

The value of bronchoalveolar lavage and bronchial washings in the diagnosis of invasive pulmonary aspergillosis

H. LEVY*, D. A. HORAK, B. R. TEGTMEIER, S. B. YOKOTA AND S. J. FORMAN

Departments of Respiratory Diseases, Clinical Pathology, Cytology, Hematology and Bone Marrow Transplantation, City of Hope National Medical Center, Duarte, California 91010, U.S.A.

The objective of this study was to clarify conflicting reports of the sensitivity and specificity of bronchoalveolar lavage or bronchial washings for diagnosing invasive pulmonary aspergillosis. The study was a retrospective review of 300 consecutive patients in a tertiary referral centre subjected to 343 fiberoptic bronchoscopic procedures for the evaluation of pulmonary infiltrates. Classification of paired fungal culture and cytologic examination of bronchoalveolar lavage or bronchial washing fluid according to clinical, radiographic, histological and autopsy evidence of invasive pulmonary aspergillosis. One-hundred and fifteen deaths occurred, with a 58% autopsy rate. A diagnosis of invasive pulmonary aspergillosis was made in 21 immunosuppressed patients with 16 deaths. Bronchoalveolar lavage cytology showed aspergillus in 19 specimens (invasive pulmonary aspergillosis in 16), cultures yielded aspergillus in 41 (invasive pulmonary aspergillosis in ten), with both tests positive in nine. Cytology sensitivity was 64.0%, specificity 99.1%, positive predictive value 84.2%, and negative predictive value 97.2%. Culture sensitivity was 40.0%, specificity 90.3%, positive predictive value 24.4%, and negative predictive value 95.0%. Concordant cytology and culture sensitivity was 32.0%, specificity 99.7%, positive predictive value 88.9%, and negative predictive value 94.9%. In conclusion, when characteristic hyphae are visualized in bronchoalveolar lavage specimens from immunosuppressed patients with compatible clinical data, it is advisable to treat for presumptive invasive pulmonary aspergillosis.

Introduction

Invasive aspergillosis continues to be a significant cause of death in immunocompromised patients with antemortem diagnosis frequently being problematical (1,2). *Aspergillus* spores are ubiquitous in the environment and can be cultured from grain, moist soil, and vegetation, or the organism can be a laboratory contaminant. While there are more than 300 species of the organism, most human infections are caused by *Aspergillus fumigatus*, followed by *A. flavus*, *A. niger*, *A. clavatus*, and *A. nidulans* (3). Although fiberoptic bronchoscopy has provided an alternative to open lung biopsy for documenting invasive pulmonary aspergillosis, many immunosuppressed patients have coagulopathy or thrombocytopenia precluding safe bronchial brushings and transbronchial biopsy. Bronchoalveolar lavage (BAL) alone is usually a safe and simple procedure in these patients, although

reports of its sensitivity and specificity for diagnosing invasive disease vary widely (4–7). We analysed the records of bronchial washings and BAL performed in immunosuppressed patients at our institution in an attempt to clarify these conflicting reports.

Materials and Methods

The City of Hope National Medical Center is a tertiary care hospital specializing almost exclusively in oncology, hematology, and bone marrow transplantation. A retrospective study was performed analysing the results of 343 sequential bronchoscopies in 300 febrile patients with new pulmonary infiltrates where bronchial washings or bronchoalveolar lavage (BAL) specimens were submitted for paired fungal culture and cytologic examination between 1 June 1986 and 30 September 1988. These immunosuppressed patients were considered to be at risk for invasive fungal disease.

The hospital records of the patients were reviewed to record the following features: age, sex, underlying disease, results of bronchial washings or BAL cytology, transbronchial lung biopsy histology, open lung biopsy

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*To whom correspondence and reprint requests should be addressed at: Department of Medicine, University of New Mexico 5ACC, 2211 Lomas Blvd NE, Albuquerque, New Mexico 87131, U.S.A.

histology and culture, percutaneous fine needle aspiration culture and cytology, corticosteroid therapy, use of broad-spectrum antibiotics, presence of leukopenia (< 1000 neutrophils mm^{-3}), absolute polymorphonuclear leukocyte count, patient outcome, and a autopsy results.

Fiberoptic bronchoscopy and BAL were performed using standard techniques as previously described (8) using ten sequential aliquots of 20 ml sterile, normal saline at 37°C (total volume 200 ml) with the bronchoscope wedged in a segmental bronchus at the site of maximal infiltrate on chest X-ray. Where diffuse disease was present, the right middle lobe or lingula was utilized. In 140 procedures, by operator preference, low volume bronchial washings were performed using 20 ml normal saline in the area of radiographic abnormality. Bronchial brushings were performed during 35 bronchoscopies. Transbronchial lung biopsy was performed when the platelet count exceeded $80\,000\ \text{mm}^{-3}$ and coagulation parameters were not significantly prolonged. The bronchial washings or BAL fluid was divided into equal volumes and submitted for routine microbiological and cytological examination.

