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Prevalence of viral infection detected by PCR and RT-PCR in patients with acute exacerbation of COPD: A systematic review

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ABSTRACT

Background and objective: **Viruses are important aetiological agents of acute exacerbation of COPD (AECOPD). Their reported prevalence varies from region to region. This systematic review calculated the prevalence of respiratory viral infections in AECOPD.**
Methods: **A systematic search was performed using Medline, and references of relevant articles and conference proceedings were hand searched. Articles for review were selected based on the following criteria: (i) prospective or cross-sectional study, (ii) original research, (iii) viral detection used the highly sensitive techniques of PCR and/or Reverse Transcriptase PCR (RT-PCR), (iv) viral prevalence in AECOPD defined, and (v) full paper available in English. We assessed the study quality and extracted data independently and in duplicate using a pre-defined data extraction form. Weighted mean prevalence (WMP) was calculated and a forest plot was constructed to show the dispersion.**
Results: **Eight studies met the inclusion criteria. The WMP of respiratory viral infection in AECOPD was 34.1% (95% CI: 23.9–44.4). picornavirus was the most commonly detected virus with WMP 17.3% (95% CI: 7.2–27.3), followed by influenza; 7.4% (95% CI: 2.9–12.0), respiratory syncytial virus; 5.3% (95% CI: 1.6–9.0), corona viruses; 3.1% (95% CI: 0.4–5.8), parainfluenza; 2.6% (95% CI: 0.4–4.8), adenovirus; 1.1% (95% CI: –1.1 to 3.3), and human metapneumovirus; 0.7% (95% CI: –0.3 to 1.8). Maximum WMP was observed in studies from Europe followed by the USA, Australia and Asia. Picorna was the most common virus detected in Western countries whereas influenza was most common in Asia.**
Conclusions: **This systematic review demonstrated that viruses are strongly associated with AECOPD, with the highest detection rates of viruses being in Europe. The geographical epidemiology of viruses may have important therapeutic implications for management of AECOPD.**

SUMMARY AT A GLANCE

Viruses are an important cause of acute exacerbation of COPD (AECOPD). This systematic review calculated the weighted mean prevalence (WMP) of respiratory viruses detected in patients with AECOPD. The overall WMP was 34.1% (95% CI: 23.9–44.4), and picornavirus was the most commonly detected virus with WMP 17.3% (95% CI: 7.2–27.3).

Key words: chronic obstructive pulmonary disease, polymerase chain reaction, respiratory virus.

INTRODUCTION

COPD is the sixth leading cause of death worldwide,[1] and the estimated population prevalence of COPD (Global Initiative for Chronic Obstructive Lung Disease Stage II and higher) is 10%.[2] The course of COPD is punctuated by episodes of acute deterioration in respiratory health, termed 'exacerbations', which account for much of the morbidity, mortality, hospital admissions and health-care costs associated with this condition.[3–5] It has been believed that the majority of exacerbations are associated with bacterial infection; however, as many episodes occur without any increase in the volume or purulence of sputum, it is also postulated that bacteria may be secondary invaders following a primary viral infection.[6] Recent studies demonstrating the presence of a virus vary widely in terms of the population studied as well as the technology used to detect the virus. Most studies have been conducted in Europe and the USA, and scant data are available from other parts of the world. Different techniques, such as serology, viral culture, PCR and RT-PCR have been used to detect viruses. Currently, PCR and RT-PCR are being widely

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used due to their better capacity for detection compared with conventional viral culture and serology.[7]

Prevalence and incidence studies are important for both health-care planning and epidemiological research as they estimate the burden of a condition within a population. The prevalence and geographical distribution of respiratory viruses in acute exacerbations of COPD (AECOPD) has not yet been clearly defined. This knowledge may assist in the management of these patients. The present study calculated the weighted mean prevalence (WMP) of respiratory viruses in AECOPD from prospective studies that utilized the most sensitive techniques (PCR and RT-PCR) to identify the presence of viruses.

