

## Opportunistic agents in bronchoalveolar lavage in 99 HIV seropositive patients

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*Opportunistic agents in bronchoalveolar lavage in 99 HIV seropositive patients. S. Durand-Amat, G. Zalzman, M-C. Mazon, C. Sarfati, B. Beauvais, F. Gerber, Y. Pérol, A. Hirsch.*

**ABSTRACT:** During a ten month period, 117 fiberoptic bronchoscopies and bronchoalveolar lavages (BAL) were performed in human immunodeficiency virus (HIV) infected patients suspected of having opportunistic pulmonary infections. The BAL were classified into 3 groups, according to clinical manifestations related to HIV infection at the time of fiberoptic bronchoscopy: pre-acquired immunodeficiency syndrome (AIDS) (n=54), AIDS with Kaposi's sarcoma (n=37), AIDS without Kaposi's sarcoma (n=26). On chest X-ray, diffuse infiltrates were most common (54%), followed by normal X-rays (24%) and localized infiltrates (18%). Amongst the 117 BAL, 68 (58%) yielded at least one opportunistic agent. In 28 BAL performed for pulmonary signs or unexplained fever with normal chest X-rays, one or several opportunistic agents were isolated in 17 samples of BAL fluid. The most frequently identified opportunistic agents were *Pneumocystis carinii* (in 38% of BAL) and cytomegalovirus (35%); these were associated in 17% of BAL. There was no statistically significant difference in opportunistic agents among the 3 groups of BAL (pre-AIDS, AIDS with Kaposi's sarcoma, AIDS without Kaposi's sarcoma). In particular, cytomegalovirus was found in BAL with the same frequency in these 3 groups.

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Opportunistic infections and Kaposi's sarcoma are the two major pathological processes involving the lung in patients with acquired immunodeficiency syndrome (AIDS) [1]. Data presented in 1984 at the National Heart Lung and Blood Institute indicated that of 1,067 patients with AIDS, 441 (41%) had pulmonary complications, including at least one opportunistic pulmonary infection. One French study [2] evaluated the opportunistic lung infections in 63 patients with AIDS and 20 patients with chronic generalized lymphadenopathy, in which bronchoalveolar lavage (BAL) yielded at least one opportunistic agent in 54 cases (65%). Fiberoptic bronchoscopy and BAL are commonly used in the diagnosis of pulmonary involvement in this disease. We investigated 99 patients known to be seropositive for human immunodeficiency virus (HIV). These patients were referred to our institution for fiberoptic bronchoscopy and BAL because of the suspicion of opportunistic pulmonary infection. In the Hôpital Saint-Louis, BAL under fiberoptic bronchoscopy is the first investigative procedure for suspected pneumonia in HIV seropositive patients, when clinical and radiographic evaluation do not suggest a routine bacterial pneumonia. The objectives of our study were: 1) to assess clinical and

radiological signs in these patients; and 2) to characterize the opportunistic agents isolated from BAL fluid among a large group of HIV seropositive patients from several medical departments.

### Materials and methods

#### Patients

Between January and October, 1987, 117 consecutive BAL were performed in 99 HIV seropositive patients because of respiratory symptoms, abnormal chest radiography or unexplained fever lasting more than 3 weeks. Repeat fiberoptic bronchoscopies in 14 patients resulted in a total of 117 procedures. Patients were referred by different hospital departments: infectious diseases (n=36), dermatology (n=21), medicine (n=19), haematology (n=16), intensive care unit (n=7). There were 92 male and 7 female subjects. The mean age of these patients was 37 yrs (range 19-62 yrs). The relative distribution amongst the groups at risk for HIV infection was homosexual and bisexual men (n=66, 67%), intravenous drug abusers (n=10, 10%), recipients of blood transfusions

haemophilia (n=1, 1%). There were 5 Haitians (5%), 2 black Africans (2%) without risk factors. Ten other patients (10%) had no known risk factor. Using this pre-BAL data, we classified the BAL into 3 groups: pre-AIDS (n=54), known AIDS with Kaposi's sarcoma (n=37), known AIDS without Kaposi's sarcoma (n=26). AIDS was diagnosed according to criteria established by the Center for Disease Control (CDC) [3]. Patients were classified as pre-AIDS when they did not fulfill CDC criteria for AIDS and presented one or more clinical signs: persistent generalized lymphadenopathy, fever persisting for more than a month, weight loss of more than 10% baseline, diarrhoea persisting for more than a month, oral hairy leukoplakia, multidermatomal herpes zoster, recurrent *Salmonella* bacteraemia, nocardiosis, tuberculosis or thrush. These signs and symptoms met the criteria for group III, and for subgroups A and C-1 of group IV. These groups were defined by the CDC in a classification system for HIV infection [4].