FUNGAL CULTURE

Ten to 15 ml of the BAL or bronchial washing sample were centrifuged in sterile conical tubes at 3000 rpm for 10 min. Portions of the pellet were inoculated on Sabouraud Dextrose Agar (SAB), in duplicate; Mycosel (MYC); and brain-heart infusion agar (BHIA) with 5% sheep blood, gentamicin, and chloramphenicol. The BHIA flask and one SAB plate were incubated at 35°C, while the MYC plate and the second SAB plate were incubated at 30°C. All cultures were incubated for 4 weeks and inspected for growth twice weekly. Aspergilli and other opportunistic moulds were identified according to morphological and cultural characteristics.

CYTOLOGY PREPARATION AND EVALUATION

The fresh, unfixed sample was transferred to conical tubes and centrifuged at 1500 rpm for 15 min. The supernatant was decanted and smears prepared by placing a small amount of the cell sediment in the centre of a poly-L-lysine coated slide. A second slide was placed over the cell sediment and the material was allowed to spread between the two slides which were then gently pulled apart to make a thin even layer on both slides. The slides were immediately placed in 95% ethyl alcohol for fixation prior to staining. Additional slides were prepared using all of the cell sediment. If the sample volume was low, smears were prepared using a cytocentrifuge.

The smears were stained with a Papanicolaou stain and with Grocott's Methenamine Silver (GMS) (10). The Papanicolaou-stained smears were evaluated for the presence of viral changes, fungi and malignancy. The GMS stained preparations were specifically examined for the presence of fungi, *Pneumocystis carinii* and other organisms. The definitive classification of fungi is not possible on cytological preparations because of morphological similarities. The criteria used for fungi consistent with aspergillus species were the identification of uniform, septate, dichotomous, 45° branching hyphae. The presence of even a single suggestive organism was considered presumptive of aspergillus for the purposes of analysis in this study.

Criteria for the diagnosis of invasive pulmonary aspergillosis were as follows:

1. Evidence of vascular and pulmonary parenchymal invasion by characteristic aspergillus hyphae in histological specimens obtained at autopsy or transbronchial lung biopsy.
2. Culture or histological evidence of disseminated (extra-pulmonary) aspergillus infection in a patient with a new diffuse radiographic infiltrate and clinical setting (neutropenia or immunosuppression, prolonged antecedent broad spectrum antibiotic therapy and fever) predisposing to invasive pulmonary aspergillosis.
3. An appropriate clinical setting and new radiographic infiltrate compatible with invasive aspergillosis in the absence of other pulmonary pathogens and resolution of the infiltrate after prolonged high-dose amphotericin B therapy.

Fusarium species and *Pseudoallescheria boydii* (*Scedosporium apiospermum*) which produce hyphae *in vivo* with morphological resemblance to aspergillus and cause clinical disease indistinguishable from invasive aspergillosis were included in our analysis.

Invasive aspergillosis was considered to be excluded when the patient did not receive antifungal therapy and was known to be alive after at least 3 months follow-up, or if there was no evidence of fungal disease at thoracotomy or autopsy if these were performed. Using these criteria for the presence or absence of true invasive pulmonary aspergillosis, clinical prediction rules for BAL and bronchial washings cytology and fungal culture were determined using standard formulae (11).

Results

A total of 343 bronchoscopic procedures were performed in 300 patients. Bronchial washings were done in 140 and BAL in 203. Bronchial brushings were also performed during 35 bronchoscopic examinations, but

none cultured fungi and were therefore not analysed separately. Cytology revealed fungal hyphae in 19 specimens (15 patients), fungi were cultured from 41 specimens (37 patients) and both investigations were positive from nine specimens (eight patients). These positive specimens were obtained from 42 BAL samples and 19 washings.

A diagnosis of invasive pulmonary aspergillosis was made in 21 patients who underwent 25 bronchoscopies (Table 1). Two patients with positive bronchoscopy were later subjected to repeat BAL to evaluate whether infection persisted. Fourteen of the 21 patients (66.7%) were detected by either cytology or culture. Six bronchoscopic procedures failed to detect aspergillus, a repeat procedure was not performed but the patient was treated on clinical suspicion of invasive disease which was subsequently confirmed at autopsy (Table 1). The group comprised 11 males, and ten females whose ages ranged from 22 to 55 with a mean of 38.0 years. The underlying diseases included hematological malignancy treated with bone marrow transplantation in 13 patients, acute leukaemia in six patients, and chronic lymphocytic leukaemia and aplastic anaemia in one patient each. Fifteen patients were receiving corticosteroids and 21 were treated with broad-spectrum antibiotics prior to the diagnosis of aspergillosis. Eight patients had leukopenia (polymorphonuclear leucocyte count of $< 1000 \text{ mm}^{-3}$). Three patients were receiving mechanical ventilation for respiratory failure. Six patients had concurrent cytomegalovirus pneumonia, two had been recently cured of cytomegalovirus infection and one patient had herpes simplex pneumonia (Table 1).

