

# Nonspecific Interstitial Pneumonitis Mimicking *Pneumocystis carinii* Pneumonia

FRED SATTLER, LARRY NICHOLS, LANCE HIRANO, ALAN HITI, FLORENCE HOFMAN, CLAIRE HUGHLETT, LICHENG ZENG, C. THOMAS BOYLEN, and MICHAEL KOSS

Departments of Medicine and Pathology, University of Southern California School of Medicine, Los Angeles County–University of Southern California Medical Center, Los Angeles, California

Bronchoalveolar lavage (BAL) and transbronchial biopsies from 351 human immunodeficiency virus (HIV)-positive patients with presumed *Pneumocystis* pneumonia were analyzed to determine the spectrum and frequency of interstitial lung disease mimicking *Pneumocystis* pneumonia. Among 67 patients without *Pneumocystis*, nonspecific interstitial pneumonitis (NSIP) was the most common histologic diagnosis (n = 16). Tissue sections from patients with NSIP were tested by *in situ* hybridization for Epstein–Barr virus, cytomegalovirus (CMV), and HIV; sections were also tested with the polymerase chain reaction (PCR) for HIV env and gag protein DNA. In patients with NSIP, Epstein–Barr virus and CMV could not be detected by *in situ* hybridization; HIV nucleic acid was amplifiable with PCR in 10 of 15 formalin-fixed, paraffin-embedded tissue sections. Symptoms, physical findings, and blood gas values were similar in patients with NSIP and matched controls with *Pneumocystis*. Patients with NSIP presented earlier in the course of HIV, with higher weight, serum albumin levels, and CD4<sup>+</sup> T-lymphocyte counts ( $492 \pm 828$  cells/mm<sup>3</sup> versus  $57 \pm 60$  cells/mm<sup>3</sup>), and more normal lactate dehydrogenase (LDH) levels ( $280 \pm 113$  IU/L versus  $432 \pm 141$  IU/L; means  $\pm$  SD). Seven to 10 d later, improvement in blood gas values was of similar magnitude for the two groups. Only one other unequivocal, treatable infection was diagnosed only with transbronchial biopsy. These results indicate that NSIP may be the most common diagnosis mimicking *Pneumocystis* pneumonia in acquired immune deficiency syndrome (AIDS), and that NSIP may improve during empiric therapy. Sattler F, Nichols L, Hirano L, Hiti A, Hofman F, Hughlett C, Zeng L, Boylen CT, Koss M. Nonspecific interstitial pneumonitis mimicking *Pneumocystis carinii* pneumonia.

AM J RESPIR CRIT CARE MED 1997;156:912–917.

In most developed countries, *Pneumocystis* pneumonia remains the most common serious initial infection in human immunodeficiency virus (HIV)-positive persons, and occurs in up to 50% of patients with acquired immune deficiency syndrome (AIDS) in the last 6 mo of their lives (1). The initial diagnostic maneuver for individuals presenting with possible *Pneumocystis* pneumonia is usually sputum induction, which is associated with a variable sensitivity of 50 to 95% (2–5). Because the test can have a negative predictive value as low as 39% (2), the absence of *Pneumocystis carinii* in induced sputum may not reliably exclude the diagnosis. Other patients are too ill to undergo sputum induction or bronchoscopy. Thus, a sizable number of patients are initially treated without a confirmed diagnosis.

It is important to know what other causes of interstitial pneumonia mimic *Pneumocystis* when patients are treated empirically for *P. carinii*. This is of considerable importance if a

sizable proportion of these patients have other treatable conditions, such as tuberculosis, fungal infection, or Kaposi's sarcoma. The problem is further complicated because in severe cases of *Pneumocystis* pneumonia, adjunctive corticosteroids should be prescribed (6). Improvement might occur initially with infections other than *Pneumocystis* if corticosteroids attenuate the inflammatory response in the lungs, but this would delay the diagnosis and specific treatment of these other disorders. Worse yet, pulmonary deterioration may occur rapidly when corticosteroids are administered to patients with pulmonary Kaposi's sarcoma (7, 8). Moreover, patients without *Pneumocystis* pneumonia may experience unnecessary drug toxicity during empiric therapy.

To ascertain the spectrum and frequency of causes of interstitial pneumonia in patients in whom *Pneumocystis* was not confirmed, we reviewed our experience with 351 patients who satisfied criteria for presumptive *Pneumocystis* pneumonia and who entered studies of experimental therapies. These patients were treated at a single institution at a time when sputum induction was not readily available. Thus, all patients subsequently underwent bronchoscopy with both bronchoalveolar lavage (BAL) and transbronchial biopsy. We report the histologic diagnoses in patients in whom *P. carinii* was not found with these procedures. We also investigated nonspecific interstitial pneumonitis (NSIP), the most common histologic

(Received in original form December 9, 1996 and in revised form April 3, 1997)

Correspondence and requests for reprints should be addressed to Dr. Fred Sattler, LAC–USC Medical Center, Rand Schrader Clinic, Room 351, 1300 North Mission Road, Los Angeles, CA 90033.

Am J Respir Crit Care Med Vol. 156, pp. 912–917, 1997

diagnosis, in HIV-positive patients without *Pneumocystis*, in order to determine its etiology and whether there were clinical features that distinguished patients with NSIP from those with *Pneumocystis*.

