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Clinical manifestations of exacerbations of cystic fibrosis associated with nonbacterial infections

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The purpose of this study was to determine whether acute pulmonary exacerbations of cystic fibrosis associated with nonbacterial infections are clinically distinguishable from other exacerbations. Eighty exacerbations in 54 patients were studied. Exacerbations associated with influenza (n = 8) were compared with those associated with other nonbacterial infections (n = 15) and those in which no nonbacterial infection was detected (n = 57). Patients with influenza had lower Shwachman scores and were more likely to be seropositive for C-reactive protein than patients in the other two groups. Patients with influenza had a mean decrease in forced expiratory volume per second of 26%, compared with test results obtained before the exacerbation. In contrast, the mean decrease in forced expiratory volume per second was 6% for other nonbacterial infections and 12% for the group without nonbacterial infection ($p < 0.05$ for both comparisons). The forced expiratory flow in first 25% of vital capacity decreased 44% in the influenza group compared with 13% and 17% in the other two groups, respectively ($p < 0.01$ for both comparisons). The influenza group also had a higher proportion of patients with at least a 20% decrease in forced expiratory volume per second and forced expiratory flow in first 25% of vital capacity than the other two groups had ($p < 0.05$ for all comparisons). These data suggest that influenza is associated with severe exacerbations in patients with cystic fibrosis and support recommendations for efforts to prevent influenza in this population.
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Studies of patients with cystic fibrosis suggest that approximately one third of acute pulmonary exacerbations are associated with nonbacterial infections.[1-4] Although the incidence of viral respiratory infection in patients with CF is comparable to that in unaffected children,[5,6] frequent infections in these patients may be associated with an increased rate of pulmonary deterioration.[5] A recent study suggests that NBI may play a role in the pathogenesis of acute exacerbations of CF.[7] Our hypothesis was that if the NBI were simply an incidental finding, the exacerbations associated with NBI should be indistinguishable from other pulmonary exacerbations of CF. The purpose of our study was to compare the clinical manifestations of exacerbations of CF associated with an NBI with those of exacerbations in which no NBI was detected.

CF	Cystic fibrosis
CRP	C-reactive protein
FEF_{25}	Forced expiratory flow in first 25% of vital capacity
FEV_1	Forced expiratory volume in 1 second
NBI	Nonbacterial infection
PFT	Pulmonary function test

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METHODS

The protocol for this study was approved by the University of Utah Medical Center Investigational Review Board, and informed consent was obtained from all participants in the study. All patients who were seen at the CF clinic at the University of Utah Medical Center between September 1986 and April 1987 with an acute pulmonary exacerbation of CF were eligible for admission to the study. Pulmonary exacerbations were defined as an acute change in health associated with fever or hemoptysis or with an increase in dyspnea, cough, or sputum production. At the time of enrollment, each patient's history was obtained, and physical examination and an evaluation of severity of the underlying CF by Shwachman score were also done. Each patient also had specimens collected for viral and bacterial culture, viral serologic studies, complete blood cell count and differential cell count, and determination of serum C-reactive protein. Patients old enough to cooperate were asked to participate in pulmonary function studies. All exacerbations for which either a viral culture or both acute and convalescent serum specimens were collected were included in the data analysis. Of 84 exacerbations, four (5%) were excluded from the data analysis because these specimens were not available.

Clinical evaluation. Specific historical information and physical findings recorded for patients in the study included the presence of upper respiratory tract symptoms, duration of illness before presentation, weight loss, household contacts with respiratory illness, and the presence of wheezing. The severity of the underlying disease was assessed by a modified Shwachman-Kulczycki scoring system[8] that employs the Brasfield score[9] for the chest roentgenogram findings. All chest roentgenograms were evaluated and scored by one investigator (P.G.B.).

Pulmonary function tests. Pulmonary function tests were done with a computerized portable spirometer (model CM-V, Cybermedic Inc., Lewisville, Colo.). Patients were asked to perform three consecutive forced vital capacity maneuvers, and the highest value was recorded. The results of the PFTs done during the acute exacerbation were compared with those of a study done during the previous 6 months, when the patient was not acutely ill. The results were analyzed by calculating the percentage of change from the baseline for each test. Groups of patients were compared by determining the mean percentage of change from baseline for each of the PFTs.

