A community-based, time-matched, case-control study of respiratory viruses and exacerbations of COPD

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Respiratory tract infections;
Respiratory viruses

Summary
Respiratory viruses are associated with severe acute exacerbations of chronic obstructive pulmonary disease (COPD) in hospitalized patients. However, exacerbations are increasingly managed in the community, where the role of viruses is unclear. In community exacerbations, the causal association between viruses and exacerbation may be confounded by random fluctuations in the prevalence of circulating respiratory viruses. Therefore, to determine whether viral respiratory tract infections are causally associated with community exacerbations, a time-matched case-control study was performed. Ninety-two subjects (mean age 72 yrs), with moderate to severe COPD, (mean FEV1 40% predicted), were enrolled. Nasopharyngeal swabs for viral multiplex polymerase chain reaction and atypical pneumonia serology were obtained at exacerbation onset. Control samples were collected in synchrony, from a randomly selected stable patient drawn from the same cohort.

In 99 weeks of surveillance, there were 148 exacerbations. Odds of viral isolation were 11 times higher in cases, than their time-matched controls (31 discordant case-control pairs; in 31 pairs only the case had virus and in three pairs only control). Picornavirus (26),
Introduction

Patients with chronic obstructive pulmonary disease (COPD), particularly those with moderate or severe disease (GOLD stage 2–illness), often experience frequent debilitating exacerbations during the course of their disease. From the patient’s perspective, acute exacerbations of COPD (AECOPD) result in acute ill health and reduced quality of life, especially the ability to perform normal activities and can contribute to accelerated decline in lung function and loss of lean muscle mass. AECOPD are also a major burden on the health care system, especially during the winter season. As much of the cost of AECOPD stems from complications, there is increasing interest in the community management of this disease to optimize outcomes and minimize costly admissions.

It is believed that the majority of AECOPD are caused by respiratory infections although exposure to respirable particulates in polluted air, pulmonary thromboembolism and other, unknown causes are also implicated. Viral respiratory infection is strongly suspected to cause AECOPD. Previous longitudinal, community-based and hospitalization studies have found that viral infections are common (39–56%) in patients with AECOPD and are most usually associated with severe presentations. Most recently, Papi and colleagues have demonstrated the association of proven viral infection with worsening of cellular and biochemical indices of inflammation. While these studies do not formally exclude bystander effects, they provide very strong evidence that viral respiratory infection is an important cause of severe AECOPD requiring hospitalization and suggest that viral infection is a cause of AECOPD in the community. In contrast, Hurst and colleagues recently studied common cold, assessed by coryzal symptoms, and AECOPD frequencies in the community, by comparing diary cards with spirometry. They concluded that exacerbation frequency is associated with an increased symptomatic cold frequency. This study does not, however, control for other causes of coryzal symptoms such as allergic rhinitis that are prevalent in the Australian context. As there may be regional differences in the type of viruses circulating through a community, it was relevant to explore the spectrum of respiratory viruses associated with AECOPD in an Australian community cohort.

To address this limitation in our understanding, we have established a long-term longitudinal cohort in Melbourne, that allows rigorous case control methodology, to address whether respiratory viruses are important causes of community AECOPD. This paper addresses the research question; is isolation of a respiratory virus more strongly associated with AECOPD than stable disease, in community-dwelling patients chosen at the same time, from the same COPD cohort. A particular advantage of the time-matched case-control method, where cases (AECOPD) are matched with controls at the time of exposure, is that it can accommodate fluctuations in the type, virulence and prevalence of respiratory viruses passing through the community.

Our data demonstrate that viruses are a major cause of AECOPD, but also that virally mediated AECOPD are not invariably severe enough to warrant hospitalization. Our data may therefore contribute to understanding the natural history of COPD and optimizing patient care in community health networks.

Methods

The study, designated the Melbourne Longitudinal COPD Cohort study, has been established as an open cohort of participants with COPD to investigate the aetiology and pathophysiology of COPD and its exacerbations.