#### Clinical and radiological features

These were evaluated for each patient at the time of the procedure. Signs of pulmonary disease (fever, cough, dyspnoea) were recorded. Prior to the diagnosis, the pattern and distribution of lung infiltrates were graded according to the following scale: 1) normal chest X-ray; 2) diffuse infiltrates (interstitial or alveolar markings); 3) localized infiltrates; and 4) other (including pleural effusions and hilar adenopathies).

#### Procedure

Fibreoptic bronchoscopy was performed with an Olympus BF type 10 bronchoscope. Patients on respiration had an endotracheal tube adaptor for bronchoscopy. After evaluation of the bronchotracheal tree, the tip of the fibroscope was wedged in the subsegmental bronchus of a radiographically abnormal region, or in the right middle lobe if the chest X-ray was normal or revealed diffuse infiltrates. A total of 150–200 ml of normal saline solution at room temperature was injected

in 3 aliquots and fluid was recovered by gravity or by gentle aspiration into sterile siliconized glass vials. The mean recovery was 50–60%. No transbronchial biopsy was performed. Several precautions were taken when performing fibreoptic bronchoscopy. A special fibreoptic bronchoscope was reserved for HIV seropositive patients. During bronchoscopy, staff wore gloves, masks and goggles. After the procedure was completed, the bronchoscope was decontaminated by soaking in a 2% glutaraldehyde solution for 20 min.

#### Bronchoscopic data in patients with Kaposi's sarcoma

Thirty seven fibreoptic bronchoscopies were performed in 30 patients with cutaneous or mucocutaneous Kaposi's sarcoma. In 9 patients, fibreoptic bronchoscopy showed, in the trachea or large bronchi, multiple, slightly raised, purple or bright red lesions, which were considered characteristic of Kaposi's sarcoma. In 4 patients, the lesions were discrete and considered suggestive of Kaposi's sarcoma.

For the diagnosis of *Pneumocystis carinii* pneumonia, 2 aliquots of 10 ml BAL fluid were centrifuged at 3,000 rpm for 5–10 min. From the resulting pellet, 4 smears were prepared on microscope slides: 2 were fixed with methanol for May-Grünwald Giemsa staining, 2 others were fixed with acetone and stained with the methanamine silver method of Gomori modified by Grocott.

For detection of cytomegalovirus (CMV), BAL fluid was centrifuged at 500 g for 10 min. The cell pellet was inoculated into human embryonic lung fibroblast monolayers (MRC<sub>5</sub>, Bio Mérieux). The monolayers were then centrifuged at 4,000 g for 45 min. CMV was detected 48 h after inoculation by an immunoperoxidase assay using the monoclonal antibody E<sub>13</sub> directed against an immediate early antigen. For diagnosis of herpes simplex virus infection, the method described above was also used, with a specific monoclonal antibody.

For bacterial evaluation, an aliquot of 10 ml of native lavage fluid was processed by the microbiology laboratory searching for *Mycobacteria* by direct examination and by cultures in appropriate media. Another aliquot was cultured for fungi.

Table 1. — Results of 117 BAL for the diagnosis of opportunistic pulmonary infections in HIV seropositive patients and clinical and radiological presentation before each BAL

Opportunistic agent(s) found in BAL (alone or in association)	n	Clinical presentation		Radiological presentation			
		Pulmonary signs*	Fever	Normal X-ray	Diffuse infiltrates	Localized infiltrates	Other†
<i>Pneumocystis carinii</i> (2.5%)	n=44	42 (95.0%)	39 (88.0%)	8 (18.0%)	34 (77.0%)	1 (2.5%)	1
Cytomegalovirus (7.0%)	n=41	35 (85.0%)	26 (63.0%)	9 (22.0%)	24 (59.0%)	5 (12.0%)	3
<i>Mycobacteria</i>	n= 8	8 (100.0%)	6 (75.0%)	3 (38.0%)	5 (62.0%)		
No opportunistic agent (8.0%)	n=49	32 (65.0%)	36 (74.0%)	11 (22.5%)	22 (45.0%)	12 (24.5%)	4

\*. Pulmonary signs include cough and/or dyspnoea with or without radiological abnormality; †: other radiological presentations include pleural effusion (n=4) and hilar adenopathy (n=4); BAL: bronchoalveolar lavage; HIV: human immunodeficiency virus.

