Early experience and difficulties with bronchoalveolar lavage and transbronchial biopsy in the diagnosis of AIDS associated pneumonia in Britain

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ABSTRACT Bronchoalveolar lavage and transbronchial biopsy have been used as adjuncts to the management of patients with pneumonia associated with the acquired immunodeficiency syndrome (AIDS) at the Middlesex Hospital and the experience gained and difficulties encountered in the first five cases are reported. Widely varying organisms were isolated from lavage aspirates, some of which may have been nasopharyngeal contaminants, and organisms cultured from the transbronchial biopsy specimens may offer a better guide to antimicrobial treatment. *Pneumocystis carinii* was found in two of the patients. In view of the potentially serious toxicity of high dose co-trimoxazole, continuation of this treatment may be inadvisable if *Pneumocystis carinii* is not identified by all available methods unless there are strong clinical grounds to suspect its presence.

The number of cases of the acquired immunodeficiency syndrome (AIDS) in the United Kingdom continues to rise; by May 1984 it was 47.[1] There is a high incidence of opportunistic lung infection in these patients and it is likely that the management of AIDS associated pneumonia will become an increasing problem in Britain. The organism most frequently identified in the United States is *Pneumocystis carinii*, which in one large series was found to be present in half the cases.[2] Pneumocystis pneumonia in patients with AIDS may be rapidly progressive, with a mortality rate of 40% despite treatment.[3] Bronchoalveolar lavage has been used to obtain diagnostic material in suspected opportunistic chest infection, both in iatrogenically immunosuppressed patients[4] and in patients with AIDS.[5] We present our findings from bronchoalveolar lavage and transbronchial biopsy in the first five patients with AIDS associated pneumonia seen at the Middlesex Hospital.

Methods

Five patients with AIDS in whom a lower respiratory tract infection was suspected on clinical and radiological grounds underwent transnasal fibreoptic bronchoscopy under intravenous sedation. Bronchoscopy was performed before the commencement of antibiotic treatment in four patients. Precautions similar to those used to prevent transmission of hepatitis B virus were taken by the operator. Topical lignocaine spray was applied to the nasopharynx, vocal cords, and carina. After insertion of the bronchoscope sterile suction apparatus was connected for the collection of specimens. The bronchoscope was wedged into the segmental bronchus that corresponded to the area of maximum radiological shadowing. Three 20 ml aliquots of warmed, sterile 0·9% saline were instilled into the segmental bronchus and immediately reaspirated. Transbronchial biopsy specimens were subsequently obtained from the same bronchopulmonary segment (four patients). The specimens were immediately taken to the microbiology laboratory, where the aspirated fluid was centrifuged and the biopsy material ground. Smears of the ground biopsy specimens and centrifuged deposit were prepared and stained with Gram's stain, auramine, Grocott's stain, and

Table   *Organisms isolated from bronchoalveolar lavage aspirates and transbronchial biopsy specimens in five cases of pneumonia associated with the acquired immunodeficiency syndrome (AIDS)**

Case No	Transbronchial biospy	Lavage fluid aspirate	Outcome of pneumonic episode
1	Not performed	Streptococcus viridans Diptheroids Candida sp ? Blastomyces‡	Died

2	Streptococcus pneumoniae
	Streptococcus pyogenes
	  (group G)
	Bacteroides melaninogenicus
		Streptococcus pneumoniae
		Streptococcus pyogenes
		  (group G)
		Bacteroides melaninogenicus
		Mycobacterium xenopi
		Proteus spp
		Cytomegalovirus
			Lived

3	Pseudomonas aeruginosa	Pseudomonas aeruginosa Streptococcus milleri Streptococcus viridans Cytomegalovirus	Lived

4	Pneumocystis carinii†
	Streptococcus pyogenes
	  (group G)
	Staphylococcus aureus
		Streptococcus pyogenes
		  (group G)
		Staphylococcus aureus
		Herpes simplex virus
			Died

5	Pneumocystis carinii† Staphylococcus epidermidis Diptheroids	Staphylococcus epidermidis Diptheroids	Died

*In cases 1–4 specimens were examined before the start of treatment with antibiotics; patient 5 was receiving high dose co-trimoxazole.
†Identified by light microscopy of Grocott stained transbronchial biopsy specimen.
‡This organism is still undergoing formal identification.

acridine orange (with and without Giemsa stain). Both specimens were cultured on blood agar (aerobically and anaerobically), cysteine lactose electrolyte deficient medium, Sabouraud's agar, and Löwenstein-Jensen slopes and the remainder of the specimens were put into Robertson's cooked meat broth, which was later cultured both aerobically and anaerobically. Material from each of the lavage and two of the biopsy specimens was inoculated into monolayers of human embryo lung, human epithelial, and baboon kidney cells for virus isolation.