Participants

Figure 1 summarizes recruitment to the cohort in 2003–2005. Inclusion criteria were diagnosis of COPD according to GOLD criteria stage II–IV, smoking history of greater than or equal to 10 pack-years, at least one inpatient admission with an exacerbation of COPD in the past 24 months, age less than 85 years and willingness to give informed consent. Patient characteristics are shown in Table 1. Exclusion criteria were living more than 50 km from the hospital, predominant asthma (FEV₁ reversibility ≥15% on spirometry), lung cancer, a primary diagnosis of bronchiectasis or idiopathic pulmonary fibrosis, chronic systemic inflammatory conditions such as rheumatoid arthritis, renal failure requiring dialysis and patients identified as requiring palliative care. The Human Research Ethics Committee (HREC) of Melbourne Health approved the study and written informed consent was obtained from all subjects.

Study design

The study design was a time-matched case control study in which an individual with an exacerbation was matched with a control randomly selected from within the cohort who was not exacerbating at that time. The study was powered to assess the primary outcome of association of viral respiratory infection with AECOPD.

COPD exacerbations were defined by the Anthonisen criteria: Type I as an increase in dyspnoea, sputum volume and sputum purulence for more than 24 h, Type II as any two...
of the above symptoms and Type III as one of the above symptoms accompanied by sore throat and nasal discharge within 5 days, fever without other cause, increased cough and an increase in respiratory rate or heart rate 20% above baseline values. Severity of exacerbations was defined according to the Burge and Wedzicha (2003) consensus report. At resolution, the exacerbation severity was coded as the maximum severity of each episode according to the consensus criteria. Stable COPD was defined as no requirement for increased treatment above maintenance therapy (other than bronchodilators) for 30 days.

**Identification of exacerbations**

Identification of exacerbations at an early stage was achieved by use of individualised patient action plans that included information about symptoms and instructions to contact the study team when key symptoms developed (increased dyspnoea, increased wheeze, decreased exercise tolerance, increased cough or change in sputum colour, rhinorrhea, nasal congestion, sore throat, myalgia or headaches, fever and or chills). This was further reinforced by fortnightly phone contact. Resolution of an exacerbation was defined as completion of treatment with antibiotics and increased steroids, and return of symptoms to baseline levels for 48h. If symptoms had not fully resolved within 30 days of exacerbation onset, symptoms needed to be stable for 48h and not require further acute treatment. A missed exacerbation was defined as symptoms not reported within 7 days of onset.

**Data and sample collection**

At baseline, demographic data, spirometry, carbon monoxide diffusing capacity (DLCO), 6-min walk test, MRC Dyspnoea Scale, St. George Respiratory Questionnaire and clinical examination data were obtained. The BODE Index at recruitment was calculated. Baseline pathology samples consisted of nose and throat swabs for respiratory virus multiplex PCR, atypical pneumonia serology, sputum (if available) for bacterial culture and serum for measurement of inflammatory indices (differential white cell count (WCC), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP)).

**Cases**

During exacerbations nose and throat swabs were collected on the day of identification and again 5–7 days later. Respiratory multiplex PCR was repeated at day 30–60 once the exacerbation had resolved.

**Controls**

Identical respiratory PCR samples were obtained from controls within 5 days of the identification of the case.
The controls were identified from within the cohort using computer generated random number selection from among the currently stable COPD patients, who did not carry the same virus as the case at recruitment.

### Pathogen detection

Respiratory virus multiplex PCR and atypical pneumonia serology were performed at the Victorian Infectious Disease Reference Laboratory (VIDRL, a WHO virology reference laboratory). The following viruses were screened: influenza A and B, picornavirus, respiratory syncytial virus (RSV), parainfluenza and adenovirus. Previous studies conducted by VIDRL have established that the multiplex PCR has high sensitivity and specificity for detection of these viruses.

