Identifying viral infections in vaccinated Chronic Obstructive Pulmonary Disease (COPD) patients using clinical features and inflammatory markers

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Background

Known inflammatory markers have limited sensitivity and specificity to differentiate viral respiratory tract infections from other causes of acute exacerbation of COPD (AECOPD). To overcome this, we developed a multi-factorial prediction model combining viral symptoms with inflammatory markers.

Methods

Interleukin-6 (IL-6), serum amyloid A (SAA) and viral symptoms were measured in stable COPD and at AECOPD onset and compared with the viral detection rates on multiplex PCR. The predictive accuracy of each measure was assessed using logistic regression and receiver operating characteristics curve (ROC) analysis.

Results

There was a total of 33 viruses detected at the onset of 148 AECOPD, the majority 26 (79%) were picornavirus. Viral symptoms with the highest predictive values were rhinorrhea [Odds ratio (OR) 4.52; 95% CI 1.99–10.29; P < 0.001] and sore throat (OR 2.64; 95% CI 1.14–6.08; P = 0.022), combined the AUC ROC curve was 0.67. At AECOPD onset patients experienced a 1.6-fold increase in IL-6 (P = 0.008) and 4.5-fold increase in SAA (P < 0.001). The addition of IL-6 to the above model significantly improved diagnostic accuracy compared with symptoms alone (AUC ROC 0.80 (P = 0.012).

Conclusion

The addition of inflammatory markers increases the specificity of a clinical case definition for viral infection, particularly picornavirus infection.

Keywords

Chronic obstructive pulmonary disease, picornavirus, exacerbations of COPD, inflammatory markers, diagnostic test accuracy.

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Background

A number of studies have established that infection by a variety of respiratory viruses can trigger exacerbations of COPD (AECOPD). Respiratory-virus detection methods such as multiplex polymerase chain reaction (PCR) and immunofluorescence usually take at least 24–48 hours to obtain a result, are relatively expensive, are not widely available in primary practice and may not identify all possible respiratory viruses. Therefore, alternative methods of rapidly and accurately differentiating viral episodes may be useful to guide treatment decision-making at the onset of AECOPD. If viral infections can be identified early after onset, there will be greater opportunity to effectively target the use of antiviral therapies such as Oseltamivir and Pleconaril, potentially improving patients’ clinical outcomes while containing health-care costs. The inflammatory response to viral infection of respiratory epithelium causes recognisable clinical features such as oedema of mucus membranes, inflamed throat, rhinorrhea, nasal congestion, swollen glands and watery eyes and in some cases systemic symptoms and signs such as chills, myalgia and fever. Influenza surveillance programmes use a clinical case-definition of viral infection to collect data on seasonal fluctuations in the incidence of acute respiratory illness in the community. Case definitions provide a rapid, non-invasive and inexpensive way to alert surveillance programmes of potential increases in the rate of circulating influenza. At times of high influenza circulation symptom-based case definitions have been reported to be 60–70% accurate. In the absence of an epidemic and in the elderly, the predictive value of symptoms to diagnose influenza drops to only 44%. The accuracy of symptoms to diagnose other viral infections in elderly
COPD patients already vaccinated against influenza is unknown.14,15

Experimental studies have established that measures of inflammation in the blood [interleukin-6 (IL-6), C-reactive protein] run parallel to clinical features,16–19 hence combining both these measures may aid accurate differential diagnosis.20–22 In this study, we used regression modelling techniques and receiver operating characteristics curve (ROC) analysis to determine whether a combination of clinical features and a measure of systemic inflammation improved diagnostic accuracy to discriminate viral infection from other causes of AECOPD in vaccinated COPD patients. In this analysis, we used acute change in IL-6 and serum amyloid A levels (SAA)23–25 as potential markers of acute amplification in inflammatory activity that might aid in the identification of acute viral infection.

Aims
To develop a COPD-specific clinical case-definition of viral illness that identifies both localised respiratory tract viral infection and systemic respiratory viral infection from data available early in the course of an AECOPD in patients already vaccinated against influenza. To evaluate whether the addition of change in IL-6, SAA increases the predictive accuracy of the multivariable clinical prediction model developed for viral infection.

