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Bronchoalveolar lavage in HIV infected patients with interstitial pneumonitis

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SUMMARY The value of taking microbiological and cytological specimens by flexible bronchoscopy and bronchoalveolar lavage under local anaesthesia was assessed on 43 occasions in 35 HIV infected children, aged 3 months to 16 years, with interstitial pneumonitis. In acute interstitial pneumonitis (n=22, 26 specimens from bronchoalveolar lavages) the microbiological yield was 73%, *Pneumocystis carinii* being the commonest infective agent (n=14). *P carinii* pneumonia was found only in children with deficient antigen induced lymphocyte proliferative responses who had not been treated with long term prophylactic co-trimoxazole. In contrast, in 13 children with chronic interstitial pneumonitis that was consistent with a diagnosis of pulmonary lymphoid hyperplasia who underwent bronchoalveolar lavage on 17 occasions, there were two isolates of cytomegalovirus and one of adenovirus, but *P carinii* was not found. Ten of the 13 children had normal antigen induced lymphocyte proliferative responses. Useful cytological data were also gleaned from bronchoalveolar lavage specimens. Lymphocytosis was significantly higher in pulmonary lymphoid hyperplasia (36(SD 11)%) than in *P carinii* pneumonia (24(19)%) whereas the percentage of polymorphonuclear neutrophils was significantly lower (3(2)% compared with 12(13)%). Flexible bronchoscopy with bronchoalveolar lavage is safe even in young infants and should reduce the necessity for open lung biopsy in the management of HIV infected children with interstitial pneumonitis.

Pulmonary complications, particularly interstitial pneumonitis, are common among HIV infected children. *Pneumocystis carinii* infection usually causes acute interstitial pneumonitis and pulmonary lymphoid hyperplasia causes chronic interstitial pneumonitis.[1 2] In adults fibreoptic bronchoscopy and bronchoalveolar lavage are useful in the investigation of interstitial pneumonitis.[3 4] Experience in children is still limited[5–8] and open lung biopsy was until recently the main method of diagnosis.[1 9 10] Our experience of flexible bronchoscopy in young children[11] and of bronchoalveolar lavage in immunocompromised children[12] led us to assess the diagnostic value of microbiological and cytological examination of bronchoalveolar lavage specimens in HIV infected children with interstitial pneumonitis.

Patients and methods

Thirty five children (24 boys and 11 girls) aged between 3 months and 16 years (mean age 3 years 2 months) underwent bronchoalveolar lavage 43 times between June 1983 and December 1987. Fourteen of these children were less than 1 year old and only five were over 7 years old. The diagnosis was made from a positive serological HIV response by western blot (Pasteur Diagnostics). For those children less than 15 months old whose mothers had positive serological responses, a virus culture or detection of the P25 antigen in the serum with a monoclonal antibody (Abbott Laboratories), or both, was required to eliminate the possibility of passively transmitted maternal antibody. HIV was transmitted from infected mothers to their offspring in 27 cases and by blood products in eight. All these children were in subgroup P2 of the Centers for Disease Control classification for HIV infections in children.[13] The immunological study included an analysis of the number of cluster determinant 4 (CD4) lymphocytes in the peripheral circulation by

immunofluorescence (Ortho), and measurement of lymphocyte proliferative capacity after stimulation by at least two different antigens.[14] Independently of their clinical state and history of infection two groups of children were identified by their proliferative response in the presence of antigen. This was positive in group 1 (n=15) and negative in group 2 (n=20), indicating a severe deficiency in cellular immunity as recently shown by Blanche *et al.*[15]

PULMONARY DISORDERS

Acute interstitial pneumonitis

Twenty six bronchoalveolar lavage specimens were taken from 22 children with acute interstitial pneumonitis diagnosed on physical findings and chest radiography. The mean (SD) interval between the onset of interstitial pneumonitis and bronchoalveolar lavage was 11 (6) days. Five of the children were from group 1 and 17 from group 2. In eight cases the pneumonitis was only moderate, but in 14 it was severe with hypoxaemia that required treatment with nasal oxygen. Four children had second bronchoalveolar lavage specimens taken two weeks after treatment had been started because of relapse of dyspnoea and fever. At the time of the first bronchoalveolar lavage 18 children were not treated or were treated for less than a week with cotrimoxazole.