Blood for atypical pneumonia serology was obtained on day one and thirty of each exacerbation and screened for Influenza A and B, Chlamydia pneumoniae and C. psittaci, Legionella pneumophila and Mycoplasma pneumoniae. An exacerbation associated with an atypical pathogen was defined as at least a four-fold rise in serum antibody titre between the day one and day thirty specimens. Atypical serology was performed by VIDRL Serology Laboratory, using the following assays; Legionella spp. using an in-house indirect fluorescence antibody (IFA) assay developed according to standard procedures. Chlamydia spp. specific serum IgG antibodies were detected using SeroELISA™ Chlamydia-IgG Enzyme-linked Immunosorbent Assay (ELISA), and C. pneumoniae, C. trachomatis and C. psittaci were identified using Chlamydia IgG SeroFIA™ IFA test kits (Savyon Diagnostics Ltd, Ashdod, Israel). M. pneumoniae was detected using SERODIA®-MYCO 11 particle agglutination test (Fujirebio Inc. Tokyo Japan), and IgM antibodies were detected by ELISA using SeroMP™ (Savyon Diagnostics Ltd). Influenza A and B antibodies were detected using an in-house complement fixation antibody test developed by VIDRL Serology Laboratory, in accordance with standard diagnostic methods.

Spontaneously expectorated sputum samples were obtained on day 1–5 after onset of AECOPD. Respiratory bacteria were identified by sputum microscopy and culture performed by Melbourne Pathology according to standard clinical laboratory procedures. Bacterial load was determined using semi-quantitative methods.

### Measurement of inflammatory serum markers

Measurement of the WCC, ESR and CRP were performed by the clinical pathology service. Serum CRP was performed by Melbourne Pathology, using an automated spectrometer (Olympus AU2700) and Olympus immuno-turbidimetric test, manufactured by Olympus Diagnostic GmbH (Irish Branch). Limits of detection were 2–300 mg/L, and values > 300 mg/L were obtained by automated onboard dilution.

### Measurement of symptoms

Severity of respiratory symptoms was scored using the MRC dyspnoea scale, the Borg dyspnoea score when performing routine daily activities and the Symptom Severity Index. AECOPD were confirmed by calculating the change in symptom severity from patients’ own stable baseline values. A score for the presence of viral symptoms was calculated on day 1 and 5 of each AECOPD. This measured the following symptoms; rhinorrhea, nasal congestion, sore throat, myalgia or headaches, and subjective fever, chills or rigors. Each symptom was recorded on a scale of zero (absent symptom) to three (severe).

### Statistical analysis

Viral infection was defined as a respiratory virus identified on day one and/or day five by respiratory PCR of nose and throat swabs. To determine whether a virus was more likely to be associated with an exacerbation (Case) or stable COPD (Control), a matched pair analysis of case-control pairs was performed and an odds ratio with 95% confidence interval was calculated (Table 2). McNamara’s chi square test for paired samples was used to measure the significance of the association.
Paired conditional logistic regression was performed to test whether the odds of virus detection was equivalent in case and controls. Potential predictors tested using univariate analysis were; age, sex, smoking status, disease severity, COPD sub-type and functional status (measured by BODE Index).

To account for varying periods of follow-up, person-weeks of observation were calculated for the surveillance periods in 2003 and 2004–2005. The incidence of atypical pneumonia infections was calculated as incidence per time at risk (incidence density). The total person-weeks of observation, minus the time in which participants had exacerbations associated with atypical organisms, was used as the denominator. The incidence density of atypical organisms is reported as incidence per 1000 person-weeks of observation.

Measurements of inflammatory indices were right skewed and were log-transformed prior to further analysis, (chi square tests were used to test the distribution was normal). To test whether there was an acute change in levels of inflammatory indices (CRP, ESR) at AECOPD onset, inflammatory indices were compared between stable baseline and AECOPD onset using paired t-tests. Results are presented as the geometric mean ratio of AECOPD onset to stable baseline values.

All analyses were performed using STATA Version 8.2.

Results

Patients

Characteristics of the study participants are shown in Table 1, (additional baseline data Supplementary Table 3). Twenty-nine percent were on long-term home oxygen. Mean pack years of smoking were 45 (Range 10–160) and 22% of patients were current smokers. Eighty-seven percent had received annual influenza vaccination for several years and were current for the multivalent pneumococcal vaccine. Twenty-eight (30%) patients who were colonized with potentially pathogenic bacteria and had frequent AECOPD (greater than 2.5 per year) were referred for diagnostic High Resolution Computerized Tomography Scan (HRCT), changes consistent with bronchiectasis were identified in 13.

