Respiratory Viruses, Symptoms, and Inflammatory Markers in Acute Exacerbations and Stable Chronic Obstructive Pulmonary Disease

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The effects of respiratory viral infection on the time course of chronic obstructive pulmonary disease (COPD) exacerbation were examined by monitoring changes in systemic inflammatory markers in stable COPD and at exacerbation. Eighty-three patients with COPD (mean [SD] age, 66.6 [7.1] yr, FEV_1, 1.06 [0.61] L) recorded daily peak expiratory flow rate and any increases in respiratory symptoms. Nasal samples and blood were taken for respiratory virus detection by culture, polymerase chain reaction, and serology, and plasma fibrinogen and serum interleukin-6 (IL-6) were determined at stable baseline and exacerbation. Sixty-four percent of exacerbations were associated with a cold occurring up to 18 d before exacerbation. Seventy-seven viruses (39 [58.2%] rhinoviruses) were detected in 66 (39.2%) of 168 COPD exacerbations in 53 (64%) patients. Viral exacerbations were associated with frequent exacerbators, colds with increased dyspnea, a higher total symptom count at presentation, a longer median symptom recovery period of 13 d, and a tendency toward higher plasma fibrinogen and serum IL-6 levels. Non-respiratory syncytial virus (RSV) respiratory viruses were detected in 11 (16%), and RSV in 16 (23.5%), of 68 stable COPD patients, with RSV detection associated with higher inflammatory marker levels. Respiratory virus infections are associated with more severe and frequent exacerbations, and may cause chronic infection in COPD. Prevention and early treatment of viral infections may lead to a decreased exacerbation frequency and morbidity associated with COPD.

Keywords: COPD exacerbation; diary cards; recovery; rhinovirus; viruses

Chronic obstructive pulmonary disease (COPD) is associated with frequent exacerbations causing considerable morbidity and mortality (1–3). These exacerbations may lead to further worsening of symptoms and increased airway resistance (4, 5), the mechanisms of which are largely unknown, although airway inflammatory markers increase at exacerbation (6). It has been suggested that the majority of exacerbations of COPD are associated with bacterial infections but many exacerbations occur without increase in volume or purulence of sputum, and bacteria may be secondary invaders after viral infection (7–11). The virus detection rates in these studies were variously stated as between 15 and 20%, with negligible rates of virus detection in the stable state. However, these earlier studies of viruses in COPD used limited methodology for diagnosis of respiratory virus infection.

Studies of childhood and adult asthma have shown that viruses can trigger up to 80 and 50%, respectively, of asthmatic exacerbations and are associated with falls in peak expiratory flow rate (12, 13). These studies have used modern methods of virus diagnosis, including the polymerase chain reaction (PCR) to detect viruses during exacerbations, with rhinovirus being the most commonly detected. We have previously shown that between 33 and 50% of COPD exacerbations may be associated with symptoms of colds (5, 6), thus it is likely that respiratory viruses are responsible for a higher proportion of COPD exacerbations than was hitherto suspected. Little is known about the symptoms and physiological changes at COPD exacerbation or the occurrence of asymptomatic virus infection in COPD and this knowledge will be important for planning treatment strategies and the development of antiviral therapies.

This study has for the first time prospectively investigated the association between exacerbations of COPD and viral infection, using PCR methodology to detect respiratory viruses and atypical bacteria, in addition to virus culture and serological methods. Nasal samples were taken from patients with COPD at baseline when stable and also at COPD exacerbation. Plasma fibrinogen and serum interleukin 6 (IL-6) were measured in the stable state and during acute infection as markers of systemic inflammation. Patients were monitored on a daily basis, using diary cards, peak flow, and symptoms, and thus changes and trends in peak flow and symptoms at COPD exacerbation were examined in relation to the presence or absence of viral infection. Factors associated with positive detection of viruses in stable COPD were examined.

METHODS

Recruitment, Exacerbations, and Stable Visits

Patients recruited from the outpatient department (London Chest Hospital, London, UK) had a forced expiratory volume in 1 s (FEV_1) less than 70% predicted for age and height and a history of β_2 agonist reversibility on predicted FEV_1 of less than 10% and/or 200 ml (14). Measurements were then made of spirometric parameters, blood gases (15), smoking habits and drug history. All patients were advised to obtain an influenza vaccination. Permission for this study was obtained from the East London and City Health Authority Ethics Committee.

