The Role of Bronchoalveolar Lavage in the Microbiological Diagnosis of Pneumonia in Patients with Haematological Malignancies

Livio Pagano¹, Gabriella Pagliari², Agostino Basso², Roberto Marra¹, Simona Sica¹, Luigi Frigieri³, Giulia Morace³, Fausta Ardito³ and Giuseppe Leone¹

In the aetiological diagnosis of pulmonary infections in patients affected by haematological malignancies we evaluated the utility of bronchoalveolar lavage (BAL). One hundred and twenty-seven BAL were performed in 119 patients. In our series, we identified the agent of pneumonia in 53.5% of episodes with the best results in aspergillosis, very common in these patients. The previous empirical anti-infective treatment was modified in 14 episodes (11%). The procedure was generally well tolerated and only one patient bled. We maintain that BAL is a useful diagnostic tool for detecting the agents of pulmonary infections in patients with haematological malignancies, especially when the routine microbiological procedures fail, and it also represents a good alternative to more invasive procedures.

Key words: bronchoalveolar lavage; pneumonia; leukaemia; lymphoma.


Introduction

Pulmonary infection is a very frequent complication in patients with haematological malignancies. In patients with acute leukaemia, the aplastic phase following chemotherapy (1, 2) represents the major cause of morbidity and mortality. In these clinical conditions (3), an empirical broad-spectrum antibiotic treatment is employed; yet in some cases in spite of this aggressive treatment a worsening of clinical conditions is observed. This worsening might be due either to a prolonged neutropenia induced by chemotherapy or to an impaired antibiotic sensitivity of the microbiological agents to the start of empirical treatment. In these cases routine microbiological procedures, i.e. sputum examination, oral washing, swabs and blood cultures, frequently fail in the identification of the pneumonia agent. For these reasons various invasive procedures have been proposed in order to achieve a correct and timely diagnosis, such as fibreoptic bronchoscopy with transbronchial biopsy, transthoracic fine-needle aspiration, open-lung biopsy, but all these diagnostic tools are associated with a high risk of bleeding, pneumothorax and superinfections (4–7). In patients with haematological malignancies, bronchoalveolar lavage (BAL), performed with fibreoptic bronchoscopy has been suggested as a good diagnostic tool for the microbiological diagnosis of pulmonary infections. The aetiological agent is identified with this procedure, in the various series reported, in a percentage ranging between 16%

Abbreviations

ALL acute lymphoblastic leukaemia
AML acute myeloblastic leukaemia
AM atypical mycobacterial
BAL bronchoalveolar lavage
CLL chronic lymphocytic leukaemia
CML chronic myeloid leukaemia
CMV Cytomegalovirus
FUO fever of unknown origin
HSV Herpesvirus
HD Hodgkin’s disease
MM multiple myeloma
NHL non-Hodgkin’s lymphoma
Pc Pneumocystis carinii
and 90% of cases with a low risk of complications (8-16).

In order to evaluate the effectiveness of this procedure and the identification of the aetiological agent that allows the start of aimed therapy, we performed BAL to a large number of patients affected by haematological malignancies and with clinical indication of pulmonary infection.

**Material and Methods**

From February 1988 to March 1996, 127 bronchoscopic procedures with BAL were performed in 119 patients affected by haematological malignancies. In 115 episodes the BAL procedure was performed in patients who presented with a developed unresponsive pneumonia, and in most cases, during the aplastic phase and after an aggressive antiblastic treatment. Diagnosis of pneumonia was made on the basis of clinical signs and symptoms (i.e. fever, thoracic pain, cough, dyspnoea) and radiological findings (lung X-ray and/or CT scan). In 12 other episodes, patients had a fever of unknown origin (FUO) unresponsive to antibiotic treatment. In these patients BAL procedure was performed in the absence of clinical or radiological findings compatible with pneumonia and with no signs of focal infection. The patients consisted of 78 males and 41 females, aged from 16 to 74 years, and the underlying diseases were: acute myeloblastic leukaemia (AML) (63 cases), acute lymphoblastic leukaemia (ALL) (8 cases), non-Hodgkin’s lymphoma (NHL) (34 cases), Hodgkin’s disease (HD) (8 cases), chronic myeloid leukaemia (CML) (4 cases), chronic lymphocytic leukaemia (CLL) (1 case) and multiple myeloma (MM) (1 case). Table 1 summarizes the patient characteristics.