Exacerbations

Patients were monitored over 3 years; from July to December 2003 (Winter–Spring) and from August 2004 to December 2005. The total number of person-weeks of observation was 4289. The median number of weeks of monitoring was 47 weeks per patient (range 1–99 weeks). There were 148 exacerbations; 63% patients had at least one exacerbation and 44% had more than one exacerbation (range 2–6 exacerbations). The mean number of exacerbations per patient per month was 0.14 and exacerbations per patient per year 1.68. Time from symptom onset to sampling was short (mean 2.4 days). Sixty-four percent of patients with exacerbations contacted the study staff, while the rest were identified on routine fortnightly phone call. Thirty-eight percent of AECOPD were mild, 43% moderate and 19% severe.

Eighty percent of AECOPD were treated in the community with oral antibiotics and/or oral corticosteroids. Table 3 summarises details of exacerbation type and severity.

Viruses at baseline

Adenovirus and RSV were detected by respiratory PCR from two patients at baseline. They did not report any symptoms of viral upper respiratory tract infection. All subsequent tests on these patients were negative.

Viruses during AECOPD

There was a total of thirty-three viruses detected by respiratory PCR at the onset of AECOPD; influenza A (3), picornavirus (26), parainfluenza 1, 2, or 3 (2), RSV (1) and adenovirus (1). Twenty-eight (84%) of viruses were detected on day 1 after symptom onset and an additional five picornaviruses were detected at day 5 after onset in patients who remained symptomatic. At the day 30 follow-up one patient remained colonised with picornavirus when stable.

Three time-matched controls had viruses detected; picornavirus (2) and adenovirus (1) although these patients had no symptoms other than slight nasal congestion or rhinorrhea. Respiratory viruses detected on day 1–5 of exacerbations in both cases and controls were included in the case-control analysis. There were 34 discordant pairs from 148 case-control pairs. The odds ratio was 11 (95%CI; 3.45–56.07, p<0.001). In 33 pairs the case was positive for virus and the control was negative and in three pairs the control had virus while the case had no virus. Results for the case control analysis are summarized in Table 2.

Paired conditional logistic regression demonstrated that none of the following were predictors of viral detection; age (p = 0.287), gender (p = 0.578), living situation

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Time-matched case-control analysis for infection with respiratory viruses.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Controls</td>
</tr>
<tr>
<td>Cases</td>
<td></td>
</tr>
<tr>
<td>PCR positive</td>
<td>0</td>
</tr>
<tr>
<td>PCR negative</td>
<td>3</td>
</tr>
</tbody>
</table>

Cases were patients suffering from AECOPD. Controls were time matched clinically stable patients drawn randomly from the same cohort. Positive means a respiratory virus was detected and negative means no virus was detected. The odds ratio for detection of a virus during AECOPD was 11 [95%CI; 3.45–56.07], (p<0.001).
Table 3 Characteristics of exacerbations.

