Significance of Isolation of Aspergillus from the Respiratory Tract in Diagnosis of Invasive Pulmonary Aspergillosis

Results from a Three-Year Prospective Study

VICTOR L. YU, M.D.
ROBERT R. MUDER, M.D.
ABBAS POORSATTAR, M.D.*
Pittsburgh, Pennsylvania

The isolation of Aspergillus species from respiratory secretions has been regarded as being of limited usefulness in the antemortem diagnosis of invasive pulmonary aspergillosis. One hundred and eight consecutive patients were evaluated in whom Aspergillus species were isolated from respiratory secretions. Invasive aspergillosis was not demonstrated in non-immunosuppressed patients or in patients with solid tumors in the absence of neutropenia. Lung tissue was examined in 17 patients with leukemia and/or neutropenia; all had invasive aspergillosis. Tissue examination was not performed in 20 neutropenic patients; of 17 not receiving antifungal therapy, 16 died. Multivariate statistical analysis showed that neutropenia and absence of cigarette smoking were significant predictors of invasive aspergillosis in patients with respiratory tract cultures yielding Aspergillus. All cases of invasive aspergillosis were associated with A. fumigatus or A. flavus. The isolation of A. fumigatus or A. flavus from the respiratory tract of a patient with leukemia and/or neutropenia is highly predictive of invasive infection. Empiric amphotericin B therapy, without the necessity for tissue diagnosis, should be considered in this patient subgroup.

The mortality rate of invasive pulmonary aspergillosis remains high, exemplified by the fact that the diagnosis is first made at autopsy in the majority of patients [1–4]. Although earlier diagnosis and therapy with amphotericin B appear to improve survival [5–7], early diagnosis is difficult. There are no pathognomonic clinical findings for invasive pulmonary aspergillosis. The commonest presentation is that of fever and pulmonary infiltrates unresponsive to antibiotics [2,3]. This presentation is, of course, compatible with any number of bacterial, fungal, viral, or protozoan infections. In addition, aspergillosis may coexist with or arise during the therapy of other infections in compromised patients, especially those due to Candida species and Pseudomonas aeruginosa [3].

Antemortem isolation of Aspergillus species from respiratory secretions in pathologically confirmed cases occurs infrequently, with sensitivity rates ranging from 13 to 34 percent [1–3,8]. Because of the low sensitivity and uncertain specificity of culture results in the diagnosis of aspergillosis [3,9,10], demonstration of tissue invasion by fungal hyphae remains the accepted standard for diagnosis [2,11]. One of us (V.L.Y.) has been impressed with the number of proved cases of invasive aspergillosis in which the diagnosis was first suspected by isolation and visualization of the organism in sputum specimens, an anecdotal personal experience gathered at two institutions in eastern and western United States. On the basis of this impression, a prospective study was initiated

From the Department of Medicine, University of Pittsburgh and Veterans Administration Medical Center, Pittsburgh, Pennsylvania. Requests for reprints should be addressed to Dr. Victor L. Yu, University of Pittsburgh School of Medicine, Scaife 968, Pittsburgh, Pennsylvania 15261.
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*Current address: Shappert Health Center, 2170 Pearl Street, Belvedere, Illinois 61008.

