogens (routine culture) were plated on blood, chocolate, and MacConkey agar, and read at both 24 and 48 hours. Specimens for fungal culture were placed on each of four slants: Sabouraud dextrose, pH 6.8, with and without gentamicin and chloramphenicol, and brain-heart infusion agar with 10% sheep blood, with and without gentamicin and chloramphenicol. Slants were incubated at 30°C and read weekly for 4 weeks. Isolates were usually reported as *Aspergillus fumigatus*, *Aspergillus* species, or *Aspergillus* species not *fumigatus*.

In determining the number of positive and negative cultures for each possible episode of IPA, all specimens obtained within 30 days before and after the first positive culture were considered. One specimen submitted for both routine and fungal culture was considered as two cultures.

Comparisons with patients with proven IPA but without positive antemortem cultures. Pathology records from 1983 to 1993 were reviewed and revealed 26 patients who had confirmed IPA and one of the five immunocompromising conditions mentioned earlier, but did not have any positive respiratory-tract cultures for *Aspergillus*. The number of cultures obtained from these 26 patients was compared to that from the definite-IPA group to determine if the absence of a positive culture antemortem could have been due to less-frequent culturing.

Positive predictive values. Calculating the PPVs for any group or subgroup requires identification of all true-positive cases. In this study, the total number of true-positive cases could not be precisely defined, because the number of patients who actually had IPA in the probable and indeterminate groups was unknown. Thus, PPVs were calculated using different estimates for the number of true-positive cultures (numerator) and number of true- plus false-positive cultures (denominator). Method A assumes that all positive cultures from definite and probable cases are true-positive results, while those from indeterminate and uninfected cases are false-positive results. Method B calculates the PPVs using only those cultures that are known with certainty to be true-positive results (defi-

TABLE I

Underlying Diseases Associated With Positive Lower-Respiratory-Tract Cultures for *Aspergillus*

Group	Underlying Disease	No. of Patients
I	Hematologic/Oncologic/BMT	47
	BMT	22
	Allogeneic	16
	Autologous	6
	Acute leukemia	17
	Other*	8
II	Solid-organ transplants	16
	Kidney	8
	Single lung	5
	Heart or heart-lung	3
III	Corticosteroid use, no transplant	8
IV	HIV infection	7
Total		78

*Chronic leukemia (n = 3), aplastic anemia (n = 2), and 1 each of refractory anemia with excess blasts, oligodendroglioma, and lymphoma. BMT = bone-marrow transplantation; HIV = human immunodeficiency virus.

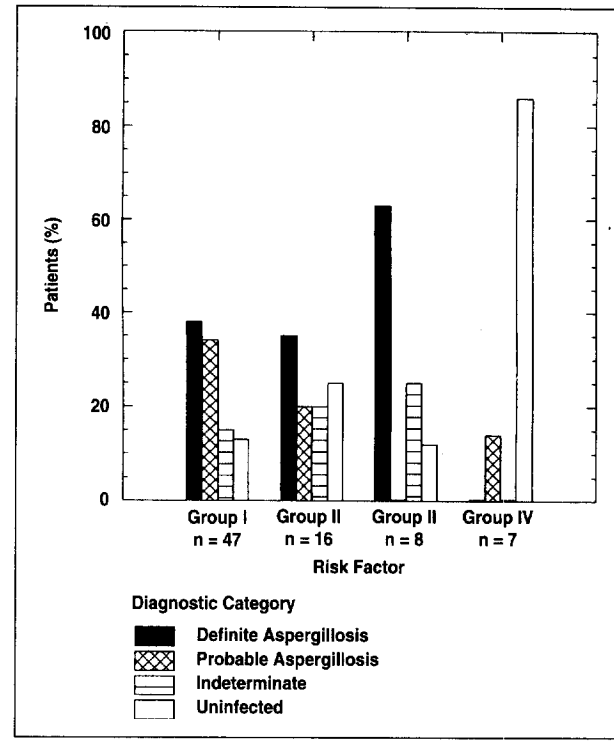

Figure. Proportion of patients in the four risk-factor groups with positive respiratory cultures for *Aspergillus* who had definite, probable, indeterminate, or no invasive disease. Risk-factor groups are: I = granulocytopenia or bone-marrow transplantation; II = solid-organ transplantation; III = corticosteroid use; IV = human immunodeficiency virus infection.

nite cases) or false-positive results (uninfected cases). Methods C and D provide extreme estimates of PPVs, assuming that all probable and indeterminate cases have either true-positive results (method C) or false-positive results (method D). It is likely that the estimates derived with methods A and B—for which values lie between the estimates derived from methods C and D—are the most accurate.