Patients recorded on daily diary cards peak expiratory flow rate (PEFR), determined with a Mini-Wright peak flow meter, and any increase relative to their chronic (stable) state during the last 24 h in "major" symptoms (dyspnea, sputum purulence, sputum amount) or "minor" symptoms (nasal discharge/congestion, wheeze, sore throat, cough).

Exacerbations were defined as an increase in any two major symptoms or increase in one major and one minor symptom on two consecutive days, the first of which was taken as the day of onset of exacerbation (3, 5, 6). When patients experienced deterioration in their daily

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symptoms they were sampled and, if required, treatment consisting of antibiotics and/or oral steroids was prescribed. Each patient was then seen at convalescence at 4 to 6 wk. Exacerbations were classified as either reported (patient seen at clinic) or unreported (3). Exacerbation frequency was taken as the total number of exacerbations in the 12 mo after recruitment. A patient was defined as stable if there was no exacerbation for the previous 4 wk and the patient was not currently having an exacerbation, the term *stable* prefixed all parameters measured at that visit.

Sampling at Clinic

Spirometry was repeated and nasal and blood samples were taken. A CH10 nasal catheter was used (16, 17), but if the volume of nasal aspirate was less than an arbitrarily chosen 0.5 ml, a throat swab was taken (16). Serum was stored at −20° C and plasma was snap frozen at −70° C.

Laboratory Analysis

Virus serology and culture. Diagnosis of acute infection required a 4-fold rise in complement-fixing antibodies at paired acute and convalescent visits for respiratory syncytial virus (RSV), adenovirus, parainfluenza virus types 1, 2, and 3, influenza virus A and B, *Mycoplasma pneumoniae*, and *Chlamydia pneumoniae*. A 0.2-ml volume of nasal sample was inoculated onto monolayers of human diploid fibroblasts and rhesus monkey kidney cells at 33 and 37° C in duplicate tubes. Cytopathic effects were confirmed by acid stability testing for rhinovirus; by immunofluorescence for RSV, parainfluenza virus, and adenovirus; and by hemadsorption for influenza virus.

RNA extraction, reverse transcription and rhinovirus PCR, and sequencing. Samples were analyzed by a reverse transcription method with random hexamers followed by PCR as described (16). Negative and positive control samples were used for each run. Rhinovirus PCR was performed on all 168 exacerbation and 83 stable samples (Figure 1). Twenty-seven of the nasal aspirates used in this study were also used in a previous study solely to compare the detection rate of rhinovirus by nasal aspirates with that in induced sputum (16). A random 18 positive picornavirus PCRs were sequenced as previously described (16, 18).

PCR methods for other respiratory organisms. Methods for other respiratory organisms were based on multiplex PCRs for parainfluenza viruses 1–3 (19), nested PCRs for coronaviruses 229E and OC43 (20), RSV (21), *C. pneumoniae* (22), and influenza viruses A and B (23), and single-stage PCRs for the adenovirus group (24) and *M. pneumoniae* (25). These PCRs were carried out only on the last 120 exacerbation samples and 68 stable samples, as the first 48 exacerbation and 15 stable samples were lost due to a freezer breakdown after the rhinovirus PCRs were completed, but before the other PCRs were carried out.

Plasma fibrinogen and serum IL-6 estimation. Thrombin-clottable fibrinogen was measured as previously described (26). Serum IL-6 was estimated by a quantitative sandwich enzyme immunoassay (ELISA, R&D Systems, Oxon, UK).