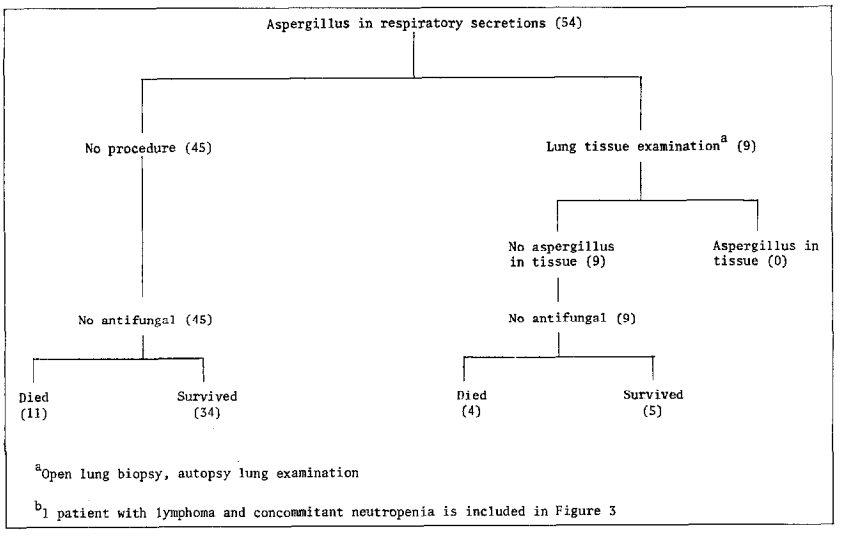

Figure 1. *Isolation of Aspergillus in 26 non-immunosuppressed patients and 28 patients with solid tumor/lymphoma. Note that invasive aspergillosis was not seen and that most patients survived without antifungal therapy.*

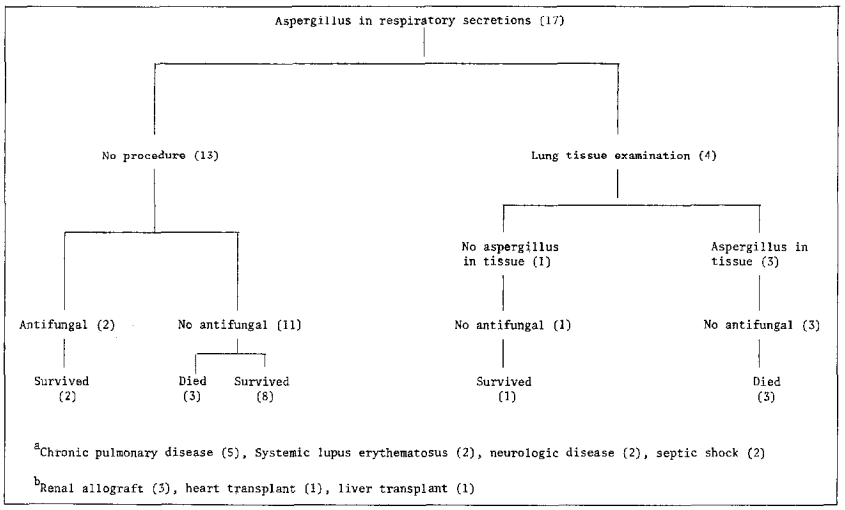

Figure 2. *Isolation of Aspergillus in 11 patients receiving steroids only (a) and six transplant recipients (b) (patients with neutropenia excluded). Note that invasive Aspergillus was documented in three of four patients undergoing an invasive procedure. However, most patients not receiving antifungal therapy survived.*

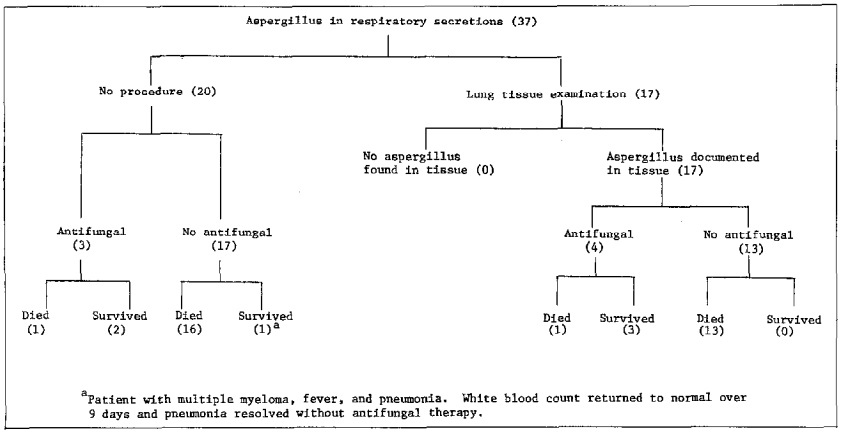

Figure 3. *Isolation of Aspergillus from 37 patients with neutropenia and/or leukemia. Note that 17 of 17 patients undergoing a diagnostic procedure showed invasive aspergillosis and that of the 20 patients not undergoing such a procedure, 17 did not receive antifungal therapy, and 16 of 17 died.*

to test the hypothesis that isolation of Aspergillus from sputum specimens might be clinically useful in selected patient subgroups in establishing the diagnosis of invasive pulmonary aspergillosis.