Procedure for sterilisation of bronchoscope   A single instrument was set aside for use in patients with AIDS. The procedure we adopted for sterilisation was as follows. The instrument was first washed with soap and water (this included brushing the lumen). After being rinsed in distilled water the instrument was immersed overnight in glutaraldehyde. After it had been rinsed in distilled water and inspected for the presence of visible dirt the bronchoscope was then subjected to ethylene oxide sterilisation and thereafter left unused for at least seven days. Before use the instrument was again rinsed in distilled water to remove any traces of ethylene oxide.

Results

Organisms of a wide variety were recovered from these patients (table). Individual case histories are reported below.

CASE 1
A 36 year old white homosexual man was admitted in mid August 1983 with rapidly advancing Kaposi's sarcoma, with which he had first presented in March 1983. He had had a dry cough for a month and was febrile, but there was no clinical or radiological evidence of lower respiratory infection. Examination showed pharyngeal Kaposi's sarcoma. The dry cough and fever persisted but serial chest radiographs remained clear until late August, when a small, soft right lower zone shadow was noted. Bronchoscopy was performed, at which oropharyngeal candidiasis and Kaposi's lesions extending into the main bronchi were seen. Bronchoalveolar lavage (but not transbronchial biopsy) was performed, and the patient immediately started treatment with high dose co-trimoxazole pending the microbiological findings. On microscopy of the lavage aspirate, fungal bodies believed to be *Candida* sp were seen. The Grocott stain was negative for *Pneumocystis carinii*. Culture of the lavage fluid grew *Streptococcus viridans,* diphtheroids, *Candida* sp, and an organism believed to be *Blastomyces* sp, which is still undergoing identification. Viral cultures were negative. There was an initial settling of the pyrexia with symptomatic improvement, but two weeks later the patient suddenly developed profound breathlessness and haemoptysis. Spontaneous bleeding and purpura were also noted. At this time the arterial oxygen tension (PaO_2) while he was breathing air was 7.2 kPa (54 mm Hg) and arterial

carbon dioxide tension (PaCO$_2$) was 3.5 kPa (26 mm Hg). A chest radiograph showed widespread diffuse shadowing throughout both lung fields compatible with infection, oedema, or intraalveolar haemorrhage. The patient was transferred to the intensive care unit and later required ventilation. He was noted to have a profound thrombocytopenia (platelets 5×10^9 l^{-1}). Bone marrow examination showed reduced numbers of megakaryocytes and megaloblastic changes in the erythroid and myeloid series. These features were thought to be compatible with, but not diagnostic of, co-trimoxazole toxicity. Further bronchoscopy, lavage, and transbronchial biopsy were not considered possible. Co-trimoxazole treatment was discontinued and replaced by tobramycin, mezlocillin, and amphotericin B and subsequently erythromycin, flucloxacillin, spiromycin, pentamidine, and acyclovir. The patient deteriorated further and died.