Sixteen of the 21 patients died (76.2%). Autopsy documentation of invasive pulmonary aspergillosis was available in 15 of these. Transbronchial biopsy was compatible with invasive aspergillosis in two patients and two additional patients had disseminated aspergillosis documented by skin biopsy. The remaining five patients had a clinical and radiological course compatible with the disease and responded to prolonged amphotericin B therapy (Table 1).

Invasive pulmonary aspergillosis was documented in ten of the 41 positive cultures (24.4%), the organism being *A. fumigatus* in three, *Aspergillus* species in three and *A. flavus* in two. Disease receiving similar therapy and behaving clinically like invasive aspergillosis was due to *Fusarium* species in one and *P. Boydii* (*S. apiospermum*) in one. Three of the 15 patients with invasive aspergillosis but negative cultures (20%) were on low-dose amphotericin B at the time BAL was performed.

Patients with false-positive culture results had similar predisposing factors [malignancy 16, bone marrow

transplant six, leukaemia four, acquired immunodeficiency syndrome (AIDS) one and miscellaneous three] for invasive fungal disease as those who had documented disease. One of these was treated with ketoconazole and two received amphotericin B in subtherapeutic total dose. Eight patients who were classified as having either false-positive cytology or culture died. Five had no evidence of fungal infection at autopsy. One patient had multiple transbronchial lung biopsies and a percutaneous fine needle aspiration showing no fungal invasion prior to death (no autopsy). One patient had evidence of fungal infection of an abscess cavity which developed 6 weeks after bronchoscopy and another had subsequent invasive aspergillosis developing 5.5 weeks after open lung biopsy documenting legionella pneumonia with no fungal disease. One-hundred and fifteen of the 300 patients in this series died and 67 were subjected to autopsy (58%) with evidence of fungal disease being seen in 15 patients (Table 1).

No respiratory failure, pneumothorax, hemoptysis or death due to bronchoscopy or BAL were documented, with all patients having routine follow-up. BAL specimens accounted for the majority of true positive specimens with only two washings yielding a correct diagnosis of invasive pulmonary aspergillosis. Clinical correlation categorized BAL cytology and culture results as listed in Table 2.

Discussion

This study documents the excellent specificity and negative predictive value of either failure to culture aspergillus or the failure to visualize organisms with aspergillus morphology in cytological preparations of BAL fluid from immunocompromized patients with invasive pulmonary aspergillosis. The sensitivity of these tests is poor but positive predictive value is satisfactory for cytological examination or concordant visualization and culture. Predictive values, however, depend upon the prevalence of disease in the population tested. Cytological examination has a superior sensitivity (64.0%) as compared with culture (40.0%) and has the added benefit of immediate availability. Fourteen of the 21 patients (67%) with invasive aspergillosis were detected by either BAL cytology or culture, which is superior to the 44–50% yield reported by Albelda *et al.* (4) and contrasts sharply with the inability to detect invasive aspergillosis reported by Saito *et al.* (6). If we confine our analysis to patients with histological confirmation of invasive pulmonary aspergillosis at autopsy or transbronchial biopsy as Saito *et al.* have done, the yield remains a satisfactory 43.8% (seven of 16) on cytology (sensitivity 40% and

Table 1 Clinical details of patients with invasive pulmonary aspergillosis

Patient No.	Age/sex	Diagnosis	BAL cytology	BAL culture	Criteria for diagnosis	Days between BAL and autopsy	Other
1	35/F	BMT	—	—	Autopsy	2	Cured CMV
2	37/M	BMT	—	—	Autopsy	27	Skin biopsy*
3	34/M	BMT	—	—	Autopsy	5	CMV
4	27/F	BMT	—	—	Autopsy	14	CMV
5	22/F	BMT	—	+†	Autopsy	35	
6	41/M	BMT	—†	—†	Autopsy	2	
7	54/F	CLL	—	—	Autopsy	17	
8	36/M	AA	—	—	Autopsy	6	
9	43/F	BMT	+	—	Autopsy	30	Skin biopsy*
10	47/M	ANLL	—	<i>A. fumigatus</i>	Autopsy	5	
11	24/M	BMT	+	<i>A. flavus</i>	Autopsy	23	
12	38/M	BMT	+	—	Autopsy	6	
13	55/F	ANLL	+	<i>A. fumigatus</i>	Clinical		
			+	<i>Fusarium</i> sp			Resolution with therapy
14	25/M	ANLL	+	<i>Aspergillus</i> sp	Autopsy	10	CMV
15	36/M	BMT	+	<i>Aspergillus</i> sp	Clinical		CMV
			+	<i>Aspergillus</i> sp			Resolution with therapy
16	22/M	BMT	+	<i>A. fumigatus</i>	Autopsy	8	CMV
17	30/F	ANLL	+	—	Transbronchial biopsy		Resolution with therapy
18	46/M	BMT	+	—	Clinical	Not done	CMV PCP
19	55/F	ALL	+	<i>A. flavus</i>	Clinical		Resolution with therapy
20	50/F	ANLL	+	—	Clinical		Resolution with therapy
21	42/F	ANLL	+	—	Transbronchial biopsy and autopsy	1	Herpes