METHODS

Search strategy

In January 2009, a systematic search was performed using Medline without language restrictions for articles and conference proceedings. The following MeSH terms were used: Chronic Obstructive Pulmonary Disease, Epidemiology and Virology. There were 1988 titles and abstracts screened and a preliminary list of 16 papers that could possibly describe viral prevalence in AECOPD was selected. Reference lists of retrieved articles were checked and provided a further three studies. The search continued until it was clear that no new references were being retrieved. As the study included previously published work, ethical approval was not sought.

Eligibility criteria

The purpose of this review was to identify methodologically rigorous studies that investigated the prevalence of respiratory viruses in AECOPD. Inclusion criteria are listed in Box 1.

Study selection

Study retrieval was conducted in duplicate by two independent reviewers in two stages. In stage one, titles and abstracts of retrieved records were screened to identify potentially relevant articles. In stage two, the full paper of the records selected in stage one were obtained for detailed evaluation. We determined the degree of inter-observer agreement for stage two and reported κ values based on 'eligible yes/no'. After making independent assessments, we resolved disagreements by consensus.

Box 1

Eligibility criteria for included studies
1. Prospective or cross-sectional study
2. Based on original research
3. Viral detection by highly sensitive techniques (PCR and RT-PCR)
4. Viral prevalence in AECOPD defined
5. Full paper available in English literature

Assessment of methodological quality

There are several validated tools for assessing quality in clinical trials but much less attention has been given to developing similar tools for observational epidemiological studies. Hence, we developed our own check-list for methodological quality assessment. The check-list has been developed keeping the STROBE statement in mind and also the needs of our study.[8] Two reviewers, working independently, assessed the quality of included studies. We dichotomized answers as 'yes' and 'no/unclear' for κ calculations (Table 1). We calculated κ values based on the total number of criteria met and subsequently resolved all disagreements by consensus.

Data extraction and analysis

All data on the methodology and results from each study included were extracted by two independent reviewers using pre-defined data extraction criteria. Information was recorded on the study's location, year of publication, number of subjects studied, technique used to identify viral infections, and the number of subjects found positive for seven common respiratory viruses: (influenza, parainfluenza (PIV), picornaviruses (includes rhino and entero virus), respiratory syncytial virus (RSV), coronaviruses,

Table 1 Quality assessment criteria

Criteria	Yes	No/unclear
1. Are the samples representative of diseases being studied?		
2. Is AECOPD defined well?		
3. Are selection criteria clearly described?		
4. Is the study setting described?		
5. Are the clinical and demographic characteristics of the studied population described?		
6. Is the severity of AECOPD in the studied population described?		
7. Is the prior vaccination coverage in the studied population described?		
8. Is the seasonal variation in prevalence mentioned?		
9. Is the sample collection and processing defined clearly?		
10. Is the technique used for viral detection defined clearly?		
11. Is the prevalence of individual viruses specified?		

AECOPD, acute exacerbation of COPD.

adenovirus and human metapneumovirus (hMPV)). The number of patients with multiple infections was also recorded. We calculated a WMP for the total respiratory viral prevalence in AECOPD and for individual virus separately. The weighted mean was calculated by dividing the sum of the product of prevalence (as percentage) and number of patients by the sum of the number of patients. Summary statistics were used to calculate 95% CIs for the prevalence and Forest plots were constructed to show the dispersion. For statistical analysis, SPSS for Windows 11.5 (SPSS Inc., Chicago, IL, USA) was used.

RESULTS

Study selection

Nineteen hundred and ninety-one articles (1988 identified from electronic searches, three additional articles from reviewing reference lists) were examined. Of these, 1983 were excluded either because they were not original descriptions of prevalence and incidence studies or did not investigate viral prevalence in AECOPD. Eighteen potentially eligible records were identified for further review based on a review of the title and abstracts ($\kappa = 0.95$). Eight records qualified for inclusion in this study and these underwent qualitative and quantitative assessment. The selection process for this review is summarized in Figure 1.

Characteristics of included studies

Brief characteristics of all the studies included for data synthesis are shown in Table 2. A total of 1134 patients studied between 2001 and 2008 were included. Four studies were published from Europe, two from the USA, one from Hong Kong and one from Australia.