CASE 2
A 33 year old white homosexual man was admitted in September 1983 with a three month history of Kaposi's sarcoma. He was febrile but had no respiratory symptoms, and examination of the chest showed nothing abnormal and a chest radiograph was clear. Three weeks later he developed a dry cough. He remained febrile. On examination he was noted to have oropharyngeal candidiasis but the chest radiograph was clear. The cough persisted and two days later a right mid zone shadow was noted on a further chest radiograph. Bronchoscopy was performed, at which oropharyngeal candidiasis and Kaposi's lesions in the airways were seen. Bronchoalveolar lavage and transbronchial biopsy were performed and the patient commenced treatment with high dose co-trimoxazole and amphotericin B pending the microbiological findings. On microscopy of the lavage deposit mixed commensals and *Candida* sp were seen. The Grocott stain was negative for *Pneumocystis carinii* on both the lavage deposit and the transbronchial biopsy specimen. The following organisms were cultured from the lavage deposit: *Streptococcus pneumoniae, Proteus* sp, *Bacteroides melaninogenicus, Streptococcus pyogenes* (group G), *Streptococcus viridans, Mycobacterium xenopi,* and cytomegalovirus. From the transbronchial biopsy specimen *Streptococcus pneumoniae, Streptococcus pyogenes,* and *Bacteroides melaninogenicus* were cultured. Fungal cultures were negative. Benzyl penicillin was added to his treatment, but further clinical and radiological deterioration occurred over the following two days, the radiographic appearances now being of right upper lobe pneumonia. PaO$_2$ and PaCO$_2$ while he was breathing air were 8.5 and 4.0 kPa (64 and

30 mm Hg). Bronchoscopy, bronchoalveolar lavage, and transbronchial biopsy were repeated. Gram stain of smears showed the presence of Gram negative rods and Gram positive diplococci. The Grocott stains were again negative. *Streptococcus pneumoniae* was subsequently cultured from both specimens and initially believed to be resistant to penicillin. Viral cultures were negative. Penicillin was discontinued and treatment with erythromycin commenced, although the organism was subsequently found to be sensitive to penicillin. There was a rapid clinical and radiological improvement. The patient was discharged, but subsequently died of pneumonia at home in January 1984.

CASE 3
A 36 year old white homosexual man presented in November 1983 with a grand mal seizure and a nine month history of malaise and lassitude. He had had a cough productive of white sputum for one week. He was afebrile and his chest was clear, but a chest radiograph showed widespread bilateral patchy shadowing. The following day he was febrile and had developed breathlessness. The arterial blood gas tensions while he was breathing air were 9.9 (PaO$_2$) and 4.0 (PaCO$_2$) kPa (74 and 30 mm Hg). A repeat chest radiograph showed an increase in the radiological shadowing. Sputum culture grew *Pseudomonas aeruginosa*. Bronchoscopy, bronchoalveolar lavage and transbronchial biopsy were performed. Oropharyngeal candidiasis was noted. Treatment with high dose co-trimoxazole, tobramycin, and ticarcillin was started pending the microbiological findings. On microscopy of the aspirate deposit Gram positive cocci could be seen. Grocott stains were negative for *Pneumocystis carinii*. On culture Streptococcus *viridans, Streptococcus milleri, Pseudomonas aeruginosa,* and cytomegalovirus were grown from the lavage deposit and *Pseudomonas aeruginosa* from the transbronchial biopsy specimen. Treatment with co-trimoxazole and ticarcillin was discontinued, with the substitution of azlocillin. The pyrexia settled with symptomatic improvement and resolution of the radiological changes. Computed tomography brain scans, however, showed at first one and subsequently multiple enhancing lesions thought to be infective in origin. The patient remained in poor health and subsequently died.

CASE 4
A 43 year old white homosexual man presented in November 1983 with a three week history of malaise, dry cough, and breathlessness. He was afebrile, but bilateral mid zone inspiratory crackles were heard in his chest. A chest radiograph showed

bilateral perihilar shadowing. Two days later his symptoms rapidly worsened and he became febrile. The blood gases while he was breathing air were 8.7 (PaO_2) and 3.5 ($PaCO_2$) kPa (65 and 25 mm Hg). Bronchoscopy, bronchoalveolar lavage, and transbronchial biopsy were performed, after which he was transferred to the intensive care unit, where his PaO_2 while he breathed air was 4.7 kPa (35 mm Hg). He required ventilation, and was treated with high dose co-trimoxazole, erythromycin, mezlocillin, and tobramycin. Gram positive cocci were seen in the lavage deposit. The Grocott stain on the transbronchial biopsy specimen was positive for *Pneumocystis carinii* but negative on the lavage aspirate. *Staphylococcus aureus* and *Streptococcus pyogenes* (group G) were subsequently cultured from both the bronchoalveolar lavage deposit and the transbronchial biopsy specimen and herpes simplex virus was isolated from the lavage fluid. The antimicrobial treatment was changed to high dose co-trimoxazole, benzyl penicillin, flucloxacillin, pentamidine, and acyclovir. There was no clinical response, and the patient died a few days later.