Statistical analysis. Means of continuous variables were compared using Student's *t*-test. Medians were compared with the Mann-Whitney *U* test. Proportions were compared with the chi-square test with Yates' correction. Values of $P < 0.05$ were considered significant.

RESULTS

Eighty patients at risk for acute IPA had 82 episodes with a lower-respiratory-tract culture positive for *Aspergillus* species. Four episodes in 4 patients were excluded because of incomplete medical records. There were 47 episodes among 46 patients from group I (leukemia, lymphoma, neutropenia, or BMT), 16 episodes from 15 patients from group II (solid-organ transplantation), 8 from group III (steroid use), and 7 from group IV (HIV infected). None of the HIV-infected patients had received corticosteroids. The underlying diseases within each group are shown in **Table I**. Mean age was 43 ± 16.4 years. There were 49 men and 27 women. Twenty-eight isolates were identified to the species level; 25 were *A fumigatus* and 3 were *A niger*.

Diagnostic category. Patients were grouped into diagnostic categories as shown in the **Figure**. Among the 78 episodes, there were 29 (37%) definite, 20 (26%) probable, and 12 (15%) indeterminate diagnoses of IPA, while in 17 patients (22%), there was no IPA. A positive respiratory-tract culture was associated with a diagnosis of definite or probable IPA in 34 of 47 (72%) episodes in group I patients. Within group I, BMT recipients with positive cultures had definite or probable IPA in 18 of 22 (82%) instances. Positive cultures were less predictive of IPA for groups II and III, only 14 of 24 (58%) episodes from these groups were classified in the definite or probable category. Only 1 of 7 (14%) HIV-infected patients had probable IPA, and none had definite IPA. The patient with probable IPA recovered with amphotericin therapy. All HIV-infected patients in the uninfected group made a complete recovery without any antifungal therapy.

Positive predictive values. The PPVs calculated by using different estimates for the number of true-positive cases (numerator) and number of true- plus false-positive cases (denominator) are shown in **Table II**.

Comparison of fungal and routine cultures. Our survey found that many positive cultures for *Aspergillus* were obtained on routine cultures. There were 132 instances when a single specimen was simultaneously plated for both fungal and routine pathogens. Routine and fungal results were concordant for 88 (67%) of these 132 specimens. However, in 42 (32%) instances, *Aspergillus* species were recovered solely from the fungal culture. Isolation exclusively from routine culture occurred only twice ($P < 0.0001$).

TABLE II

Estimates of Positive Predictive Value (PPV) of Lower-Respiratory-Tract Cultures for *Aspergillus* by Method of Calculation of PPV*

Patient Group	N	Method (%) A	B	C	D
Neutropenic or BMT	47	72	75	87	38
BMT alone	22	82	86	91	55
Solid-organ transplant	16	56	60	75	38
Steroid use	8	63	83	88	63
HIV infected	7	14	0	14	0
Total	78	63	63	78	37

*A: Definite and probable = true positives; indeterminate and uninfected = false positives. B: Definite = true positives; uninfected = false positives. C: Definite and probable and indeterminate = true positives; uninfected = false positives. D: Definite = true positives; probable and indeterminate and uninfected = false positives.
BMT = bone-marrow transplantation; HIV = human immunodeficiency virus.

Also, the mean percentage of fungal cultures positive per patient (67% ± 37%) was significantly greater than the mean percentage of routine cultures positive per patient (31% ± 37%; $P < 0.001$).

Culture results by IPA diagnostic category. The rate of recovery of *Aspergillus* from all cultures (routine and fungal) from patients with definite or probable IPA was compared with the rate of recovery from uninfected patients. The number of fungal or routine cultures submitted from patients in these groups was not significantly different. The number of positive routine cultures per patient was identical in these two groups. In contrast, the number of positive fungal cultures per patient with definite or probable IPA was nearly two fold greater than that from uninfected patients (1.7 ± 1.1 per patient, versus 0.9 ± 0.5 per patient, $P = 0.02$). Similarly, the mean percentage of positive fungal cultures per patient was significantly greater in definite or probable IPA (75% ± 33% per patient, versus 50% ± 35% per patient for uninfected patients, $P = 0.02$). Multiple positive fungal cultures were also associated with definite or probable IPA. Only 1 of 17 (6%) patients in the uninfected group had more than one positive fungal culture (this patient had bronchiectasis of a transplanted lung). In contrast, 18 of 49 (37%) patients with definite or probable IPA had multiple positive fungal cultures ($P < 0.05$).

