Pulmonary infiltrates in immunocompromised patients: diagnosis by cytological examination of bronchoalveolar lavage fluid

JENNIFER A YOUNG, JM HOPKIN, WP CUTHBERTSON

From the Departments of Pathology and Medicine, Queen Elizabeth Hospital and University of Birmingham, Birmingham

SUMMARY Thirty pulmonary infiltrates in 26 patients were investigated by bronchoalveolar lavage. Sixteen of the patients were on therapeutic immunosuppression for renal disease or transplant and 10 had leukaemia, lymphoma, or allied conditions. A rapid specific diagnosis was made in 21 (70%) episodes by cytological examination of the fluid and in 28 (93%) by a combination of cytology and microbiology. No complications from haemorrhage or pneumothorax ensued. Pneumonia due to *Pneumocystis carinii* was the most common diagnosis (27%), but opportunistic infections from cytomegalovirus, candida, aspergillus, zygomycetes, and acid fast bacilli were also identified by cytology. Two episodes were caused by occult pulmonary haemorrhage and five patients had malignant infiltration of the lung from leukaemia, myeloma, Hodgkin's disease, and lymphoplasmacytoid lymphoma. In two of these there was also evidence of infection. In seven cases with non-diagnostic cytology infections due to *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *pneumococcus*, *micrococcus*, and *Aspergillus fumigatus* were identified on culture. In two patients (7%) no specific diagnosis was established by lavage: one had serological evidence of legionella infection and the second had *P aeruginosa* septicaemia. Twelve (75%) of the renal patients and six (60%) of those with leukaemia, lymphoma, and allied conditions recovered.

Pulmonary infiltrates in immunocompromised patients are common and have a varied aetiology and a high mortality.^{1,2} Opportunistic infection is a common cause but must be distinguished from other conditions such as occult haemorrhage, drug or radiation toxicity, malignant disease, and non-specific interstitial pneumonitis. In addition, if optimum treatment is to be given the pathogenic organism must be identified. Non-invasive investigations are seldom helpful,^{3,4} and the diagnostic accuracy of biopsy, whether transbronchial or open lung, varies considerably.⁵ Also some doctors are reluctant to advise biopsy in severely ill immunocompromised patients.⁶ Fine needle aspiration of the lung causes fewer serious complications than biopsy but is associated with the same hazards of haemorrhage and pneumothorax.⁷

The technique of bronchoalveolar lavage has been developed for the investigation of interstitial lung disease.^{8,9} Published reports show it to be a safe¹⁰

procedure which obtains plentiful cellular material from the lungs.¹¹ The purpose of the present study was to assess the value of cytological examination of bronchoalveolar lavage fluid in the investigation of immunocompromised patients with pulmonary infiltrates of unknown aetiology.

Material and methods

Thirty pulmonary infiltrates in 26 patients were investigated by bronchoalveolar lavage. The patients comprised two groups. Sixteen were from the Department of Nephrology and were receiving therapeutic immunosuppression, 11 after renal transplant and five for kidney disease (rapidly progressive glomerulonephritis, systemic lupus erythematosus, and Goodpasture's syndrome). Ten patients were from the Departments of Haematology and Oncology. These included five patients with leukaemia, one of whom had received a bone marrow transplant, and two patients with aplastic anaemia, one of whom had also undergone bone marrow transplant. There was one patient with

non-Hodgkin's lymphoma and one with Hodgkin's disease. The remaining patient was an elderly woman who was admitted as an emergency with severe respiratory distress. She was found to have dysglobulinaemia, the cause of which had not been established at the time of bronchoscopy.

The patients who had undergone renal or bone marrow transplant were all receiving either cyclosporin A or prednisolone and azathioprine. The other renal patients were taking prednisolone and cyclophosphamide, and the patients with leukaemia and lymphoma were on a range of therapeutic regimens which included steroids, cytotoxic drugs, and radiotherapy. Both patients who had received bone marrow transplants had undergone total body irradiation. The indications for bronchoalveolar lavage were severe illness, with cough or dyspnoea, and abnormal chest x ray film in patients in whom no specific microbial diagnosis had resulted from examination of sputum (if available) and blood. The patient with Hodgkin's disease had already had a percutaneous fine needle aspiration of the lung, which was non-diagnostic both cytologically and on culture.