PATIENTS AND METHODS

All patients at the Veterans Administration Medical Center and Presbyterian University Hospital, Pittsburgh, in whom Aspergillus was isolated from sputum samples or the respiratory tract were prospectively followed by one of us. The duration of the study was from July 1979 to June 1982. Follow-up study at three and six months after initial data collection consisted of determination of outcome as well as examination of clinical and autopsy records for such factors as administration of antifungal therapy, course of underlying disease, and results of antemortem biopsies.

Patient data were entered into a computer data bank (Prophet System, Division of Research Resources, National Institutes of Health). The Fisher exact test was used for univariate analysis. A stepwise logistic regression method was used for multivariate analysis (BMDP statistical software, University of California). The Mann-Whitney rank sum test was used for assessing the association of severity of neutropenia with invasive aspergillosis.

RESULTS

In the three-year period from July 1979 to June 1982, 108 consecutive patients with Aspergillus isolated from antemortem respiratory tract culture samples were observed. Seventy-seven patients were hospitalized at Presbyterian University Hospital and 31 at the Pittsburgh Veterans Administration Medical Center. Ninety-one of the Aspergillus isolations were from sputum, 11 from bronchoscopy specimens, five from transtracheal aspirates, and one from a nasotracheal swab specimen.

Fifty-three isolates were A. fumigatus, 42 were A. flavus, and 10 were A. niger. One patient had both A. flavus and A. niger isolated from the respiratory tract. The species of two isolates were not identified. Patients' ages ranged from 18 to 82 years, with a mean of 57 years.

Figures 1 to 3 summarize the outcome with and without antifungal therapy for the entire study group of 108 patients. In 30 patients, lung tissue was examined for evidence of invasive pulmonary aspergillosis either by open lung biopsy or by postmortem lung examination. **Table I** and Figures 1 to 3 delineate the outcome of the study group subclassified by increasing degree of immunosuppression. The right side of the flow diagrams in Figures 1 to 3 depicts the results for patients undergoing tissue examination (open lung biopsy or postmortem examination). Note that as the degree of immunosuppression increases, the finding of Aspergillus in the respiratory tract ante mortem heralds the presence of invasive pulmonary aspergillosis (Table I). The left side of the flow diagrams in Figures 1 to 3 depicts the outcome in patients without definitive diagnosis. "Survived" is survival to discharge from the hospital. "Died" is death during hospitalization. Since the study group consisted of patients hospitalized for a variety of reasons, death was often due to multiple causes including infection, cardiac and respiratory failure, and so on. No attempt was made to attribute death to a specific cause. However, Figures 1 to 3 show that as immunosuppression increases, survival without administration of empiric antifungal chemotherapy becomes increasingly unlikely. Invasive aspergillosis was not seen in non-immunosuppressed patients or in patients with solid tumors and lymphoma (neutropenic patients excluded) (Table I). Invasive aspergillosis developed in one patient receiving steroids for systemic lupus erythematosus (Table I). Of six transplant recipients without neutropenia, two of three patients undergoing lung tissue examination had invasive aspergillosis (Table I). Of three patients who did not undergo a diagnostic procedure, two survived without antifungal therapy and presumably did not have invasive aspergillosis (data not shown). Figure 3 evaluates the outcome in the group of patients with the greatest degree of immunosuppression: neutropenic and leukemic patients. In this group, 92 percent (34 of 37) of the patients were also receiving concomitant steroids and/or cytotoxic chemotherapy. All (17 of 17) patients undergoing lung tissue examination in this group were shown to have invasive aspergillosis. The 20 patients in this group who did not undergo tissue examination constitute an interesting subgroup; 17 of these 20 did not receive an antifungal agent, and only one survived hospitalization.