In an attempt to control for the tendency to order more cultures from patients with a greater suspicion of IPA, only those patients who had two or more specimens submitted were considered. Multiple positive cultures remained predictive of IPA. Sixteen of 32 (50%) patients with definite or probable IPA, versus 1 of 12 (8%) uninfected patients, had two or more positive fungal cultures ($P < 0.05$). Thus, multiple positive fungal cultures predicted IPA, though this criterion was satisfied in only one half of the cases.

Culture results from bronchoscopy. Forty-one of the 76 patients had 51 bronchoscopic evaluations. Overall, 28 of the 41 (68%) patients had a positive culture for *Aspergillus* (routine or fungal) from BAL, bronchial washings, or brushings. The yield from bronchoscopic cultures was 20 of 26 (77%) from patients with definite or probable IPA versus 8 of 11 (73%) from uninfected patients (P = NS). Thus, bronchoscopic cultures did not help distinguish between infected and uninfected patients. Specimens obtained at bronchoscopy also did not appear to be more sensitive than sputum samples. Of the 26 patients with definite or probable IPA who underwent bronchoscopy, 12 had at least 1 sputum sample submitted within 10 days before or after bronchoscopy. Five (42%) of these 12 were sputum-negative, but bronchoscopy-positive, whereas 4 (33%) had positive sputum cultures, but negative bronchoscopic cultures.

Clinical correlation. The presence of clinical findings suggestive of IPA was determined for each group. These included hemoptysis, pleuritic chest pain, and pleural rub (**Table III**). At least one of these signs or symptoms was found in 29 (59%) of the 49 patients with definite or probable IPA compared to 4 of 17 (24%) of the uninfected patients ($P < 0.025$). The presence or absence of infiltrates highly suggestive of IPA (nodules, cavities, or wedge-shaped infiltrates) also was analyzed. Alveolar infiltrates, suggestive of, but not specific for, IPA, were excluded. Thirty-six of 49 patients (73%) with definite or probable IPA had at least one highly suggestive infiltrate, while 1 of 17 uninfected patients (6%) had such infiltrates ($P < 0.0001$); this patient had tumor nodules that were present prior to transplantation.

Relationship to granulocytopenia. Twenty-seven patients in group I had granulocytopenia (<1,000/mm^3 neutrophils) within 10 days prior to the isolation of *Aspergillus*. A diagnosis of definite or probable aspergillosis was not more common in patients with recent neutropenia than in patients without neutropenia (70% versus 75%; P = NS). There was also no difference in the onset time or frequency of clinical or radiographic findings between those patients with and without recent neutropenia. Twenty-one patients had <500/mm^3 neutrophils and 14 had <100/mm^3 neutrophils at the time of isolation of *Aspergillus*, but these low white blood cell counts were not associated with a significantly higher probability of the diagnosis of definite or probable aspergillosis. Among granulocytopenic patients, there was no significant difference in the median duration of neutropenia before isolation of *Aspergillus* in patients with definite or probable aspergillosis versus those with indeterminate or no aspergillosis (24 days versus 13.5 days; $P = 0.26$).

Impact of cultures on diagnosis and outcome of IPA. To determine whether respiratory-tract cultures contributed to the early diagnosis of IPA, the timing of the first positive culture in patients with definite or

TABLE III
Clinical and Radiographic Findings in Patients at Risk for Invasive Aspergillosis Who Had Lower-Respiratory-Tract Cultures Yielding *Aspergillus*

Diagnostic Category	Number (%) with Findings		
	Radiographic	Clinical	Both
Definite (n = 29)	22 (76)*	16 (55)†	16 (55)‡
Definite or probable (n = 49)	36 (73)§	29 (59)‡	29 (59)¶
Indeterminate (n = 12)	7 (58)	5 (42)	5 (42)
Uninfected (n = 17)	1 (6)*§	4 (24)†‡	3 (18)‡¶

Radiographic findings were nodular, cavitary, or wedge-shaped infiltrates. Clinical findings were hemoptysis, pleural rub, or pleuritic chest pain.
*P <0.0001.
†P <0.1.
‡P <0.05.
§P <0.0001.
¶P <0.02.