Statistical Analysis

Normally distributed data were summarized by mean (standard deviation, SD), skewed data by median (interquartile range, IQR), and categorical data by percentages. Normally distributed continuous variables were compared by *t* test, otherwise the Mann–Whitney U test or Wilcoxon signed ranks test was used. Daily symptoms were binary coded and summed to give a total daily symptom count for each COPD exacerbation. Baseline total daily symptom count was the mean value of total daily symptom count over Days 14 to 8 before onset of exacerbation and the difference between this and the total daily symptom count at onset of exacerbation was called the change in total daily symptom count at exacerbation. Total daily symptom count recovery time was the time from exacerbation onset by which a 3-d moving average was equal to or exceeded the baseline value (5), and the ratio of this to the change in total daily symptom count at exacerbation was called the total daily symptom count recovery rate (5). Seasonal differences were examined by using a Wald equation (27). Statistical analysis was carried out with computer software SPSS (Chicago, IL) 10.0 for Windows.

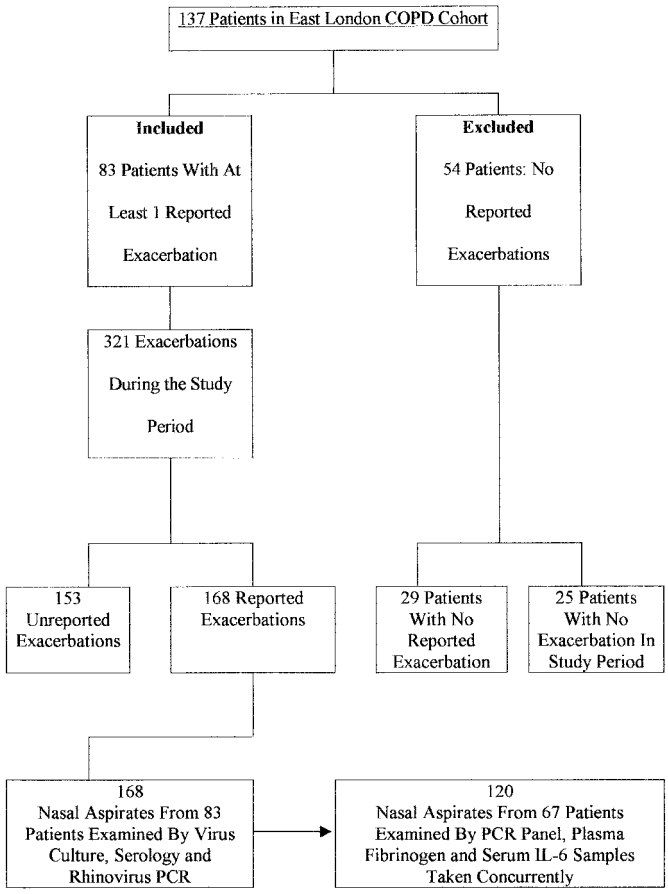

Figure 1. Study profile: flow of patients and exacerbations in the study.

RESULTS

Patients

Figure 1 shows that there were 137 patients monitored in the East London COPD Cohort during the study period, 83 patients had at least one reported exacerbation and were included in this study. Twenty-five patients had no exacerbations during the study period and 29 patients had exacerbations, but were not sampled because of failure to report exacerbations to the study team. These 29 patients had a total of 49 unreported exacerbations. The characteristics for all patients in the study are shown in Table 1. The 83 patients included in this study did not differ from the other 54 patients in any of the characteristics shown in Table 1. Eighty-one of the 83 patients used a mean (SD) 1.12 (0.75) g of inhaled steroid per day. Of the patients, 74% were immunized against influenza during each year of the study. Fourteen patients remained for less than 12 mo in the study because of the following: death, 6; personal reasons such as change of address, 5; and poor compliance, 3.

Exacerbations

The 83 patients had a total of 321 exacerbations over the 16 mo of the study or 2.9 exacerbations per patient per year of the study period. During the period of the study there were 168 (52.3%) reported exacerbations as shown in Figure 1. Reported and unreported exacerbations did not differ in any of the physiological or clinical parameters measured in this study, $p > 0.27$ in all cases. Thus there was no difference in severity between reported and unreported exacerbations.