## METHODS

Three hundred and fifty one patients with clinical features deemed highly suggestive of *Pneumocystis* pneumonia (fever, nonproductive cough, dyspnea, interstitial radiographic infiltrates, elevated serum lactate dehydrogenase [LDH] levels, and hypoxemia) were empirically enrolled in studies at Los Angeles County–University of Southern California Medical Center to test new therapies for *Pneumocystis* pneumonia. These studies were conducted between November 1986 and October 1990.

To reduce the likelihood of including patients with mycobacterial or fungal infections or pulmonary Kaposi's sarcoma, potential study candidates whose chest radiographs showed miliary patterns, hilar adenopathy, or pleural effusions were excluded from enrollment. None of the study patients had received aerosolized pentamidine for prophylaxis of *Pneumocystis* pneumonia or chemotherapy for Kaposi's sarcoma or lymphoma.

Experimental therapies for *Pneumocystis* pneumonia that were evaluated in the studies included trimetrexate plus leucovorin (AIDS Clinical Trials Group [ACTG] protocol 029/031), aerosolized pentamidine (ACTG protocol 040), adjunctive corticosteroids (California Collaborative Treatment Group protocol 509), and primaquine plus clindamycin (ACTG 044). These studies were not limited to patients with first episodes of *Pneumocystis* pneumonia; rather, each evaluated therapies based on severity of *Pneumocystis* pneumonia at presentation. ACTG 029/031 and CCTG 509 selected patients with moderate-to-severe *Pneumocystis* pneumonia (i.e., baseline [A-a]DO<sub>2</sub> > 30 mm Hg), whereas ACTG 040 and 044 selected patients with mild-to-moderate pneumocystis pneumonia (i.e., baseline [A-a]DO<sub>2</sub> < 40 mm Hg).

Each patient provided written informed consent, and was scheduled for bronchoscopy with BAL and to have four to six transbronchial biopsies. Bronchoscopy was performed within 5 d after the start of empiric therapy for *Pneumocystis* pneumonia. Transbronchial biopsies were done in all cases unless there was a coagulopathy or endobronchial Kaposi's sarcoma. The BAL sample from each patient was routinely processed for bacterial, fungal, and mycobacterial cultures, and was stained with Gomori's methenamine silver for cytologic demonstration of *P. carinii* and other fungi.

Cytologic diagnosis of *Pneumocystis* pneumonia was confirmed in 284 (81%) of the patients. In the remaining 67 (19%) patients, BAL and transbronchial biopsies revealed no evidence of *P. carinii*. Experimental therapy was continued for 21 ± 3 d, as mandated by the protocols for patients with confirmed *Pneumocystis* pneumonia. For subjects without documentation of *P. carinii*, experimental therapy for *Pneumocystis* pneumonia was discontinued as soon as the results of examination of BAL and transbronchial specimens were complete.

Clinical information gathered prospectively, culture results, and histopathology of biopsy specimens from the patients without *Pneumocystis* pneumonia served as the primary data set for this study.

## Histopathology

The histopathology of transbronchial biopsies not showing *P. carinii* was independently reviewed by an AIDS pathologist (L.N.) and a pulmonary pathologist (M.K.), who were blinded to the clinical aspects of the study cases. Biopsy specimens were considered adequate for review if there were at least 30 total alveoli in sections for each patient. All tissue sections were stained with methenamine silver (Gomori method) for *P. carinii* and other fungi, and with the Ziehl-Neelsen method for mycobacteria.

NSIP was defined by the presence of chronic inflammation in the interalveolar septa in the absence of a specific etiologic agent identified through standard histopathologic, histochemical, cytologic, and culture techniques. Specific histologic abnormalities of NSIP, namely interstitial inflammation, interstitial fibrosis, interstitial edema, pneumocyte hyperplasia, alveolar edema, alveolar hyaline membranes, alveolar inflammation, bronchial inflammation, and vasculitis were

sought. The extent of each finding was graded numerically as 0 (absent), 1 (plus/minus, < 10% involvement), 2 (mild, 10 to 25% involvement), 3 (moderate, 26 to 50% involvement), or 4 (severe, > 50% involvement). Cytomegalovirus (CMV) pneumonia was diagnosed only if sections showed typical nuclear inclusions with surrounding halos, plus adjacent interstitial inflammation. Bronchitis was defined by acute or chronic inflammatory infiltrates in the bronchial wall, with normal lung parenchyma.

## *In situ* Hybridization and Tissue Polymerase Chain Reaction Studies

In 15 of the 16 biopsies showing NSIP, Epstein-Barr virus (EBV), CMV, and HIV nucleic acid was sought through *in situ* hybridization. Biopsies from two HIV-positive patients with pulmonary lymphoid hyperplasia (PLH), and biopsies from three HIV-positive patients with normal lung tissue were also tested. For EBV and CMV, biotinylated probes were used (Enzo Biochem, New York, NY). The *in situ* probes from CMV, EBV, and HIV have a limit sensitivity of 10 to 20 copies of target per cell.