probable IPA was compared with the onset of suggestive signs, symptoms, and pulmonary infiltrates. Hemoptysis, pleuritic chest pain, or a pleural rub occurred a mean of 11.8 ± 16.4 days before the first positive culture, while infiltrates suggestive of IPA occurred a mean of 8.8 ± 15.0 days prior to the first positive culture. In only 4 patients with definite or probable IPA did the positive culture antedate both clinical and radiographic findings. Of the 36 patients with definite or probable IPA who died, the median survival after the first positive culture was 7.5 days, and 42% of all deaths occurred within 4 days of culture positivity. Thus, positive cultures were late findings in many patients. However, this may have reflected delay in obtaining specimens rather than an inability to recover *Aspergillus* early in the course of infection. Indeed, 32 (65%) of the patients with definite or probable IPA had *Aspergillus* grown from their first submitted specimen. Mortality was 72% among these 32 patients with no earlier specimen submission, compared to 53% among patients in the definite or probable groups who had earlier specimens (P >0.2). Taken together, these observations show that an aggressive strategy of culturing for *Aspergillus* was not undertaken for many patients early in the course of IPA, despite the presence of suggestive clinical findings.

To determine whether culture data influenced treatment decisions, the timing of administration of amphotericin in relation to first positive cultures was analyzed. Forty-five of the 49 patients with definite or probable IPA received amphotericin, beginning a mean 8.3 ± 14.5 days prior to the first positive cultures. High-dose (≥0.8 mg/kg per day) amphotericin was begun for 27 of these patients a mean of 2.6 ± 12.8 days after the first positive culture. Eighteen patients were considered to have had inadequate treatment in that they never received amphotericin (4 patients), or never received high-dose amphotericin (14 patients). In 12 of the 18 patients, however, death occurred within a week of positive cultures. There was a trend toward better outcomes in patients who received high-dose amphotericin. Thirteen (76%) of 17 survivors with definite or probable IPA received high-dose amphotericin versus 14 (44%) of 32 patients who died (chi-square = 3.5, P <0.1).

Recovery rate of *Aspergillus* species in relation to culture frequency. The patients we identified with definite or probable IPA likely represented only a fraction of all cases of IPA occurring at our institution during the study period. To determine if isolation of *Aspergillus* might be improved by more frequent submission of specimens, two subsets of patients with histologically proven IPA were compared. Data for 29 patients with histologic evidence of *Aspergillus* and positive antemortem respiratory cultures (definite IPA, group A) were compared to data for 26 patients who had IPA at postmortem examination or on biopsy, but did not have any positive respiratory cultures (group B). The group of patients with documented aspergillosis and positive antemortem cultures for *Aspergillus* had significantly more specimens submitted than the group of patients with documented aspergillosis but no positive cultures before tissue diagnosis (5.8 ± 4.7 versus 2.1 ± 2.2 cultures submitted; P <0.001). If patients who did not have any specimens submitted were excluded from group B, the difference remained significant. Submission of three specimens was needed to detect IPA in >90% of the patients in group A.

COMMENTS

Previous studies have established that the isolation of *Aspergillus* from sputum or nonbiopsy bronchoscopic cultures in patients with recognized risk factors for acute IPA frequently reflects invasive infection rather than airway colonization.[10,12,29,32,34,36,37] Nalesnik and coworkers[29] demonstrated a significant association between the isolation of *Aspergillus* species from patients with severe granulocytopenia, leukemia, or corticosteroid usage, and the presence of IPA. In ad-

dition, the detection of *Aspergillus* in multiple positive cultures,[10,29,36] speciation of the fungus as *A fumigatus* or *Aspergillus flavus*,[10,29] and the appearance of moderate-to-heavy growth[36] have been reported to distinguish between invasive disease and colonization or contamination. In the largest of these series, Yu and colleagues[37] studied 108 patients who had *Aspergillus* isolated from sputum or bronchoalveolar lavage fluid (BAL). These patients were classified into three groups: nonimmunocompromised patients (n = 54); patients being treated with corticosteroids, including solid-organ–transplant recipients (n = 17); and leukemic and/or granulocytopenic patients (n = 37). Among those patients who eventually had tissue examination, IPA was documented in 17 out of 17 patients with leukemia/granulocytopenia, but in none of 9 immunocompetent patients. Of the 54 patients from the two immunocompromised groups, 20 (37%) had tissue-proven IPA, 9 (17%) survived without antifungal therapy, and 1 did not have aspergillosis on tissue examination. In the remaining 24 patients, the likelihood of IPA could not be rigorously assessed. Nineteen of these 24 patients did not receive antifungal therapy and died within 6 months. Thus, the PPV of respiratory-tract cultures, while possibly high, could not be definitively established. Kusne et al[12] found that the predictive value of positive respiratory-tract cultures for *Aspergillus* was 72% in liver-transplant recipients. In another recent study of solid-organ recipients, Weiland et al[10] found that the PPV of respiratory-tract cultures was only 45%. In summary, while all of the above studies show a strong association of positive respiratory-tract cultures with IPA, particularly in granulocytopenic patients, the PPVs for individual high-risk groups, including BMT recipients, remains uncertain. In addition, the use of clinical criteria to augment the predictive value has not been investigated.