Quality assessment of included studies

The assessment of quality based on the criteria listed in Table 1 gave an inter-observer agreement of 98.9% ($\kappa = 0.95$). Four of the eight studies did not describe the severity of AECOPD and viral aetiology.[9,12] Four studies failed to report or account for seasonal bias.[9,10,13,14] Three studies did not consider prior vaccination coverage for influenza, which is a potential confounder.[12,14,15] Although the severity of AECOPD has been shown to be associated with viral aetiology, three studies did not stratify viral prevalence according to severity.[11,12,14] The mean of number of quality assessment criteria met by individual studies was 9.4.

Heterogeneity

There was substantial heterogeneity between studies with regard to the viral detection technique used, sample collection and the characteristics of the subjects studied. Four studies had used RT-PCR as the viral detection tool,[11,12,14,15] while the remaining four had used PCR.[9,10,13,16] Studied subjects had variable

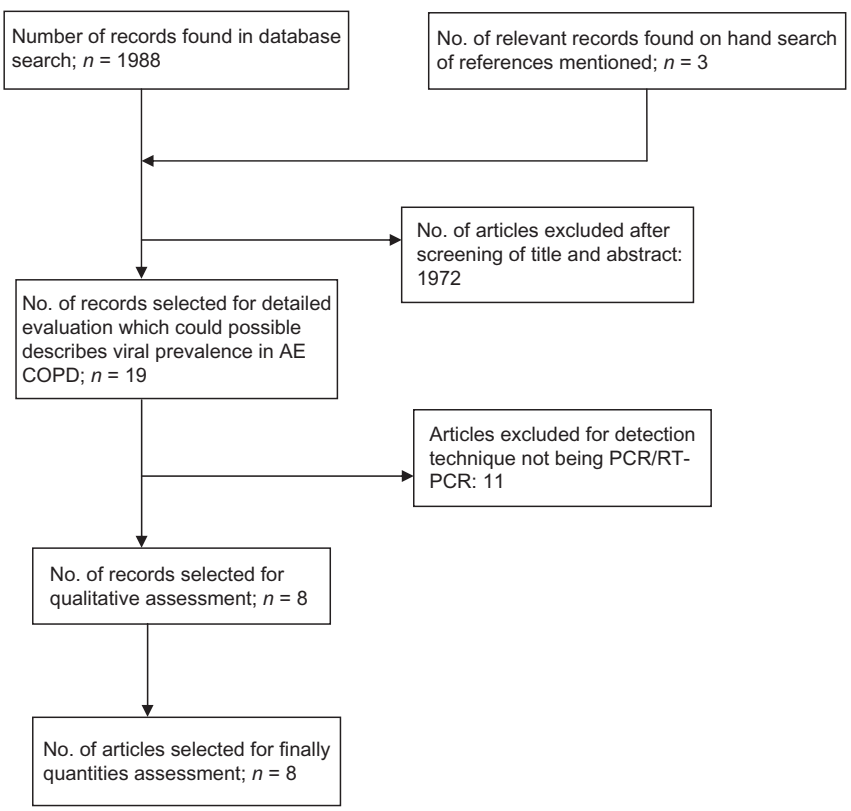

Figure 1 The selection process for the systematic review.