CASE 5
A 41 year old white homosexual man presented in February 1984 with a two month history of malaise, fever, and weight loss. During the three weeks before presentation he had a dry cough and increasing breathlessness. A chest radiograph at presentation showed diffuse bilateral pulmonary shadowing. The arterial blood gases while he was breathing air were 6.0 (PaO_2) and 3.7 ($PaCO_2$) kPa (45 and 28 mm Hg respectively). High dose co-trimoxazole treatment was started and he was referred to this hospital for bronchoscopy after three days of treatment, as there has been no clinical or radiological improvement. Bronchoscopy, bronchoalveolar lavage and transbronchial biopsy were performed. The Grocott stain on the transbronchial biopsy specimen but not the lavage deposit was positive for *Pneumocystis carinii*. Gram positive cocci and Gram negative rods were seen on microscopy of stained material from both the aspirate and the biopsy specimen, from both of which *Staphylococcus epidermidis* and diphtheroids were subsequently cultured. Viral cultures were negative. Flucloxacillin and pentamidine were added to the treatment regimen but the patient continued to deteriorate, required ventilation, and died in February 1984.

Discussion

The number of cases of AIDS in the United States has increased exponentially, over 1600 cases having been notified by 1983.[6] Although we do not know whether this pattern will be repeated in Britain, the management of patients with AIDS associated pneumonia is likely to present an increasing problem. We have reported our findings from bronchoscopy, bronchoalveolar lavage and transbronchial biopsy in the first five patients with AIDS to be seen at this hospital with clinical and radiological evidence of lower respiratory tract infection. Although we believe these techniques to be of value in the management of probable opportunistic chest infections, we have encountered several problems that require further experience before they are resolved.

The cause of AIDS is unknown, but the transmission of the disease appears to follow an epidemiological pattern similar to that of hepatitis B[6] and a viral cause is therefore suspected. The *New England Journal of Medicine* has recommended that measures consistent with those suggested for the prevention of hepatitis B are used when dealing with AIDS patients.[7] While performing these bronchoscopies we have therefore worn gloves, gowns, masks, and goggles as coughing can cause nasopharyngeal secretions to be sprayed into the eye of the operator through the endoscope suction channel. We have additionally set aside a bronchoscope for exclusive use in patients with suspected AIDS, as although we follow a special sterilisation procedure after each examination we do not think this instrument should be used in other patients. We have been unable to use other equipment—for example, for the measurement of carbon monoxide transfer factor in case 1 to help to establish whether the pulmonary shadowing was the result of intra-alveolar haemorrhage, infection, or oedema—because of our inability to subject it to adequate sterilisation procedures.

The optimum timing of bronchoscopy in these patients with suspected opportunistic lung infection is not clear. We have withheld the procedure from patients with a dry cough until there were clinical or radiological signs of a lower respiratory tract infection, but a recent series from the United States reported the presence of *Pneumocystis carinii* in bronchoalveolar lavage fluid in eight of 25 patients with AIDS who had a dry cough but a normal chest radiograph. The arterial PO_2 was found to be reduced in these patients, despite the normal chest radiograph.[8] It may therefore be necessary to perform more frequent estimations of blood gas tensions and consider bronchoscopy if the PaO_2 falls. Possibly the very rapid clinical and radiological deterioration which can occur in pneumocystis pneumonia would be in part prevented by earlier therapeutic intervention. By contrast, Venet *et al*[5] identified *Pneumocystis carinii* in the lavage aspirate only of those patients in whom the chest radiograph was abnormal. Additionally, this group has reported