Viral cultures. All specimens for viral culture were obtained within 48 hours of patients' enrollment in the study. Each patient had a nasopharyngeal and a throat specimen obtained with calcium alginate swabs. The swabs were placed in viral collecting broth (Earle balanced salt solution with 0.5% bovine serum albumin) supplemented with penicillin (500 U/ml) and streptomycin (250 μg/ml) and kept at 4° C until inoculated into cell culture. Within 24 hours of collection the specimens were inoculated into tube cultures of human foreskin fibroblast, HEp-2, and primary cynomolgus monkey kidney cells. Madin-Darby canine kidney cells were also inoculated during the winter months, when influenza virus was prevalent. These tubes were incubated in stationary racks at 36° C. Two tube cultures of human lung fibroblast cells (MRC-5) were inoculated and incubated in a roller drum at 33° C for isolation of rhinoviruses. All viral isolates were identified by standard immunologic or virologic methods.

Serologic determinations. Serum specimens obtained within 4 days of enrollment in the study and serum specimens collected at convalescence 2 to 6 weeks after enrollment were tested for the presence of specific antiviral antibody. All specimens were stored at −20° C from the time of collection until the antibody assays were performed. Antibodies to influenza A and B, parainfluenza types 1 to 3, adenovirus, respiratory syncytial virus, and *Mycoplasma pneumoniae* were detected by complement fixation. Antibody to coronavirus 229E was detected by a microtiter neutralization assay.[10] A fourfold or greater rise in antibody titer between the paired serum samples was considered evidence of a viral or mycoplasmal infection.

Bacterial cultures. Sputum specimens were obtained from all patients able to produce adequate samples. In young children who were not able to provide a sample, specimens of respiratory secretions were obtained by sampling the hypopharynx with a cotton swab. All specimens were inoculated onto sheep blood agar plates and incubated at 37° C.

C-reactive protein determination. Concentrations of CRP were determined in the acute serum specimens obtained at the time of enrollment in the study. CRP assays were done by rate nephelometry (Beckman Immunochemistry Systems, Beckman Instruments Inc., Fullerton, Calif.). Values >0.59 mg/dl were considered positive.

Statistical analysis. Proportions were compared by the Fisher Exact Test. Continuous variables in groups of patients were compared by a two-sided Mann-Whitney U test. Statistical analyses were performed with commercial software (NWA Statpak, Northwest Analytical, Inc., Portland, Ore.).

RESULTS

Eighty exacerbations in 54 patients were included in the data analysis. Thirty-five patients had one exacerbation, 13 had two exacerbations, five had three exacerbations, and one patient had four exacerbations during the 9-month study period. The patients ranged in age from 10 months to 32 years; the mean age was 15.4 years. Twenty-eight (52%)

Table I. Nonbacterial diagnoses in patients with exacerbations of CF

Diagnosis	Culture	Serologic finding	Total
Influenza A	1	4	5
Influenza B	0	4	4
Coronavirus 229E	0	6	6
Respiratory syncytial virus	1	2	3
Rhinovirus	2	—	2
Parainfluenza 1	0	1	1
Enterovirus	1	—	1
Mycoplasma pneumoniae	—	4	4
TOTAL	5	21	26*

*Twenty-three exacerbations, three combined infections (1 influenza A/influenza B, 1 respiratory syncytial virus/229E, 1 *M. pneumoniae*/respiratory syncytial virus).