Table 2 Brief characteristics of all the included studies

Author, country (publication year)[ref]	No. in cohort (%men)	Mean age, years (SD/range)	Current, ex-smoker (%), mean pack-years (SD/range)	FEV_1 (L); mean (SD)	Quality assessment score	Detection technique	Influenza vaccine coverage (%)	Most common virus	Remarks
Ko *et al.*, Hong Kong (2007)[8]	262 (81.6)	75.7 (7.5)	16.8, 82.1, —	0.8(0.4)	10	PCR	40.3	Influenza	Viral infection related to severe airway obstruction No reduction in influenza-A in vaccinated patients
Beckham *et al.*, USA (2004)[9]	96 (49.0)	63.1 (9.2)	35.0, 49.0, —	—	9	RT-PCR	73	Picorna	RT-PCR has higher sensitivity than viral culture and serology.
Hutchinson *et al.*, Australia (2007)[10]	92 (63.0)	72.0 (49–85)	22.0, 87.0, 53.0 (10–160)	0.9 (0.4–1.9)	10	RT-PCR	87	Picorna	Running nose and sore throat were associated with viral positive AECOPD Severity was more with non-picorna associations
McManus *et al.*, Ireland, UK (2008)[11]	136 (47.1)	70.2 (9.4)	—, —, 48.0 (39.2)	0.8 (0.5)	10	PCR	—	Picorna	Viral positivity associated with severe disease and fever 12% of stable COPD patients were virus positive Viral positive AECOPD had earlier hospitalization and higher prevalence of fever
Rohde *et al.*, Germany (2003)[12]	85 (80.0)	70.0 (43–83)	37.7, 49.4, median; 35 (1–100)	Median (range); 1.0 (0.4–2.5)	9	RT-PCR	—	Picorna	No relation with increased episodes of AECOPD Sample from lower respiratory tract had higher virus yield than upper respiratory airway
Camargo *et al.*, USA (2008)[15]	76 (68.0)	72.0 (9.0)	29.0, 71.0, 64.0(32.0)	—	10	PCR	87	RSV	Viral aetiology identified in 25% of AECOPD presenting in the Emergency Department, of which 1/3 were RSV-related

AECOPD, acute exacerbation of COPD; RSV, respiratory syncytial virus.

Table 3 Weighted mean prevalence (WMP) of individual viruses associated with an acute exacerbation of COPD

	WMP	95% CI upper bound	95% CI lower bound
Picorna	17.3	7.2	27.3
Influenza	7.1	2.5	11.6
Parainfluenza	2.6	0.4	4.8
RSV	5.3	1.6	9.0
Adeno	1.1	–1.1	3.3
Corona	3.1	0.4	5.8
Human Metapneumonia virus	0.7	–0.3	1.7

vaccination coverage for influenza ranging from 40.3%[11] to 87%.[13] Most studies had recruited hospitalized patients.[9,11–13,15,16] Papi *et al.*[14] recruited all subjects from an outpatient clinic, whereas 55% patients were hospitalized patients in the study by Beckham *et al.*[9] and 80% in the study by Camargo *et al.*[10] Most studies used nasal swabs and/or throat swabs for sample collection and subsequent viral isolation.[10,11,13,15,16] Two studies[10,15] used sputum along with nasal swabs and/or throat swabs, whereas Papi *et al.*[14] used only induced sputum for sample collection and viral detection. Five studies[9–11,13] defined AECOPD as per the criteria set by Anthonisen *et al.*,[17] two studies[11,14] used GOLD definitions,[18] while Seemungal *et al.*[16] used American Thoracic Society criteria.[19]

Respiratory viral prevalence and bacterial co-infection

The WMP of respiratory viral infections in AECOPD was 34.1% (95% CI: 23.9–44.4). Table 3 shows WMP of individual respiratory viral infections in AECOPD. The absolute viral detection in each study is depicted in Figure 2. The highest viral positivity rate was found in a German study (56%).[12] Only one study reported the details regarding bacterial positivity, including atypical bacteria.[13] and found evidence of viral and bacterial co-infection in 9% of patients with AECOPD at admission, rising to 13.6% by day five. In addition, 71% of patients who had bacterial infection at onset also reported some viral symptoms at the onset of the current episode, underlining the high incidence of bacterial superinfection following viral infection.

Geographical variations in viral prevalence

There was significant geographical variation in viral prevalence. As shown in Table 4, maximum WMP was observed in studies from Europe followed by the USA, Australia and Asia. Picornavirus was the most common virus detected in Australia, followed by Europe and the USA, whereas influenza was the most commonly detected virus in Asian studies. Corona virus had the maximum prevalence in a study from USA.[9]

