The Role of Bronchoalveolar Lavage in the Diagnosis of Invasive Pulmonary Aspergillosis

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Cultures and histochemical stains for fungi were performed on concentrated, cytocentrifuged bronchoalveolar lavage (BAL) samples from 82 immunocompromised patients undergoing bronchoscopic evaluation of new pulmonary infiltrates. Aspergillus hyphae were identified in 9 of 17 BAL samples from patients with invasive pulmonary aspergillosis and from 3 of the remaining 65 study patients without this diagnosis. Thus, the presence of Aspergillus hyphae in BAL samples had a 53% sensitivity, 97% specificity, and 75% positive predictive value for the diagnosis of invasive pulmonary aspergillosis. BAL fungal cultures were positive in only 4 of 17 cases (23% sensitivity). A combination of fungal stains and cultures yielded a diagnostic sensitivity of 58% and a specificity of 92%. Results of routine fiberoptic bronchoscopy procedures in the 17 patients with aspergillosis who had BAL and in a retrospectively identified group of 10 patients with invasive aspergillosis who had only routine bronchoscopy were tabulated. Routine bronchoscopy procedures yielded a diagnosis in 5 of 27 cases (22% overall yield), with washings diagnostic in 4 of 27 cases (15%), and transbronchial biopsies positive histologically in 2 of 11 cases (18%). BAL is a valuable first procedure for diagnosing invasive pulmonary aspergillosis in the compromised host. (Key words: Pulmonary aspergillosis; Bronchoalveolar lavage; Bronchoscopy; Diagnosis; Transbronchial biopsy) Am J Clin Pathol 1986; 86:518-523

INVASIVE PULMONARY ASPERGILLOSION is an important cause of morbidity and mortality in immuno-
compromised hosts. The rising incidence of invasive aspergillosis has not been met with a corresponding improvement in methods of diagnosing this infection, despite the fact that the outcome of aspergillosis has been directly correlated with early diagnosis and institution of therapy. Recent reports continue to document mortality rates approaching 70-90% in patients with hematopoietic malignancies and invasive aspergillosis.

Rapid diagnosis of invasive pulmonary aspergillosis is difficult. Analysis of expectorated sputa lacks both sensitivity and specificity for the diagnosis. Serologic detection of circulating fungal antigens, while an appealing and promising technic, has not gained widespread application. Invasive diagnostic technics, such as transtracheal aspiration, percutaneous needle biopsy, and fiberoptic bronchoscopy with bronchial brushings, have all been reported to be useful in establishing a diagnosis of pulmonary aspergillosis. However, the overall usefulness of these technics, particularly fiberoptic bronchoscopy, has not been documented. In many published reports the diagnosis was ultimately established only by an open lung biopsy or necropsy.

Recently, the bronchoscopic technic of bronchoalveolar lavage (BAL) has been demonstrated to be effective in diagnosing respiratory infections in the immunocompromised host. To date, there have been few reports describing its use in diagnosing invasive pulmonary aspergillosis. Relatively simple and rapid staining technics can detect fungal elements in concentrated, cytocentrifuged BAL samples obtained from immunocompromised patients. As BAL becomes more common, the clinical laboratory will be asked to process BAL specimens for histochemical staining and the pathologist or microbiologist solicited for interpretation of BAL slides. In this article, we describe our experience in using BAL for diagnosing invasive pulmonary aspergillosis in the compromised host. For patients with invasive aspergillosis, we compare the results obtained by BAL with those obtained with “routine” fiberoptic bronchoscopy studies, specifically bronchial washings and transbronchial biopsy.

Materials and Methods

Patients Selected for Study.

Forty cases of invasive pulmonary aspergillosis among patients hospitalized at the University of Wisconsin Clinical Sciences Center (CSC) or the William Middleton Veterans Administration Hospital between January 1, 1981, and May 30, 1985, were identified by a retrospective review of autopsy records, a computer-assisted search of patient records, and review of the mycology laboratory record book. Thirty-six patients with invasive pulmonary aspergillosis were identified based on histologic evidence of tissue invasion by characteristic Aspergillus hyphae with or without culture confirmation. An additional four cases were included for analysis based on the presence of positive cultures for Aspergillus from a normally sterile site (transstracheal aspirate; bronchial brushing) and appropriate clinical and radiographic evidence of invasive aspergillosis in the absence of other pulmonary pathogens.