In our study group, we found that 49 (63%) of the 78 episodes with a positive, lower–respiratory-tract culture were associated with definite or probable IPA. The strongest association was among BMT recipients, in whom 18 of 22 (82%) patients with a positive culture had definite or probable IPA. To accurately determine the PPVs, both the total number of positive cultures and the proportion which are true- and false-positive results must be known. Although the presence or absence of IPA with a positive culture could not be determined with certainty for 33 (42%) of our patients (ie, those with probable and indeterminate IPA), reasonable estimates and ranges could be calculated.

We believe that the majority of the probable group truly had IPA. Nodules, cavities, wedge-shaped infiltrates, and a syndrome mimicking pulmonary embolus with infarction have all been shown to be highly associated with IPA, especially in patients with leukemia.[22,26,39,40] Sterile pulmonary embolism is rare in neutropenic or BMT patients.[41,42] Bacterial pathogens such as *Staphylococcus aureus* can cause nodular or cavitary infiltrates, but patients with the recovery of bacterial pathogens or a response to broad spectrum antibiotics would have been placed into the indeterminate or uninfected groups. Lastly, other angioinvasive fungi such as *Zygomycetes* and *Pseudallescheria* can be clinically and histologically indistinguishable from *Aspergillus*.[38,43] However, our patients were identified initially by cultures, and simultaneous invasive disease due to a different fungus would be unusual. If all of the definite and probable group patients truly had IPA, the PPV of respiratory-tract cultures from the entire study group would range from 63% to 78%, depending on how many of the indeterminate group patients actually had IPA. Five of 12 cases were classified as indeterminate because of concomitant bacteremia. Pneumonia due to concurrent bacterial and *Aspergillus* infection was initially reported in up to a third of cases of IPA diagnosed at autopsy in leukemic patients, although more recent data, collected largely from BMT populations, suggest that the incidence of simultaneous bacteria and *Aspergillus* pneumonia is lower.[1,4,17,33,39,44-46]

Previous studies have shown that clinical and radiographic findings such as hemoptysis, pleuritic chest pain, pleural rub, and nodular, cavitary, or wedge-shaped infiltrates are highly suggestive of IPA in patients with leukemia.[26,27,38,39] However, in these studies, the use of respiratory cultures was not evaluated. Likewise, studies of positive cultures for *Aspergillus* in high-risk patients have not reported on the use of clinical findings to help interpret culture results. Our analysis showed that highly suggestive clinical signs and symptoms increased the PPV to 73%, and suggestive radiographic findings increased the PPV to 82%. Although these increases in PPV are modest, they may force a decision to treat in some instances.

Ninety-two percent of patients with definite or probable IPA had their first positive culture obtained more than a week after the onset of suggestive clinical findings, and in the majority of cases, just a few days prior to death. While these results suggest that respiratory cultures do not yield information early enough to affect outcome, they may also reflect delayed or infrequent specimen submission. Indeed, 32 (65%) of the patients with definite or probable IPA had *Aspergillus* recovered from their first submitted specimen. Some of these patients may have had positive cultures earlier, if specimens had been submitted at the onset of fever. Although submission of seven specimens was required to detect *Aspergillus* for the group of all 49 patients with definite or probable IPA, submission of only 3 specimens detected 91% of the cases. Thus, submission of at least 3 fungal cultures would be reasonable when *Aspergillus* infection is suspected. Moreover, a group of patients with definite IPA but without posi-

tive antemortem cultures had significantly fewer specimens submitted. These findings suggest that multiple specimens should be obtained as early in the course of suspected infection as possible. These should be prompted by any clinical abnormalities, such as unexplained fever, without waiting for findings more suggestive of aspergillosis. Finally, some patients with fatal outcomes did not receive aggressive amphotericin therapy, despite positive culture results and other findings suggestive of *Aspergillus*. Some of these patients may have been saved by more aggressive treatment.