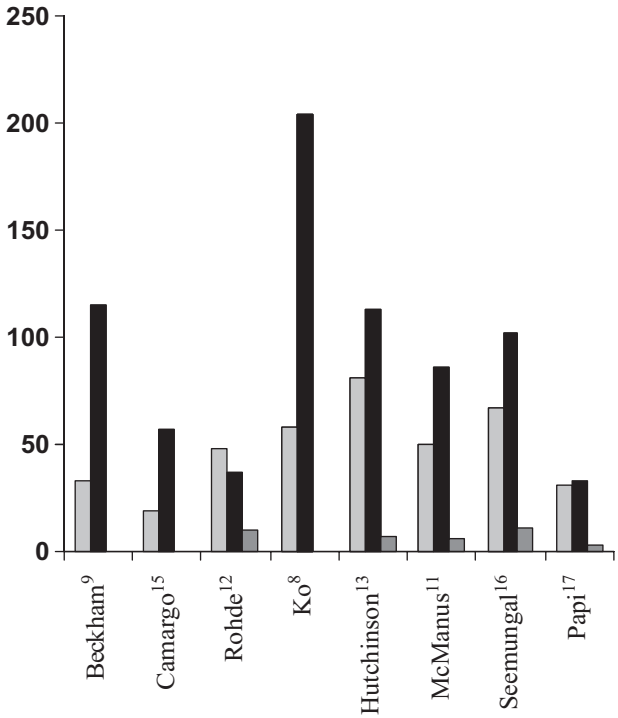

Figure 2 Bar chart showing the number of patients reported to have (▫) viral positivity, (■) viral negativity and (▪) infection with more than one virus by various authors.

Strain variation

Influenza A contributed more than 80% of the total influenza burden. All the influenza A viruses detected by Ko and colleagues[15] and McManus's team were of H3 subtype.[11] The most commonly detected strain of corona virus was OC43 and PIV 3 in PIV.[9]

The presence of viruses in AECOPD has been found to be associated with earlier hospitalization, prolonged hospitalization, high prevalence of fever, upper respiratory tract infection features, severe exacerbation, greater reduction in FEV_1 and sputum eosinophilia compared with episodes of non-viral aetiology.[11,12,14–16] (Box 2) Non-picornavirus-associated episodes of AECOPD were more severe than episodes associated with picornavirus.[13]

DISCUSSION

Infections are associated with a significant proportion of acute exacerbations of COPD. The role of viruses in the aetiology of AECOPD was underestimated until recently. Recent studies have detected viruses in up to 56% of all exacerbations.[12] The use of RT-PCR techniques has increased the detection of respiratory viruses by 2–3 times that of the conventional/rapid viral culture methods.[10,11,16] However, the geographical distribution and clinical significance of these viral infections has not been systematically reviewed. We found that the WMP of respiratory viruses in AECOPD was 34.1% (95% CI: 23.9–44.4). There was wide

Table 4 Geographical variation in overall viral prevalence

Group	WMP† (overall)	95% CI for WMP		WMP (Picorna)	95% CI for WMP		WMP (influenza)	95% CI for WMP		WMP (RSV)	95% CI for WMP	
		Lower bound	Upper bound		Lower bound	Upper bound		Lower bound	Upper bound		Lower bound	Upper bound
USA	0.370	–0.491	1.231	0.159	–0.603	0.922	0.070	–0.151	0.291	0.048	–0.172	0.268
Europe	0.432	0.304	0.560	0.240	0.220	0.260	0.070	–0.069	0.210	0.081	–0.005	0.168
Asia‡	0.221			0.031			0.099			0.023		
Australia‡	0.359			0.283			0.033			0.011		
Overall	0.359	0.257	0.461	0.173	0.072	0.273	0.074	0.029	0.120	0.053	0.016	0.090

†The confidence intervals are constructed by assuming a Normal distribution for the ratios.
‡The confidence interval was not calculated for Asia and Australia as there was only one study for each of these two continents.
WMP, weighted mean prevalence.

Box 2

Clinical features suggesting viral positivity in AECOPD

- Severe episode of AECOPD
- Greater reduction in FEV_1, FEV_1%, FEV_1/FVC
- Presence of fever
- Clinical features of upper respiratory tract infection
- Sputum eosinophilia
- Exposure to young children

geographical variation in the total viral prevalence as well as the type of viruses. Overall, picornavirus was the most commonly detected virus followed by influenza and RSV.