The bronchoalveolar lavage were collected during fiberoptic bronchoscopy by the method previously described.¹² The washings were obtained from the middle lobe or lingula in patients with diffuse radiological infiltrates and as appropriate where the abnormality was localised. Two or three 20 ml aliquots of sterile saline were instilled and aspirated, roughly half of the volume being returned. A sample of fluid was submitted to the Department of Microbiology and the remaining material sent for immediate cytological processing.

The lavage fluid was centrifuged for 5 min at 3000 rpm and smears were prepared from the deposit. These were stained by the Papanicolaou, May-Grünwald-Giemsa, Ziehl-Neelsen, and Grocott methenamine silver methods. When the pigment laden macrophages were numerous a Perls' stain for haemosiderin was included. If organisms suggestive of nocardia or actinomyces were seen a Gram stain was also carried out. The specimen from the patient with dysglobulinaemia was examined for immuno-

globulins (IgG, IgA, IgM, and kappa and lambda light chains) by the unlabelled antibody peroxidase-antiperoxidase method.¹³ Antisera were obtained commercially from Mercia-Brocades Ltd, West Byfleet, Surrey, England.

The cytology was all reported by one author (JAY). The fluid was examined for the presence of abnormal cells, haemosiderin laden macrophages, acid fast bacilli, and evidence of viral, parasitic, or mycotic infection. Apart from acid fast bacilli, nocardia, and actinomyces, bacteria were not looked for in the cytological specimens.

Results

The number of specific diagnoses and the sensitivity of cytology, microbiology, and the combined techniques are shown in Table 1. On six occasions a specific diagnosis was made by both cytology and microbiology. Four times this was the same, but on two occasions different aetiological factors were identified. In nine patients the cytology was non-diagnostic, although three displayed non-specific cellular abnormality. Tables 2 and 3 show the findings in the two groups of patients in more detail together with the primary medical condition and eventual outcome.

A definitive diagnosis was made in 21 (70%) of the 30 episodes by cytology. These included 15 opportunistic infections, five cases of malignant infiltration of the lung (in one patient there was cytological evidence of both infection and malignancy), and two episodes of occult pulmonary haemorrhage. In seven patients in whom cytology was non-diagnostic a specific diagnosis resulted from culture, increasing the overall sensitivity to 93% (28 episodes). In two of the patients who had undergone renal transplant the overall bronchoalveolar lavage results were non-diagnostic. In one of these a retrospective diagnosis of legionella infection was made on serology. The second patient was severely ill with graft rejection and ascites in addition to respiratory distress. The bronchoalveolar lavage was hypercellular and contained, in addition to numerous macrophages, mixed inflammatory cells and large single bizarre cells, which were interpreted as hyperplastic alveolar lining cells. Some of the macrophages contained haemosiderin. These changes were considered consistent with interstitial pneumonitis but no cause was identified. Ferruginous bodies were also found in the fluid, which raised the possibility of pre-existing fibrosis, although there was no known history of exposure to asbestos. This patient did not recover. At necropsy areas of bronchopneumonia coexisted with interstitial pneumonitis. There was oedema, fibrosis, and thick-

Table 1 Results of bronchoalveolar lavage in 30 episodes

Result	Cytology	Microbiology	Combined
Diagnostic	21*	13*	28
Non-diagnostic			
Non-specific abnormality	3		1
No abnormality	6	17	1
Total	30	30	30
Sensitivity	70%	42%	93%

*Both diagnostic in six episodes.