the persistence of *Pneumocystis carinii* in the lavage aspirate three weeks after high dose co-trimoxazole treatment and a return to normal of the chest radiograph, from which they inferred that the presence of this organism may not always be an indication for treatment. We failed to identify *Pneumocystis carinii* in the bronchoalveolar lavage aspirates, but positively identified it in the transbronchial biopsy specimens from two patients. This finding is in agreement with early reports from the United States, in which the diagnostic yield of *Pneumocystis carinii* was higher from transbronchial biopsy than bronchoalveolar lavage specimens.[9] Possibly therefore *Pneumocystis carinii* was present in patient 1, in whom transbronchial biopsy was not performed. We have cultured many other organisms from the lavage aspirates in all five cases. In general, fewer were cultured from the transbronchial biopsy specimens, and the organisms found there were also present in the lavage aspirates (table). Probably some of the organisms found in the lavage fluid were contaminants from the nasopharynx. Bartlett examined this problem in 16 patients undergoing diagnostic bronchoscopy in whom there was no clinical evidence of infection, and isolated an average of five bacterial organisms per lavage aspirate. Methylene blue dye sprayed into the pharynx of 10 of these patients immediately before bronchoscopy was visible in the lavage aspirate of eight patients. The authors concluded that nasopharyngeal contamination of the lavage aspirate was a potential source of misleading results.[10] Although the transbronchial biopsy specimens are withdrawn through the suction channel of the endoscope and are therefore not immune from contamination, we believe that the organisms isolated may be more representative of those responsible for infection. This consideration, together with our failure to identify *Pneumocystis carinii* in the bronchoalveolar lavage aspirates, suggests that transbronchial biopsy may offer a better guide to antimicrobial treatment. Necropsies were not performed on those patients who died in hospital because of the possible hazards to the pathologist. We were therefore unable to obtain firmer evidence to support this suggestion.

The wide variety of organisms isolated from each patient led to treatment with many antimicrobial drugs, as it was not always clear which organism was the principal pathogen. Because *Pneumocystis carinii* has been reported as the organism most commonly responsible for pneumonia in these patients, we began treatment with high dose co-trimoxazole in four patients immediately after bronchoscopy (patient 5 was already receiving this treatment). This treatment was reported to cause potentially serious side effects in eight out of 28

patients with AIDS.[11] Patient 1 developed a profound thrombocytopenia associated with possible intra-alveolar haemorrhage after 14 days of co-trimoxazole, which may have contributed to his clinical course. We feel that it may be inadvisable to continue this treatment if *Pneumocystis carinii* is not identified by all available means unless there are strong clinical grounds to suspect its presence. In this context it must be remembered that these patients may also develop more "conventional" infections, as exemplified by the pneumococcal lobar pneumonia in case 2.

In conclusion, we have experienced several difficulties in the management of these patients. The incidence of AIDS associated pneumonia will almost certainly increase and further experience is required before these problems can be satisfactorily resolved and firm recommendations made.

References

1 Public Health Laboratory Service. *Communicable Diseases Report* 1984/05.
2 Curran JW. AIDS–two years later. *N Engl J Med* 1983;**309**:609–11.
3 Kaye SB. Pneumocystis pneumonia. *Br Med J* 1983;**286**:499.
4 Hopkin JM, Turney JH, Young JA, Adu D, Michael J. Rapid diagnosis of obscure pneumonia in immunosuppressed renal patients by cytology of alveolar lavage fluid. *Lancet* 1983;ii:299–301.
5 Venet A, Dennewald G, Sandron D, Stern M, Jaubert S, Leibowitch J. Bronchoalveolar lavage in acquired immunodeficiency syndrome. *Lancet* 1983;ii:53.
6 Weller IVD. Acquired immune deficiency syndrome. *Br Med J* 1984;**288**:136–7.
7 Anonymous. Infection-control guidelines for patients with the acquired immunodeficiency syndrome (AIDS). *N Engl J Med* 1983;**309**:740–4.
8 Tierstein AS, Rosen MJ. Management of opportunistic pneumonias in AIDS. *Ann N Y Acad Sci* (in press).
9 Schwiep F, Garay SM, Aranda C, Greene J. Pneumocystis carinii pneumonia in the acquired immune deficiency syndrome (AIDS). Diagnostic yield utilizing fibreoptic bronchoscopy. *Am Rev Respir Dis* 1983;**127**:189.
10 Bartlett JG, Alexander J, Mayhew J, Sullivan-Sigler N, Gorbach SL. Should fibreoptic bronchoscopy aspirates be cultured? *Am Rev Respir Dis* 1976;**114**:73–8.
11 Jaffe HS, Abrahams DI, Ammann AJ, Lewis BJ, Golden JA. Complications of co-trimoxazole in treatment of AIDS-associated Pneumocystis carinii pneumonia in homosexual men. *Lancet* 1983;ii:1109–11.