Studies from Western countries have shown picornavirus to be the most common virus associated with AECOPD, whereas the single Asian study detected influenza most frequently. This disparity in prevalence of influenza in acute exacerbation could be related simply to the strain circulating at that particular year and time, or could reflect the wider coverage of influenza vaccination in Western countries compared with Asian. In an older cohort of patients with chronic lung disease in the USA, studied by Nichol *et al.*,[18] patients who were not vaccinated for influenza had twice the hospitalization rate in the influenza season than in the non-influenza season.[20] In addition, influenza vaccination was associated with a lower risk of death. It was further observed in a systematic review by Poole and colleagues that vaccination of COPD patients resulted in a significant reduction in the total number of exacerbations per vaccinated subject compared with those who received placebo (Weighted Mean Difference (WMD) 0.37).[21]

Human metapneumovirus was discovered in 2001 and initially detected in young children with respiratory illness.[12] However, in two subsequent studies, hMPV was detected in 2.3% and 4.69% of patients with AECOPD, respectively, thereby confirming the presence of hMPV in adults.[14,22] Rohde and colleagues detected a high hMPV load in their patients, more so in nasal washings than in induced sputum, and postulated that hMPV may be a trigger for exacerbation in COPD.[22]

Viral infection and morbidity and mortality

Exacerbations associated with viral infection appeared to be more severe, as reflected by length of hospitalization, decrease in FEV_1, FEV_1%, FEV_1/FVC% and diffusion capacity, and with a trend towards greater hypoxaemia. In a study by Seemungal and colleagues, exacerbations associated with respiratory viruses had a longer median symptom recovery time than did non-viral exacerbations (13 and 6 days, respectively).[16] Viral positivity is also associated with a greater degree of systemic inflammation and higher serum fibrinogen levels, which are independent risk factors for cardiovascular related mortality.[16,23] A

recent study of influenza immunization in older patients has shown that immunization is associated with a reduction in the risk of hospitalization for heart disease and stroke, thus providing more evidence for the link between respiratory viral infection and vascular disease.[24]

Symptoms and viral prevalence

Viral presence in AECOPD is associated with a higher number of patients with symptoms of upper respiratory tract infection and fever as well as earlier hospitalization.[12,13] The likelihood of viral infection can also be predicted from the sputum cell count. The presence of neutrophils predicts infection, bacterial or viral, whereas eosinophils favour a probable viral aetiology.[14] It has been noted that patients with repeated viral infections tended to have had exposure to infants or young children, indicating that children might be a source of repeated exposure to circulating respiratory viruses.[13] Viral isolation was highest in samples taken within 2 days of the onset of symptoms and was greater in sputum than in the upper airways,[12,13] except in one study where the viral load of hMPV in nasal lavage was three and one half times higher than from induced sputum.[25]

Vaccination and viral positivity

Cohorts with higher vaccine coverage for influenza have been found to have a lower prevalence of influenza.[10,13] Beckham *et al.*[9] found that the subgroup of their studied population with low influenza vaccine coverage in the previous year had higher influenza infections and detection. Moreover, none of the influenza-positive patients in their group received vaccination in the previous year. On the other hand, Ko *et al.*[11] did not observe any beneficial effect of influenza vaccination on the prevalence of virus infections. Nevertheless, inadequate use of influenza vaccine remains an important modifiable risk factor in patients hospitalized with COPD.[20]

In summary, this study supports the hypothesis that a significant proportion of patients with AECOPD have associated respiratory viral infections. Overall, picornavirus has been the most common viral infection detected, followed by influenza and RSV. There is a significant geographical variation in viral epidemiology, the reasons for which may be multifactorial. The association between viral infections and mortality and morbidity as well as the level of immunization may have important therapeutic implications for the future management of AECOPD.