<table>
<thead>
<tr>
<th>Total exacerbations</th>
<th>148</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean time to sampling*</td>
<td>2.4 days (1–12 days)</td>
</tr>
<tr>
<td>Exacerbations reported by patients</td>
<td>64%</td>
</tr>
<tr>
<td>Pathogens detected</td>
<td></td>
</tr>
<tr>
<td>Only virus</td>
<td>21</td>
</tr>
<tr>
<td>Only bacteria</td>
<td>30</td>
</tr>
<tr>
<td>Co-infection</td>
<td>12</td>
</tr>
<tr>
<td>Atypical organism</td>
<td>3</td>
</tr>
<tr>
<td>Virus detected in cases</td>
<td></td>
</tr>
<tr>
<td>Influenza A</td>
<td>3</td>
</tr>
<tr>
<td>Rhinovirus</td>
<td>26</td>
</tr>
<tr>
<td>Adenovirus</td>
<td>1</td>
</tr>
<tr>
<td>Parainfluenza 1, 2, 3</td>
<td>2</td>
</tr>
<tr>
<td>RSV</td>
<td>1</td>
</tr>
<tr>
<td>Bacteria detected in cases at onset</td>
<td></td>
</tr>
<tr>
<td>Hemophilus influenzae</td>
<td>16</td>
</tr>
<tr>
<td>Pseudomonas spp.</td>
<td>9</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>7</td>
</tr>
<tr>
<td>Moraxella catarrhalis</td>
<td>3</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>3</td>
</tr>
<tr>
<td>Stenotrophomonas maltophilia</td>
<td>3</td>
</tr>
<tr>
<td>Other Gram negatives</td>
<td>8</td>
</tr>
<tr>
<td>Atypical organisms on serology</td>
<td></td>
</tr>
<tr>
<td>Chlamydia pneumoniae</td>
<td>1</td>
</tr>
<tr>
<td>Chlamydia psittaci</td>
<td>1</td>
</tr>
<tr>
<td>Mycoplasma pneumoniae</td>
<td>1</td>
</tr>
<tr>
<td>Severity of exacerbations*</td>
<td></td>
</tr>
<tr>
<td>Mild</td>
<td>38%</td>
</tr>
<tr>
<td>Moderate</td>
<td>43%</td>
</tr>
<tr>
<td>Severe</td>
<td>17%</td>
</tr>
<tr>
<td>Life-threatening</td>
<td>2%</td>
</tr>
<tr>
<td>Anthonisen type*</td>
<td></td>
</tr>
<tr>
<td>Type I</td>
<td>33%</td>
</tr>
<tr>
<td>Type II</td>
<td>16%</td>
</tr>
<tr>
<td>Type III</td>
<td>51%</td>
</tr>
</tbody>
</table>

*Burge and Wedzicha Consensus Report. 18

(p = 0.328), current smoker or not (p = 0.165), diagnosis of chronic bronchitis (p = 0.544) or childhood asthma (p = 0.277). On the other hand, less severe disease (measured by FEV1 % predicted) was predictive of viral infection. The odds of having a positive viral PCR increased by 6% for each 1-unit improvement in FEV1 % predicted (OR 1.06, [95% CI: 1.03–1.09], p < 0.001). Functional status was also predictive PCR positivity with a 34% decrease in the odds of having a positive viral PCR per 1-unit increase in the BODE Index (OR 0.66, [95% CI 0.54–0.80], p < 0.001). All the above predictors were analysed using univariate regression models.

Contact patterns with children

Twenty-eight patients (30%) had AECOPD of viral aetiology confirmed by respiratory PCR. Eight patients (32%) of the cohort had regular contact (3–7 days per week) with infants or children less than 10 years of age. Seven (88%) of these patients had at least one PCR positive AECOPD. There were four patients who had multiple viral AECOPD confirmed by PCR, three of the four (75%) had regular contact with children.

Association between viral symptoms and rates of viral detection

A virus was identified on respiratory PCR in 21.0% of exacerbations overall and 31% of cases with three or more viral symptoms and an overall score of ≥4. The individual symptoms with the highest predictive values were the presence of rhinorrhoea (Odds ratio [OR] 4.86, [95% CI: 1.82–14.31], p = 0.004), and sore throat (OR 2.24, [95% CI: 0.93–5.55], p = 0.05). Other symptoms associated with viral infection were not statistically significant predictors of PCR positivity in this study; nasal congestion (OR 1.46, [95% CI 0.58–3.57], p = 0.37), fever or chills (OR 1.92, [95% CI 0.80–4.63], p = 0.11) and headaches or myalgia (OR 1.36, [95% CI 0.57–3.26], p = 0.45).

Bacteria at AECOPD onset

Patients were able to produce spontaneous sputum samples at the onset of 88 (60%) of AECOPD (Refer Supplementary Table 1). Bacteria cultured from spontaneous sputum samples obtained at AECOPD onset of 42 AECOPD (Day 1) are listed in Table 3. The most common organisms were Hemophilus influenzae (16) Streptococcus pneumoniae (7) and Pseudomonas spp. (9). Additional bacterial isolates obtained at Day 5–7 after AECOPD onset were H. Influenzae (7), S. pneumoniae (1), Pseudomonas spp. (5) Staphylococcus aureus (2), and other gram negatives (4).