TABLE I **Increase in Specificity of Aspergillus Isolation from Respiratory Secretions with Increasing Degree of Immunosuppression**

	Total Number	Lung Tissue Examined	Aspergillus Documented
Non-immunosuppressed patients	26	4	0% (0/4)
Solid tumor or lymphoma*	28	5	0% (0/5)
Steroid therapy*	11	1	100% (1/1)
Transplantation*	6	3	67% (2/3)
Neutropenia/leukemia	37	17	100% (17/17)

* Neutropenic patients excluded.

Patients were further analyzed by classification into the categories of "invasive aspergillosis" and "no disease." *Invasive aspergillosis (20 patients):* Nineteen patients were shown to have hyphal invasion characteristic of Aspergillus on examination of lung tissue. Subsequent isolation by culture was demonstrated for 18 of 19 patients. An additional patient with A. fumigatus isolated from the sputum was classified as having invasive aspergillosis on the basis of isolation of the organism from liver samples during limited postmortem examination in which examination of the chest was not permitted. Of the respi-

TABLE II Clinical Characteristics of 72 Evaluable Patients with Antemortem Respiratory Tract Culture for Aspergillus

	Invasive Aspergillosis (n = 20)	No Disease* (n = 52)	Fisher p Value
Underlying disease			
Chronic lung disease†	10%	54%	<0.01
Solid tumor‡	0%	40%	<0.01
Transplantation§	10%	6%	NS
Leukemia**	50%	0%	<0.01
Neutropenia††	80%	2%	<0.01
Risk factors			
Steroid therapy‡‡	100%	21%	<0.01
Cigarette smoking	40%	81%	<0.05
Prior antibiotics	95%	57%	<0.01
Clinical features			
Fever	95%	61%	<0.01
Cough	75%	70%	NS

* Includes 10 patients with negative results of tissue examination and 42 patients who survived without antifungal therapy.
† Chronic obstructive pulmonary disease and cystic fibrosis.
‡ Carcinoma of the lung (10), head and neck (four), breast (one), prostate (one), carcinoid (one), and lymphoma (three); neutropenic patients excluded.
§ Renal (four) and liver (one); three renal transplant recipients and one heart transplant recipient not included here, but included in the "neutropenic" category.
** Acute myelogenous (four), acute promelocytic (two), chronic myelogenous (two), and hairy cell (two).
†† Neutrophil count below 1,800/mm^3: leukemia (nine), transplantation (four), lymphoma (one), multiple myeloma (two), and Felty's syndrome (one).
‡‡ Leukemia (nine), transplantation (nine), chronic obstructive pulmonary disease (three), collagen vascular disease (three), lymphoma (two), solid tumor (two), myeloma (two), and neurologic syndrome (one).

ratory tract isolates, 13 were A. fumigatus, and seven were A. flavus. *No Aspergillus disease (52 patients):* In 10 patients, examination of lung tissue failed to reveal evidence of hyphal invasion, and fungal culture was nonrevealing. An additional 42 patients were classified as having "no Aspergillus disease" on a presumptive basis, in that these patients showed no signs of clinical deterioration despite the absence of antifungal chemotherapy, and all survived their hospitalization.

Table II shows that patients with invasive aspergillosis were significantly more likely to have leukemia and neutropenia, and to have received steroids or prior antibiotic therapy ($p < 0.01$). In contrast, chronic lung disease, solid tumor, and cigarette smoking were significantly associated with the presence of colonization ($p < 0.05$). **Table III** details the significant association of invasive aspergillosis with neutropenia (Fisher exact test, $p < 0.001$). Severity of neutropenia was significantly associated with the presence of invasive aspergillosis (Mann-Whitney rank sum test, $p < 0.001$).