Chronic interstitial pneumonitis

Seventeen bronchoalveolar lavage specimens were taken from 13 children with chronic interstitial pneumonitis that had been present for a mean (SD) 9 (6) months and the procedure was carried out when they were stable clinically. Ten children were from group 1 and three from group 2. They all had stable radiographs showing diffuse nodular or reticulonodular lung patterns. This type of interstitial pneumonitis was associated with cervical lymphadenopathy (all cases), parotitis (9/13), hepatosplenomegaly (9/13), clubbing of the fingers (5/13), and shortness of breath on exertion (7/13). Hypergammaglobulinaemia (>30 g/l) was present in all cases. This picture was consistent with the diagnosis of pulmonary lymphoid hyperplasia.[1]

PROCEDURES

Flexible bronchoscopy, bronchoalveolar lavage, and lavage fluid analysis were carried out as previously described.[12] All except one of the bronchoscopies were carried out under local anaesthesia after premedication that usually comprised atropine (0·01 mg/kg given subcutaneously) one hour before the procedure and midazolam (0·3–0·5 mg/kg) or diazepam (0·5 mg/kg) given rectally 10–15 minutes before examination. Two fibreoptic bronchoscopes were used, the Olympus BF 3C10 for children aged less than 7 years and the Olympus BF 4B2 for older children. In the youngest children, and whenever interstitial pneumonitis was severe, the procedures were carried out while the patients were receiving oxygen through the other nostril. Once the tracheobronchial tree had been examined, the bronchoscope was wedged in a lobar or segmental bronchus, usually the middle lobe or the right lower lobe. An initial sample (5–10 ml) of prewarmed physiological saline was injected and then aspirated. This initial sample was discarded as being of bronchial origin. Several samples were then injected and aspirated up to a maximum volume of 10% of the functional residual capacity. The recovered liquid was used for microbiological and cytological studies: part of it was used for immunofluorescence screening (for *Legionella pneumophila,* respiratory syncytial virus, parainfluenza virus, herpes simplex virus, cytomegalovirus, and adenovirus), and cultures for viruses, bacteria, fungi, and mycobacteria. A total cell count of nucleated cells was done on the lavage fluid, and 12 Cytospin preparations were made by cytocentrifugation at 30 *g* for 10 minutes on a Cytospin 2 (Shandon Southern Instruments). These were stained by May Grünwald Giemsa, periodic acid Schiff (PAS), Gram, Grocott, Ziehl, and Perls, and examined for cytological evidence of fungal, viral, and parasitic infection and for the presence of siderocytes. The remaining fluid was cytocentrifuged for 10 minutes at 60 *g,* the sediment was fixed in Bouin's solution and embedded in paraffin, and histological sections were cut and stained with haematoxylin and eosin, PAS, and Grocott.

Data were analysed by Student's *t* test and the χ^2 test. A p value of less than 0·05 was accepted as significant.

Results

TOLERANCE

No complications were encountered during or after flexible bronchoscopy and bronchoalveolar lavage. The mean fluid return was 65%. No case of respiratory decompensation or of transfer to the intensive care unit could be attributed to the procedure. Treatment with oxygen by nasal tube prevented drops in arterial oxygen pressure in even the most hypoxic and youngest children. A transient spike of fever occurred in 13 (30%) of the children four to six hours after the bronchoalveolar lavage.

MICROBIOLOGICAL RESULTS

Acute interstitial pneumonitis

Bronchoalveolar lavage specimens gave diagnostic

Table 1 *Organisms isolated from the bronchoalveolar lavage fluid in 26 patients with acute interstitial pneumonitis*

Organism	*No of patients*
P carinii	17
L pneumophila	1
Cytomegalovirus	3
Respiratory syncytial virus	1
Parainfluenza 3 virus	1
Mixed infections	3

information in 19 of the 26 examinations (73%) (table 1). The most common organism isolated was *P carinii* (n=17), which was found in 14 of 17 children in group 2 and in none of the five children in group 1 ($p<0.001$). *P carinii* was still present in three of the four treated children who were re-examined on the 15th day, and in 14 of the 18 children who were not treated or treated for less than a week with co-trimoxazole. It was not found in the four children treated for over a month. Three mixed infections were observed: *P carinii* and cytomegalovirus in two cases, and *P carinii*, *L pneumophila* and parainfluenza 3 virus in the other.

Chronic interstitial pneumonitis

Only two isolates of cytomegalovirus and one of adenovirus were found in 17 bronchoalveolar lavage specimens; no *P carinii* was identified.