Patient records were analyzed for the following features: age; underlying disease; use of broad-spectrum antibiotics preceding the diagnosis of pulmonary aspergillosis; presence and duration of neutropenia (<500 neutrophils/mm³); admission and prebronchoscopy hemoglobin and platelet counts; maximum serum creatinine, alkaline phosphatase and bilirubin levels; prebronchoscopy chest x-ray appearance; the timing of bronchoscopy in relation to change in chest x-ray, fever onset and institution of amphotericin B therapy; total dose and duration of antifungal therapy; the results of routine bronchoscopy procedures i.e., washings, brushings and transbronchial biopsies; results of BAL studies; results of fungal stains and cultures of expectorated sputa; results of invasive diagnostic procedures; and autopsy results.

Twenty-seven of the 40 study patients underwent bronchoscopy and had results available for analysis. Patients undergoing bronchoscopy were divided into two groups: Group 1 (10 patients) consisted of patients with invasive pulmonary aspergillosis who had routine bronchoscopy studies performed, including bronchial washings, brushings, and/or transbronchial biopsies; Group 2 comprised 17 patients with the diagnosis of invasive pulmonary aspergillosis found in a study population of 82 immunocompromised patients undergoing BAL in addition to routine bronchoscopy studies for the evaluation of new pulmonary infiltrates. There were 65 patients in the BAL study population without pulmonary aspergillosis; these patients were called Group 2 controls. Protocols for BAL were approved by the University of Wisconsin Human Subjects Committee.

Bronchoscopic Technics

Fiberoptic bronchoscopy and BAL were performed as previously described. Briefly, after atropine and meperidine preanesthesia, topical nasal and laryngeal anesthesia was applied. An Olympus model BF-1T or equivalent bronchoscope (Olympus Corporation of America, New Hyde Park, NY) was passed transnasally into the trachea. Further topical anesthesia was applied, and the bronchoscope was advanced and wedged into a segmental bronchus. Alveolar lavage was then performed by sequential instillation and suctioning of 50 mL volumes of physiologic saline. This procedure was repeated three to four times. If less than 25 mL was recovered from the
first two lavages or if the patient was not tolerating the procedure, lavage was terminated. Routine bronchoscopy studies, including brushings, low-volume washings (using 20–40 mL saline), and biopsies were then performed from an appropriate lung subsegment supplying an area of radiographic abnormality. Transbronchial biopsies were done only if coagulation studies were normal and the platelet count exceeded 50,000/mm³.

**Laboratory Studies**

Following the bronchoscopy procedure, lavage samples were immediately submitted for bacterial, fungal, viral, mycobacterial, and Legionella cultures. A portion of the lavage sample was centrifuged at 1,500 × g for 15 minutes. The supernate was decanted and the cell pellet was resuspended in Hank’s salt solution to a final volume of 10 mL. Cell counts were performed on the cell pellet by hemocytometer. The cell pellet was cytocentrifuged with an ethanol fixative (Shandon Instruments, Sewickley, PA), and the slides were gently heat-fixed and stained with Giemsa’s, Gram’s, toluidine blue-O, and auramine–rhodamine histochemical stains and examined for microorganisms. A cellular differential was obtained by counting a minimum of 200 cells on the Giemsa-stained slide.

Bronchial washings and biopsies were routinely submitted for bacterial, fungal, viral, mycobacterial, and Legionella cultures and stains, and cytologic studies (Papanicolaou). Examination for fungi in bronchial washings included potassium hydroxide (KOH) and Gram’s staining performed in a laboratory (Wisconsin State Laboratory of Hygiene) separate from the site where the BAL studies were performed.