Table 2. - Opportunistic agents in 117 BAL in HIV seropositive patients

Findings in BAL	Number of BAL
<b>Opportunistic agent</b>	
<i>Pneumocystis carinii</i>	44
without coexisting infection	22
with coexisting infection	22
Cytomegalovirus	20
<i>Mycobacterium</i>	2
Other	2
Cytomegalovirus	41
without coexisting infection	18
with coexisting infection	23
<i>Pneumocystis carinii</i>	20
<i>Mycobacterium</i>	2
Other	3
<i>Mycobacterium</i>	8
<i>M. tuberculosis</i>	3
<i>M. avium intracellulare</i>	2
<i>M. kansasii</i>	2
<i>M. gordonae</i>	1
Herpes simplex virus	3
Fungi	2
<i>Cryptococcus neoformans</i>	1
<i>Aspergillus niger</i>	1
<b>No opportunistic agent</b>	49

BAL: bronchoalveolar lavage; HIV: human immunodeficiency virus.

(n=4, 2%), and hilar adenopathies (n=4, 2%). Pleural effusions and hilar adenopathies were only seen in patients presenting with Kaposi's sarcoma. In 28 BAL chest X-rays were normal. Among these, in 17 BAL (61%), one or several opportunistic agents were isolated: cytomegalovirus (n=9), *Pneumocystis carinii* (n=8), herpes simplex virus (n=2), *Mycobacterium tuberculosis avium intracellulare* (n=1), *Mycobacterium kansasii* (n=1). In 3 BAL performed for prolonged fever without respiratory signs and without chest radiographic abnormalities, no opportunistic agent was isolated.

#### Opportunistic agents isolated by BAL

The opportunistic agents found in BAL fluid are summarized in table 2. BAL yielded at least one opportunistic infection in 68 cases (58%). Two pathogens were found in 24 BAL, and three pathogens in 2 BAL. The most frequently identified pulmonary pathogens were *P. carinii* (n=44) and CMV (n=41). *P. carinii* and CMV coexisted in 20 BAL. In 49 BAL (42%), no opportunistic agent was found in the BAL fluid. We compared the opportunistic agents isolated by BAL in the 3 pre-BAL classified groups: pre-AIDS, known AIDS with Kaposi's sarcoma and AIDS without Kaposi's sarcoma (table 3). The distribution of pulmonary opportunistic agents was not significantly different between these 3 groups ( $\chi^2$ , p<0.05).

Table 3. - Opportunistic agents isolated in BAL in 3 groups of HIV seropositive patients (classified before each BAL)

BAL (n=117)	Pre-AIDS n=54 (46.0%)	AIDS with Kaposi's sarcoma n=37 (32.0%)	AIDS without Kaposi's sarcoma n=26 (22.0%)
<i>Pneumocystis carinii</i> n=44 (38.0%)	24 (20.0%)	11 (10.0%)	9 (8.0%)
Cytomegalovirus n=41 (35.0%)	10 (8.5%)	15 (13.0%)	16 (13.5%)
Atypical <i>Mycobacteria</i> n=5 (4.0%)		2 (1.5%)	3 (2.5%)
<i>M. avium intracellulare</i> n=2		2	
<i>M. kansasii</i> n=2		1	1
<i>M. gordonae</i> n=1		1	
<i>M. tuberculosis</i> n=3 (2.5%)	3 (2.5%)		
Herpes simplex virus n=3 (2.5%)	1 (1.0%)	1 (1.0%)	1 (1.0%)
Fungi n=2 (2.0%)	1 (1.0%)		1 (1.0%)

BAL: bronchoalveolar lavage; HIV: human immunodeficiency virus; AIDS: acquired immune deficiency syndrome.