Methods
Patients were recruited from the Melbourne Longitudinal COPD Cohort, a prospective cohort of community-dwelling patients with moderate to severe COPD who are identified as at risk of requiring hospitalization for management of COPD exacerbations. Patients who had been admitted to the Royal Melbourne Hospital for management of an acute exacerbation (ICD-10 Codes J44.0–J44.9) were recruited into the cohort following discharge from acute care. Ninety-one patients were included in this study with a mean age of 72 years, 63% were male. All patients had a history of smoking (mean pack years = 50, range 10–160) and 22% were current smokers. Eight-four per cent of patients had chronic bronchitis and 13 patients had a diagnosis of bronchiectasis confirmed on HRCT. Cardiac disease was the most common comorbidity including ischaemic heart disease (29%), arrhythmias (12%), hypertension (31%) and chronic heart failure (21%).

Definition of exacerbation
COPD exacerbations (AECOPD): were defined according to the Anthonisen criteria: (Anthonisen, Manfreda et al. 1987; Rodriguez-Roisin 2000) Anthonisen type-I is defined as an increase in dyspnoea, sputum volume and/or sputum purulence for more than 24 hours, type-II as any two of the above symptoms and type III as one of the above symptoms accompanied by symptoms of viral upper respiratory tract infection. Exacerbation Severity was defined according to the American Thoracic Society Exacerbation Severity Criteria; level I is treated at home, level II requires hospitalisation and level III leads to respiratory failure (ATS COPD Guidelines 2005).

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. This was further reinforced by fortnightly phone contact. Viral symptoms (increased rhinorrhea, nasal congestion, sore-throat, myalgia or headaches, fever and or chills)26 were measured at stable recruitment, AECOPD onset and post-resolution and compared with the viral detection rates on PCR. Each symptom was recorded on a scale of zero (absent symptom) to three (severe).

Detection of respiratory viruses
Pathogen detection
Nasal and oropharyngeal swabs for respiratory RT-PCR were obtained according to the VIDRL Influenza Surveillance protocol.7 Nose and throat swabs were pooled in viral transport medium and transported to the testing laboratory within 2 hours in a refrigerated transport container.

Respiratory virus multiplex PCR was performed at the Victorian Infectious Disease Reference Laboratory.7 A panel of nested PCR assays capable of detecting 10 respiratory viruses was used for amplification of nucleic acid sequences and viral identification. The following viruses were screened; influenza A (H1N1 and H3N2 subtypes) and B, picornavirus (with primers specific to enteroviruses and rhinoviruses), respiratory syncytial virus (RSV), parainfluenza (subtypes 1, 2 & 3) and adenovirus.

Measurement of inflammatory serum markers
Serum for measurement of inflammatory markers was obtained at recruitment (stable baseline), AECOPD onset and post-recovery (Day 30 to 60), in a sub-set of patients. Interleukin-6 (IL-6) was measured using ELISA for Human IL-6 (OptEIA) ELISA Set (Serial Number #555220) (BectonDickson OptEIA ELISA, San Diego, USA). The lower limit of detection was 4.7 pg/ml. Quantitative determination of SAA was also measured using a commercial ELISA sandwich kit (Anogen, Ontario Canada) with a minimal detection limit 1·1 ng/ml. SAA comprises four family members (SAA1-SAA4), with only SAA1 and SAA2 being induced during the acute response.6 The assay used identi-
fies both SAA1 and SAA2 and reports the sum of both. Measurement of all inflammatory markers was performed independently from the clinical and microbiological assessment of exacerbations.

Statistical analysis

Predictive accuracy of the viral symptom score

The predictive value of individual symptoms to predict PCR positivity associated with the onset of an AECOPD was assessed using logistic regression. Univariate logistic regression models were developed for each symptom individually, symptoms that had an overall odds ratio (OR) greater than one, whether statistically significant or not, were retained in the multivariable model. To determine which cut-off on the 4-point severity scale had the highest predictive value, the odds and 95% confidence interval at each cut-off were tabulated. Logistic regression models were compared sequentially to determine how much the addition of different predictive variables incrementally increased the log-likelihood ratio. The diagnostic sensitivity and specificity of viral symptoms versus PCR-defined infection was evaluated using Area Under the Receiver Operating Characteristics Curve (AUC ROC) analysis. Statistically the AUC ROC is a non-parametric test, similar to the Wilcoxon (Mann-Whitney U test) that is not influenced by the underlying population distribution of values. A statistically significant result has an AUC ROC > 0.5, with a lower bound of the 95% confidence interval that does not include 0.5.