In 71 pneumonia episodes, the lung X-ray or CT study showed a focal or multifocal pulmonary infiltration. In 44 episodes diffuse bilateral pulmonary infiltrate was present. In 12 episodes, all patients with FUO, and in a deep and prolonged neutropenic phase (neutrophil count < 0.1 x 10^9/L), the lung X-ray and/or CT scan were/was negative. All patients underwent bronchoscopic procedure, microbiological examinations of sputum and urine, of nasal and pharyngeal plugs and serological studies for fungal or viral agents, and at least three sets of blood cultures were performed.

At the onset of neutropenia (neutrophil count < 0.5 x 10^9/L), all patients were given a prophylactic treatment with oral quinolones (ciprofloxacin 500 mg twice a day) and topical Amphoterica B. ALL and NHL patients were also treated with prophylactic administration of cotrimoxazole (960 mg twice a day) twice a week against *Pneumocystis carinii* (Pc).

At the onset of the fever, all patients were treated with an empirical large-spectrum antibiotic association of two or three drugs (aminoglycoside and β-lactamic with or without glycopeptide) (3). After 3-5 days of antibiotic therapy without improvement or recovery, endovenous antifungal therapy with Amphoterica B was added. Furthermore, in those cases with a diffuse interstitial infiltration suspected for a *Cytomegalovirus* (CMV) infection, an antiviral treatment (Ganciclovyr) was started. Even in the presence of a specific diagnosis therapeutic doses of cotrimoxazole (100 mg/day) towards Pc were administered to 15 patients.

**Bronchoscopy Technique**

After topical anesthaesia, a flexible fibreoptic bronchoscope (Ø5.6 mm) was passed into the trachea transnasally. After the examination of the tracheobronchial tree, the bronchoscope was wedged in the airway of the segment that showed the worst radiographic picture. When a pulmonary diffuse infiltration was present, bronchoalveolar lavage was performed in the right middle lobe and the lingula. An aspiration of bronchial secretion was performed before washing. A volume of 240 mL in 60 mL aliquots of normal saline at 37°C was instilled. A brush biopsy was also performed after BAL to nine patients with focal pneumonia who presented a normal platelet count.

**Microbiological Studies**

BAL fluids were all submitted to the microbiology laboratory for bacterial, fungal, parasitic and virological examinations.

BAL fluids for bacteria were stained with Gram and cultured onto blood, chocolate and McConkey agar plates. If bacterial growth was detected after 24 hours of incubation at 37°C, bacteria were identified into species level using Sceptor panels for Gram+ and Gram- bacteria system (Becton Dickinson, Cockeysville, MD, USA). Antibiotic susceptibility assays were performed with the same system. In the case of mycobacteria, the specimens were decontaminated with NaOH (2%), then stained with Ziehl–Neelsen and cultured onto Löwenstein–Jensen and Middlebrook agar slants. At the same time, cultures were also performed onto 7H12B and BACTEC 460 radiometric system (Becton Dickinson, Cockeysville, MD, USA). Mycobacteria were identified using biochemical tests; recently we also used a commercial molecular method (ACCPROBE, Gene Probe Inc., San Diego, CA, USA).
In case of viruses BALs were centrifuged, treated with mucolytic and antibiotic agents and cultured. Detection of CMV and Herpesvirus (HSV) was obtained using specific monoclonal antibodies. Detection of CMV antigenemia was obtained by using P65 monoclonal antibodies on lymphocytes separated from blood.

The presence of Pc was demonstrated with toluidine blue stain and, in the last 22 cases, using monoclonal fluorescent antibody and PCR (17).

In the diagnosis of fungal infections all BAL fluids were microscopically observed with potassium hydroxide (10%); during the last 5 years all BALs have been stained with a commercial fluorescent reagent, 'Fungiflora Y' (Biomate Co Ltd, Tokio, Japan), which specifically reacts with fungal cell wall polysaccharides with \( \beta \) linkages (i.e. glucans and chitin) and observed with a fluorescent microscope. Fungi are clearly distinguishable for more marked fluorescence, especially in the septa, although aspecific background can be observed. Cultures were performed onto Sabouraud dextrose agar with chloramphenicol (50 mg/mL), and cicloeximide (500 mg/mL) slants and Bacto Candida growth agar plates. Yeasts were identified on the basis of the biochemical assays performed with the Vitek system (BioMerieux Italy, Rome), germ tube production in foetal bovine serum and micromorphological characteristics were seen in Rice extract agar. On the basis of macro- and microscopic features of the colony using the slide culture technique, filamentous fungi were identified.