## Results

### Clinical and radiological features

These are summarized in table 1. Fever, cough and dyspnoea were present in 78%, 64% and 50% of cases, respectively. The chest radiographic findings included isolated or associated diffuse infiltrates (n=64, 54%), localized infiltrates (n=21, 18%), pleural effusions

## Discussion

The diagnosis of pulmonary disease in AIDS patients presents several specific problems. The lung is commonly involved in infectious and non-infectious processes, including Kaposi's sarcoma, non-specific interstitial pneumonitis and lymphoid interstitial pneumonitis [5, 6]. More than one pathological process may be present. The presenting symptoms are non-specific and develop more

gradually than in other immunosuppressed patients [7]. Repeated episodes of pneumonia occur. Recent studies have assessed the role of fiberoptic bronchoscopy with BAL as the initial invasive procedure for diagnosis of pulmonary disease in AIDS [8-10]. The interest in BAL is increased given the complications associated with transbronchial biopsy (haemorrhage and pneumothorax). Moreover, BAL can be performed in patients with coagulopathies or who are being mechanically ventilated.

This study reports clinical and radiological data, the opportunistic agents isolated from BAL fluid, and endoscopic bronchial aspects observed among 99 HIV infected patients who were at different stages of HIV infection.

Chest X-ray patterns were non-specific. As previously reported [11], diffuse infiltrates were the commonest finding and were seen in 54% of cases. When BAL was performed for pulmonary symptoms or unexplained fever with normal chest radiography, one or more opportunistic agents were isolated in 61% of cases. Among patients with *P. carinii* pneumonia, diffuse infiltrates were the commonest finding (77% of cases) and chest X-ray was normal in 18% of cases. Normal chest radiography in *P. carinii* pneumonia has usually been considered uncommon [11, 12]. However, in some recent studies [6, 13], chest radiography was normal in about 25% of AIDS patients presenting with *P. carinii* pneumonia. These cases may increase if patients are investigated earlier in the course of pneumonia, because AIDS patients have a prolonged mild prodromal illness [7, 14]. Earlier BAL allows earlier diagnosis, which increases the likelihood of successful treatment [14].

In 58% of BAL fluid, we isolated at least one opportunistic agent. *P. carinii* was the most common pathogen (38%). *P. carinii* is known as the most frequent cause of infectious pulmonary involvement in AIDS patients [1]. However, the frequency of *P. carinii* observed in our study is low in comparison with other studies which used BAL as the only diagnostic procedure for pneumonia in AIDS patients [2, 8, 9]: in these studies, *P. carinii* was found in 60-80% of patients with pneumonia. In one recent study [6], the rate of *P. carinii* pneumonia, diagnosed by BAL and transbronchial biopsy was 41%. How frequently clinicians report *P. carinii* pneumonia may depend on the characteristics of HIV seropositive patients seen at their hospitals (stage of HIV infection, risk factors and ethnic background). This may also depend greatly on the clinical indications for BAL. Most often, American studies focus on the pulmonary infections in patients with known AIDS. Fiberoptic bronchoscopy and BAL are often performed after several procedures such as the measurement of single-breath diffusing capacity for carbon monoxide and gallium lung scanning, especially in cases of normal chest X-ray. In our study, 54 BAL (46%) were performed in pre-AIDS patients, and BAL was the first procedure for suspected pneumonia in HIV seropositive patients. We think that the low rate of *P. carinii* pneumonia observed in our study could be due partly to the fact that 14 patients with known AIDS had received trimethoprim-sulphamethoxazole (TMP-SMX) prophylaxis because of a

previous episode of *P. carinii*. There is no randomized control study with TMP-SMX prophylaxis with TMP preventing *P. carinii* lymphocytic leukaemia. Adverse reaction in immunosuppressed subjects may carry a risk.

The high frequency of *C. carinii* (19%) is apparent in this pathogen was very often CMV, as previously reported.

[1]. CMV was isolated in 35% of BAL, by conventional cell culture. This technique has the same specificity as conventional cell culture and at least equal sensibility for detection of CMV in BAL fluid [17, 18], without having to wait for the production of a cytopathic effect. The significance of CMV in BAL fluid and the prognosis related to this finding is unclear [10, 19]. CMV cultures of BAL are positive in 50-60% of HIV seropositive patients [6, 20]. However, a high incidence of CMV infection in homosexual men and AIDS patients has been documented [21, 22]. Thus, the CMV found in BAL could be due to blood or oropharyngeal contamination. Moreover, it is possible that the lung acts as a reservoir for CMV [19]. The detection of characteristic cytological changes due to CMV, with intracytoplasmic inclusions and cytomegaly is necessary to distinguish CMV lung infection and CMV pneumonia [23]. Among bone marrow transplant recipients with pneumonia, the identification of CMV by cytology and specific monoclonal antibodies in cells recovered by BAL seems to be closely related [24]. Moreover, immunofluorescence with specific monoclonal antibodies seems to provide a quantifiable system for detection of CMV in BAL, and diagnosis of CMV pneumonia [25]. However, studies including careful clinico-pathologic correlations are required to extend these results in AIDS patients and to discover the significance of CMV in BAL amongst HIV seropositive patients.