Abbreviations: BMT, bone marrow transplant; CLL, chronic lymphocytic leukemia; AA, aplastic anemia; ANLL, acute non-lymphocytic leukemia; ALL, acute lymphoblastic leukemia; CMV, cytomegalovirus pneumonia; PCP, *Pneumocystis carinii* pneumonia.

*Skin biopsy on day of bronchoscopy positive for *Aspergillus*.

†*Pseudallescheria boydii* (*Scedosporium apiospermum*).

‡BAL done on right lower lobe, *Aspergillus* in right upper lobe at autopsy.

Table 2 Patient categorization and clinical prediction rules

Test	Cytology	Culture	Both cytology and culture
Sensitivity (%)	64.0	40.0	32.0
Specificity (%)	99.1	90.3	99.7
Positive predictive value (%)	84.2	24.4	88.9
Negative predictive value (%)	97.2	95.0	94.9

specificity 100%) and 37.5% (six of 16) on culture. As our patient populations are comparable in other respects, we cannot account for their poor results, other than that only nine of their patients had invasive aspergillosis.

Albelda *et al.* (4) reported that bronchoscopy suggested the diagnosis in 50% of patients and appeared to be less successful when performed early in the disease. Bronchoscopy and BAL were performed within 24 h of the appearance of an infiltrate in our series with a 67% yield. We would caution against delay in performing bronchoscopy if invasive pulmonary aspergillosis is suspected in immunosuppressed patients.

It should be noted that in several patients with negative BAL cytology and fungal culture but invasive aspergillus documented at autopsy, disease may well have developed during the interval between BAL and death. If patients dying more than 10 days after BAL are excluded, the sensitivity of BAL cytology and fungal cultures would improve to 72.2 and 44.4%, respectively.

Rapid diagnosis of invasive pulmonary aspergillosis has been difficult in the past. Isolation of *A. fumigatus* or *A. flavus* from expectorated sputum or nose cultures have been reported to have sensitivity and specificity for diagnosis in the appropriate clinical setting, but incurs a delay before the organism is cultured and identified (2, 12–15). The culture of other aspergillus species does not exclude the diagnosis (Table 1). Serological detection of circulating aspergillus antigen is a promising technique for early diagnosis but is not widely available (16,17). Invasive diagnostic techniques including trans-bronchial biopsy and bronchial brushings, trans-thoracic needle aspiration and open lung biopsy are often not safely feasible in immunosuppressed patients with coagulopathy or thrombocytopenia. Bronchoalveolar lavage is a relatively safe technique in these patients but the sensitivity for detecting the disease has been questioned. Detection of aspergillus antigen in BAL fluid is promising but its sensitivity and specificity remain undetermined (18). Oxalic acid levels in BAL fluid is significantly higher

in patients with aspergillosis but can also be elevated in the presence of cytomegalovirus infection, lactic acidosis and *Pseudomonas aeruginosa* infection (19).

Many authors consider the visualization of septate hyphae in respiratory tract secretions in an appropriate clinical and radiographic setting as presumptive evidence for invasive pulmonary aspergillosis (1, 2, 4, 12). Our study demonstrates a positive predictive value for cytology of 84.2 with a 99.1% specificity, further documenting the validity of this association. This series yielded superior results in a larger group of patients than that reported by Kahn *et al.* (5), and serve to bolster their conclusion that BAL is a valuable procedure for the diagnosis of invasive pulmonary aspergillosis. The possibility that lavage at more than one site may increase the sensitivity of BAL was not addressed in this study but may be warranted if this life threatening disease is suspected. Our data provide information concerning the relative contributions of BAL cytology and culture, alone or together allowing us to conclude that cytology is the most valuable test because of its superior sensitivity, specificity, rapidity and wide availability compared with other procedures and tests.

The institution of anti-fungal therapy is therefore advised when characteristic hyphae are visualized in BAL fluid, especially when transbronchial lung biopsy and other invasive procedures are contraindicated.

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