**Diagnostic Criteria**

According to previous reports (8, 13), the isolated micro-organism was considered the aetiological agent of pneumonia only when at least one of the following criteria was noted:

- presence of the same micro-organism in BAL and blood, in lung autopic tissue or in other extrapulmonary tissues.
- isolation of the same micro-organism also in bronchial brush biopsy or in transbronchial biopsy.
- microscopy positive for the suspected agent (i.e. Aspergillus) and response of the patient to an appropriate specific treatment.

**Results**

BAL study was diagnostic in 68 episodes (53.5%). Table 2 reports the microbiological agents identified. In 22 cases aspergillosis was diagnosed by the mycological (microscope and culture) examination of BAL fluids. In 17 patients, Aspergillus spp. were isolated and identified (Table 3). The isolates were considered aetiological agents because the diagnosis was also confirmed by other procedures. In four cases, the same species was present in brushing, in three cases at the autopsy (histology), in three cases in cutaneous biopsies, in two cases in transbronchial biopsy and in the remaining five cases by the presence of typical pulmonary lesions at the lung X-ray examination, and the same species (three patients with *Aspergillus versicolor* and two with *Aspergillus terreus*) were also isolated from sputa. For these five remaining patients, only microscopy of the BAL fluids demonstrated the presence of dichotomous branching septate hyphae compatible with the diagnosis of aspergillosis. In three cases aspergillosis was confirmed by the presence of typical pulmonary lesions at the lung X-ray examination, associated with a good response to specific antifungal treatment, and by *Aspergillus fumigatus* isolated from sputa. In one episode by pulmonary lobectomy, the culture of lung biopsy was negative probably because the patient underwent surgical removal of the lung lesion after prolonged treatment with itraconazole (400 mg/day for 1 month), that could have had an inhibitory effect on the culture. Only one patient had unresponsive fever as a symptom. Only four of these 22 patients presented nasal colonization by *Aspergillus*. All patients but two had a good response to specific antifungal therapy (Amphotericin B with or without following itraconazole).

In 36 patients *Candida* spp. were detected in BAL, but only in three cases was the diagnosis of *Candida* pneumonia made: in two cases a positive blood culture for *C. albicans* and *C. krusei*, respectively, was associated to a diffuse colonization of mucosae; in the third case *Candida* pneumonia was detected at autopsy. In the other cases detection of *Candida* spp. in BAL was considered contamination by a rhinopharyngeal colonization.

Pc pneumonia was diagnosed in five patients, four NHL and one ALL. All these had previously refused cotrimoxazole prophylaxis. Special stains performed for Pc resulting in a negative diagnosis was possible only by the use of monoclonal immunofluorescence and polymerase chain reaction.

In two patients with ALL, CMV was isolated in BAL fluid and the diagnosis was confirmed by the use of serology and antignaemia detection with P65 monoclonal antibodies. In two patients the pneumonia agent was an HSV type 1. In the first case the diagnosis was confirmed by autopsy, in the second by the presence of specific IgM and IgG antibodies at significant titres and by improvement with a specific therapy.

*Mycobacterium tuberculosis* was the agent of pulmonary infection in four cases. It was confirmed by autopsy in two cases, and in the other two cases by transbronchial biopsy and by the resolution of the focal lesion with the use of specific three drugs therapy.

Three cases of atypical mycobacterial (AM) pneumonia (2 *Mycobacterium terrae* complex and 1 *Mycobacterium avium*) were diagnosed. We determined that these agents were responsible for the pneumonia because of the contemporaneous presence of the same species in urine.

The presence of bacterial saprophytic flora was detected in 65 (48%) BAL procedures. In the other 21 episodes, BAL fluid microbiological study was considered diagnostic for bacterial pneumonia (12 Gram+ and 9 Gram-). The most frequent bacterial
agents were *Staphylococcus epidermidis* and *Pseudomonas* spp. In six cases a mixed bacterial flora with two or more pathogens were present in BAL fluid. (Table 2).

The empirically administered anti-infective treatment, including antifungal therapy, was modified on the basis of BAL fluid microbiology in 14 cases. A specific treatment was added in the following cases: aspergillosis two cases; *Mycobacterium tuberculosis* three cases; *Mycobacterium terrae* complex one case; HSV1 two cases; *Pc* five cases; teicoplanin-resistant *Staphylococcus aureus* one case.