Positive cultures for *Mycobacteria* were found in 7% of BAL. *Mycobacterium avium intracellulare* is one of the most frequently reported opportunistic infections in AIDS patients [1], but we found it in BAL fluid from only 2 cases, in association with another pathogen. As previously reported in a French study on BAL in AIDS [2], this may be related to geographical features of *Mycobacteria* in France, where *M. avium intracellulare* is rarely encountered [2]. Diagnosis of pulmonary infection with *M. avium intracellulare* is difficult and needs evaluation for disseminated disease [10].

The techniques used in this study were not designed to diagnose bacterial infections: quantitative bacteriological studies of BAL are required to differentiate oropharyngeal contamination from pulmonary infection [26]. For the same reason, we did not take account of *Candida albicans* because of the great frequency of oral thrush in HIV seropositive patients.

Forty two percent of BAL did not yield any opportunistic agent: 65% of these non-diagnostic BAL were performed for clinical pulmonary symptoms and 69.5%

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for diffuse or localized infiltrates on chest X-ray. These pulmonary signs, without identifiable infectious pathogens, could be related to non-specific interstitial pneumonitis. SUFFREDINI *et al.* [6] recently emphasized the frequency of non-specific interstitial pneumonitis, which accounts for 32% of the pneumonitis in AIDS patients in their study. However, in our study, the histopathologic findings needed to assess the real frequency of non-specific pneumonitis are not available.

The comparison between the 3 groups of BAL (pre-AIDS, AIDS with Kaposi's sarcoma, AIDS without Kaposi's sarcoma) does not demonstrate a difference as regards the opportunistic agents isolated. In the pre-AIDS group the most frequent pathogen was *P. carinii*.

*P. carinii* pneumonia was the first manifestation of AIDS in 24 cases. CMV was isolated in 10 BAL from this group, in association with *P. carinii* in 7 cases and alone in 3 cases. Since evidence of the cytological changes due to CMV infection was not available, we did not consider this to be a first symptom of AIDS. Thus, CMV can be found in BAL at an early stage of HIV infection. An association between CMV infection and Kaposi's sarcoma is known to exist [27], but there is no proven causal relationship between CMV and Kaposi's sarcoma. In our study CMV was isolated in BAL with the same frequency in the 2 groups of known AIDS, with or without Kaposi's sarcoma.

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Agents opportunistes décelés dans le lavage broncho-alvéolaire chez 99 patients séro-positifs VIH. S. Durand-Amat, G. Zalzman, M.C. Mazon, C. Sarfati, B. Beauvais, F. Gerber, Y. Pérol, A. Hirsch.

RÉSUMÉ: 117 fibroscopies bronchiques et lavages broncho-alvéolaires (LBA) ont été pratiqués en 10 mois pour une suspicion de pneumopathie opportuniste, chez des patients ayant une sérologie VIH positive. Les données cliniques et radiologiques, les aspects endoscopiques, et les agents opportunistes

isolés par LBA ont été étudiés. Les manifestations cliniques liées à l'infection VIH, établies avant chaque fibroscopie, ont permis de classer les LBA en 3 groupes: pré-SIDA (n=54), SIDA avec syndrome de Kaposi (n=37), SIDA sans syndrome de Kaposi (n=26). Les anomalies les plus fréquentes à la radiographie thoracique étaient des opacités diffuses (54%), et des opacités localisées (18%). 24% des radiographies thoraciques étaient normales. 68 des 117 LBA (58%) ont permis d'isoler un ou plusieurs agents opportunistes. Les plus fréquents étaient *Pneumocystis carinii* et cytomégalovirus, qui étaient associés dans 17% des LBA. Parmi les 28 LBA pratiqués alors que la radiographie thoracique était normale, 17 ont ramené un ou plusieurs agents opportunistes. Il n'a pas été trouvé de différence significative concernant le type d'agent opportuniste isolé dans les 3 groupes de LBA (pré-SIDA, SIDA avec syndrome de Kaposi, SIDA sans syndrome de Kaposi). En particulier, le cytomégalovirus a été isolé avec la même fréquence dans ces 3 groupes.

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