The diagnostic utility of different prediction models were then compared using the Stata ‘ROCCOMP’ command, which compares the AUC ROC between two models while taking into account expected correlations that occur in the data where two tests are compared using the same dataset.

Inflammatory markers

The distributions of SAA and IL-6 were approximately log-normal. To control for raised inflammatory marker levels in stable disease a difference score was generated (between log-transformed values at AECOPD onset minus those during stable state). Exponentiation of the mean difference in the natural log (log_e) values yields the geometric mean ratio of SAA or IL-6. Logistic regression was used to determine whether the difference (between AECOPD onset and stable), in log_e(IL-6) and log_e(SAA) were predictive of viral infection. Cluster analysis by patient was used to adjust the regression models for repeated AECOPD episodes, from the same patient, where these occurred. The diagnostic utility of log_e(IL-6) and log_e(SAA) was then assessed using the same statistical techniques as described above for the viral symptom score data. The final stage of this analysis was to combine clinical prediction models based on symptoms with the inflammatory marker data to assess whether this increased diagnostic accuracy. This was done using logistic regression techniques and AUC ROC analysis. Differences between models were assessed by comparing the log-likelihood ratios for the regression models and differences in the shape of the ROC curve and AUC ROC.

Results

AECOPD

Ninety-one patients were monitored over three years; from July to December 2003 (Winter – Spring) and from August 2004 to December 2005. The median number of weeks of monitoring was 47 weeks per patient (range 1–99 weeks). There were 148 exacerbations included in this study analysis. Sixty-four per cent of patients with exacerbations contacted the study staff while the rest were identified on routine fortnightly phone call. The time from symptom onset to sampling was short (mean 2.4 days), in the self-report group median time was 1.5 days and in the phone contact group the median time was 6 days. Eighty per cent of AECOPD were treated in the community with oral antibiotics and/or oral corticosteroids. 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 AECOPD onset and an additional five picornaviruses were isolated at day-5 after onset. Viral detection rates by respiratory PCR was higher in the group that self-reported their AECOPD, only four viruses were detected in AECOPD identified by phone follow-up, no doubt reflecting the delay between infection onset and obtaining the viral PCR.

Symptoms when stable

At recruitment, participants were interviewed about the presence of upper respiratory symptoms and symptoms of viral infection. Nasal congestion and rhinorrhea were commonly reported when well; eight (28%) of patients using long-term home oxygen experienced nasal congestion and blocked nose when well and six (9.5%) of those not using home-oxygen therapy. Intermittent rhinorrhea not associated with colds was reported by 15 (17%) of the cohort, possibly indicating intermittent allergic rhinitis. Participants commonly reported headaches and myalgias when well eight (9%) possibly reflecting the high prevalence of osteoarthritis 22 (24%) and of osteoporosis 21 (23%) in this older patient group.

Viral symptoms at AECOPD onset and viral detection by multiplex PCR

Rhinorrhea (82%) and sore throat (59%) were the most common viral symptoms reported at AECOPD onset and
all cases of sore throat also reported rhinorrhoea. In the group who self-reported their AECOPD, 55% reported rhinorrhoea at the onset of the episode and 47% reported sore-throat. In the group whose AECOPD were identified by follow-up phone call, 42% rhinorrhoea and 48% reported sore throat, these differences in symptom reporting rates were not statistically significant. These two symptoms were commonly associated with the detection of picornaviruses. Forty-seven per cent also reported subjective fevers, chills and myalgia associated with picornavirus infection. Parainfluenza infection was only associated with upper respiratory symptoms; sore throat (33%) and rhinorrhoea (33%). In contrast, the small number of patients with influenza infection (3) all reported systemic symptoms in addition to upper respiratory symptoms; headaches and/or myalgia (100%) and subjective fever (66%).

**Individual clinical symptoms and prediction of PCR positivity**

The individual symptoms with the highest predictive values were the presence of rhinorrhoea [Odds ratio (OR) 4.52; 95% CI 1.99–10.29; \( P < 0.001 \)], sore throat (OR 2.99; 95% CI 1.99–10.29; \( P < 0.001 \)] and nasal congestion (OR 4.52; 95% CI 1.10–7.42; \( P = 0.032 \)). Subjective fever (OR 1.04; 95% CI 0.69–1.56; \( P = 0.85 \)) and myalgia or headaches (OR 1.01; 95% CI 0.73–1.44; \( P = 0.17 \)) were not predictive of detection of respiratory viruses overall. However, when influenza cases were considered separately from the other viruses, myalgia was predictive of influenza detection (OR 10.15; 95% CI 1.06–97.73; \( P = 0.05 \)).