BAL was diagnostic in three of 12 episodes of FUO. In one case the agents identified were *Aspergillus fumigatus*, and *Pseudomonas* spp. in the other two episodes.

The bronchoscopic procedure was well tolerated and in only one case, of a patient with a low platelet count, the examination was halted because of bleeding during

### Table 2. Most common pathogens detected by bronchoalveolar lavage fluid study.

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>Number of</th>
<th>Responsible</th>
<th>Found as single agent</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fungi</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus ssp.</td>
<td>22</td>
<td>22</td>
<td>5*</td>
</tr>
<tr>
<td>Candida ssp.</td>
<td>36</td>
<td>3</td>
<td>2†</td>
</tr>
<tr>
<td>Pneumocystis carinii</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td><strong>Viruses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herpesviruses type 1</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Gram-negative bacilli</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas maltophilia</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>3</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Gram-positive bacilli</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>6</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>5</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td><strong>Mixed bacteric flora</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas spp. + Enterococcus spp.</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus epidermidis</em> + Enterococcus faecalis</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Escherichia coli + Enterococcus faecalis</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecium + Klebsiella pneumoniae</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Mycobacterium tuberculosis</strong></td>
<td>4</td>
<td>4</td>
<td>3‡</td>
</tr>
<tr>
<td><em>Mycobacterium terrae complex</em></td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><em>Mycobacterium avium</em></td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

*In 10 cases associated with *Neisseria sicca* and/or *Streptococcus alfa-haemolyticus*; in five cases associated with *Staphylococcus epidermidis*; in two cases associated with *Candida* spp.

†In only one case associated with *Enterococcus faecalis* (interstitial pneumopathy).

‡In one case associated with *Staphylococcus epidermidis*.

### Table 3. Cases of aspergillosis diagnosed by mycological examination of the bronchoalveolar lavage fluids.

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Microscopy positive n = 17</th>
<th>Culture positive n = 17</th>
<th>Species identified</th>
<th>Sputa positive n = 15</th>
<th>Nasal colonization n = 4</th>
<th>Lung X-ray positive n = 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>4</td>
<td>7</td>
<td>Aspergillus flavus</td>
<td>4</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>5</td>
<td>Aspergillus fumigatus</td>
<td>3</td>
<td>2</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>Aspergillus versicolor</td>
<td>3</td>
<td>—</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>2</td>
<td>Aspergillus terreus</td>
<td>2</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>5 (Fungiflora Y)</td>
<td>3</td>
<td>None</td>
<td>3</td>
<td>—</td>
<td>3</td>
</tr>
</tbody>
</table>
transbronchial biopsy, transthoracic needle aspiration or follow chemotherapy. The aetiological agents of this viruses and parasites (1, 2, 15). To date, general agree- that includes the use of two or more drugs towards haematological malignancies, or generally in the treatment; also, the prolonged empirical antibiotic with a normal platelet count (4-7).

the nasal introduction of the bronchoscope. The patient underwent platelet concentrates transfusion without further respiratory problems related to the procedure.

Discussion

Pneumonia is the major cause of death in haematological patients, especially during the aplasia that follows chemotherapy. The aetiological agents of this complication can be bacteria, mycobacteria, fungi, viruses and parasites (1, 2, 15). To date, general agreement exists about empirical therapeutic approaches, that includes the use of two or more drugs towards infective agents (2, 18, 19). On the other hand, antibiotics add toxicity to that caused by antiblastic treatment; also, the prolonged empirical antibiotic therapy against pulmonary infection favours superinfections by agents such as mycoplasmas, mycobacteria or, most frequently, fungi.

For these reasons timely identification of the microorganism responsible for pneumonia will be useful in starting a selective and effective antimicrobial treatment. For this purpose a series of diagnostic tools, unfortunately burdened by a high complication rate, have been proposed in the literature. Fibreoptic bronchoscopy with transbronchial biopsy, transthoracic needle aspiration or open lung biopsy could be performed only if associated with a rather good performance status and, above all, with a normal platelet count (4–7).

BAL appears to be an easy and well-tolerated procedure. The diagnostic sensitivity of fibreoptic bronchoscopy and bronchial washing in patients with haematological malignancies, or generally in the immunocompromised host, in pulmonary infections ranges between 16% and 90% (8–16). In our opinion, this wide variability is due above all to the lack of a standard technique both in the BAL procedure and in the microbiological tests performed.