Fungal cultures were superior to routine cultures for the recovery of *Aspergillus*. They also discriminated better than routine cultures between patients who had definite infection and those who were proved free of infection. Only 1 patient without IPA had more than one positive fungal culture, and she had bronchiectasis, a known risk factor for fungal colonization.

In series reporting ≥10 cases of IPA, the sensitivity of respiratory-tract cultures has ranged from 15% to 69%.[1-3,17,24,25,29-31] This wide range probably reflects variability in inclusion criteria as well as the small number of cases in some series. At our hospital, 48 cases of IPA were documented histologically between 1983 and 1993. In 39 of these, respiratory-tract specimens were submitted antemortem, and 22 had positive cultures, yielding a sensitivity of 56%. Most of the above data reflect the results of sputum cultures. Nonbiopsy specimens obtained during bronchoscopy have yielded sensitivities ranging from 21% to 50%.[31-35] In our study, BAL and bronchial washings or brushings were positive in 20 (77%) of 26 patients with definite or probable IPA. Interestingly, bronchoscopic cultures were also positive in 8 (73%) of 11 uninfected patients. This might suggest that specimens obtained during bronchoscopy are no more specific than sputum specimens. However, the decision to perform bronchoscopy was not controlled, and patients with positive sputum cultures and obvious clinical disease may have been less likely to undergo bronchoscopy. Some authors have found that careful cytologic analysis on BAL specimens enhanced the sensitivity for detection of IPA.[34] We could not evaluate cytology in our series, because examination was performed infrequently and positive results were reported as "fungal elements" without clear distinction between *Candida* and *Aspergillus*.

Surveillance cultures during a defined high-risk period, for example, chemotherapy-related granulocytopenia, would theoretically enable earlier recovery of *Aspergillus*. With the exception of one study,[47] surveillance cultures of the nares or throat have not been useful in predicting invasive aspergillosis.[7,17,18,48] Data on the usefulness of sputum cultures either have been drawn from small numbers of patients or do not clearly distinguish between true surveillance cultures and aggressive culturing prompted by fever or respiratory findings.[22,24,30,49] Strategies aimed at early detection may be facilitated by antigen or polymerase chain reaction assays currently being developed.[28,50]

Several limitations of a retrospective study of aspergillosis are apparent in this report. The timing and frequency of specimen submission were uncontrolled, and the frequency of signs, symptoms, and radiographic findings may have varied considerably between observers. Furthermore, many of our results were derived from patients with BMT or profound granulocytopenia. These patients are more likely to receive empiric therapies and less likely to undergo definitive procedures than are other patients at risk for IPA. Thus, the impact of culture data in these patients could also be more difficult to demonstrate. Also, the lack of speciation and quantitation of growth may have eliminated potentially useful information for interpreting culture data.

In summary, the isolation of *Aspergillus* species from nonbiopsy, lower-respiratory-tract cultures was associated with acute, invasive infection among patients with granulocytopenia, hematologic malignancies, BMT, solid-organ transplantation, or corticosteroid use in nearly two thirds of instances. The estimated predictive value for positive cultures varied according to risk group, being highest (82%) for BMT recipients, 72% for hematologic malignancies, and 56% to 63% for patients with solid-organ transplants or corticosteroid usage, respectively. Fungal culture media were superior to routine bacterial culture media for the recovery of *Aspergillus*. The correlation of culture data with clinical and radiographic findings helped to distinguish invasive infection from colonization or contamination. We suggest that at least three sputum specimens should be submitted for fungal culture whenever fungal infection is suspected.

ACKNOWLEDGMENT

We acknowledge Steven Wolff, MD, and James B. Dale, MD, for their cogent and helpful comments and Todd Shay for his assistance in preparation of the manuscript.

REFERENCES

1. Young RC, Bennett JE, Vogel CL, et al. Aspergillosis. *Medicine*. 1970;49: 147–173.

2. Bodey GP. Fungal infections complicating acute leukemia. *J Chron Dis*. 1966;19:667–687.

3. Fisher BD, Armstrong D, Yu B, Gold JWM. Invasive aspergillosis. *Am J Med*. 1981;71:571–577.

4. Meyer RD, Young LS, Armstrong D, Yu B. Aspergillosis complicating neoplastic disease. *Am J Med*. 1973;54:6–15.

5. Gerson SL, Talbot GH, Hurwitz S, et al. Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. *Ann Intern Med*. 1984;100:345–351.

6. Sherertz RJ, Belani A, Kramer BS, et al. Impact of air filtration on nosocomial *Aspergillus* infections. *Am J Med*. 1987;83:709–718.