Viral–bacterial co-infection

Eight (19%) of the 42 AECOPD that had a bacterial isolate on Day 1 also had a virus isolated on respiratory PCR and an additional four had both viruses and bacteria isolated by Day 5. The viral–bacterial coinfections were Influenza A and S. pneumoniae and H. influenzae (1), Picornavirus with Streptococcus pneumoniae (3), H. influenzae (4), Pseudomonas sp. (2) and Enterobacter sp. (1) and adenovirus with S. pneumoniae and Acinetobacter sp. (1). Thirty-six percent percent of exacerbations in which a virus was detected at onset developed secondary bacterial infection over the following 7 days. In 30 (71%) of AECOPD in which bacteria were isolated at onset, the patients had also reported some viral symptoms at AECOPD onset. Overall 78% of AECOPD in which H. influenzae was isolated in the first 5 days after onset were preceded by viral symptoms.

Atypical pneumonia organisms and AECOPD

There were three AECOPD associated with at least a four-fold rise in atypical pneumonia serology titres; C. pneumoniae (1), C. psittaci (1) and Mycoplasma sp. (1) These exacerbations were associated with a positive viral symptom score, including symptoms of sore throat, rhinorrhoea, fever...
and chills and myalgia. The incidence density of new infections with atypical organisms was low, at 0.70 per 1000 person-weeks. However, AECOPD associated with atypical organisms were severe or life threatening and had a prolonged recovery time.

**Community acquired pneumonia (CAP)**

This study did not exclude AECOPD complicate by CAP. Chest X-rays were clinically indicated in 26% of AECOPD, (criteria were; clinical signs of pneumonia, patients attending the emergency department or requiring inpatient admission). Review of these chest X-rays showed that six AECOPD were associated with acute collapse and consolidation. Organisms identified were Influenza A (1), *Moraxella catarrhalis* and *Pseudomonas aeruginosa* coinfection (1), *H. influenzae* (1). No organisms were identified in the other three cases.

**Clinical severity and inflammatory indices**

AECOPD associated with rhinovirus were nine (35%) mild, 15 (58%) moderate and two (7%) severe. AECOPD associated with RSV and adenovirus were mild, while one parainfluenza AECOPD was mild, and the other severe complicated by hypercapnic respiratory failure. The three influenza A AECOPD were of moderate severity, with the initial infection resolving within 2 weeks. At 3 weeks, two of these patients developed mixed viral and bacterial exacerbations that required inpatient admission.

Serum inflammatory indices were obtained at the onset of 121 (82%) of exacerbations. There was an acute rise in serum CRP levels at AECOPD onset compared to baseline. Table 4 summarises inflammatory indices according to the laboratory confirmed pathogens detected at AECOPD onset. As the distribution of CRP and ESR values was right skewed, data was log-transformed prior to further analysis, chi square tests confirmed that the natural log of these markers was normally distributed. At AECOPD onset there was an acute increase in serum CRP levels whether or not a virus was detected. In PCR positive AECOPD there was a 4.5 fold increase (geometric mean ratio 4.491 [95%CI: 2.256–8.939], p < 0.001) and in PCR negative a 2.7 fold increase (2.709 [95%CI: 1.958–3.747], p < 0.001).

**Discussion**

In this study, discordant case-control pairs were 11 times more likely to have the case positive for viral infection than the control positive. Only three time-matched controls were positive for virus. The present study demonstrates that infection with respiratory viruses, specifically picornavirus, parainfluenza and influenza, is strongly associated with the development of COPD exacerbations (AECOPD).

The need for rigorous infectious disease epidemiological study designs that can establish whether or not a novel virus is pathogenic in humans has been identified as an important methodological issue. The strength of this community-based, case-control study design is time-matching controlled for seasonal fluctuations in viral prevalence. This presents a solution to the problem of establishing causation when exposure patterns are random, seasonal or unknown. The estimated strength of the association between AECOPD and viral detection will probably change with the prevalence, type and virulence of virus circulating in a particular community. In the present study, the lower limit of the 95% confidence interval around the odds ratio is greater than three, establishing that exposure to respiratory viruses increases the risk of an AECOPD. The use of controls from within the cohort controlled for both susceptibility to viral infection and susceptibility to symptoms indicative of an AECOPD.