TABLE 1. PATIENT CHARACTERISTICS FOR ALL PATIENTS IN THE STUDY (n = 137) AND THOSE WHO WERE SAMPLED DURING REPORTED EXACERBATIONS (n = 83)*

	n = 137 Patients		n = 83 Patients		
	Mean	SD	Mean	SD	p Value
Age, yr	67.7	8.1	66.6	7.1	0.07
FEV_1, L	1.07	0.45	1.06	0.61	0.55
FEV_1, %pred	41.3	18.5	42.4	19.8	0.51
FEV_1 reversibility, %†	3.10	4.30	2.90	5.1	0.51
FVC	2.45	0.80	2.44	0.63	0.79
PEFR, L/min	201	88	212	87	0.09
Pa_{O_2}, kPa	8.90	1.04	9.05	1.13	0.17
Smoking, pack-years‡	46.9	33.3	47.6	34.9	0.92
	n	%	n	%	
Sex, males	96	70.1	59	71.1	0.74
Smokers	45	34.6	28	33.7	0.78
Daily sputum production	75	54.9	49	59.0	0.22
Daily cough	68	49.6	45	54.2	0.19
Daily dyspnea	56	40.9	33	39.8	0.74
Daily wheeze	51	37.2	32	38.6	0.69

* Chronic stable symptoms were compared by χ^2 test.
† FEV_1 reversibility as percent predicted for age and height.
‡ (Number of cigarettes per day × number of years of smoking)/20.

At the time of presentation 128 (76%) exacerbations were associated with increased dyspnea, 69 (39%) with increased sputum purulence, 104 (62%) with increased sputum volume, 85 (51%) with colds, 83 (49%) with increased wheeze, 31 (18%) with sore throat, and 80 (48%) with increased cough. One hundred and seven exacerbations (64%) could be associated with a cold that occurred up to 18 d before onset of exacerbation. At presentation 33 exacerbations were associated with 3 major symptoms, 77 with 2 major symptoms, and 58 with 1 major symptom. For reported exacerbations, the median total daily symptom count recovery time was 8 (3 to 15) d and the median total daily symptom count recovery rate was 0.200 (0.090 to 0.375)/d.

Detection of Respiratory Viruses and Atypical Bacteria at Exacerbation

The median (IQR) time to sampling was 2.5 (1 to 5) d which was not related to virus detection (p = 0.75). Throat swabs were required in 62 reported exacerbations and was the sole sample in 26 exacerbations (15.4%). Table 2 shows that 66 (39.2%) of all reported exacerbations were associated with respiratory viruses or atypical bacteria detected by PCR, virus culture, or serology. All 18 randomly chosen picornavirus-positive PCRs sequenced were shown to be due to rhinovirus; thus it was likely that all PCR-positive picornavirus bands were due to rhinovirus and this was assumed to be true for purposes of analysis.

A total of 39 (23.3%) reported exacerbations or 58.2% of viral exacerbations were associated with rhinovirus; coronaviruses, 7 (4.2%); influenza A, 6 (3.6%); influenza B, 3; parainfluenza, 1; RSV, 19; adenovirus, 1; *C. pneumoniae*, 1; and *M. pneumoniae*, 0. In 11 (6.5%) exacerbations there was coinfection involving rhinovirus and another virus (coronavirus, 2; influenza A, 2; RSV [by serology], 1; RSV [by PCR], 6).

At least one respiratory virus at exacerbation was detected in 53 (64%) of the 83 patients in this study and these patients had a higher exacerbation frequency (geometric mean 3.1 versus 2.3 exacerbations per patient per year), p = 0.038.

Patient reported exacerbation rates were (reported exacerbations per patient = 1, number of patients = 40) as follows: (2, 22), (3, 7), (4, 9), (5, 3), and (6, 2). Of the 43 patients with 2 or more reported exacerbations, only 6 had the same virus at 2 different exacerbations. No virus was detected more than twice in the same patient at different exacerbations.

RSV detected by PCR was the only virus detected at a greater frequency when stable than during exacerbation (23.5 versus 14.2%), and thus from here on in the RESULTS we exclude RSV detected by PCR and present the latter later.

Virus Detection, Lung Function, and Symptom Recovery from COPD Exacerbation

Virus detection at exacerbation was unrelated to baseline age, sex, PEFR, FEV_1, FEV_1%pred, forced vital capacity (FVC), or baseline Pa_{O_2}, Pa_{CO_2}, or smoking habit, p > 0.190 in all cases. At presentation median (IQR) total daily symptom count for viral exacerbations was 3 (2 to 4) versus 2 (2 to 3) for nonviral exacerbations, p = 0.009. Table 3 shows that colds, sore throat, and increased dyspnea concurrently with a cold were associated with positive virus detection in nasal aspirates.