Table II. Change in pulmonary function in patients with pulmonary exacerbations of CF

	No NBI (n = 36)	NBI except influenza (n = 9)	Influenza (n = 6)
FEV_1	−12*	−6	−26†
FEF_{25}	−17	−13	−44
FVC	−9	−1	−18
PEFR	−11	−8	−23
FEF_{25-75}	−17	−9	−32

FEF_{25-75}, Forced expiratory flow in middle 50% of vital capacity; *FVC*, forced vital capacity; *PEFR*, peak expiratory flow rate.
*Mean percentage of decline from baseline.
†$p < 0.05$ for comparison of patients with influenza with either of the other two groups.

of the patients were male. There was a broad spectrum of disease severity; the modified Shwachman scores ranged from 30 to 99, with a mean of 67. Of the 80 exacerbations, 44 (55%) required hospitalization in the judgment of the physicians caring for the patient. Uniform criteria for hospital admission were not used. Two deaths occurred during the study period.

Viral culture specimens were obtained during 71 (89%) of the 80 exacerbations, and paired sera for viral and mycoplasmal serologic studies were available from the patients with 40 (50%) of the exacerbations. Twenty-three (29%) of the 80 exacerbations were associated with an NBI. Five patients had positive viral cultures, and 18 had an NBI detected by serologic study (Table I). Three exacerbations were associated with serologic evidence of infection by two different pathogens.

Bacterial cultures revealed that 61 (76%) of the pulmonary exacerbations occurred in patients who were colonized with *Pseudomonas aeruginosa,* and 17 (21%) were in patients colonized with *Pseudomonas cepacia.* There was no correlation between colonization with either of these organisms and the detection of an associated NBI.

Clinical characteristics. History and physical examination findings in the 23 exacerbations associated with NBI were compared with the findings in the 57 exacerbations with no associated NBI. The clinical findings in the two groups were similar. Of the 23 patients with NBI, 10 (43%) were admitted to the hospital, compared with 34 (60%) of the patients with no NBI. Further examination revealed that those patients with a diagnosis of influenza infection were more likely to be admitted to the hospital (6/8) than patients with other NBIs (4/15; $p < 0.04$). The mean Shwachman score in the patients with influenza was 52 ± 11 (mean ± SD), compared with 72 ± 11 in patients with other NBIs and 67 ± 14 in patients with no NBI ($p < 0.01$ for both comparisons).

Laboratory findings. Complete blood cell counts were done on enrollment in the study for 21 (91%) of the exacerbations associated with NBI and for 51 (89%) of the remaining exacerbations. The complete blood cell counts were similar in the two groups.

Quantitative CRP measurements were done on enrollment in the study in 18 (78%) of the patients with exacerbations of NBI and in 38 (67%) of those with the remaining exacerbations. The CRP finding was positive in 30 (54%) of the 56 exacerbations tested. Of the patients with NBI, 12 (67%) had positive CRP findings, compared with 18 (47%) of the patients with no NBI ($p > 0.05$). All six of the patients with influenza-associated exacerbations who were tested had positive CRP findings, compared with 6 (50%) of the 12 patients with other NBIs and 18 (47%) of the 38 patients with no NBI ($p < 0.05$ for both comparisons). The mean quantitative CRP value was higher (3.8 ± 4.0 mg/dl) in the patients with influenza than in the patients with no NBI (1.7 ± 2.0 mg/dl; $p < 0.05$). The Shwachman scores were significantly lower in patients with positive CRP findings (61.9 ± 14.6) than in patients without CRP (72.4 ± 13.1; $p < 0.005$). There was no association between a positive CRP finding and the magnitude of decline in the pulmonary function test results.

Pulmonary function testing. Pulmonary function testing was done after 15 (65%) of the 23 exacerbations associated with NBI and after 36 (63%) of the 57 exacerbations with no NBI. The mean percentage of change in the PFT results did not differ significantly for the two groups. Pulmonary function was tested in six of the eight patients with influenza. Comparison of the patients with influenza either with the patients with no NBI or with those with NBI other than influenza revealed a significantly greater decline in the forced expiratory volume in 1 second and in the forced expiratory flow in the first 25% of vital capacity in the patients