Table 2 Primary medical condition, results of bronchoalveolar lavage, and outcome in 16 patients (18 episodes) from the Department of Nephrology

Primary medical condition	Cytology	Microbiology	Outcome
SLE	<i>P. carinii</i>	—	Recovered
SLE	<i>P. carinii</i>	—	Recovered
Glomerulonephritis (i)	Cytomegalovirus	—	Recovered
(ii)	Zygomycetes	Mucor	Died
Glomerulonephritis	Haemorrhage	—	Recovered
Goodpasture's syndrome	Haemorrhage	—	Recovered
Renal transplant	<i>P. carinii</i>	—	Recovered
Renal transplant	<i>P. carinii</i>	—	Recovered
Renal transplant	<i>P. carinii</i>	—	Recovered
Renal transplant	<i>P. carinii</i>	—	Recovered
Renal transplant (i)	<i>P. carinii</i>	—	Recovered
(ii)	AFB	<i>M. tuberculosis</i>	Recovered
Renal transplant	Candida	<i>C. albicans</i>	Recovered
Renal transplant	—	<i>S. aureus</i>	Died
Renal transplant	—	<i>S. aureus</i> and <i>P. aeruginosa</i>	Died
Renal transplant	—	Micrococcus	Recovered
Renal transplant	Non-specific pneumonitis	—	Died*
Renal transplant	—	—	Recovered†

**P. aeruginosa* septicaemia.

†Serological evidence of legionella.

SLE = systemic lupus erythematosus; AFB = acid fast bacilli.

Table 3 Primary medical condition, results of bronchoalveolar lavage, and outcome in 10 patients (12 episodes) from the Departments of Haematology and Oncology

Primary medical condition	Cytology	Microbiology	Outcome
AML	Leukaemic infiltration	—	Died
ALL (i)	<i>P. carinii</i>	—	Recovered
(ii)	Candida	<i>C. albicans</i>	Recovered
ALL + BMT	Degeneration	Micrococcus	Recovered*
CGL	Leukaemic infiltration	<i>P. aeruginosa</i>	Died
CLL	Aspergillus	—	Recovered
Aplastic anaemia (i)	—	<i>A. fumigatus</i>	Recovered
(ii)	—	<i>S. aureus</i> and <i>P. aeruginosa</i>	Died
Aplastic anaemia + BMT	Hypereosinophilia	Pneumococcus	Recovered*
Hodgkin's disease	Infiltration by Hodgkin's disease	—	Recovered
Non-Hodgkin's lymphoma	CMV and infiltration by lymphoplasmacytoid lymphoma	<i>P. aeruginosa</i>	Died
Hyperglobulinaemia	Infiltration by myeloma	—	Recovered*

*Died within six months.

AML = acute myeloid leukaemia; ALL = acute lymphoblastic leukaemia; BMT = bone marrow transplant; CGL = chronic granulocytic leukaemia; CLL = chronic lymphocytic leukaemia; CMV = cytomegalovirus.

ening of the alveolar walls. Pneumocyte hyperplasia, atypia, and desquamation were demonstrated by immunocytochemical staining for epithelial membrane antigen. *Pseudomonas aeruginosa* was grown from the blood culture.

The most common finding in patients with a definitive diagnosis was opportunistic infection due to *Pneumocystis carinii*. The eight cases occurred in a cluster shortly after the start of the project. All the patients recovered after treatment with high dose cotrimoxazole. The lavage fluid contained much amorphous debris and few alveolar macrophages. Characteristic cystic forms of *P. carinii* containing one or two comma shaped structures together with small numbers of collapsed empty cysts were identified on the slides stained with Grocott

methenamine silver (Fig. 1). Three of these patients had high antibody titres for cytomegalovirus infection but no viral inclusions were found in the bronchoalveolar lavage fluid. Two of the eight patients became ill a second time (tuberculosis and candidiasis) during the study.

Classic "owl's eye" inclusions (Fig. 2) indicating pulmonary cytomegalovirus infection were found in the macrophages from two patients. One of these also had cytological evidence of infiltration of the lung by malignant cells from lymphoplasmacytoid non-Hodgkin's lymphoma in addition to *P. aeruginosa* on culture. This patient died suddenly from myocardial infarction 8 h after bronchoscopy. The cytological findings were all confirmed at necropsy. The other patient with cytomegalovirus infection,

Fig. 1 Cystic forms of *Pneumocystis carinii* with foamy exudate in background. Grocott methenamine silver $\times 1060$.