In the presence of viruses, the median daily total symptom count recovery time was 13 d (IQR, 5 to 20 d; n = 45), which was longer than the 6 d (3 to 13 d; n = 105) for nonviral exacerbations. Similarly, the median total daily symptom count recovery rate was slower (0.143 versus 0.222/d) in the presence of viral infection, p = 0.031. Recovery times could not be calculated where data was missing for 18 of the 168 exacerbations. Figure 2 illustrates the effect of viral infection on total daily symptom count recovery time.

Virus Detection, Plasma Fibrinogen, and Serum IL-6 at COPD Exacerbation

Plasma fibrinogen and serum IL-6 were available for the last 120 consecutive exacerbations with corresponding baselines in

TABLE 2. VIRUSES OR ATYPICAL BACTERIA DETECTED IN NASAL ASPIRATES DURING 66 OF 168 EXACERBATIONS REPORTED BY 83 PATIENTS WITH COPD*

	Detection Method			Total
Virus	PCR	Culture	Serology	Detected
Rhinovirus	39	2	ND	39
Coronavirus	7	ND	ND	7
Influenza A	0	1	5	6
Influenza B	2	0	3	3
Parainfluenza	1	0	0	1
Adenovirus	0	1	0	1
Chlamydia pneumoniae	1	ND	0	1
RSV	17	0	2	19
Mycoplasma pneumoniae	0	ND	0	0

Definition of abbreviation: ND = not done.
* The predominant virus was rhinovirus, occurring in 39 viral exacerbations (58.2%).

TABLE 3. EFFECT OF SYMPTOMS AT PRESENTATION ON DETECTION OF VIRUSES IN NASAL SAMPLES DURING 168 EXACERBATIONS IN 83 PATIENTS WITH COPD*

Symptom at Presentation	Odds Ratio	p Value
Colds†	3.55	< 0.001
Increased dyspnea and colds†	3.27	0.001
Sore throat	2.27	0.043
Increased sputum volume	1.59	0.182
Increased dyspnea	1.38	0.420
Increased purulent sputum	1.31	0.421
Increased cough	1.22	0.552
Increased wheeze	1.09	0.786

* Odds ratios calculated from univariate logistic regression with virus detected as the outcome variable. See text for details.
† Colds, Increased nasal congestion and/or increased rhinorrhea.

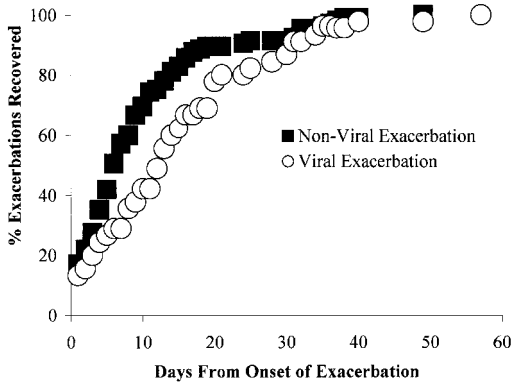

Figure 2. Graph showing the cumulative percentage of viral and nonviral exacerbations recovering with respect to time after onset during 150 COPD exacerbations. Recovery times could not be calculated for 18 exacerbations (see text for explanation). (p = 0.006, Mann–Whitney U test.)

67 patients. Both plasma fibrinogen and serum IL-6 have been shown to rise during COPD exacerbation (26). Mean plasma fibrinogen rose by 0.56 g/L during viral exacerbations, and 0.27 g/L in nonviral exacerbations; difference was 0.29 g/L (95% confidence interval, −0.01 to 0.58 g/L), p = 0.056. Also, during viral exacerbations, median (IQR) serum IL-6 rose to 8.30 (1.80 to 12.10) pg/ml, which was greater than the median serum IL-6 concentration of 5.50 (1.30 to 8.95) pg/ml seen in nonviral exacerbations, although the difference just failed to reach statistical significance (p = 0.064).