with influenza (Table II). When the analysis was limited to exacerbations in patients with Shwachman scores less than 70, the results of the comparisons were the same except that the difference in the magnitude of decline of FEV_1 in patients with influenza, compared with those with no NBI, was no longer statistically significant. The data were also analyzed by comparing the proportion of patients in each group who had at least a 20% decline in the results of each of the PFTs. Four (67%) of the six patients with influenza, 8 (22%) of the 36 patients with no NBI, and 1 (11%) of the 9 patients with NBI other than influenza had at least a 20% decline in FEV_1 ($p < 0.05$ for both comparisons). The proportion of patients in each group with at least a 20% decline in FEF_{25} was 6/6, 20/36 (56%), and 4/9 (44%), respectively ($p < 0.05$ for both comparisons). There was no correlation between the Shwachman score and the magnitude of the change in PFT results during the acute exacerbations ($r = 0.11$ and $r = 0.007$ for correlation between the Shwachman score and the change in FEV_1 and FEF_{25}, respectively).

DISCUSSION

Taken as a whole, the pulmonary exacerbations of CF associated with NBI in our study were generally indistinguishable from exacerbations in which no NBI was detected. The various nonbacterial pathogens, however, appeared to have different implications for pulmonary function. Exacerbations associated with influenza virus were generally more severe than those associated with other NBIs or those in which no NBI was detected.

Most patients with CF have colonization of the lower respiratory tract with bacterial pathogens that are assumed to play a role in the pathogenesis of acute pulmonary exacerbations of CF. Although strong arguments have been made against such a role and a recent controlled study did not detect a significant benefit from antibiotic therapy,[11, 12] the acceptance of bacterial infection as a cause of the acute exacerbations persists.[13] The assumption that bacterial infections were the cause of the exacerbations may have discouraged examination of other possible precipitating agents.

The association of viral infection with acute exacerbations of CF has been examined in several studies[1-4]; the incidence of viral infection in patients with exacerbations has been 13% to 32%. The predominant viral pathogens have been influenza, parainfluenza, and respiratory syncytial virus. In our study, 29% of the exacerbations were associated with NBI. Coronavirus 229E, which has not been sought in previous studies, was detected in 8% of the exacerbations.

Two controlled studies have indicated that the incidence of viral respiratory disease in patients with CF is similar to that in healthy children. Wang et al.[5] found that the incidence of respiratory viral infections documented by serologic study was 1.7 per patient per year in both groups. Ramsey et al.[6] found the incidence of viral respiratory infections to be 1.3 per patient per year in the CF patients and 1.4 per patient per year in the control subjects; only one third of the viral infections in the subjects with CF were associated with respiratory symptoms. These two studies allow the conclusion that patients with CF are not unusually susceptible to either viral infection or illness caused by viral pathogens, but the results differ with regard to the long-term implications of viral infection. Wang et al. found a significant association between the annual incidence of viral infection and the rate of pulmonary disease progression. Ramsey et al. were unable to confirm this observation.

None of the previous studies in CF patients have been designed to examine the clinical manifestations of acute exacerbations of disease associated with NBIs. We found that the exacerbations associated with the influenza viruses were more severe than either those associated with other NBI or those with no detectable NBI. The variation in the clinical manifestations of pulmonary exacerbations associated with different nonbacterial pathogens suggests that the results of studies of the effects of NBI in CF will need to be interpreted in light of the specific viral pathogens detected in the study population.

The serum CRP concentration has been suggested as a means to differentiate viral from bacterial infections.[14] There was no correlation between CRP positivity and the presence or absence of NBI in our study. Glass et al.[15] recently reported that the serum CRP concentration is a sensitive indicator of pulmonary exacerbations in patients with CF. Only 54% of patients with pulmonary exacerbations by our definition had positive CRP findings. Furthermore, we were unable to demonstrate any correlation between CRP and the degree of change in pulmonary function. Our data appear to confirm those of Moreton and Kennedy,[16] that a positive CRP finding in a patient with CF is best correlated with the severity of underlying disease as determined by Shwachman score.

Our study was not designed to establish a cause-and-effect relationship between NBI and pulmonary exacerbations of CF. The results of our study, however, support a role for influenza infection in these illnesses. Immunization for prevention of both influenza A and influenza B and the use of amantadine for prevention of influenza A are recommended for some high-risk children.[17] Whether intensive efforts to prevent influenza infections in patients with CF can have a beneficial effect remains to be determined.

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