When both acute and chronic interstitial pneumonitis were considered together, *P carinii* was found in none of the 15 children in group 1 and in 14 of the 20 in group 2 ($p<0.001$).

CYTOLOGICAL RESULTS

The data are summarised in table 2. In both acute and chronic interstitial pneumonitis there was an increase in the total cell count with a significant increase in lymphocytes, and a moderate increase in neutrophils.[14] Lymphocytosis (SD) (36(11)%) was significantly higher in the patients with pulmonary lymphoid hyperplasia than in acute interstitial pneumonitis (17(19)%, $p<0.0005$), in acute interstitial pneumonitis with *P carinii* (24(19)%, $p<0.05$), and in acute interstitial pneumonitis without *P carinii* (13(19%), $p<0.0005$). The mean percentage of neutrophils (12(13)%) was significantly higher in acute interstitial pneumonitis with *P carinii* than in acute interstitial pneumonitis without *P carinii* (2(3)%, $p<0.05$) and in pulmonary lymphoid hyperplasia (3(2)%, $p<0.005$).

OUTCOME OF ACUTE INTERSTITIAL PNEUMONITIS

Co-trimoxazole treatment was effective in 12 of the 14 children with *P carinii* pneumonitis, and two children died.

Discussion

These results confirm that flexible bronchoscopy and bronchoalveolar lavage carried out by an experienced paediatric bronchoscopist can be performed safely even in young infants and is well tolerated and useful in children infected with HIV with acute and chronic interstitial pneumonitis.[5 12 17]

During acute interstitial pneumonitis, the overall microbiological yield of bronchoalveolar lavage was 75%, and *P carinii* was the predominant opportunistic infective agent (63%), which is similar to results obtained in AIDS related interstitial pneumonitis in adults[3 18 19] as well as in children.[5 20] In the series of Bye *et al*,[5] microbiological analysis of 14 aspirates from bronchoalveolar lavage from 29 children with AIDS and AIDS related complex and acute respiratory illness grew *P carinii*. The diagnostic yield of *P carinii* pneumonia can be improved in adults by doing transbronchial biopsies.[4] Transbronchial biopsy, however, cannot be carried out safely in children younger than 7 years old.[21] Only five children in the present series could have benefited from such exploration, but all had *P carinii* in the bronchoalveolar lavage. None of the children taking part in this study had open lung biopsy, and all those who had had a negative bronchoalveolar lavage (25%) recovered completely. The episodes of interstitial pneumonitis in which no infective agent was identified may have several explanations: bronchoalveolar lavage may have been carried out in a

Table 2 *Results of cytology of 43 samples of bronchoalveolar lavage fluid from 35 children with HIV infection; 26 had acute interstitial pneumonitis and 17 chronic pulmonary lymphoid hyperplasia. Results are expressed as mean (SEM)*

	Cells (10^3/ml)	*% Macrophages*	*% Lymphocytes*	*% Neutrophils*
Pulmonary lymphoid hyperplasia (n=17)	413 (48)	62 (2·7)	** 36 (2·7) *	** 3 (2·9)
Interstitial pneumonitis with *P carinii* (n=17)	383 (49)	63 (5·8)	** 24 (4·6)	12 (3·2) *
Interstitial pneumonitis without *P carinii* (n=9)	474 (119)	84 (3·0)	13 (3·0)	2 (1·0)

*$p<0.05$; **$p<0.005$.

child who had been on co-trimoxazole for at least a month, there may have been undocumented viral infection, or interstitial pneumonitis may have been due to the HIV itself. The sensitivity and specificity of bronchoalveolar lavage compared with open lung biopsy cannot be evaluated in our HIV infected children, but our results confirm those of Suffredini *et al*[18] and those of Bye *et al*.[5] In the study of Suffredini *et al*, 32% of 152 episodes of interstitial pneumonitis diagnosed by bronchoalveolar lavage and transbronchial biopsy had non-specific lesions without a detectable infective agent. In the study of Bye *et al* 12 of 29 children with acute respiratory illnesses had sterile bronchoalveolar lavage fluid: open lung biopsy (n=4) or necropsy (n=3) did not show any false negative lavages. The association between the patient's immunological state and the presence of *P carinii* should be emphasised. *P carinii* pneumonia was observed only in children in group 2 (with deficient cellular immunity) who were either insufficiently treated or not treated. In eight of 14 children *P carinii* pneumonia was the symptom leading to the diagnosis of HIV infection. All these immunological and retrospective data suggest that prophylactic treatment with co-trimoxazole is justified and it is noteworthy that during the same period none of 34 children admitted for HIV infection and given prophylactic co-trimoxazole because of their altered immune state, developed *P carinii* pneumonia (S Blanche, unpublished observations).

