Utility of percutaneous lung biopsy for diagnosing filamentous fungal infections in hematologic malignancies

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Background and Objectives. The incidence of invasive filamentous fungal infections in hematologic patients is increasing as a consequence of high dose chemotherapy and bone marrow transplant procedures. Mortality is usually very high. The diagnosis is often difficult and yet a fast, accurate diagnosis is of fundamental importance for treating the infection and planning subsequent management of the hematologic disease. We evaluated the sensitivity of computed tomography (CT)-guided percutaneous biopsy in diagnosing pulmonary fungal infections.

Design and Methods. Between 1997 and 2002 we performed 17 CT-guided percutaneous transthoracic lung biopsies in 17 hematologic patients with suspected filamentous fungi infection with negative BAL, to obtain a certain diagnosis and to know what species of fungi was responsible for infection. In all cases suspected mycosis began during the post-chemotherapy aplastic period. Patients were receiving antifungal therapy at the time of all biopsies. When the platelet count rose above 50×10^9/L, CT-guided percutaneous lung biopsy with fine-needle aspiration for cytology was performed.

Results. Twelve of 17 patients had histologic confirmation of the fungal infection (70.5%), 8 with *Aspergillus spp.* 4 with *Mucorales spp.* Biopsies provided non-specific results in 4 cases; in 2 of these cases, clinical course and response to therapy confirmed the diagnosis of mycosis; in the last case bronchoalveolar carcinoma was found as a new diagnosis. Cultures were positive in only 6 cases, all for *Aspergillus spp.* The sensitivity of CT-guided percutaneous lung biopsy was 70.6% and its positive predictive value (PPV) was 100%. This procedure provided an immediate diagnosis and only one side-effect (1 pneumothorax, without complications).

Interpretation and Conclusions. Histologic discrimination between aspergillosis and mucormycosis is very important for deciding secondary prophylaxis during transplant procedures, because Mucor is usually resistant to azoles.

Key words: percutaneous lung biopsy, aspergillosis, mucormycosis, hematologic malignancies.

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The presence of invasive filamentous fungal infections is increasing in hematologic patients, due to high dose chemotherapy and bone marrow transplant procedures.[1] Mortality is usually very high, particularly in recipients of bone marrow transplants.[2]

The diagnosis is very difficult in neutropenic patients because cultures are often negative and diagnosis can only be said to be probable. Nevertheless, it is important to confirm the fungal etiology of pulmonary lesions, which are defined *probable aspergillosis*, and also to determine the causative fungal species. Prolonged antifungal treatment, correct secondary prophylaxis and a possible subsequent decision about pulmonary resection can be indispensable in scheduling bone marrow transplantation in leukemic patients.[3,4] In these patients computed tomography (CT) scanning of the chest, performed early, is very useful to identify a probable pulmonary aspergillosis by detecting the presence of the *halo sign* and *air crescent sign* which are characteristic of fungal infection.[3] Bronchoalveolar lavage (BAL) is usually performed in these patients, but the sensitivity of this method in detecting aspergillosis is only 50% according to some authors[5] and also in our experience.[4] These bronchoscopic procedures may detect colonization but not invasion from fungi. Transbronchial biopsy, performed only when the platelet count is $> 50\times10^9$/L, improves the diagnostic accuracy by only 6.5%.[5] There are, therefore, many patients in whom the diagnosis of mycosis must simply be considered possible, and only the clinical course helps in the choice of therapy; every diagnostic procedure that converts a *possible* mycotic infection to a *probable* or *proven* one is useful for programming the following therapies.

In the last six years (1997-2002), we performed percutaneous lung biopsy in hematologic patients with a pulmonary picture of filamentous fungal infection with negative BAL, in order to obtain a certain diagnosis and to identify the species of fungi that was responsible for the infection.

Design and Methods

We retrospectively reviewed the results of 17 CT-guided percutaneous lung biopsies from hematologic patients (13 with acute myeloid leukemia, 1 with acute lymphoid leukemia, 2 with non-Hodgkin's lymphoma, and 1 with aplastic anemia) performed between 1997-2002.