Multivariate analysis was performed using a stepwise logistic regression method. The following parameters found to be statistically significant by univariate analysis were entered: neutropenia, chronic obstructive pulmonary disease, history of cigarette smoking, fever, and prior antibiotic usage. Four models were evaluated: immunosuppression (transplantation, hematologic malignancy, neutropenia) and neutropenia alone were entered into the regression method separately. Fever and prior antibiotic usage were entered together as well as separately. Chronic obstructive pulmonary disease and history of cigarette smoking were entered in all models. Results from all four models were consistent: neutropenia plus the absence of smoking history were significant parameters in predicting the presence of invasive aspergillosis (odds ratio 61.79, 95 percent confidence interval 9.93 to 409.1).

COMMENTS

Sputum culture is regarded as not useful for the diagnosis of invasive aspergillosis. For example, in most patients with invasive aspergillosis, sputum samples fail to yield the organism. Likewise, the specificity of the sputum culture result is equally unsatisfactory; most patients with Aspergillus on sputum culture will *not* have invasive aspergillosis. Our study confirms this view: of 30 patients who had respiratory tract culture samples that grew Aspergillus and who underwent an invasive procedure, 10 (33 percent) had no evidence of invasive aspergillosis.

However, we hypothesized that there may exist important subsets of patients for which antemortem isolation of Aspergillus may still be diagnostically useful. This study shows that underlying disease is a critical factor in selecting patients in whom antemortem sputum cultures may be useful. Table I and Figures 1 to 3 show sequentially that, as the degree of immunosuppression increases, the diagnostic specificity and clinical applicability of isolation of Aspergillus from respiratory tract samples increases. For example, none of the patients with positive respiratory tract culture results among the non-immunosuppressed patients or among the patients with solid tumors or lymphoma group were shown to have invasive pulmonary aspergillosis. One of 11 patients among the steroid-treated group and two of six among the transplant recipients without neutropenia had invasive aspergillosis (Table I). However, for neutropenic/leukemic patients, 17 of 17 (100 percent) undergoing tissue examination were ultimately shown to have invasive aspergillosis (Figure 3). It is also interesting to note the outcome of the 20 patients with neutropenia/leukemia in whom no diagnostic procedure was performed (Figure 3); 17 received no antifungal therapy, and 94 percent (16 of 17) died. In the group of non-immunosuppressed patients who did not undergo a definitive procedure, only 14 percent (three of 22) died. Although this undoubtedly is due to the fact that immuno-

suppressed patients have a poorer overall prognosis, it is also consistent with the thesis that these leukemic/neutropenic patients without a tissue diagnosis may well have had invasive pulmonary aspergillosis.

A weakness of this study is the relatively small number of patients with definitive tissue diagnosis (30 of 108), although this number remains larger than many other comparative series reported in the literature. We attempted to offset this deficiency with the following analysis: we assumed that patients with positive results of respiratory tract culture did *not* have invasive aspergillosis if they did not show signs of clinical deterioration and survived without receiving antifungal therapy. The evidence supporting this assumption is persuasive. Survival among patients with invasive pulmonary aspergillosis, even when given antifungal therapy, is uncommon, although it has been suggested that early diagnosis accompanied by therapy will improve prognosis. Survival without antifungal therapy is virtually unrecognized. In a review of seven reports of invasive aspergillosis [1,2,6,7,9,12,13], we found only one patient who survived without antifungal therapy [9]. This particular patient had an aspergilloma with lung abscesses. He was lost to follow-up, so that long-term outcome was never reported. Thus, we believe the 42 patients who survived without antifungal therapy can reasonably be included in the "no Aspergillus disease" category. Given these criteria, 52 patients were characterized as having "no disease"—10 on the basis of negative results of tissue examination and 42 on the basis of the aforementioned clinical criteria. Twenty patients were characterized as having "invasive aspergillosis" on the basis of tissue demonstration of the organism. Table II summarizes the results of this analysis. Again, the presence of leukemia and neutropenia was noted significantly more frequently in patients with invasive aspergillosis. Administration of steroids and antibacterial agents and the presence of fever were noted significantly more frequently in patients with invasive aspergillosis. However, Gerson et al [14] have suggested that these associations may be artifactual, occurring as a result of confounding factors in which duration of neutropenia was the primary factor. In our study, multivariate analysis showed that neutropenia and the *absence* of a history of cigarette smoking were associated with the presence of invasive aspergillosis.