To establish that respiratory viruses have a causative role in triggering exacerbations it is essential to determine whether viruses are present at the onset of AECOPD. Previous studies of AECOPD requiring hospitalization have reported an association, but are not able to establish whether viral infection precedes the development of AECOPD. This is important because finding a virus in an established exacerbation (e.g. more than a week old) may reflect a secondary rather than a primary event. Basing the study in the community facilitated early identification of an exacerbation and measurement of potential triggers shortly after onset. We found that the majority of viruses were detected in samples obtained within two days of symptom onset and only a small number of viruses (five picornaviruses), could still be detected in exacerbating patients, 5 days after onset of viral symptoms.

<table>
<thead>
<tr>
<th>Pathogen identified</th>
<th>Viral PCR positive</th>
<th>Bacterial</th>
<th>Coinfection</th>
<th>No pathogen identified</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viral PCR positive</td>
<td>10.9 (4.9–30.1)</td>
<td>12.36 (7.9–19.2)</td>
<td>10.13 (4.9–16.7)</td>
<td>9.9 (5.9–18)</td>
<td>10.7 (4.9–30.1)</td>
</tr>
<tr>
<td>Neutrophils (10⁹/L)</td>
<td>8.39 (2.1–25.6)</td>
<td>9.7 (3–16.4)</td>
<td>7.48 (2.5–15.6)</td>
<td>7.3 (3–15.2)</td>
<td>8.2 (2.1–25.6)</td>
</tr>
<tr>
<td>Lymphocytes (10⁹/L)</td>
<td>1.74 (0.5–2.8)</td>
<td>1.3 (0.3–3.3)</td>
<td>1.32 (0.8–3.1)</td>
<td>1.5 (0.2–2.9)</td>
<td>1.4 (0.2–3.3)</td>
</tr>
<tr>
<td>Eosinophils (10⁹/L)</td>
<td>0.14 (0–0.6)</td>
<td>0.12 (0–0.7)</td>
<td>0.28 (0–1)</td>
<td>0.15 (0–0.5)</td>
<td>0.15 (0–1)</td>
</tr>
<tr>
<td>ESR (mm/1h)</td>
<td>27.9 (9–71)</td>
<td>36.46 (&lt;1–97)</td>
<td>32.11 (&lt;1–65)</td>
<td>33.19 (&lt;1–111)</td>
<td>33.02 (&lt;1–111)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>37.6 (&lt;2–160)</td>
<td>65 (&lt;2–341)</td>
<td>63 (&lt;2–171)</td>
<td>26 (&lt;2–226)</td>
<td>41 (&lt;2–341)</td>
</tr>
</tbody>
</table>

Mean and range of values for each test grouped according to pathogen detection on microbiological tests.

WCC, white cell count; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.
Respiratory viruses were detected at the onset of 21% of AECOPD, and all but one exacerbating patient had cleared the virus from the upper respiratory tract by day 30. The overall detection rates in this study were lower than those reported in other studies, this may relate to the time between symptom onset and patient report of symptoms or the detection technique used or the number of viruses circulating through our community at that time. The sensitivity and specificity of respiratory PCR for the detection of respiratory virus infection is better than traditional methods using viral culture and serology, particularly for viruses such as picornavirus,22,42 There remains some controversy over whether to use nasopharyngeal swabs,43 nasal lavage samples44 or induced sputum samples, for viral detection using PCR techniques. We chose to use nasopharyngeal swabs, as these are the standard specimens used by our diagnostic laboratory, are easily obtained in the community setting, and have the advantage of capturing virus in the upper respiratory tract shortly after exposure. In other studies viral detection in nasopharyngeal swabs is well correlated with infection.22,45 The strength of the case control methodology is that while some viral infections may have gone undetected using this technique, we were still able to establish a strong association between viral detection and the onset of AECOPD.

In our study picornavirus, RSV and adenovirus were detected in low numbers in stable COPD. Reported rates of virus detection in stable COPD vary, with chronic carriage of RSV, picornavirus and adenovirus identified in different patient cohorts.11,12,42 The London COPD cohort detected virus in 39% of stable COPD patients, with RSV accounting for 23% of these.12 In contrast, a recent study found that most RSV infections were associated with respiratory symptoms and measurable immune responses and did not find evidence for persistent RSV infection in stable COPD.43 As it is possible that viral mRNA may be retained for lengthy periods in induced sputum, in the absence of active infection this may also influence the higher detection rate reported in other studies.