Formalin-fixed, paraffin-embedded tissue sections were deparaffinized in successive rinses with xylene and alcohol, followed by blocking of endogenous peroxidase activity with 3% H<sub>2</sub>O<sub>2</sub> in 100% methanol for 15 min at room temperature. A solution of probe was then applied to the tissue sections and heated to 92° C for 10 min. The tissue sections were then incubated at 37° C for 25 min, covered with posthybridization solution for 10 min at 37° C, and then rinsed with wash buffer. Avidin-biotin-peroxidase complex was then applied for 15 min, followed by aminoethyl carbazole solution for 10 min. The sections were then rinsed and counterstained with Mayer's hematoxylin for 3 min. Positive control cells provided by the manufacturer, as well as negative and positive control tissue sections, were processed with the transbronchial biopsies.

For *in situ* hybridization of HIV nucleic acid, an alkaline phosphatase-linked RNA probe (DuPont, Boston, MA) was used. Briefly, the tissue sections were rehydrated in 100 mM MgCl<sub>2</sub> for 10 min at room temperature. They were then prehybridized in formamide for 10 min at 70° C, rinsed in 5× standard citrate saline (SSC), heated to 55° C, incubated with probe solution for 60 min at 55° C, and treated with detection buffer for 4 h in the dark at 37° C; the development was stopped by rinsing the slides in distilled water. The sections were counterstained with methyl green for 25 s, rinsed with distilled water, and allowed to air-dry in the dark. Positive control cells infected with HIV, as well as negative and positive control retinal tissue sections, were processed with the transbronchial biopsies.

The HIV *gag* and *env* genes were each sought separately in these same formalin-fixed, paraffin-embedded tissues with the polymerase chain reaction (PCR) (9). In brief, 10-μm-thick sections were suspended in 90 μl PCR buffer with 0.25% Tween 20 and 10 μl proteinase K (10 mg/ml) prior to incubation at 56° C overnight. After boiling, DNA templates were amplified with 2.5 units Taq polymerase (Perkin Elmer-Cetus) in a standard 100-μl PCR reaction for 40 cycles at 94° C for 30 s, 42° C for 15 s, and 72° C for 1 min. Primer pairs (1 μM each) were SK38 and SK39 (HIV *gag*) and SK68 and SK39 (HIV *env*). The PCR products were denatured, fixed to nylon membranes, and subsequently hybridized to <sup>32</sup>P-labeled probes (SK19, SK70, or K12, ~ 1 × 10<sup>9</sup> cpm/μg). Washed dot blots (50° C, 1× SSC, 0.2% sodium dodecylsulfate [SDS]-[final stringency]) were exposed on XAR-5 film (Kodak, Rochester, NY). All experiments included positive and negative control templates, deoxyribonucleic acid (DNA) control reactions, and reagent and water negative controls, with the expected results.

The PCR technique is capable of identifying less than 10 DNA templates per reaction. Diluted positive control templates at the level of 100 copies per PCR reaction were demonstrably positive in this system. Therefore, the limit sensitivity was considered to be less than 100 copies per PCR reaction under the described experimental conditions.

Extended formalin fixation can decrease the availability of targets for PCR amplification, by cross-linking. PCR experiments in this study were done on surgical specimens that were routinely formalin fixed for less than 24 h. Comparisons between DNA extracted from fresh and formalin-fixed, paraffin-embedded sections were not done to assess yield or variation in priming efficacy.

### Flow Cytometry for Lymphocyte Phenotyping

All 16 patients with NSIP and 32 case controls had lymphocyte phenotyping done within three days of initiating experimental therapies for *Pneumocystis* pneumonia. The tests were done in the University of Southern California Flow Cytometry Laboratory, which is certified by the College of American Pathologists and the National Institutes of Health AIDS Clinical Trials Group.

### Case Control Study

For each patient with NSIP, two patients with *Pneumocystis* pneumonia were selected from the same treatment study and matched with the index case for age to within 3 yr, and for prior use of zidovudine (the only licensed antiretroviral therapy at the time these studies were conducted). The age of control subjects was determined from their medical record identification cards, and prior use of zidovudine was determined from a computerized list of medications for patients that was generated by the hospital pharmacy. Case controls were matched for these variables and selected from the same treatment study in order to provide control subjects with pneumonia of similar severity to that of patients with NSIP.

Thirty-nine clinical descriptors present when patients entered the treatment studies were evaluated and compared for patients with NSIP and *Pneumocystis* pneumonia. Differences between arterial blood gas values at baseline and 7 to 10 d later were also compared in the two groups. Data was not collected for subjects without *Pneumocystis* pneumonia after they were discharged from the hospital. Continuous variables in the two groups were compared with Student's *t* test, and categorical variables were compared through contingency tables (chi-square and Fisher's exact tests). Differences were considered significant if for values of  $p < 0.05$ .

## RESULTS

### Pathologic Diagnoses

Table 1 shows the pathologic diagnoses in the 67 patients who did not have *Pneumocystis* pneumonia based on the absence of *P. carinii* by silver staining and absence to the telltale intraalveolar eosinophilic exudate from the cell-wall matrix of *P. carinii*.