Bronchoalveolar lavage also provides useful cytological information. The increase in total cell counts and lymphocytes and the moderate increase in polymorphonuclear neutrophils, are well known in adults,[22-25] but have not previously been reported in children. Cytologically, *P carinii* pneumonia produces clumps of cellular debris, lymphocytosis (24(19)%) and an increase in polymorphonuclear neutrophils (12(13)%). These anomalies were significantly different from those seen in acute interstitial pneumonitis in which *P carinii* has not been identified. In contrast with the results of Smith *et al*,[26] the increase in polymorphonuclear neutrophils was not associated with a more severe clinical or radiological course.

Pulmonary lymphoid hyperplasia represents a special entity. Although this diagnosis has been, until now, histological,[13] the clinical, radiological, and laboratory picture and the slow progressive course are now so characteristic that open lung biopsy is no longer routinely required.[1 20 27] In pulmonary lymphoid hyperplasia, bronchoalveolar lavage provides supplementary diagnostic evidence, both confirming the absence of opportunistic infection[1 27] and showing a large increase in lymphocytes without a corresponding increase in polymorphonuclear neutrophils.[24-27] Mean percentages of lymphocytes and neutrophils were significantly different from those found in children with *P carinii* pneumonia. In none of the 17 bronchoalveolar lavages was *P carinii* identified; only two grew cytomegalovirus, and one an adenovirus. This absence of *P carinii* infection should be correlated with the immune state of this group of children: 10 of 13 were in group 1 and had normal antigen induced lymphocyte proliferation. Here again, although the Centers for Disease Control criteria include *P carinii* pneumonia and pulmonary lymphoid hyperplasia in the same P2 subgroup, the two diseases are quite different in several points (especially immunological), and the better medium term prognosis for cases of pulmonary lymphoid hyperplasia.[1 15] The mechanism of this lymphocytosis is not yet known. There is a latent lymphocytic alveolitis that is probably specific and linked to the HIV virus itself.[28 29] The development of pulmonary lymphoid hyperplasia might be the result of an increase in this alveolitis and occur under the influence of a cofactor, such as Epstein-Barr virus.[29]

In conclusion, flexible bronchoscopy and bronchoalveolar lavage should be advocated as a primary procedure in the exploration of interstitial pneumonitis in children infected with HIV. The detection of opportunistic infections especially *P carinii* should decrease the indications for open lung biopsy, which should be reserved for children in whom bronchoscopy was not diagnostic and whose clinical condition was deteriorating. Similarly, the presence of lymphocytosis alone in the absence of *P carinii* and the absence of an increased proportion of neutrophils should also provide additional evidence in children with clinical and radiological suspicion of pulmonary lymphoid hyperplasia.

References

1 Barbour SE. Acquired immunodeficiency syndrome in childhood. *Pediatr Clin North Am* 1987;**34**:247–68.

2 Rubinstein A, Morecki R, Silverman B, *et al*. Pulmonary disease in children with acquired immune deficiency syndrome and AIDS-related complex. *J Pediatr* 1986;**108**:498–503.

3 Orenstein M, Webber CA, Cash M, *et al*. Value of bronchoalveolar lavage in the diagnosis of pulmonary infection in acquired immune deficiency syndrome. *Thorax* 1986;**41**:345–9.

4 Murray JF, Garay SM, Hopewell PC, *et al*. Pulmonary complications of the acquired immunodeficiency syndrome: an update. *Am Rev Respir Dis* 1987;**135**:504–9.

5 Bye MR, Bernstein L, Shah K, *et al*. Diagnostic bronchoalveolar lavage in children with AIDS. *Pediatr Pulmonol* 1987;**3**:425–8.

6 de Blic J, McKelvie P, Le Bourgeois M, *et al*. Bronchoalveolar lavage in children with acquired immunodeficiency syndrome. *Am Rev Respir Dis* 1987;**135**:A72.

7 Frankel LR, Smith DW, Lewiston NJ. Bronchoalveolar lavage for diagnosis of pneumonia in the immunocompromised child. *Pediatrics* 1988;**81**:785–8.

8 Pattisthall EN, Noyes BE, Orenstein DM. Use of broncho-

alveolar lavage in immunocompromised children with pneumonia. *Pediatr Pulmonol* 1988;**5**:1–5.