Results of BAL and bronchoscopy studies were considered positive for Aspergillus when characteristic septate, 3–5 μm diameter, 45-degree branching hyphae without a yeast phase were seen by Giemsa’s, Gram’s, or toluidine blue-O stain (Fig. 1) or when the fungus was isolated from fungal cultures. Histologic sections of formalin-fixed, paraffin-embedded lung tissue obtained from diagnostic procedures, such as open lung biopsy, or autopsy were stained with Gomori methenamine silver nitrate and reviewed for the presence of fungi.

**Ancillary Studies**

Routine prebronchoscopy studies performed on all study patients included a chest radiograph, complete blood count, platelet count, serum creatinine, arterial blood gases, bilirubin, prothrombin time, and partial thromboplastin time, as well as cultures of blood, urine, and sputum (if available).

Chest radiographs were routinely read and reported by the staff radiologists at the University of Wisconsin CSC. Pulmonary infiltrates were characterized as unilateral or bilateral and as having one of the following patterns: (1) alveolar infiltrate; (2) nodule(s); (3) pleural-based defect (infarct pattern); or (4) nonspecific alveolar or interstitial pattern.

**Statistical Analysis**

Data were entered into a standard data base, using an IBM-PC® computer and were analyzed using a statistical program (Statplan®, Futures Group, Gastenbury, CT). For interval scaled variables, correlation coefficients and Student’s t-test values were calculated as required. Relationships between frequencies of various occurrences were studied by cross-tabulation (chi-square) analysis.

**Results**

The patients with invasive pulmonary aspergillosis included 27 men and 13 women with a mean age of 53 years (range of 18–78 years). Underlying diseases included: acute or chronic leukemia (32 patients), lymphoma (2
patients), renal transplant (2 patients), and diabetes melitus (1 patient). In three, steroids and/or cytotoxic drugs were administered for an autoimmune condition. An overall mortality rate of 77% was found, as only 9 of the 40 patients survived their infection. Surprisingly, statistical analysis revealed that likelihood of survival or death could not be predicted by a number of clinical parameters described previously in "Material and Methods." Only the use of corticosteroids in combination with anti-leukemic cytotoxic drugs was found to be significantly correlated with death (P < 0.01).

Twenty-seven of the 40 study patients had fiberoptic bronchoscopy performed during the study period. Ten patients (group 1) had routine fiberoptic bronchoscopy studies performed between 1981 and 1984. Starting on 2/1/84, we entered immunocompromised patients into a prospective evaluation of the diagnostic yield of BAL compared with routine fiberoptic bronchoscopy studies for pneumonitis. Between 2/1/84 and 5/30/85, 82 immunocompromised patients had BAL performed in addition to routine bronchoscopy studies in the evaluation of new pulmonary infiltrates. Seventeen patients from this population were found to have invasive pulmonary aspergillosis (Group 2). None of the patients undergoing BAL had complications related to the procedure. Tables 1 and 2 summarize the results of bronchoscopic procedures.

Histochemical stains were positive in 9 of 17 BAL samples from patients with the diagnosis of invasive pulmonary aspergillosis (7 by toluidine blue stain, 1 by Giemsa's stain, and 1 by Gram's stain). In three of the remaining patients studied, Aspergillus hyphae were seen in the BAL sample. None of these patients had clinical or histologic evidence of aspergillosis, and each eventually had another pulmonary diagnosis established (herpes simplex infection, pulmonary edema, and Pseudomonas pneumonia). Cytocentrifugation and staining were readily performed within two to three hours of receipt of the BAL sample (mean time to diagnosis = 6.6 hours). Differential counts of Giemsa-stained slides revealed a predominance of alveolar macrophages and did not demonstrate eosinophilia (defined by >5% eosinophils) in any of the BAL specimens from patients with invasive aspergillosis.

We analyzed clinical and laboratory parameters of study patients undergoing bronchoscopy and BAL in order to determine if any factors were predictive for positive results. There was no chest x-ray pattern strongly associated with a positive BAL result. While duration of neutropenia preceding diagnosis was greater than seven days in six of nine patients with positive BAL results, this association did not reach statistical significance. Duration of fever or chest x-ray changes more than five to ten days prior to bronchoscopy did not increase the likelihood of obtaining a positive BAL result. Although the administration of amphotericin B more than 5 days prior to bronchoscopy correlated with a positive BAL stain (P < 0.01), total dose of amphotericin B prior to bronchoscopy and use of ancillary anti-fungal drugs (rifampin or flucytosine) prior to bronchoscopy did not influence the likelihood of obtaining a positive BAL culture for Aspergillus. Perhaps because many patients had very prolonged, profound neutropenia, the total amount of amphotericin B given to patients after bronchoscopy did not influence their survival.