7. Wingard JR, Beals SU, Santos GW, et al. *Aspergillus* infections in bone marrow transplant recipients. *Bone Marrow Transplant.* 1987;2:175–181.
8. Meyers JD. Fungal infections in bone marrow transplant patients. *Sem Oncol.* 1990;17:10–13.
9. Gurwith MJ, Stinson EB, Remington JS. *Aspergillus* infection complicating cardiac transplantation. *Arch Intern Med.* 1971;128:541–545.
10. Weiland D, Ferguson R, Peterson PK, et al. Aspergillosis in 25 renal transplant patients. *Ann Surg.* 1983;198:622–629.
11. Castaldo P, Stratta RJ, Wood PR, et al. Clinical spectrum of fungal infections after orthotopic liver transplantation. *Arch Surg.* 1991;126:149–156.
12. Kusne S, Torre-Cisneros J, Manez R, et al. Factors associated with invasive lung aspergillosis and the significance of positive *Aspergillus* culture after liver transplantation. *J Infect Dis.* 1992;166:1379–1383.
13. Bodey GP, Vartivarian S. Aspergillosis. *Eur J Clin Microbiol Infect Dis.* 1989;8:413–437.
14. Denning DW, Follansbee SE, Scolaro M, et al. Pulmonary aspergillosis in the acquired immunodeficiency syndrome. *NEJM.* 1991;324:654–662.
15. Lortholary O, Meyohas M-C, Dupont B, et al. Invasive aspergillosis in patients with acquired immunodeficiency syndrome: report of 33 cases. *Am J Med.* 1993;95:177–187.
16. Pursell KJ, Telzak EE, Armstrong D. *Aspergillus* species colonization and invasive disease in patients with AIDS. *Clin Infect Dis.* 1992;14:141–148.
17. Pannuti CS, Gingrich RD, Pfaller MA, Wenzel RP. Nosocomial pneumonia in adult patients undergoing bone marrow transplantation: a 9-year study. *J Clin Oncol.* 1991;9:77–84.
18. Karp JE, Merz WG, Charache P. Response to empiric amphotericin B during antileukemic therapy-induced granulocytopenia. *Rev Infect Dis.* 1991; 13:592–599.
19. O'Donnel MR, Schmidt GM, Tegtmeier BR, et al. Prediction of systemic fungal infection in allogeneic marrow recipients: impact of amphotericin prophylaxis in high-risk patients. *J Clin Oncol.* 1994;12:827–834.
20. Aisner J, Schimpff SC, Wiernik PH. Treatment of invasive aspergillosis: relation of early diagnosis and treatment to response. *Ann Intern Med.* 1977; 86:539–543.
21. Pennington JE. *Aspergillus* pneumonia in hematologic malignancy. *Arch Intern Med.* 1977;137:769–771.
22. Sinclair AJ, Rossof AH, Coltman CA. Recognition and successful management in pulmonary aspergillosis in leukemia. *Cancer.* 1978;42:2019–2024.
23. Rotstein C, Cummings KM, Tidings J, et al. An outbreak of invasive aspergillosis among allogeneic bone marrow transplants: a case-control study. *Infect Control.* 1985;6:347–355.
24. Burch PA, Karp JE, Merz WG, et al. Favorable outcome of invasive aspergillosis in patients with acute leukemia. *J Clin Oncol.* 1987;5:1985–1993.
25. Shpilberg O, Douer D, Goldschmied-Reouven A, et al. Invasive aspergillosis in neutropenic patients with hematological disorders. *Leukemia and Lymphoma.* 1991;4:257–262.
26. Gerson SL, Talbot GH, Lusk E, et al. Invasive pulmonary aspergillosis in adult acute leukemia: clinical clues to its diagnosis. *J Clin Oncol.* 1985;3: 1109–1116.
27. Gerson SL, Talbot GH, Hurwitz S, et al. Discriminant scorecard for diagnosis of invasive pulmonary aspergillosis in patients with acute leukemia. *Am J Med.* 1985;79:57–64.
28. Andriole VT. Infections with *Aspergillus* sp. *Clin Infect Dis.* 1993;17(suppl 2):S481–S486.
29. Nalesnik MA, Myeroqitz RL, Jenkins R, et al. Significance of *Aspergillus* species isolated from respiratory secretions in the diagnosis of invasive pulmonary aspergillosis. *J Clin Microbiol.* 1980;11:370–376.
30. Jeffery GM, Beard MEJ, Ikram RB, et al. Intranasal amphotericin B reduces the frequency of invasive aspergillosis in neutropenic patients. *Am J Med.* 1991;90:685–692.