REFERENCES

1 World Health Organization. *The 10 leading causes of death by broad income group, 2004*. Fact sheet No 310/November 2008.
2 Buist AS, Vollmer WM, McBurnie MA. Worldwide burden of COPD in high- and low-income countries. Part I. The burden of obstructive lung disease (BOLD) initiative. *Int. J. Tuberc. Lung. Dis.* 2008; **12**: 703–8.
3 Connors AF Jr, Dawson NV, Thomas C *et al.* Outcomes following acute exacerbations of severe chronic obstructive lung disease. *Am. J. Respir. Crit. Care Med.* 1996; **154**: 959–67.
4 Seemungal TAR, Donaldson GC, Paul EA *et al.* Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 1998; **157**: 1418–22.
5 Sullivan SD, Ramsey SD, Lee TA. The economic burden of COPD. *Chest* 2000; **117** (Suppl. 2): 5S–9S.
6 Sethi S. Infectious etiology of acute exacerbations of chronic bronchitis. *Chest* 2000; **117** (5 Suppl. 2): 380S–5S.
7 Kuypers J, Campbell AP, Cent A *et al.* Comparison of conventional and molecular detection of respiratory viruses in hematopoietic cell transplant recipients. *Transpl. Infect. Dis.* 2009; **11**: 298–303.
8 Von Elm E, Altman DG, Egger M *et al.* STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J. Clin. Epidemiol.* 2008; **61**: 344–9.
9 Beckham JD, Cadena A, Lin J *et al.* Respiratory viral infections in patients with chronic, obstructive pulmonary disease. *J. Infect.* 2005; **50**: 322–30.
10 Camargo CA Jr, Ginde AA, Clark S *et al.* Viral pathogens in acute exacerbations of chronic obstructive pulmonary disease. *Intern. Emerg. Med.* 2008; **3**: 355–9.
11 Ko FW, Ip M, Chan PK *et al.* Viral etiology of acute exacerbations of COPD in Hong Kong. *Chest* 2007; **132**: 900–8.
12 Rohde G, Wiethege A, Borg I *et al.* Respiratory viruses in exacerbations of chronic obstructive pulmonary disease requiring hospitalisation: a case-control study. *Thorax* 2003; **58**: 37–42.
13 Hutchinson AF, Ghimire AK, Thompson MA *et al.* A community-based, time-matched, case-control study of respiratory viruses and exacerbations of COPD. *Respir. Med.* 2007; **101**: 2472–81.
14 Papi A, Bellettato CM, Braccioni F *et al.* Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. *Am. J. Respir. Crit. Care Med.* 2006; **173**: 1114–21.
15 McManus TE, Marley AM, Baxter N *et al.* Respiratory viral infection in exacerbations of COPD. *Respir. Med.* 2008; **102**: 1575–80.
16 Seemungal T, Harper-Owen R, Bhowmik A *et al.* Respiratory viruses, symptoms, and inflammatory markers in acute exacerbations and stable chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 2001; **164**: 1618–23.
17 Anthonisen NR, Manfreda J, Warren CP *et al.* Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann. Intern. Med.* 1987; **106**: 196–204.
18 Rabe KF, Hurd S, Anzueto A *et al.* Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am. J. Respir. Crit. Care Med.* 2007; **176**: 532–55.
19 American Thoracic Society. ATS statement: standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. *Am. J. Respir. Crit. Care Med.* 1995; **152**: S78–S83.
20 Nichol KL, Baken L, Nelson A. Relation between influenza vaccination and outpatient visits, hospitalization and mortality in elderly persons with chronic lung disease. *Ann. Intern. Med.* 1999; **130**: 397–403.
21 Poole PJ, Chacko E, Wood-Baker RW *et al.* Influenza vaccine for patients with chronic obstructive pulmonary disease. *Cochrane Database Syst. Rev.* 2006; (1): CD002733.
22 Rohde G, Borg I, Arinir U *et al.* Relevance of human metapneumovirus in exacerbations of COPD. *Respir. Res.* 2005; **6**: 150.
23 Wedzicha JA, Seemungal TA, MacCallum PK *et al.* Acute exacerbations of chronic obstructive pulmonary disease are accompanied by elevations of plasma fibrinogen and serum IL-6 levels. *Thromb. Haemost.* 2000; **84**: 210–5.
24 Nichol KL, Nordin J, Mullooly J *et al.* Influenza vaccination and reduction in hospitalizations for cardiac disease and stroke among the elderly. *N. Engl. J. Med.* 2003; **348**: 1322–32.
25 Van den Hoogen BG, de Jong JC, Groen J *et al.* A newly discovered human pneumovirus isolated from young children with respiratory tract disease. *Nat. Med.* 2001; **7**: 719–24.