Fig. 3 Pseudohyphae and budding spores of *Candida* sp. Grocott methenamine silver $\times 825$.

who had rapidly progressive glomerulonephritis, made a slow recovery but died during a second episode of infection (mucormycosis).

Mycotic infections were found either alone or in association with other abnormalities on six occa-

sions. Three were due to candida, two to aspergillus, and one to mucor. *Candida* generally presents as a mixture of yeast cells (blastospores) and filamentous elements, which may be true hyphae or pseudohyphae. Budding spores originating at points of constriction on the filaments are characteristic (Fig. 3). The aspergillus was not seen in the cytological preparations in one of the cases but was found on culture, and in the second instance septate hyphae with dichotomous branching were seen in the fluid but there was no fungal growth on culture. Both these patients recovered after anti-fungal treatment, though one later died from pyogenic infection. Long hyphae, branching at angles up to 90° , were found in one patient and were interpreted as probable zygomycetes (Fig. 4). Speciation from culture confirmed mucormycosis. The infection was fatal.

The two cases of occult pulmonary haemorrhage occurred in patients with Goodpasture's syndrome and rapidly progressive crescentic IgA nephritis. On each occasion the macrophages, when stained by the Papanicolaou technique, were filled with large granules of golden brown pigment. Perls' stain confirmed the presence of haemosiderin.

Malignant infiltration of the lung was diagnosed in five patients. One had acute myeloid leukaemia and

Fig. 2 Cytomegalovirus infection. Macrophage containing intranuclear inclusion body. Papanicolaou $\times 825$.

Fig. 4 Ribbon-like hyphae of zygomycetes (*mucor*). Grocott methenamine silver $\times 330$.

another had chronic granulocytic leukaemia, in whom *P aeruginosa* was also found. Malignant cells were identified in the fluid from the patient with lymphoplasmacytoid non-Hodgkin's lymphoma, who also had multiple infections (cytomegalovirus, *P aeruginosa*). In a girl with mediastinal Hodgkin's disease, lymphocytes, Reed-Sternberg cells, and mononuclear Hodgkin's cells were found in the fluid, confirming pulmonary involvement. The elderly woman with dysglobulinaemia was clinically suspected to have pneumocystis infection. The cytological specimens showed a monoclonal (IgG, lambda) proliferation of morphologically abnormal plasma cells, including many unusually large Mott type cells (Fig. 5). There was no evidence of infection. The bronchoalveolar lavage diagnosis of respiratory distress owing to infiltration of the lungs by malignant cells from a plasma cell tumour was substantiated when similar cells were found in the bone marrow. Pulmonary function tests and chest x ray film improved considerably on treatment with melphalan, but she died from myelomatosis four months later.

In two cases with bacterial infections on culture, the cytology, although non-diagnostic, showed cellular abnormality. The patient who had undergone bone marrow transplant had grade 2/3 graft versus host disease at the time of bronchoscopy. The lav-

age fluid showed severe cellular degeneration especially of the bronchial epithelium. The same feature was seen in sputum specimens. The degeneration gradually cleared with improvement of graft versus host disease and treatment of infection. The specimens obtained from the patient who had received a bone marrow transplant for aplastic anaemia also showed abnormality. There were abundant eosinophils and strikingly numerous Charcot-Leyden crystals. Thorough investigation failed to identify any parasitic or fungal cause for the eosinophilia and there was no history of bronchial asthma in the patient or donor. We concluded that the pulmonary findings were part of a generalised hypereosinophilia associated with considerable regeneration of the marrow after transplantation. This patient recovered from the bacterial (pneumococcus) infection but later developed rising antibody titres to cytomegalovirus and died.

Bronchoalveolar lavage was well tolerated by all patients. No pulmonary haemorrhage or pneumothorax ensued. Twelve (75%) of the 16 renal cases and six (60%) of the 10 from the departments of haematology and oncology recovered.