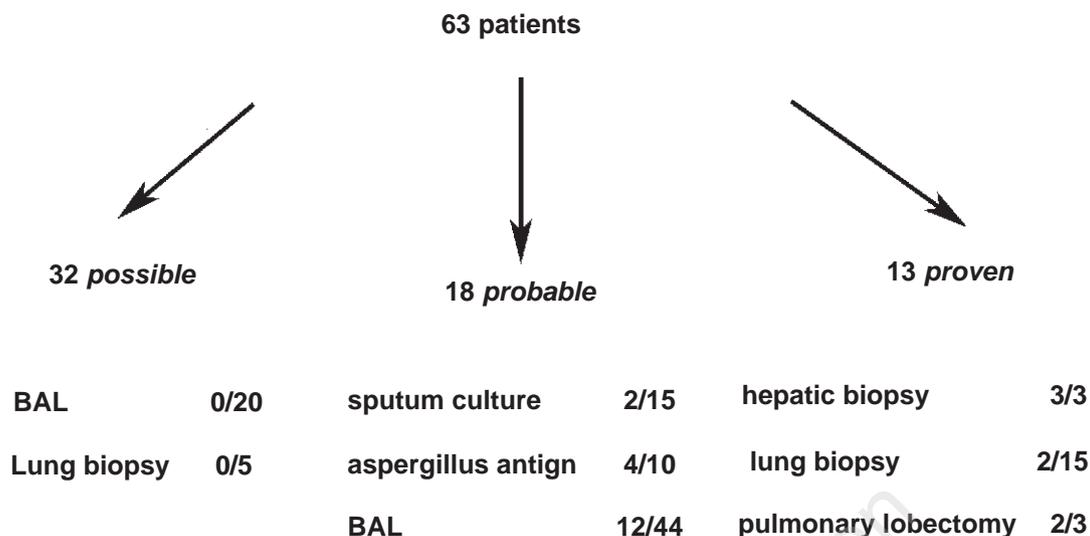Microbiological surveillance cultures (nose, throat,

63 patients

32 *possible*

18 *probable*

13 *proven*

BAL	0/20	sputum culture	2/15	hepatic biopsy	3/3
Lung biopsy	0/5	aspergillus antign	4/10	lung biopsy	2/15
		BAL	12/44	pulmonary lobectomy	2/3

Figure 1. Positive results from diagnostic procedures in the 63 patients with high resolution CT scan suggestive of aspergillosis.

stools and vagina) were performed every week during the post-chemotherapy period of aplasia. All patients were receiving antifungal prophylaxis with oral itraconazole 300 mg/day. Empirical antifungal therapy (amphotericin B deoxycholate 1 mg/Kg/day) was begun in febrile neutropenic patients who did not respond to broad-spectrum antibiotic therapy and had negative cultures or in patients with persistent neutropenia who had a subsequent episode of fever with negative cultures. Liposomal amphotericin (Ambisome) was administered if the patient's creatinine concentration was > 2 mg/dL. As reported in literature[3] early high resolution CT scan was performed to identify possible fungal infection; BAL was also usually done as soon as possible. If the BAL was negative for fungi, when platelet count rose above 50×10^9/L, CT-guided percutaneous lung biopsy with fine-needle aspiration for cytology was performed, preferably on peripheral lesions.[6,7] A 22-gauge needle was preferred to minimize the risk of pneumothorax; the median number of passes taken to pleura was 3 (range 2-5), sufficient to ensure an adequate specimen. Aspirated material was immediately inspected by a pathologist to judge the adequacy of the sample and was then usually immediately fixed in 10% formalin solution. The hyphae were visualized in tissue sections stained with periodic acid-Schiff reaction or Grocott-Gomori methanamine-silver nitrate. A histologic diagnosis of aspergillosis was made when biopsy specimens showed typical septate hyphae with dichotomous branches; mucormycosis was diagnosed when irregularly shaped, broad, non-septate hyphae with branches occurring at right-angles were found. All samples were cultured for bacteria and fungi. The cross-tabulation parameters were calculated using the standard Bayesian method.

Results

Between 1997 and 2002 we cared for 70 patients with a clinical picture compatible with filamentous fungal infection; of these patients 7 had mucormycosis, 32 were defined as having a *possible infection*, according to EORTC/ NIAID Mycoses Study Groups criteria[8] and 31 had probable or proven aspergillosis. Mucormycosis was diagnosed by autopsy (3 cases) and pulmonary biopsy (4 cases). Proven aspergillosis was diagnosed by autopsy (3 cases), by hepatic biopsy (3 cases), pulmonary biopsy (8 cases) and pulmonary lobectomy (2 cases). Aspergillosis was considered probable in 18 patients because of a suggestive lung CT scan with sputum culture (2 cases), positivity for Aspergillus antigen (4 cases) and positive BAL (12 cases). Among 32 cases of possible aspergillosis 25 diagnostic procedures (BAL in 20 patients and percutaneous lung biopsy in 5) were negative (Figure 1). The following information was evaluated: 1) duration of antifungal therapy prior to biopsy; 2) results of histology and cultures; 3) clinical course after percutaneous biopsy.