### NSIP

NSIP, as strictly defined by the absence of histologic evidence of any disease that could result in an interstitial inflammatory

reaction, was detected in 16 (24%) cases. This was the most common pathologic diagnosis and was found in 5% of the 351 patients with presumed *Pneumocystis* pneumonia. All cases of NSIP showed chronic interstitial inflammation, ranging from mild to severe. On a scale of 0 to 4, the average score for interstitial inflammation was 2.5. The second most consistent histologic feature of NSIP was type II pneumocyte hyperplasia, which was demonstrable in all cases and averaged 2.4 on a scale of 0 to 4. The inflammation of NSIP generally "spilled over" into the alveoli, with a severity ranging from plus/minus to moderate, and with an average score of 2.3. All but one of the cases of NSIP showed interstitial edema, ranging from plus/minus to moderate, with an average numerical grading score of 2.1. Ten of the cases of NSIP showed bronchial inflammation, which was generally mild, but was moderate in two cases and severe in one case. Seven cases showed interstitial fibrosis, which was plus/minus in one case and mild in the other six. Four cases showed alveolar edema, ranging from plus/minus to moderate. Only one of the cases showed alveolar hyaline membranes, and none showed vasculitis.

Normal histology was present in 16 other cases; this does not entirely exclude pulmonary disease, since the limited size of biopsies may have allowed diagnoses to be missed. The inadequate-histology category contained 13 cases. Although inadequacy was based on the presence of fewer than 30 total alveoli, there were in fact no diagnoses that could have been made from the limited amount of tissue available from slides of the sections in these cases.

The next most common diagnosis was acute and/or chronic bronchitis with normal lung parenchyma. Since all patients had either abnormal blood gas values or chest radiographs that showed infiltrates, the explanation for this finding is not clear. The "normal" parenchyma may represent sampling errors away from areas of ventilation-perfusion mismatch.

Sections from the remaining 15 cases showed a variety of abnormalities. The only biopsies for which the histologic diagnosis had unequivocal therapeutic implications involved a case of tuberculosis with caseating granulomas containing acid-fast bacteria (AFB) and a case that met the ACTG definition of CMV pneumonia. The patient with tuberculosis also had AFB in sputum, and thus the diagnosis of tuberculosis would have been established without bronchoscopy. The patient with clinical and pathologic evidence of CMV pneumonia had no retinitis on dilated fundoscopic examination. Thus, transbronchial biopsy resulted in the diagnosis of only one treatable infection other than *P. carinii* that would have been diagnosed by noninvasive testing. However, long-term follow-up of the two patients with caseating and one patient with non-caseating granulomas without AFB and fungi was not available to permit a more confident assessment of whether these three patients also had pulmonary infections.

### In situ Hybridization and Tissue PCR Results

*In situ* hybridization studies of the tissues from patients with NSIP showed no cells positive for EBV or CMV in any of the transbronchial biopsies studied. *In situ* hybridization showed rare cells positive for HIV in four of the 15 cases of NSIP, in neither of the biopsies from HIV-positive patients with PLH, and in one of three biopsies from HIV-positive patients with normal histology. The few cells positive for HIV generally appeared to be alveolar macrophages or alveolar lining cells (pneumocytes).

PCRs for the HIV *gag* and *env* genes showed variable degrees of positivity in 10 of the 15 cases of NSIP, weak reactivity in sections from both patients with PLH, and weak reactiv-

TABLE 1  
HISTOLOGY OF LUNG BIOPSIES NOT REVEALING  
*Pneumocystis carinii* PNEUMONIA

Number of Cases (n = 67)	Histologic Description
16	Nonspecific interstitial pneumonitis
16	Normal histology
13	Inadequate histology (< 30 alveoli)
7	Bronchial abnormalities:
	Acute or chronic bronchitis
	No alveolar or interstitial disease
2	Pyogenic pneumonia
2	Pulmonary lymphoid hyperplasia
2	Caseating granulomas*
2	Talc pneumonitis
1	Eosinophilic pneumonia
1	Pulmonary anthracosis
1	Squamous-cell dysplasia
1	Pulmonary hemosiderosis
1	Non-caseating granulomas*
1	Caseating granulomas with acid-fast bacilli†
1	Cytomegalovirus pneumonia‡

\* Negative stains and cultures for fungi and acid-fast bacilli.

† Cultures of lavage fluid grew *Mycobacterium tuberculosis*.

‡ Intranuclear inclusions and interstitial inflammatory changes were present; culture of biopsy grew cytomegalovirus.

TABLE 2

CLINICAL FEATURES OF PATIENTS WITH NONSPECIFIC INTERSTITIAL PNEUMONITIS AND *PNEUMOCYSTIS* PNEUMONIA

Features	NSIP (n = 16)	<i>Pneumocystis</i> Pneumonia (n = 32)
Sex, male/female	16/0	32/0
Age, yr + 1 SD	33.9 ± 7.3	33.5 ± 7.6
Ethnicity		
White, non-Hispanic	8	15
Hispanic	5	8
African-American	3	8
Asian	0	1
Risk factors for AIDS		
Homosexual	14	29
IVDU	1	2
Heterosexual	0	1
Transfusion	1	0
Prior AIDS diagnosis		
<i>Pneumocystis</i> pneumonia	1	7
Kaposi's sarcoma	2	7
Esophageal candidiasis	2	1
CMV retinitis	0	1
CMV colitis	1	1
Cryptococcal meningitis	0	1
History of fever	11	20
Days of fever	16.5 ± 44.0	13.0 ± 16.0
Days of cough	12.8 ± 13.6	18.8 ± 16.5
Days of dyspnea	14.9 ± 18.3	15.7 ± 14.9
Days of chest pressure	9.1 ± 14.9	5.0 ± 6.5
Weight, kg	70.9 ± 13.4	63.2 ± 10.0
Admission temperature	100.9 ± 2.1	100.5 ± 1.8
Respiratory rate, per min	26 ± 7	27 ± 10
Oral thrush	9	22
Lymphadenopathy	3	5
Rales	8	16

Definition of abbreviation: IVDU = intravenous drug use.

ity in one and strong reactivity in two of the three samples from HIV-positive patients with normal lung histology.