[9] Scott GB, Buck BE, Leterman JG, *et al.* Acquired immunodeficiency syndrome in infants. *N Engl J Med* 1984;**310**:76–81.

[10] Shannon KM, Amman AJ. Acquired immune deficiency syndrome in childhood. *J Pediatr* 1985;**106**:332–42.

[11] de Blic J, Scheinnmann P, Paupe J. Successful treatment of persistent neonatal interstitial emphysema by flexible bronchoscopy. *Lancet* 1984;**ii**:1389–90.

[12] de Blic J, McKelvie P, Le Bourgeois M, *et al.* Value of bronchoalveolar lavage in the management of severe acute pneumonia and interstitial pneumonitis in the immunocompromised child. *Thorax* 1987;**42**:759–65.

[13] Centers for Disease Control. Classification system for human immunodeficiency virus (HIV) infection in children under 13 years of age. *MMWR* 1987;**36**:225–36.

[14] Durandy A, Wioland M, Savolovic D, *et al.* Electrophoretic characteristics and membrane receptor of lymphocytes in primary immunodeficiency diseases. *Clin Immunol Immunopathol* 1975;**4**:440–4.

[15] Blanche S, Le Deist F, Fischer A, *et al.* Longitudinal study of 18 children with perinatal LAV/HTLV III infection: attempt at pronostic evaluation. *J Pediatr* 1986;**109**:965–70.

[16] Grimfeld A, Garcia J, Sardet A, *et al.* Technique et résultats cytologiques normaux du lavage broncho-alvéolaire chez l'enfant. *Revue Francaise des Maladies Respiratoires* 1983;**11**:332–3.

[17] Leigh MW, Henshaw NG, Wood RE. Diagnosis of Pneumocystis carinii pneumonia in pediatric patients using bronchoscopic bronchoalveolar lavage. *Pediatr Infect Dis* 1985;**4**:408–10.

[18] Suffredini AF, Ognibene FP, Lack EE, *et al.* Nonspecific interstitial pneumonitis: a common cause of pulmonary disease in the acquired immunodeficiency syndrome. *Ann Intern Med* 1987;**107**:7–13.

[19] Rosen MJ, Tow TWY, Teirstein AS, *et al.* Diagnosis of pulmonary complications of the acquired immune deficiency syndrome. *Thorax* 1985;**40**:571–5.

[20] Novick BE, Rubinstein A. AIDS—the paediatric perspective. *AIDS* 1987;**1**:3–7.

[21] Fitzpatrick SB, Stokes DC, Marsh B, Wang KP. Transbronchial lung biopsy in pediatric and adolescent patients. *Am J Dis Child* 1985;**139**:46–9.

[22] Wallace JM, Barber RG, Oishi JS. Cellular and T lymphocyte subpopulation in bronchoalveolar lavage fluid from patients with AIDS and pneumonitis. *Am Rev Respir Dis* 1984;**130**:786–90.

[23] Venet A, Dennewald G, Sandron D, *et al.* Bronchoalveolar lavage in acquired immunodeficiency syndrome. *Lancet* 1983; **ii**:53.

[24] Fouret P, Touboul JL, Picard F, *et al.* Apport de l'examen cytologique du liquide de lavage bronchoalvéolaire chez les patients atteints de syndrome d'immunodéficience acquise et de syndrome associé. *Ann Pathol* 1986;**6**:45–52.

[25] Randall YK. Bronchoalveolar lavage cells and proteins in patients with the acquired immunodeficiency syndrome. *Ann Intern Med* 1985;**103**:522–33.

[26] Smith RL, El-Sadr W, Lewis ML. Correlation of bronchoalveolar lavage cell populations with clinical severity of pneumocystis pneumonia. *Chest* 1988;**92**:60–4.

[27] Couderc LJ, Hervé P, Solal-Celigny P, *et al.* Pneumonie lymphoïde interstitielle et polyadénopathies chez des sujets infectés par le virus LAV/HTLV III. *Presse Med* 1986;**15**:1127–30.

[28] Plata F, Autran B, Martins LP, *et al.* AIDS virus-specific cytotoxic T lymphocytes in lung disorders. *Nature* 1987;**328**:348–51.

[29] Ho DD, Pomerantz RJ, Kaplan JC. Pathogenesis of infection
with immunodeficiency virus. *N Engl J Med* 1987;**317**:278–86.

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