CT scanning was performed a median of 8 days after the beginning of antifungal therapy and was

Table 1. Results of percutaneous lung biopsies in hematologic patients with negative BAL.

Age (yrs.) sex	Hematologic disease	Days of neutropenia <100/mm³ before biopsy	Chest CT scan	Days of antifungal therapy before biopsy	Results of biopsy /histology	Surgery	Outcome
28/M	AML	28	multiple nodules	15	Mucor		alive after autoBMT
34/F	Aplastic anemia	0	two nodules	20	Aspergillus		alive
55/F	AML	27	multiple nodules	7	Aspergillus		alive after autoBMT
56/M	AML	11	one cavitation	15	Aspergillus	lobectomy	alive after autoBMT
58/F	AML	14	multiple cavitations	27	Aspergillus		dead due to leukemia relapse and mycosis
40/M	NHL	0	one cavitation	0	Aspergillus		alive
58/F	AML	11	one cavitation	14	Mucor		dead due to leukemia
60/F	AML	12	multiple nodules	33	Mucor		alive after autoBMT
54/M	ALL	10	one cavitation	0	Mucor		dead due to leukemia and mycosis
36/F	NHL	0	one cavitation	0	Aspergillus		alive
47/M	AML	20	two cavitations	22	Aspergillus	lobectomy	alive
64/F	AML	14	multiple nodules	24	Aspergillus		dead due to leukemia and mycosis
35/F	AML	17	multiple nodules	20	negative		alive after alloBMT
43/F	AML	15	multiple nodules	30	negative		alive
63/M	AML	16	multiple nodules	23	negative		alive
49/F	AML	17	multiple nodules	13	negative		alive
64/F	AML	15	three nodules	90	carcinoma		dead due to carcinoma and leukemia

repeated at the time of percutaneous biopsies. BAL was done after a median of 10 days and percutaneous biopsies were taken, preferably from peripheral lesions, after a median of 15 days (range 0-90) of empirical antifungal treatment. Neutropenia (<0.5×10⁹/L) during the post-chemotherapy aplastic phase lasted a median of 14 days (range 1-27)

The clinical characteristics and results of our patients submitted to percutaneous pulmonary biopsy are given in Table 1.

Twelve of the 17 patients had histologic demonstration of a fungal infection, an *Aspergillus* in 8 cases, and *Mucorales* in the other 4 cases. Biopsies provided non-specific results in 4 cases; in 2 of these, the clinical course and response to therapy confirmed the diagnosis of mycosis. In the last case bronchoalveolar carcinoma was found as a new diagnosis . Overall, clinically useful information was obtained in 13 of the 17 biopsies performed, 12 being diagnostic for fungal infection (accuracy 70.5%) and 1 for carcinoma. We determined the accuracy of percutaneous lung biopsies in 2 cases by subsequent surgical procedures (lobar resection); histologic examination of the resected tissue material confirmed the results of the biopsies. Of the 4 patients with negative biopsies, two had progressive radiologic signs of mycosis. The sensitivity of CT-guided percutaneous lung biopsy for the detection of fungal infections was 70.6% and its PPV was 100%. Cultures were positive in only 6 cases, all with aspergillosis. Only one patient had pneumothorax, which required further treatment with tube insertion. The biopsy procedure did not cause hemorrhage or other complications in any of the cases.

Twelve patients are alive and well after a median follow-up of 23 months (range 3-60); one patient died because of leukemia, 3 patients died because of progression of hematologic disease and mycosis and 1 because of carcinoma and leukemia. Five patients underwent bone marrow transplantation (1 allogeneic, 4 autologous) without recurrence of the fungal infection.

Discussion

Aspergillosis and mucormycosis infections are caused by filamentous fungi are usually very difficult to identify because the etiological pathogens grow with difficulty even in appropriate media. Serologic studies for diagnosing invasive aspergillosis have been proposed but their results are still under discussion. CT scans have proven to be very useful for detecting specific signs of mycotic lesions in the lung.[9] In our experience chest CT scans were indispensable: 1) to orientate diagnosis by showing the halo and/or air crescent signs, 2) to verify the spread of infection, 3) to locate the position of fungal lesions with respect to big vessels and to determine risk of hemoptysis and, finally 4) to choose where percutaneous biopsy could be done. Unfortunately radiologic signs alone define pulmonary aspergillosis as possible and these are not able to discriminate between different fungal species.