#### Case Control Analysis

Table 2 shows that patients with NSIP were generally comparable with patients who had *Pneumocystis* pneumonia. Risk factors for AIDS, prior AIDS-case-defining conditions, the proportion of patients with pulmonary symptoms and the duration of these symptoms, and physical findings were similar in the two groups. However, evaluation of other clinical descriptors did reveal significant differences ( $p < 0.05$ ) between the two groups (Tables 2 and 3). At entry into the treatment studies, patients with NSIP weighed more (70.9 kg versus 63.2 kg), had lower serum LDH values (280 IU/L versus 432 IU/L), more often had normal LDH (69% versus 19% of patients), had higher serum albumin concentrations (3.8 g/dl versus 3.4 g/dl), had higher white blood cell counts ( $9.5 \times 10^9/L$  versus  $5.4 \times 10^9/L$ ), had appreciably higher CD4<sup>+</sup> lymphocyte counts (492 cells/ $\mu$ l versus 57 cells/ $\mu$ l), and were less likely to have CD4<sup>+</sup> cell counts  $< 200/\mu$ L (31% versus 97% of patients), with  $p < 0.05$  for each comparison. Moreover, patients with NSIP as a group had less extensive infiltration on chest radiographs, (i.e., they more often had only one or two quadrants involved, as compared with diffuse involvement of multiple lobes in patients with *Pneumocystis* pneumonia;  $p = 0.003$ ).

#### Response to Therapy

Of the 16 patients with NSIP, eight were initially randomized to receive treatment with "blinded" trimetrexate or intrave-

TABLE 3

LABORATORY TESTS IN PATIENTS WITH NONSPECIFIC INTERSTITIAL PNEUMONITIS AND *PNEUMOCYSTIS* PNEUMONIA

Test Result	NSIP (n = 16)	<i>Pneumocystis</i> Pneumonia (n = 32)
Lactate dehydrogenase, IU/L	280 ± 113	432 ± 141*
LDH abnormal, $> 300$ IU/L	5	26 <sup>†</sup>
Serum albumin, g/dl	3.8 ± 0.6	3.4 ± 0.5 <sup>‡</sup>
Serum hemoglobin, g/dl	11.9 ± 2.1	11.9 ± 1.8
Total WBC count, $\times 10^3/\mu$ l	9.5 ± 5.8	5.4 ± 2.5
Neutrophil count, $\times 10^3/\mu$ l	6.3 ± 4.0	4.0 ± 2.1
Platelet count, $\times 10^3/\mu$ l	273 ± 79	277 ± 112
CD4 lymphocyte count, per $\mu$ l	492 ± 828	57 ± 60 <sup>§</sup>
No. with CD4 <sup>+</sup> counts, $< 200/\text{mm}^3$	5	31 <sup>¶</sup>
Alkaline phosphatase, IU/L	135 ± 123	101 ± 64
Alanine aminotransferase, IU/L	32 ± 15	40 ± 35
PaO <sub>2</sub> , mm Hg	73.3 ± 7.0	69.2 ± 14.9
Pco <sub>2</sub> , mm Hg	32.1 ± 3.9	33.7 ± 4.4
(A-a)DO <sub>2</sub> , mm Hg	35.0 ± 7.8	38.6 ± 14.0
Chest radiographic abnormalities**		
Diffuse interstitial	6	25
Diffuse alveolar	1	0
$< 2$ quadrants involved	8	6
Apical infiltrates	0	1
Pleural effusions	0	0
Hilar adneopathy	0	0
None	1	0

\*  $p = 0.0002$ .

<sup>†</sup>  $p = 0.0001$ .

<sup>‡</sup>  $p = 0.0048$ .

<sup>§</sup>  $p = 0.006$ .

<sup>¶</sup>  $p = 0.000001$ .

\*\*  $p = 0.003$ .

nous trimethoprim-sulfamethoxazole (ACTG 029/031), six to open label therapy with clindamycin plus primaquine (ACTG 040), one to blinded therapy with aerosolized pentamidine or intravenous trimethoprim-sulfamethoxazole (ACTG 044), and one to open label trimethoprim-sulfamethoxazole without prednisone (CCTG 509). Once the results of stains of BAL and transbronchial biopsy specimens were reported to have been negative for *P. carinii*, experimental therapy was discontinued. This occurred between treatment Days 3 and 10. In none of the 16 cases was open-label, nonstudy therapy for *Pneumocystis* pneumonia initiated during the respective hospitalization.

Of the 16 patients with NSIP, eight were sufficiently ill to have a blood-gas analysis done while breathing room air at 7 to 10 d after their baseline evaluation. The other eight patients were discharged from the hospital without repeat blood gas analyses. Thirty-one of 32 patients with *Pneumocystis* pneumonia had blood gas analysis done at Days 7 to 10 as directed by study design. Table 4 shows that both the PaO<sub>2</sub> and (A-a)DO<sub>2</sub> improved after 7 to 10 d in patients with NSIP. Although these improvements appeared greater than for patients with *Pneumocystis* pneumonia, the differences in response between the two groups were of similar magnitude ( $p > 0.05$  for both comparisons).