In our 9 years of experience the BAL procedure proved positive in 53.5% of episodes. All patients but one, which was in poor clinical conditions, tolerated the BAL procedure well. In 14 infective episodes (11%) this diagnostic procedure allowed us to modify the empirical antibiotic treatment, with an improvement of the clinical course of the infection. Our result is lower than that reported by von Eiff et al. (12) and is probably due to the wide empirical antimicrobial approach that we used.

Our series included a very selected, homogeneous group of patients: all had previously undergone aggressive chemotherapy followed by prolonged aplasia. All were treated with the same prophylaxis and empirical antibiotic treatment at the onset of fever. BAL and microbiological tests were performed with the same procedure.

The most common pathogen detected by BAL in our study was Aspergillus spp. In our limited series, pulmonary aspergillosis was detected in 18.4% of the cases. It is interesting to note that, in the study period, the years with the major incidence of aspergillosis (4 cases/year) were 1992, 1993 and 1994. In these years there was building renovation of our hospital, which could have influenced our data. In fact, in the same year we had also the major prevalence of Aspergillus spp. isolated from sputa of haematological patients (25 of a total of 56 patients). Our data were comparable with previously reported data identifying Aspergillus as responsible for pneumonia in 15% and 50% of BAL procedures performed (13, 20, 21). It is probable that this positive result was related, as suggested by Kahn et al. (20), to the fact that starting Amphotericin B therapy before bronchoscopy does not adversely affect the likelihood of obtaining a positive culture for Aspergillus spp. as is also demonstrated by the high percentage of Aspergillus isolation in our series (77.2%).

Candida pneumonia was diagnosed only in three cases (2%). In the other cases the presence of Candida spp. in BAL fluid was considered a contamination. Our experience confirmed the previously reported data (13, 22); in fact, the incidence of Candida pneumonia in patients with acute leukaemia ranged from 0 to 15% (13, 22).

The culture of several bacterial agents in the same BAL fluid presents low reliability because most haematological patients who undergo aggressive chemotherapy have a polymicrobial colonization, as already suggested by Stover et al. (8). Gram- bacteria were recognized as the only aetiological agent in nine episodes, and in another five associated with other pathogens. They represented 12% of all the infections but, on the whole, the incidence of this infection was low, and could be due to the prophylactic use of quinolones (23). On the other hand, the use of this kind of prophylaxis induced an increase in Staphylococcus epidermidis and Enterococcus faecium pneumonia (24). In our experience, the increase of coagulase negative staphylococci pneumonia was related strictly to increasing septicemia due to the same agent (25).

We never detected Legionella pneumonia. This was probably because of the prolonged hospitalization of our patients before the onset of the pulmonary infection. Moreover, this agent had not been epidemiologically demonstrated at our institution during the time of this study.

We observed five cases of Pneumocystis carinii pneumonia. The rarity of this infection in our study may be due to various factors but, above all, to the use of correct prophylactic treatment with cotrimoxazole. In all these patients, as in patients with Mycobacterium tuber- culosis infection, BAL procedure was particularly useful because it allowed a successful change of therapeutical strategy with rapid improvement of their clinical conditions, independently from neutrophil count.

Our results suggest that BAL is an easy and useful diagnostic procedure for the identification of microbiological agents of pulmonary infections in patients with haematological malignancies. It is indicated especially when results of conventional microbiological procedure are negative.

In our opinion, the use of bronchoscopy with BAL results are more efficacious, particularly in the diagnosis of pulmonary aspergillosis. Microscopic examination of this specimen, when positive for dichotomous branch-
ing septate hyphae, allows earlier diagnosis of aspergillosis with consequent timely treatment. Moreover, advanced techniques such as specific nucleic acid amplification and antigen detection (galactomannan) directly on BAL fluids, as suggested by Verweij et al. (26), could enhance the possibility of a rapid and accurate diagnosis of aspergillosis, making useful a more extensive application of the BAL procedure in haematological patients.

Finally, we advocate the use of bronchoscopy with BAL fluid study because it is a useful diagnostic tool for detecting the agents of pulmonary infections in patients with haematological malignancies, and we firmly believe that currently, and still more in the future, it could represent a good alternative to more invasive procedures.

This work was supported by Contract No 96.00602 PF39 Targeted Project ACRO of National Research Council (CNR).

References