Stable COPD and Respiratory Virus Detection

All 83 patients, who had at least 1 reported exacerbation, had stable nasal aspirates and serum that were all negative for respiratory viruses by virus culture and serology. Sixty-eight of these patients had paired nasal aspirate and blood samples during stable COPD and these samples were analyzed by PCR for the various respiratory viruses and inflammatory markers, respectively. Viruses were found by PCR in a total of 11 (16.2%) of these 68 samples from patients with stable COPD (excluding RSV detected by PCR, which is discussed later) with rhinoviruses in 5, coronaviruses in 4, and parainfluenza virus and *Chlamydia pneumoniae* occurring in 1 patient each. Comparison with results at exacerbation showed that five of these patients had the same virus at stable and exacerbation sampling: one *C. pneumoniae*, three parainfluenza, and three either coronavirus or rhinovirus.

There was no relation between virus detection in stable COPD and age, sex, FEV_1, FEV_1%pred, season during which sample was taken, or presence of single symptoms (whether upper or lower respiratory tract) during the 4 wk preceding sampling, p > 0.20 in all cases. In particular, there was no relationship between virus detection in stable COPD and the prior occurrence of single respiratory symptoms up to 1 mo before sampling. When all baselines were used (n = 83 samples from 83 patients), patients in whom viruses were detected (n = 11) gave a history of more frequent exacerbations than those in whom no virus was detected (4.0 [2.0–7.0] versus 2.5 [1.0 to 4.0]), p = 0.043.

Respiratory Syncytial Virus Detection by PCR in Patients with COPD

RSV detection at COPD exacerbation. Detection of RSV was not related to any of the parameters discussed above at COPD exacerbation apart from recovery time, which was still longer during viral exacerbations that included RSV (p = 0.011), but not in exacerbations involving RSV alone. These data suggest that detection of RSV by itself during an exacerbation was not related to the severity or time course of the exacerbation.

RSV detection during stable COPD. RSV was also detected in 16 (23.5%) of the stable COPD patients (n = 68), including 3 (27.5%) of the 11 samples in which other respiratory viruses were detected. In view of the high rate of detection in both exacerbation and stable samples, and the high sensitivity of the PCR (one virus copy) (21), these results were interpreted as likely to represent detection of low-grade asymptomatic infection. To investigate whether some of the exacerbation samples had high levels of virus load (thus indicating acute infection) relative to the stable samples, repeat RSV PCRs were carried out with reducing numbers of cycles to determine rates of detection at reduced sensitivities. With 25 and 23 cycles in the first and second rounds, respectively, the detection rates were decreased, but exactly in proportion to those observed with higher cycle numbers (8% in stable COPD and 5% during COPD exacerbation), indicating that there was no association of exacerbation samples with higher virus loads and that these results were indeed likely to reflect low-grade infection.

Table 4 shows that mean plasma fibrinogen was elevated in patients in whom RSV was detected when stable, as was median serum IL-6 and median arterial carbon dioxide. There was a tendency for these patients to have smoked for more years 59 (33–65 yr) as compared with the non-RSV patients (35 [20–63] yr), p = 0.065, although pack-years of smoking was unrelated to plasma fibrinogen. RSV detection was not related to season during which the sample was taken (p > 0.24, Wald test) or to FEV_1 or FEV_1%pred.

RSV detection during either exacerbation or stable COPD. Four (25%) of the 16 patients who were RSV positive at stable COPD were also RSV positive at COPD exacerbation. Twenty-five (36.8%) of the 68 patients were positive for RSV either at exacerbation or at stable sampling, and 43 (63.2%) of patients were negative both at baseline and at exacerbation. Patients who were RSV positive at least once (n = 25) had greater stable plasma fibrinogen (4.18 [0.78] vs. 3.76 [0.72] g/L, p = 0.028) and higher stable median arterial CO_2 (6.72 [6.29 to 8.03] vs. 6.10 [5.71 to 6.83]) kPa, p = 0.021].