#### DISCUSSION

It is important to know the frequency of various conditions that may mimic *Pneumocystis* pneumonia, since treatment for *P. carinii* is often begun empirically, especially when tachypnea or hypoxemia is present. Results of our evaluation, at an institution where more than 200 cases of *Pneumocystis* pneumonia have been confirmed annually for almost a decade, and

TABLE 4  
RESPONSE TO THERAPY IN PATIENTS WITH NSIP AND *PNEUMOCYSTIS* PNEUMONIA

	NSIP			<i>Pneumocystis</i> Pneumonia			p Value*
	Baseline	Follow-up	Difference	Baseline	Follow-up	Difference	
Number of patients with tests	16	8		32	31		
Blood gases <sup>†</sup>							
Pa <sub>O</sub> <sub>2</sub> , mm Hg	73 ± 7.0	85 ± 14	8.5	69 ± 15	75 ± 16	7.7	> 0.05
(A-a)D <sub>O</sub> <sub>2</sub> , mm Hg <sup>‡</sup>	35 ± 8.1	20 ± 14	-13.6	38 ± 14	30 ± 17	-8.1	> 0.05

\* Comparison of differences between baseline and follow-up at Days 7 to 10.

<sup>†</sup> All tests were done with patients breathing room air for at least 20 min.

<sup>‡</sup> Normal value is ≤ 14 mm Hg.

where practitioners have had considerable experience in diagnosing this infection, indicate that almost 20% of patients with typical features of *Pneumocystis* pneumonia do not have *P. carinii* confirmed by BAL and transbronchial biopsy. It would be desirable to avoid the expense and toxicities of therapies for *Pneumocystis* in such a sizable number of patients in whom the diagnosis cannot be confirmed.

The most common histologic diagnosis in patients with clinical and radiographic features mimicking *Pneumocystis* was NSIP. NSIP was detected in 24% of the 67 patients without *Pneumocystis* and 5% of the original 351 patients presumed to have *Pneumocystis*. The prevalence of NSIP in other studies of HIV-positive patients with pulmonary disease has varied from 11 to 38% (10–14). Selection of patient populations could explain these differences. In a study in which 41 (38%) of 110 patients with AIDS had NSIP, either biopsies showed concurrent pulmonary Kaposi's sarcoma or patients had received previous experimental therapies or had a history of intravenous drug use (in 28 of the 41 cases) (10). Diffuse alveolar damage and chronic interstitial disease are reported to occur in patients who use intravenous drugs (15). In addition, histologic changes in pulmonary Kaposi's sarcoma may resemble those of NSIP (16). By contrast, our patients were selected for evidence highly suggestive of *Pneumocystis* pneumonia. They had not received prior experimental therapy, and the diagnosis of NSIP was not made if there was histologic evidence of other diseases such as Kaposi's sarcoma. Thus, differences in study populations and a more stringent definition of NSIP may explain why the occurrence of NSIP in our patients was rarer than in several other reports (10, 11, 13).

Our case control analysis indicated that certain clinical descriptors might be helpful in distinguishing NSIP from *Pneumocystis* pneumonia. In particular, patients with NSIP were more likely to have less advanced HIV with better weight, serum albumin levels, and CD4<sup>+</sup> lymphocyte counts. In addition, patients with NSIP appeared to have less lung inflammation, since they were more likely to have normal LDH values and fewer lobes with interstitial disease on chest radiographs. It is unlikely that these differences were caused by patient-selection bias, since controls were matched by enrollment in treatment studies based on severity of hypoxemia and ventilatory abnormality. In fact, Pa<sub>O</sub><sub>2</sub> and (A-a)D<sub>O</sub><sub>2</sub> at baseline were similar in the patients with NSIP and *Pneumocystis* pneumonia. Unfortunately, there was considerable overlap of features between the groups, and thus none of the clinical descriptors was sufficiently predictive to be of clinical value in diagnosis.

The natural history of NSIP is yet to be determined. However, in our study, results of follow-up blood-gas determinations done 7 to 10 d after the baseline evaluation showed ap-

preciable improvement in at least half the patients with NSIP during empiric therapy for *Pneumocystis* pneumonia. The improvement was of similar magnitude to that which occurred in patients with documented *Pneumocystis* pneumonia. The other patients with NSIP were sufficiently improved clinically to have therapy for *Pneumocystis* discontinued, and their primary physicians did not feel it was necessary to document improvement in their blood gas values. In a series of seven patients with NSIP reported from Europe, a subacute course and spontaneous improvement was also noted (17). The reason for improvement in patients with NSIP is not clear. It is unlikely that more than a few patients with NSIP in our study could have had *Pneumocystis* pneumonia that was not detected by bronchoscopy, since the combined yield of BAL and transbronchial biopsy for identifying *P. carinii* is generally in excess of 95% (18). In addition, most patients had anti-*Pneumocystis* therapy discontinued after 3 to 5 d when the results of BAL and biopsies were reported not to show *P. carinii*. It is likely that most patients with *Pneumocystis* pneumonia would relapse quickly after such abbreviated therapy.