Fig. 5 Plasma cells, including large binucleate Mott cell, mixed with macrophages. Lavage fluid from patient with pulmonary infiltrate due to myelomatosis. May-Grünwald Giemsa $\times 825$.

Discussion

The development of a pulmonary infiltrate in an immunocompromised patient is an urgent clinical problem. Arriving at an exact diagnosis is made difficult by the frequently non-specific nature of clinical and radiological findings, the variety of possible causes, and the problem of obtaining adequate representative material from the lungs of severely ill immunosuppressed patients.¹⁴

A survey of the results of open lung biopsy⁵ found the mean diagnostic yield to be 69%, but in many studies^{4,15} the percentage of specific diagnoses falls below this figure, though 91% was achieved by Rosen *et al*¹⁶ in the diagnosis of *P. carinii*. The overall complication rate was 11%.⁵ Limitations of open lung biopsy include the need for general anaesthetic, delay encountered in organisation, and the reluctance of doctors to use it in patients with recurrent infiltrates.¹⁷ In eight series of fiberoptic bronchoscopies the mean diagnostic yield from transbronchial biopsy was 41%, from bronchial brushing 27%, and from a combined approach 47%.⁵ Matthay *et al*¹⁸ obtained a diagnosis in 84% of their cases by the combined use of biopsy and brushing, but the study contained a high incidence of focal carcinoma, which may have augmented the sensitivity. The mean incidence of haemorrhage (> 25 ml) and pneumothorax was 7% for each complication in seven series of bronchoscopies.⁵ Cunningham *et al*,³ however, found that 26% suffered from haemorrhage and 19% from pneumothorax. Fewer studies on fine needle aspiration of the lung in immunosuppressed patients have been published, but Sagel *et al*¹⁹ reported a diagnostic rate of 77% in 31 such patients and Castellino and Blank²⁰ achieved a rate of 73% in 108 infectious episodes. The most common complication is pneumothorax, which occurs in about a quarter of all patients.⁷ Castellino and Blank²⁰ reported this complication in 28%, of which 13% required thoracostomy. The present investigation shows the sensitivity of cytological examination of bronchoalveolar lavage fluid (70%) to be comparable to that of published reports on biopsy⁵ and fine needle aspiration^{19,20} in immunosuppressed patients. In contrast, however, there were no complications due to haemorrhage or pneumothorax.

There is increasing recognition of the value of morphological assessment of histological and cytological material for the rapid identification of unusual pathogens,²¹⁻²³ especially *P. carinii* and certain genera of opportunistic viruses and fungi. This can be carried out on the same preparations and at the same time as the evaluation of specimens for non-infectious causes. This is especially valuable in patients who are immunodeficient as a result of

malignant disease. Cytology, however, has little to contribute to the diagnosis of bacterial infections. Bronchoalveolar lavage provides abundant material which is suitable for microbiology as well as cytology. By using both techniques on the fluid the sensitivity rate was increased from 70% to 93%. Two patients had evidence of both neoplastic infiltration of the lung and infection, which emphasises the value of the combined approach to diagnosis. Opportunistic infection was diagnosed by cytology as the cause of the infiltrate in 15 (50%) episodes. In eight instances (27%) the pathogen was *P. carinii*. An incidence of 32-38% has been found in other series,^{4,24} confirming the increasing importance of this organism as a cause of pneumonia in immunocompromised hosts. *P. carinii* is often associated with cytomegalovirus.²⁵ Three of these patients had serological evidence of cytomegalovirus infection but no indication of pulmonary disease in the bronchoalveolar lavage fluid. The specimens from patients with *P. carinii* pneumonia contained much amorphous exudate and only small numbers of alveolar macrophages, which may perhaps have resulted in false negative findings. The patients all recovered after treatment with cotrimoxazole. This is unlikely to have occurred if pulmonary cytomegalovirus was a contributory factor in the respiratory disease. In interpreting cytological specimens stained with Grocott methenamine silver care must be taken not to mistake clusters of fungal spores or artefactual deposition of silver on leucocytes and erythrocytes as *P. carinii*. Adequate control material is essential.