DISCUSSION

This study has investigated the relationship between respiratory virus infection, COPD exacerbation, symptoms and phys-

TABLE 4. PARAMETERS RELATED TO RSV DETECTION IN 68 PATIENTS WITH STABLE COPD*

	RSV		
	−	+	p Value
N	52	16	
Fibrinogen, g/L			
Mean	3.82	4.24	0.05
SD	0.77	0.66	
IL-6, pg/ml			
Median	3.75	8.5	0.018
IQR	2.33–5.80	3.50–9.70	
Pack-years			
Median	35	59	0.065
IQR	20–63	33–65	
Pa_{CO_2} kPa			
Median	5.71	6.34	0.014
IQR	5.43–6.29	5.77–6.82	

Definition of abbreviations: IQR = interquartile range; SD = standard deviation.
* Unpaired *t* test for fibrinogen; Mann–Whitney U test for other parameters.

iological changes. We have shown that almost 40% of acute exacerbations of COPD are associated with respiratory virus infection. This is a much higher detection rate than has been found in other studies in which virus culture and virus serology alone have been used (8–11), including two studies in which detection rates of 19 and 14% were found in COPD exacerbation (28, 29). Of the viruses found, rhinovirus is the most common, occurring in 58% of viral exacerbations. Increased dyspnea, cold symptoms, and sore throat were associated with viral exacerbations. Exacerbations associated with change in sputum characteristics (increased volume or purulence) at presentation were not related to the detection of viruses in nasal secretions. Viruses were associated with prolonged symptom recovery from COPD exacerbation and exacerbation frequency. There was also evidence of low-grade infection in stable COPD in that viruses other than RSV were identified in 16% of stable patients by PCR and RSV was identified in 24%. RSV was the only virus found in a greater proportion of patients with stable COPD than patients experiencing COPD exacerbation. Patients with virus detection when stable were more likely to have a greater concentration of stable plasma fibrinogen and serum IL-6 and increased exacerbation frequency than those in whom the virus was not detected. These data indicate that chronic viral infection may play a role in stable COPD.

In this study there were 321 COPD exacerbations, of which 52% were reported and thus sampled for respiratory viruses. This reporting rate is consistent with our previous experience (3). Because unreported exacerbations were similar in lung function and symptom changes and recovery times to reported exacerbations, our conclusions may be generalized to all COPD exacerbations in this cohort.

The PCR technique used for rhinovirus detection in this study used primers against the picornavirus group validated in previous work (12, 17). Although all picornavirus PCR-positive specimens were not sequenced, a random 18 specimens were sequenced and all were positive for rhinovirus; thus it is likely that all picornavirus PCR-positive bands were due to rhinovirus. Rhinovirus was the predominant respiratory virus detected in nasal samples in this study. In addition, we also detected influenza viruses during nine viral exacerbations (16.4%), although 74% of our patients had received influenza immunization before the study. Thus influenza vaccination may have helped to reduce the impact of influenza on this cohort.

Rhinovirus infection is widespread in the community and the major cause of the common cold. A previous study of elderly patients showed that lower respiratory tract syndromes of rhinovirus or unknown etiology were associated with greater health burden, compared with those associated with other known respiratory viruses (30). Previous studies of picornavirus infections in adults have found that rhinovirus is mainly associated with respiratory tract infection and few enteroviruses were detected (13, 30–32). Thus it is unlikely that enteroviruses contributed significantly to exacerbations in this study. Coronaviruses were associated with a proportion of exacerbations similar to influenza virus but parainfluenza virus was associated with only 0.6% of exacerbations, consistent with previous work (13). *Chlamydia pneumoniae* was detected by PCR in one COPD exacerbation (0.6%), similar to previous results using serological techniques (33). We found no evidence of *M. pneumoniae* infection, consistent with previous findings in adults with asthma (13) and patients with COPD (11).