To determine whether seasonal variation in virus circulation changed the predictive value of symptoms, a comparison was made of the predictive value of symptoms in winter and spring compared with summer and autumn (Figure 1). In winter and spring, the predictive value of clinical symptom was similar to the values for the year overall (Table 1). No combination of symptoms was a significant predictor of viral detection in summer and autumn, although there was a trend for the presence of sore throat to be predictive (OR 6.29).

**Development of a multivariable clinical case definition for viral infection**

Symptoms included in multivariate clinical prediction model were rhinorrhoea, sore throat, subjective fever and myalgias. Subjective fever and myalgias were not significant predictors but were retained, as they were clinically important. Symptoms were only coded as positive if they had worsened from the severity recorded at the baseline interview. The clinical model based on patient report of viral symptoms had an AUC ROC 0.72 (95% CI 0.61–0.84; \( P = 0.64 \)). At a cut-off of 0.22, the model correctly identified viral infection in 71% of cases with a sensitivity of 68% and specificity of 71%. When the cut-off was raised to 0.43, the model was 79% accurate with a sensitivity of 29% and a specificity of 94%.

**Results Part 2: Inflammatory markers to predict viral infection**

**Participants**

A total of 78 AECOPD from 37 patients were included in the inflammatory marker sub-study, with 63% of patients

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**Table 1. Odds of predicting PCR positive AECOPD each season**

|                  | All seasons | Winter/spring | Summer/autumn |
|------------------|-------------|---------------|---------------|
|                  | Odds ratio  | \( P \)-value (95% CI) | Odds ratio  | \( P \)-value (95% CI) | Odds ratio  | \( P \)-value (95% CI) |
| Sore throat      | 2.64        | 0.022 (1.14–6.080) | 2.76         | 0.038 (1.06–7.25) | 6.29         | 0.06 (0.94–41.96) |
| Nasal congestion | 4.52        | 0.032 (1.10–7.42) | 2.89         | 0.07 (0.93–9.03) | 1.88         | 0.47 (0.34–10.33) |
| Rhinorrhoea      | 2.99        | <0.001 (1.99–10.29) | 5.18         | 0.001 (1.95–13.80) | 3.17         | 0.14 (0.68–14.81) |
| Subjective fever | 1.04        | 0.85 (0.69–1.56) | 0.96         | 0.16 (0.56–1.63) | 1.03         | 0.91 (0.53–2.04) |
| Myalgia and/or headaches | 1.01 | 0.17 (0.73–1.44) | 1.05         | 0.22 (0.69–1.59) | 1.06         | 0.86 (0.55–2.05) |
| Combined throat/rhinorrhoea | 5.46 | <0.001 (2.60–8.31) | 5.56         | <0.001 (2.46–8.66) | 4.34         | 0.05 (0.08–8.75) |
exacerbating more than once during the study period. The 37 participants had a mean FEV₁ of 42% predicted (range 15–69%), and mean FEV₁/FVC ratio of 46% (24–74%). Twenty-four per cent were on long-term home oxygen. Mean pack years of smoking was 43 (range 10–115), with 8% current smokers. Respiratory viruses were detected by multiplex PCR in 22 (17%) of exacerbations. Picornavirus (21%) and parainfluenza (3%) were the most common viruses detected.

Interleukin-6 and serum amyloid A levels
Mean time from symptom onset to obtaining serum for measurement of inflammatory markers was 2–4 days. IL-6 and SAA were raised at exacerbation onset compared with stable disease with a median IL-6 of 3.57 pg/ml (IQR 1.98–5.96) versus 5.56 pg/ml (IQR 2.37–14.06) (P < 0.024), SAA median was 7.62 mg/l (IQR 4.56–12.65) versus 28.20 mg/l (IQR 10.3–163.0) (P < 0.001); and they returned to baseline with clinical recovery. Comparing IL-6 levels between the self-reported AECOPD to those identified by phone follow-up; in stable COPD median IL-6 levels were 3.57 versus 3.17 pg/ml, respectively, and at AECOPD onset 5.56 versus 4.56 pg/ml, respectively, these differences were not statistically significant.