Currently, the etiology of NSIP is unknown. The cytokine profile of BAL fluid (BALF) samples from patients with NSIP differs from that of those with *Pneumocystis* pneumonia, suggesting that NSIP is a distinct entity and not merely a diagnosis of exclusion in cases in which *P. carinii* or other typical pathogens cannot be detected (19, 20). Whether HIV *per se* is the cause of NSIP in some patients is uncertain. HIV RNA in lung tissue was detected by *in situ* hybridization in rare cells of four of our 15 patients with NSIP, and in four of 46 patients with NSIP in another study (21). In our investigation, the more sensitive PCR testing for HIV *gag* and *env* DNA in tissue sections was positive in 10 of the 15 patients, but was also positive in the HIV control sections from patients with PLH and normal lung histology. One interpretation of these results is that HIV *per se* may, in some cases, result in an immunologic pulmonary reaction associated with histologic findings of NSIP.

We also sought evidence for EBV and CMV by *in situ* hybridization, but all 15 specimens from our patients with NSIP were negative for these viruses. Another herpes virus, human herpes virus-6 (HHV-6), has been associated with interstitial pneumonitis in some patients with AIDS (22), and DNA of this virus has been detected in high concentrations in the lungs of bone-marrow transplant recipients with pneumonia (23). Whether pneumonia due to HHV-6 would improve spontaneously and give the false impression that a patient was responding to therapy for *Pneumocystis* is unknown. It remains to be determined whether some cases of NSIP are due to HHV-6 or to other, yet undefined viruses.

There was a remarkable near absence of unequivocal treatable disorders (other than *Pneumocystis* pneumonia) diagnosed solely by transbronchial biopsy. Only the single case of CMV pneumonia would have been missed without transbronchial biopsy, although mycobacterial or fungal infection could not be entirely excluded in the three cases with lung granulomas. However, the study population was highly selected, since subjects with chest radiographs showing miliary patterns, hilar adenopathy, or pleural effusions were excluded from enrollment in the treatment studies. Because these findings may be associated with pulmonary tuberculosis, fungal infection, or Kaposi's sarcoma, it is likely that many patients with granulomatous infections or Kaposi's sarcoma were excluded from our study.

These results should not be construed as justification for not doing bronchoscopy in less selected patients, especially those failing to improve with empiric therapy. In a retrospective study of patients with negative sputum examinations for *P. carinii*, bronchoscopy resulted in a diagnosis of *P. carinii* pneumonia in more than half of the cases; the most frequent diagnoses were *Pneumocystis* pneumonia (192 cases), Kaposi's sarcoma (93 cases), tuberculosis (28 cases), and cryptococcosis (9 cases) (24). Thus, treatable neoplastic and granulomatous conditions may be detected more commonly in less selected populations than in our study subjects. In addition, the argument can be made that a negative bronchoscopy for *P. carinii* in many cases allows potentially toxic drugs to be discontinued. We therefore believe that the use of bronchoscopy still needs to be considered on a case-by-case basis, but our results suggest that the diagnostic yield of treatable causes of interstitial lung disease other than *P. carinii* in patients with HIV will be low in the absence of radiographic changes more suggestive of other diseases.

In summary, our results suggest that NSIP may be the most common diagnosis mimicking *Pneumocystis* pneumonia in patients with HIV, and may improve during empiric therapy for *P. carinii* pneumonia. Although NSIP more often occurred earlier in the course of HIV, when patients had better immunologic and nutritional function, and was more often associated with normal LDH levels, the overlap in presentations was too great for differentiating NSIP from *Pneumocystis* pneumonia on the basis of these clinical parameters alone. The study also demonstrated that other treatable conditions are unlikely to be detected by transbronchial biopsy when radiographs are carefully screened to exclude patients with findings more consistent with granulomatous causes of interstitial lung disease or pulmonary Kaposi's sarcoma.