Aspergillus is commonly found as a commensal in the respiratory tract flora of patients with chronic pulmonary disease [15,16]. Chronic obstructive pulmonary disease constituted the largest patient category in Strimlan et al's [16] series of 169 patients with Aspergillus in the respiratory tract. This predisposition to pulmonary disease is significant; in our study, chronic lung disease was seen more frequently in patients with "no Aspergillus disease" than in those with invasive pulmonary aspergillosis (Table II). Similarly, there were more elderly patients (data not shown) and cigarette smokers in the "no Aspergillus disease" category (Table II). Thus, the diagnostic utility of Aspergillus isolation from sputum appears to be compromised in an elderly, cigarette-smoking patient with chronic lung disease.

TABLE III Significant Association of Invasive Aspergillosis with Presence of Neutropenia in Patients with Respiratory Tract Culture Yielding Aspergillus

Neutrophil Count	Invasive Aspergillosis	No Disease	Total
$<1,800/mm^3$	16	1	17
$\geq 1,800/mm^3$	4	51	55
Total	20	52	72

$p < 0.001$, Fisher's exact test. Increasing severity of neutropenia is significantly associated with invasive aspergillosis ($p < 0.01$, Mann-Whitney rank sum test). Range of neutropenia was 16 to $1,800/mm^3$.

Given the results of our prospective study, a re-review of the literature is also revealing. We found 14 patients described in recent studies with antemortem sputum culture evidence of Aspergillus who would fall into our high-risk category of neutropenia/leukemia [1,6,7,13,17]. All 14 were ultimately shown to have invasive aspergillosis. In most of these patients, the significance of the initial isolation went unappreciated, and antifungal therapy was not given or was not given until shortly before death. On the other hand, investigators from Memorial Hospital have had a different experience. Six patients with "leukemia or lymphoma" [9] and three patients with "hematologic malignancy" [3] had positive results of sputum cultures and were found not to have evidence of invasive pulmonary aspergillosis. How can their findings be reconciled with our data? There are a number of possible explanations. One is that the epidemiology of Aspergillus dissemination in their institutions is different from that in ours. It is also possible that our findings and theirs may not be contradictory, in that their patients were not categorized by discrete subgroups (leukemia, lymphoma, neutropenia) and comparative statistical analysis was not performed. Finally, it is conceivable, but unlikely, that rising neutrophil counts during the course of infection may have led to resolution without antifungal therapy in some of their patients.

In summary, the results of this three-year prospective study suggest the following conclusions and courses of action:

- The isolation of Aspergillus from respiratory secretions in non-immunosuppressed patients is rarely indicative of invasive pulmonary aspergillosis. The presence of chronic lung disease and cigarette smoking makes colonization significantly more likely.
- Likewise, the isolation of Aspergillus in patients with solid tumors (in the absence of neutropenia) rarely is associated with invasive aspergillosis. Although these

patients should be monitored by chest radiography and clinical follow-up, invasive diagnostic procedures are not usually indicated.

- In immunosuppressed but non-neutropenic patients with a transplant, lymphoma, or steroid therapy, isolation of Aspergillus from respiratory secretions is a cause for concern. Our data would indicate that empiric amphotericin B is not warranted because of the low specificity of the respiratory tract culture; however, in the presence of pulmonary infiltrates, aggressive diagnostic procedures including lung biopsy by bronchoscopy or open thoracic operation must be strongly considered.
- For patients with neutropenia or leukemia (chronic lymphocytic excluded), isolation of Aspergillus from respiratory secretions was virtually diagnostic of invasive pulmonary aspergillosis. This is our most important finding. We now believe that positive results of respiratory tract culture in this select patient group are sufficient indication for immediate initiation of amphotericin B therapy at our institution. Invasive diagnostic procedures to obtain lung tissue need not be performed. Until our results are confirmed by other investigators, however, invasive diagnostic procedures (e.g., open lung biopsy) for definitive diagnosis may be a preferred course at other institutions.

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