In all 78 AECOPD inflammatory markers at AECOPD onset were compared with samples obtained in stable COPD. At AECOPD onset, patients experienced a 14–5-fold increase in SAA: IL-6 (geometric mean ratio 1.63; 95% CI 1.14–2.33; P = 0.008) and SAA (4.53; 95% CI 7.01–2.93; P < 0.001).

PCR positive versus negative AECOPD
The geometric mean ratio (AECOPD onset/stable) logeIL-6 in PCR-positive AECOPD was 3.81 and in negative 1.26. Comparing PCR-positive and -negative AECOPD, the difference in geometric mean ratio of logeIL-6 was 3.02 (95% CI 1.40–6.54; P = 0.006). The difference between onset logeIL-6 and baseline logeIL-6 levels was predictive of PCR positivity of nose and throat swabs (OR 1.54; 95% CI 1.14–2.06; P = 0.004). The ratio of SAA levels at AECOPD onset to baseline was in PCR-positive AECOPD a mean of 34.72 (range 0.26–153.63) and in PCR negative AECOPD a mean of 26.54 (range 0.23 to 424.65). In contrast to IL-6, differences between logeSAA at AECOPD onset compared with baseline were not strong predictors of PCR positivity (OR 1.23; 95% CI 0.96–1.58; P = 0.110).

Multivariable prediction models including inflammatory markers
There was no significant difference in the AUC ROC analysis between change in IL-6 and clinical-model (P = 0.96). The predictive accuracy of viral symptoms alone was then compared with a model containing viral symptoms and acute change in IL-6 levels. The addition of IL-6 significantly improved accuracy (clinical model AUC ROC 0.67; 95% CI 0.52–0.83 versus IL-6 & clinical model AUC ROC 0.80; 95% CI 0.70–0.91; P = 0.012) (Figure 2). Importantly combining IL-6 with viral symptoms increased the exclusion of episodes with minor symptoms that may have been of non-viral origin. At a cut-off of 0.41–0.59, the specificity was 87–96% with 78% of true viral infections correctly identified, giving a positive predictive value of 6/84 and a negative predictive value of 0.77.

To validate these results, the analysis was repeated excluding AECOPD in which viruses other than picornavirus were detected on respiratory PCR. The AUC ROC for change in IL-6 at AECOPD onset to discriminate picornavirus infection from other causes of AECOPD was 0.65 (95% CI 0.50–0.81) and the combination of IL-6 and viral symptoms had an AUC ROC 0.88 (95% CI 0.79–0.98).

Combining IL-6 and SAA into a single prediction model (not containing any clinical information) improved the predictive accuracy by 7% compared with the null (empty) model. An alternative model combining the two inflammatory markers and the clinical-model increased the predictive accuracy by 34% compared with the null model. Using AUC ROC analysis to compare accuracy of the different models, the combination of clinical symptoms with both inflammatory markers (AUC ROC 0.82) was significantly

Figure 2. Diagnostic accuracy of models to identify respiratory viral infection. The blue scale displays the AUC ROC for the clinical model to discriminate viral infection defined by positive multiplex PCR (AUC ROC 0.67; 95% CI 0.52–0.83) and the red scale displays the AUC ROC for clinical model combined with IL-6 (AUC ROC 0.80; 95% CI 0.70–0.91). The diagnostic accuracy of the clinical prediction model was significantly increased by the addition of IL-6 (P = 0.012).
The predictive value of viral symptoms for the detection of respiratory viruses on PCR was modest in this study. It is noteworthy that patients reported only mild to moderate symptoms associated with viral illness and the number of significant systemic viral illnesses was low. Consistent with the predominance of picornavirus detection by PCR, the clinical symptoms with the highest predictive value were rhinorrhea and sore throat. In contrast to the Picornavirus Index developed by Monto and colleagues, we removed ‘nasal congestion’ as non-predictive. This may be explained by the use of domiciliary oxygen devices that cause drying of the nares and symptoms of congestion in the absence of infection. During the summer, the predictive value of sore throat increased compared with winter and spring (OR 6.29 versus 2.64). This indicates that while rhinorrhea is associated with allergic rhinitis and may occur through the year that sore throat had greater specificity for viral infection. In contrast to influenza surveillance studies, few patients in this study reported ‘fever and myalgia’. This undoubtedly reflects the very low levels of influenza infection in our vaccinated COPD patient population.