## References

- Chan, I. S. F., J. D. Neaton, L. D. Saravolatz, L. R. Crane, J. Osterberger, for the Community Programs for Clinical Research for AIDS. 1995. Frequencies of opportunistic diseases prior to death among HIV-infected persons. *AIDS* 9:1145-1151.
- Bigby, T. D., D. Margolskee, J. L. Curtis, P. F. Michael, D. Sheppard, W. K. Hadley, and P. C. Hopewell. 1986. The usefulness of induced sputum in the diagnosis of *Pneumocystis carinii* pneumonia in patients with the acquired immunodeficiency syndrome. *Am. Rev. Respir. Dis.* 133:515-518.
- Kovacs, J. A., V. L. Ng, H. Masur, G. Leoung, W. K. Hadley, G. Evans, H. C. Lane, F. P. Ognibene, J. Shelhamer, and J. E. Parrillo. 1988. Diagnosis of *Pneumocystis carinii* pneumonia: improved detection in sputum with use of monoclonal antibodies. *N. Engl. J. Med.* 318:589-593.
- Leigh, T. R., C. Hume, B. Gazzard, P. Parsons, O. A. N. Husain, and J. V. Collins. 1989. Sputum induction for diagnosis of *Pneumocystis carinii* pneumonia. *Lancet* 2:205-206.
- O'Brien, R. F., J. L. Quinn, B. T. Miyahara, R. B. Lepoff, and D. L. Cohn. 1989. Diagnosis of *Pneumocystis carinii* pneumonia by induced sputum in a city with a moderate incidence of AIDS. *Chest* 95:136-138.
- The National Institutes of Health—University of California Expert panel for Corticosteroids as Adjunctive Therapy for *Pneumocystis* pneumonia 1990. Consensus statement on the use of corticosteroids as adjunctive therapy for *Pneumocystis* pneumonia in the acquired immunodeficiency syndrome. *N. Engl. J. Med.* 323:1451-1457.
- Gill, P., C. Loureiro, M. Bernstein-Singer, M. Rarick, R. Sattler, and A. Levine. 1989. Clinical effect of glucocorticoids on Kaposi's sarcoma related to the acquired immunodeficiency syndrome (AIDS). *Ann. Intern. Med.* 110:937-940.
- Salahuddin, S. Z., S. Nakamura, P. Biberfeld, M. H. Kaplan, P. D. Markham, L. Larrson, and R. C. Gallo. 1988. Antigenic properties of Kaposi's sarcoma-derived cells after long-term culture *in vitro*. *Science* 242:430-433.
- Saiki, R. K., T. L. Bugawan, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1986. Analysis of enzymatically amplified  $\beta$ -globulin and HLA-DQ $\alpha$  DNA with allele-specific oligonucleotide probes. *Nature* 324:163-166.
- Suffredini, A. F., F. P. Ognibene, E. E. Lack, J. T. Simmons, M. Brenner, V. J. Gill, H. C. Lane, A. S. Fauci, and J. E. Parrillo. 1987. Nonspecific interstitial pneumonitis: a common cause of pulmonary disease in the acquired immunodeficiency syndrome. *Ann. Intern. Med.* 107:7-13.
- Batungwanayo, J., H. Taelman, S. Lucas, J. Bogaerts, D. Alard, A. Kagame, P. Blance, J. Clerinx, P. van de Perre, and S. Allen. 1994. Pulmonary disease associated with human immunodeficiency virus in Kigali, Rwanda. *Am. J. Respir. Crit. Care Med.* 149:1591-1596.
- Stover, D. E., D. A. White, P. A. Romano, R. A. Gellene, and W. A. Robeson. 1985. Spectrum of pulmonary disease associated with the acquired immune deficiency syndrome. *Am. J. Med.* 78:429-437.
- Barrio, J. L., C. Harcup, J. B. Horst, and A. E. Pitchenik. 1987. Value of repeat fiberoptic bronchoscopies and significance of nondiagnostic bronchoscopic results in patients with the acquired immunodeficiency syndrome. *Am. Rev. Respir. Dis.* 135:422-425.
- Prober, C. G., H. Whyte, and C. R. Smith. 1984. Open lung biopsy in immunocompromised children with pulmonary infiltrates. *Am. J. Dis. Child.* 138:60-63.
- Ramaswamy, G., V. Jagadha, and V. Tchertkoff. 1985. Diffuse alveolar damage and interstitial fibrosis in acquired immunodeficiency syndrome patients without concurrent pulmonary infection. *Arch. Pathol. Lab. Med.* 109:408-412.
- Im-Hof, V., T. Cerny, and A. Burkhardt. 1988. Involvement of the trachea and lungs with Kaposi's sarcoma. *J. Suisse Med.* 118:134-138.
- Griffiths, M. H., R. F. Miller, and S. J. G. Semple. 1995. Interstitial pneumonitis in patients infected with human immunodeficiency virus. *Thorax* 50:1141-1146.
- Hopewell, P. C. 1988. *Pneumocystis carinii* pneumonia: diagnosis. *J. Infect. Dis.* 157:1115-1119.
- Denis, M., and E. Ghadirian. 1994. Dysregulation of interleukin 8, interleukin 10, and interleukin 12 release by alveolar macrophages from HIV type 1-infected subjects. *AIDS Res. Hum. Retrovir.* 10:1619-1627.
- Lipschik, G. Y., M. E. Doerfler, J. A. Kovacs, W. D. Travis, V. A. Andrawis, M. G. Lawrence, J. R. Dichter, F. P. Ognibene, and J. H. Shelhamer. 1993. Leukotriene B<sub>4</sub> and interleukin-8 in human immunodeficiency virus-related pulmonary disease. *Chest* 104:763-769.
- Travis, W. D., C. H. Fox, K. O. Devaney, L. M. Weiss, T. J. O'Leary, and F. P. Ognibene. 1992. Lymphoid pneumonitis in 50 adult patients infected with the human immunodeficiency virus: lymphocytic interstitial pneumonitis versus nonspecific interstitial pneumonitis. *Hum. Pathol.* 23:529-541.
- Knox, K. K. and D. R. Carrigan. 1994. Disseminated active HHV-6 infections in patients with AIDS. *Lancet* 343:557-558.
- Cone, R. W., R. C. Hackman, M. W. Huang, R. A. Bowden, J. D. Meyers, M. Metcalf, J. Zeh, R. Ashley, and L. Corey. 1993. Human herpes virus 6 in lung tissue from patients with pneumonitis after bone marrow transplantation. *N. Engl. J. Med.* 329:156-161.
- Huang, L., F. M. Hecht, J. D. Stansell, R. Montanti, W. K. Hadley, and P. C. Hopewell. 1995. Suspected *Pneumocystis carinii* pneumonia with a negative induced sputum examination. *Am. J. Respir. Crit. Care Med.* 151:1866-1871.