Many viruses produce no specific cytological changes. Several which cause opportunistic respiratory infection are associated with characteristic cytological markers, however, which permit rapid preliminary identification at genus level. In addition to cytomegalovirus, these include, *Herpesvirus hominis* (Herpes simplex virus), *Herpesvirus varicellae* (varicella zoster), and adenovirus. While some features produced by cytomegalovirus, herpes simplex virus, and varicella zoster do show overlap, basophilic Cowdry type A²⁶ intranuclear inclusions, intracytoplasmic inclusions, and cytomegaly characterise cytomegalovirus infection. The eosinophilic inclusions, "ground glass" nuclei, and cell fusion with multinucleation and moulding found in herpes simplex virus and varicella zoster are not a feature of cytomegalovirus infection. Basophilic inclusions occur with adenovirus, but these are associated with "smudge" cells,²² which are not seen with cytomegalovirus.

Proof of the presence of mycotic infection is ideally established by culture, but this is slow and the fungus may fail to grow.^{23,27} There are also many

limiting factors in the use of immunologically specific fluorescent antibody stains.²³ Preliminary morphological identification using empirical staining methods can therefore be a rapid and valuable aid in patient management. Both candida and aspergillus, however, occur in the upper respiratory tract unassociated with lower respiratory tract infection. Fungal elements which are not closely intermingled with alveolar macrophages should therefore be interpreted with caution. Speciation of candida, aspergillus, and zygomycetes is not satisfactory when based on morphology alone, but identification at genus or class level is adequate for appropriate treatment to be instituted. Culture showed the case of zygomycosis to be due to mucor. Aspergillus was found in two patients, once by cytology and once on culture. Both patients responded to antifungal treatment, confirming the diagnoses and the complementary nature of the investigations.

In two patients, one with Goodpasture's syndrome and the other with rapidly progressive IgA crescentic glomerulonephritis, no infection was identified, but there was cytological evidence of occult pulmonary haemorrhage. Antimicrobial treatment was withheld, both x ray films cleared, and the patients recovered. Macrophages laden with haemosiderin may also be found in severe pulmonary oedema, but this is clinically diagnosable and responds to diuretics. In the cases of infective pneumonia and also in the patient with pneumonitis and pseudomonas septicaemia smaller numbers of Perls' positive macrophages were present but they did not approach the numbers seen in the two cases with pulmonary haemorrhage.

Five patients had malignant infiltration. The likelihood of this is influenced by the general state of the disease—pulmonary dissemination is unlikely without evidence of advancing malignancy elsewhere. Both patients with leukaemia were in haematological relapse, the patient with Hodgkin's disease had mediastinal node disease, and the patient with lymphoplasmacytoid lymphoma had recurrent lymphadenopathy. The elderly woman with myelomatosis, who presented as an urgent case of respiratory distress, also had widely disseminated disease. Evidence of malignant infiltration of the lung does not, however, preclude the possibility of coexisting infection, as was found in two cases in this series.

Pneumonia accounts for half of the deaths in some transplant programmes,¹ but mortality is highest in the first six months and long term prognosis is generally good if early infection is survived. The potential for recovery is increased by rapid diagnosis and the institution of prompt specific antimicrobial treatment.²⁸ This is especially true of infection by *P*

carinii. In the present series the 12 renal patients who survived all remained alive and well at follow up one year later. Six (60%) of the patients with leukaemia, lymphoma, and allied conditions recovered. Owing to the nature of their primary disease, however, the long term outlook of this group is much less favourable and at six months survival had fallen to 30%.

As a result of this study, we believe that cytological examination of bronchoalveolar lavage fluid is a safe, rapid, and helpful diagnostic aid in the management of immunocompromised patients with pulmonary infiltrates.

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Requests for reprints to: Dr Jennifer A Young, Department of Pathology, The Medical School, University of Birmingham, Birmingham B15 2TJ, England.