**Table 1. BAL Analysis in Diagnosis of Invasive Pulmonary Aspergillosis**

<table>
<thead>
<tr>
<th>Analysis Procedure</th>
<th>No. Positive/No. Specimens</th>
<th>No. Positive/No. Specimens</th>
<th>Sensitivity</th>
</tr>
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<tbody>
<tr>
<td>Stain</td>
<td>9/17 (53)</td>
<td>3/65 (5)</td>
<td>95</td>
</tr>
<tr>
<td>Culture</td>
<td>4/17 (23)</td>
<td>3/65 (5)</td>
<td>95</td>
</tr>
<tr>
<td>Stain and/or culture</td>
<td>10/17 (59)</td>
<td>5/65 (8)</td>
<td>92</td>
</tr>
</tbody>
</table>

Numbers in parentheses indicate percent positive. Specificity and predictive values are given as percent values.

Bronchial washings were performed in all 27 aspergillosis patients undergoing bronchoscopy. Fungal stains were positive in three cases (11% sensitivity) and cultures were positive in four cases (15% sensitivity). Because of an underlying coagulopathy and/or respiratory distress, transbronchial biopsies could be performed in only 11 of 27 cases and were positive by histologic examination in only 2 cases, thus yielding an 18% sensitivity. In one case, bronchial brushings were performed and revealed fungal elements as well as positive cultures for *Aspergillus fumigatus*.

Sputum cultures were eventually culture positive in 13 of 27 patients from Groups 1 and 2 in this series. However, spuata were positive only relatively late in the course of infection (mean time after change in chest x-ray = 15 days), thus limiting their diagnostic usefulness.

**Table 2. Efficacy of Bronchial Washings, Transbronchial Biopsy, and Sputum Culture in Diagnosis of Invasive Pulmonary Aspergillosis**

<table>
<thead>
<tr>
<th>Analysis Procedure</th>
<th>No. Positive/No. Specimens</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture of bronchial washings</td>
<td>4/27</td>
<td>15</td>
</tr>
<tr>
<td>Transbronchial biopsy</td>
<td>2/11</td>
<td>18</td>
</tr>
<tr>
<td>Washings and/or transbronchial biopsy</td>
<td>5/2</td>
<td>22</td>
</tr>
<tr>
<td>Sputum culture</td>
<td>13/27</td>
<td>48</td>
</tr>
</tbody>
</table>

Data are derived by considering specimens obtained from all study patients with invasive pulmonary aspergillosis having bronchoscopy (Groups 1 and 2 combined, n = 27).
Discussion

The diagnostic sensitivity of fiberoptic bronchoscopy in the immunocompromised host with new pulmonary infiltrates has been reported to range from 5% to 85%. A variety of factors, including the timing of the procedure and the types of diagnostic technics performed during bronchoscopy, have been postulated to affect the diagnostic yield. Numerous reports have described the use of fiberoptic bronchoscopy in diagnosing invasive pulmonary aspergillosis, with diagnoses established by bronchial brushings, washings, and transbronchial biopsies. Recently, Albelda and co-workers reported that fiberoptic bronchoscopic washings, brushings, and biopsies yielded a diagnosis in 50% of patients with pulmonary aspergillosis at their institution. However, to date, the number of reported patients has been small and no consensus can be reached to the overall efficacy of bronchoscopy for diagnosing invasive aspergillosis.