31. Kahn FW, Jones JM, England DM. The role of bronchoalveolar lavage in the diagnosis of invasive pulmonary aspergillosis. *Am J Clin Pathol.* 1986;86: 518–523.
32. Albelda SM, Talbot GH, Gerson SL, et al. Role of fiberoptic bronchoscopy in the diagnosis of invasive pulmonary aspergillosis in patients with acute leukemia. *Am J Med.* 1984;76:1027–1034.
33. Saito H, Anaissie EJ, Morice RC, et al. Bronchoalveolar lavage in the diagnosis of pulmonary infiltrates in patients with acute leukemia. *Chest.* 1988;94:745–749.
34. Levy J, Horak DA, Tegtmeier BR, et al. The value of bronchoalveolar lavage and bronchial washings in the diagnosis of invasive pulmonary aspergillosis. *Respir Med.* 1992;86:243–248.
35. McWhinney PHM, Kibbler CC, Hamon MD, et al. Progress in the diagnosis and management of aspergillosis in bone marrow transplantation: 13 years' experience. *Clin Infect Dis.* 1993;17:397–404.
36. Treger TR, Visscher DW, Bartlett MS, Smith JW. Diagnosis of pulmonary infection caused by *Aspergillus*: usefulness of respiratory cultures. *J Infect Dis.* 1985;152:572–576.
37. Yu VL, Muder RR, Poorsattar A. Significance of isolation of *Aspergillus* from the respiratory tract in diagnosis of invasive pulmonary aspergillosis. *Am J Med.* 1986;81:249–254.
38. Libshitz HL, Pagani JJ. Aspergillosis and mucormycosis: two types of opportunistic fungal pneumonia. *Radiology.* 1981;140:301–306.
39. Orr DP, Myerwitz RL, Dubois PJ. Patho-radiologic correlation of invasive pulmonary aspergillosis in the compromised host. *Cancer.* 1978;41: 2028–2039.
40. Kuhlman JE, Fishman EK, Siegelman SS. Invasive pulmonary aspergillosis in acute leukemia: characteristic findings on CT, the CT halo sign, and the role of CT in early diagnosis. *Radiology.* 1985;157:611–614.
41. Needleman SW, Aschenbrener CA, Armitage JO. Occult pulmonary thromboembolism presenting as diffuse interstitial pneumonitis in cancer patients. *Postgrad Med J.* 1983;59:508–511.
42. Uderzo C, Marraro G, Riva A, et al. Pulmonary thromboembolism in leukaemic children undergoing bone marrow transplantation. *Bone Marrow Transplant.* 1993;11:201–203.
43. Vartivarian SE, Anaissie EJ, Bodey GP. Emerging fungal pathogens in immunocompromised patients: classification, diagnosis and management. *Clin Infect Dis.* 1993;17(suppl 2):s487–s491.
44. Cordonnier C, Bernaudin J-F, Bierling P, et al. Pulmonary complications occurring after allogeneic bone marrow transplantation. *Cancer.* 1986;58: 1047–1054.
45. Mori M, Glavin JR, Barloon TJ, et al. Fungal pulmonary infections after bone marrow transplantation: evaluation with radiography and CT. *Radiology.* 1991;178:721–726.
46. Barloon TJ, Galvin JR, Mori M, et al. High-resolution ultrafast chest CT in the clinical management of febrile bone marrow transplant patients with normal or nonspecific chest roentgenograms. *Chest.* 1991;99:928–933.
47. Aisner J, Murillo J, Schimpff SC, Steere AC. Invasive aspergillosis in acute leukemia: correlation with nose cultures and antibiotic use. *Ann Intern Med.* 1979;90:4–9.
48. Sandford GR, Merz WG, Wingard JR, et al. The value of fungal surveillance cultures as predictors of systemic fungal infections. *J Infect Dis.* 1980;142: 503–509.
49. Schwartz RS, MacKintosh FR, Schrier SL, Greenberg PL. Multivariate analysis of factors associated with invasive fungal disease during remission induction therapy for acute myelogenous leukemia. *Cancer.* 1984;53: 411–419.
50. Tang CM, Holden DW, Aufauvre-Brown A, Cohen J. The detection of *Aspergillus* sp. by the polymerase chain reaction and its evaluation in bronchoalveolar lavage fluid. *Am Rev Respir Dis.* 1993;148:1313–1317.