Consistent with recent studies using PCR techniques, picornavirus was the most common virus associated with AECOPD in our cohort and was detected throughout the year.43–46 There were very low levels of influenza infection detected in the cohort, reflecting low to normal seasonal influenza activity and that 98% of the cohort was vaccinated against influenza. Influenza surveillance reports indicate that in 2003–2005, there was good vaccine coverage for circulating influenza strains in Australia.47,48 Detection rates of both picornavirus and parainfluenza increased in synchrony with increased detection of these viruses in the general community, as reported by influenza surveillance.47 Upper respiratory symptoms of viral infection (rhinorrhoea and sore throat) were predictive of PCR positivity while systemic symptoms were not, probably because infection with viruses such as influenza, that typically cause severe systemic illness were uncommon.

There was a trend for cohort patients with less severe disease and better functional capacity to have virus isolated. Possibly patients with better functional status were more active in the community, had higher rates of social contact, resulting in greater exposure to circulating respiratory viruses. It appears virus exposure rates, rather than severe lung function impairment, determined the likelihood of a viral AECOPD. This finding is supported by Hurst’s study that found that frequent exacerbations were associated with more frequent colds, rather than an increased susceptibility to exacerbate once infected with a cold.14 It is noteworthy that patients in our study with repeated viral infections, tended to be those providing childcare to infants or young children, indicating that children were one source of repeated exposure to circulating respiratory viruses. This is consistent with studies that have found a correlation between the rates of admission for acute respiratory illness in children and hospital admission for AECOPD,49,50 and that older adults and children are usually infected with the same circulating virus strain.51 Laboratory confirmed viral infection preceded the isolation of bacteria in 29% of AECOPD in which bacteria were isolated in the first five days after exacerbation onset. The association between influenza virus and bacterial infection is well documented.52–54 New evidence is emerging that other respiratory viruses interact with bacteria in the lower airway in a virus specific, cell-type specific manner.55 Recent clinical studies indicate that Picornaviridae may interact with new or colonising bacteria in the lower airway, resulting in increased bacterial load and heightened inflammatory responses.14,56 Measuring viral–bacterial interactions was not the primary aim of this study, however we did observe that confirmed viral infection or probable viral infection (based on symptoms) preceded 71% of laboratory confirmed bacterial infections in the first week after AECOPD onset and 78% of H. influenzae infections. This highlights the importance of studying the onset of AECOPD in the community setting, where initial exposures and infective triggers can be accurately measured. It is clear that studying hospitalised AECOPD alone will underestimate the importance of viral infection in triggering AECOPD and the effect of viral infection on host susceptibility to bacterial infection. Further research should address the impact of viral infections on the delicate balance between colonising bacteria and host immunity.

Given the feasibility of reaching patients rapidly after the onset of AECOPD and the suggestive evidence that that early therapeutic intervention may reduce burden of disease,57 it would be highly desirable to accurately and rapidly differentiate between viral and other causes of AECOPD. The capacity to identify exacerbations early and differentiate between viral pathogens by PCR, suggests the possibility of prospective trials of newer antiviral therapies in the management of AECOPD.58 Community-based studies are needed to evaluate whether effective early management of viral exacerbations may decrease the incidence of secondary bacterial infection and the overall severity and duration of the AECOPD episode.

This study demonstrates that the presence of virus in upper airway secretions is strongly associated with the development of AECOPD. The strength of the methodological approach used in this study is that it controlled for random and seasonal fluctuations in the levels of virus circulating in the community. By conducting this study in the community, we have shown that respiratory viruses can trigger the onset of AECOPD and that picornaviruses are the most common viral trigger for AECOPD. These data support the causative role of viruses in AECOPD.
Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rmed.2007.07.015.

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References

42. Victorian Infectious Diseases Laboratory (VIDRL). Fortnightly surveillance reports <>, 2005.