In this study we have demonstrated that viruses are detected in almost 40% of exacerbations of COPD. We believe for a number of reasons that this may be an underestimate of the true importance of respiratory viral infection. First, sampling in this study was carried out within 48 h of the occurrence of COPD exacerbation. It is well recognized clinically that colds precede exacerbations of lower airway disease by a number of days and that late sampling for viruses may result in poor detection rates. We therefore believe that by sampling only when exacerbations had already started, a number of virus detections may have been missed as a result of late sampling. While 51% of exacerbations were associated with colds at the time of reporting, a retrospective look at the diary cards revealed that in a further 13% of exacerbations a cold had occurred between 6 and 18 d before onset of exacerbation. Thus 64% of exacerbations were associated with cold symptoms and are therefore likely to have been precipitated by viruses. Second, there is increasing evidence that rhinoviruses directly infect the lower airway (34–37). We have shown that rhinovirus can be detected in induced sputum at COPD exacerbation and that frequency of detection in sputum was greater than in nasal aspirates taken from the subjects at the same time (16). These data confirm that lower airway respiratory viral infection occurs with rhinoviruses, and taken together suggest that viruses would likely have been detected more frequently if upper airway sampling had been carried out earlier, at the peak of upper respiratory symptoms, and if lower airway sampling had been done at the time of lower respiratory symptoms.

Exacerbations were associated with a median recovery time of 8 d. Viruses were associated with a longer median symptom recovery time of 13 d compared with 6 d for nonviral exacerbations. When the change in total symptom score was taken into account and a symptom recovery rate was calculated, viruses were found to be associated with a slower rate of symptom score resolution. Previous studies have found weak relationships with lung function changes but have not considered symptom changes and recovery to baseline (8, 10, 38). Thus viruses are associated with more severe exacerbations and with greater morbidity. Further, most virus infections are generally more common during the winter, when hospitalization for COPD exacerbation is more frequent. Thus measures to prevent viral infection may be of importance in patients with COPD.

Fibrinogen and IL-6 are acute-phase proteins and have been shown to rise acutely at COPD exacerbation (26). IL-6 was higher during viral exacerbations than during exacerbations in which viruses were not detected. Plasma fibrinogen is synthesized in the liver in increased amounts during the acute-phase response and this is mediated by the cytokine IL-6. Respiratory virus infection can increase IL-6 production by blood monocytes and thus increase the plasma fibrinogen levels (39). IL-6 is released from bronchial epithelial cells after rhinovirus infection and has been found to rise during COPD exacerbation (6, 35, 36). Local production of IL-6 in the lung may also lead to an increase in plasma fibrinogen levels by the liver, which can predispose to thrombosis at distant sites (26). Thus virus infection may predispose to thrombotic events in the systemic circulation.

Viruses apart from RSV were detected in 16.2% of patients with stable COPD by PCR, with rhinoviruses and coronaviruses most common. We have shown that the detection of viruses in stable COPD was not related to the occurrence of single symptoms in the 4 wk before sampling and thus was unlikely to represent recent acute (nonexacerbation) infection. There was a tendency for patients in whom these viruses were detected in stable COPD to give a history of more frequent exacerbations in the year before recruitment. RSV was the only virus detected in a larger proportion of patients with stable COPD (23.5%) than in patients experiencing COPD exacerbation (14.2%). In 25% of patients in whom RSV was detected in stable COPD, this virus was also detected at COPD exacerbation. However, in none of these samples was RSV de-

tected by virus culture or virus serology, suggesting that the virus may have been present at low levels. The fact that RSV detection also disappeared from exacerbation and stable samples similarly when the sensitivity of the PCR was reduced supports a hypothesis that there may be chronic low-grade infection with RSV in these patients. Further, the patients in whom RSV was detected in stable COPD, or at least once either during stable COPD or COPD exacerbation, were more likely to have higher stable plasma fibrinogen or higher stable serum IL-6. These patients were also more likely to be hypercarbic. Thus our findings for the first time suggest a relationship between low-grade virus infection and disease severity in stable COPD.

We have shown that substantial proportions of COPD exacerbations are associated with acute respiratory viral infection and that viral exacerbations have a prolonged recovery phase. The prevention or early treatment of viral infection in patients with COPD may attenuate the severity and frequency of COPD exacerbations and should lead to a decrease in health burden and thus an improvement in health-related quality of life (3). We have also presented evidence that virus infection may cause chronic infection in patients with COPD and that this may be related to disease severity. The health impact of preventing viral infection in COPD requires future exploration.

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