The bronchoscopic technic of BAL has recently been reported to be effective in the diagnosis of respiratory infection in the immunocompromised host. This procedure, employing relatively high-volume alveolar lavage (100–300 mL), has unique advantages over more conventional bronchoscopy technics, including its simplicity, safety, and enhanced diagnostic yield. A limited number of reports have described the use of BAL to diagnose invasive pulmonary aspergillosis. Andrews and Weiner described detection of Aspergillus antigen(s) in BAL samples from two patients with this diagnosis. While Stover and associates described finding Aspergillus in BAL samples from four patients with invasive pulmonary aspergillosis, it is unclear how the fungus was detected in their patients. In one other series, Hopkin and co-workers found fungal elements in a BAL sample from a patient with this diagnosis.

The visualization of characteristic hyphae in bronchial brushings or washings has been considered sufficient evidence of invasive aspergillosis to warrant institution of anti-fungal therapy. Our results support these previous reports, and we suggest that, in the right clinical setting, the visualization of Aspergillus hyphae in BAL samples also supports a diagnosis of invasive pulmonary aspergillosis. We found that direct detection of Aspergillus hyphae was diagnostic in 53% of patients with pulmonary aspergillosis. Furthermore, this test was specific and had a high positive predictive value for the diagnosis, in contrast to BAL cultures that were relatively insensitive and that had a lower predictive value. One potential source of bias of these data was the inclusion of four cases without histologic verification of the diagnosis. However, these cases were included based on a strong clinical and radiographic suspicion of the diagnosis, which was supported by growth of Aspergillus from a normally sterile site (transtracheal aspirate) in three cases and from bronchial brushings in one case. All three cases in which transtracheal aspirates were performed were from the BAL study group (Group 2). However, BAL studies were nondiagnostic in two of the three cases, and exclusion of these cases would have biased sensitivity of BAL in a positive manner. Exclusion of these four cases from analysis would not have changed the overall conclusions of this study.

In contrast to the recent data of Albelda and associates, we found that routine bronchial washings and biopsies were insensitive for the diagnosis of pulmonary aspergillosis. However a number of variables could have accounted for these differences, including the manner in which specimens were collected, the types of stains performed, and the fact that we did not perform bronchial brushings in our patients. Seventy-three percent of patients studied here were receiving amphotericin B therapy prior to bronchoscopy; however, use of amphotericin prior to bronchoscopy did not appear to effect adversely the likelihood of demonstrating Aspergillus hyphae histochemically or to influence the likelihood of obtaining a positive culture for Aspergillus. It is our impression that at our medical center, patients with prolonged, profound neutropenia who develop pulmonary infiltrates while receiving anti-bacterial drugs promptly begin to receive amphotericin B therapy pending further diagnostic tests. Thus, the positive correlation between use of amphotericin B therapy prior to bronchoscopy and a positive BAL stain for Aspergillus may reflect the fact that such patients are more likely to have aspergillosis. Analysis of a variety of other factors failed to reveal any significant correlations that could be used to predict positive from negative BAL results. Our results are in contrast to those of others who have asserted that bronchoscopy is more often positive when performed at least five days after chest x-ray changes in patients with invasive pulmonary aspergillosis. We suggest that BAL can be performed soon after evolution of an unexplained pulmonary infiltrate without decreasing the likelihood of diagnosing aspergillosis.

In conclusion, we have determined that direct detection of fungal elements in BAL samples is a rapid and effective technic for diagnosing invasive pulmonary aspergillosis in the immunocompromised host. In this series, BAL was clearly superior to more conventional bronchoscopic technics, perhaps because BAL sampled a larger number of alveoli than was possible with a transbronchial biopsy or a routine bronchial washing. We feel that BAL should be performed early in a patient's course when invasive pulmonary aspergillosis is suspected, and anti-fungal therapy instituted when histochemical stains reveal characteristic mycelial elements of Aspergillus. If BAL analysis is negative for Aspergillus and another cause for the patient's pulmonary infiltrate cannot be found, further di-
Four technics were compared to find the most suitable screening test for the cytomegalovirus (CMV) antibody status in blood donors. One hundred thirty-five donor samples were tested by two enzyme immunoassays, EIA(Litton) and EIA(Abbott), and by latex agglutination (LA) and complement fixation (CF). The seroreactivity of the tests were judged by concordance of three agglutination technics, such as open lung biopsy, should be considered.

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References

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