Bronchoalveolar lavage (BAL) is usually performed in neutropenic patients if the radiologic picture suggests a fungal infection, but the sensitivity of this method's detection of invasive aspergillosis in a leukemic population is < 50%[5] confirmed by a recent report from Caillot et al.[3] and also by our own experience.[4] It should, however, be noted that we performed BAL a median of 10 days after the beginning of empiric antifungal therapy, which could have caused our negative results. Regarding mucormycosis, ante-mortem diagnosis in neutropenic patients is unusual because blood cultures are invariably negative; bronchial washings have yielded hyphal forms only on rare occasions. Berns et al.[10] noted that a mucoraceous fungus was recovered from only 1 of 1,440 consecutive bronchial brushing specimens, but that 1 instance was an autopsy-proven case of mucormycosis. Aspergillus is a common saprophyte and a positive culture is not proof of infection. Hence the gold standard is mycologic and/or histologic evidence of tissue invasion.

Until now percutaneous pulmonary biopsy has rarely been performed in hematologic patients; sporadic cases and rare series are described.[6,11] More often, this method has been used successfully to diagnose pulmonary neoplasms and to identify pulmonary infections in radiologically suspicious nodules.[12] In our experience the sensitivity (70.6%) of percutaneous pulmonary biopsy was high, even in patients who had been receiving antifungal therapy for many days; in this case it is possible to obtain false negative results, which have also been reported using open–lung biopsy.[13] On the other hand, it is usual to begin antifungal therapy in an empirical manner in leukemic patients in the post-chemotherapy aplastic phase, because fungal infections in such patients are very aggressive and mortality is very high.[2] As these patients also have severe thrombocytopenia, it is necessary to wait for the platelet count to increase (> 50×10^{9}/L) before attempting invasive procedures. Despite this wait, which in our experience was a median of 15 days, biopsy showed a diagnostic result in a high number of cases (76.4%), and allowed aspergillosis and mucormycosis to be discriminated. This histologic discrimination was very important for our patients because *Mucorales spp.* are usually resistant to azoles and echinocandins, so only amphotericin B can be used as treatment and secondary prophylaxis during transplant procedures. Mucormycosis was not rare in our experience[14] and is probably generally underevaluated because the clinical pictures of this infection and aspergillosis are similar.

A major advantage of percutaneous lung biopsy is the speed of the procedure, which provides an immediate diagnosis and usually does not have important side-effects. Pneumothorax[15] and fatal pulmonary hemorrhage[16] have, however, been previously described. The increased risk of morbidity when performing an invasive procedure in a compromised patient is obvious, particularly in leukemic patients with a low number of platelets. Nevertheless, in our patients this procedure, always performed when the platelet count was > 50×10^{9}/L, was well tolerated and only one patient had pneumothorax (6%) without severe complications. Dissemination of the fungal infection has also been described after transthoracic lung biopsy;[17] however we usually continue empirical antifungal therapy until confirmation of fungal infection and/or disappearance of lesions in the absence of a certain diagnosis; all the biopsies were done under antifungal therapy and no dissemination was seen.

Two patients underwent pulmonary lobectomy, one because of hemoptysis and the other before transplant; biopsy results are certainly useful also for the surgeon when deciding on a possible pulmonary resection,[11] which is often necessary in cases of hemoptysis and/or when big cavitations are present in scheduled bone marrow recipients. In fact fungal relapses are possible in bone marrow transplant recipients both during the early post-transplant neutropenic phase and if acute and chronic graft-versus-host disease occurs.[18]

In conclusion, an accurate diagnosis of fungal species allows the correct duration and choice of antimycotic therapy to be planned, and an adequate secondary prophylaxis to be given both during subsequent chemotherapy courses and in the post-transplant period, in particular in patients who have undergone allogeneic and one fully mismatched HLA haplotype transplant,[19] who are considered at very high risk of fungal infection.

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Pre-publication Report

Contributions

AN was responsible for the conception of the study, analysis and interpretation of data and for drafting the manuscript. LM, MM, GM and SR were responsible for analysis and interpretation of data. MA was involved in the collection of the clinical data. GC and AV performed biopsies and radiologic studies and were responsible for their interpretation. CG performed histologic studies. EM gave her critical contribution and approved the final version of the paper.

Disclosures

Conflict of interest: none
Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by an Associate Editor. The final decision to accept this paper for publication was taken by the Editors. Manuscript received March 11, 2003; accepted October 8, 2003.