The prediction models using composite of clinical features had a predictive value of approximately 70%. The relatively poor predictive value of our clinical model for identifying viral infection reflects the added complexity that chronic disease and the natural ageing process add to differential diagnosis of viral illness in older adults with COPD. Symptoms of COPD, such as chronic productive cough overlap with symptoms of viral infection. Comorbidities such as osteoarthritis and rheumatoid arthritis that cause intermittent joint-pain and swelling make the significance of symptoms such as myalgia difficult to interpret. Symptom severity in response to viral infection may also be muted in this older adult population. It is noteworthy that very few patients experienced objectively measurable fever response associated with AECOPD.

As the clinical symptoms of acute viral infection occur as the direct effect of up-regulation of the acute phase immune response, it was possible that measuring inflammatory activity might assist in excluding non-inflammatory triggers for reported symptoms. In this study, there was an acute increase in both IL-6 and SAA at onset of AECOPD compared with baseline and IL-6 levels were higher in viral versus non-viral events. When IL-6 levels were combined with clinical symptoms the combined model was significantly more accurate than either parameter in isolation. The addition of SAA to our prediction models did not significantly improve diagnostic accuracy but did exclude more mild events. This demonstrates that SAA may have greater utility for identifying severe viral infection such as influenza and SARS.

Respiratory viruses such as rhinovirus that may cause relatively mild upper respiratory symptoms trigger AECOPD. A clinical case-definition of viral illness in an older,
Identifying viral infections in COPD patients

References

1. Hutchinson A, Ghimire AK, Thompson MA et al. A community-based, time-matched, case-control study of respiratory viruses and exacerbations of COPD. Respir Med 2003; 107:1161–1167.

2. Saip K, Stockley RA. COPD exacerbations. 2: aetiology. Thorax 2006; 61:208–213.

3. Falsey A, Walsh E. Respiratory Syncytial Virus infection in adults. Curr Microbiol Rev 2000; 13:371–384.

4. Seemungal T, Wedzicha J. Viral infections in obstructive airway diseases. Curr Opin Pulm Med 2003; 9:111–116.

5. Beckham J, Cadena A, Lin J et al. Viral respiratory infections in children with chronic obstructive pulmonary disease. J Infect 2005; 50:322–330.

6. Martinello R, Espel F, Weibel C, Ferguson D, Landry ML, Kahn JS. Human metapneumovirus and exacerbations of chronic obstructive pulmonary disease. J Infect 2005; 53:248–254.

7. Druce J, Tran T, Kelly H et al. Laboratory diagnosis and surveillance of human respiratory viruses by PCR in Victoria, Australia, 2002–2003. J Med Virol 2005; 75:122–129.

8. Seemungal T, Harper-Owen R, Bhowmik A, Jeffries D, Wedzicha J. Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. Eur Respir J 2000; 16:677–682.

9. Anzueto A, Niederan M. Diagnosis and treatment of rhinovirus respiratory infections. Chest 2003; 123:1664–1672.

10. Shorman M, Moorman J. Clinical manifestations and diagnosis of influenza. South Med J 2003; 96:739–743.

11. Monto A, Gravenstein S, Elliott M, Colopy M, Schweinle J. Clinical signs and symptoms predicting influenza infection. Arch Intern Med 2000; 160:3243–3247.

12. Bolvin G, Hardy J, Tellier G, Maziade J. Predicting influenza infections during epidemics with use of a clinical case definition. Clin Infect Dis 2000; 31:1166–1169.

13. Ebell M, White L, Casault A. A systematic review of the history and physical examination to diagnose influenza. J Am Board Fam Pract 2003; 17:1–5.

14. Gorse G, O’Connor T, Young SL et al. Efficacy of live, cold-adapted and inactivated influenza vaccines in older adults with chronic obstructive pulmonary disease: a VA cooperative study. Vaccine 2003; 21:2133–2144.

15. Neuzil K, O’Connor T, Gorse G, Nichol K. Recognizing influenza in older patients with chronic obstructive pulmonary disease who have received influenza vaccine. Clin Infect Dis 2003; 36:169–174.

16. Caramori G, Ito K, Contoli M et al. Molecular mechanisms of respiratory virus-induced asthma and COPD exacerbations and pneumonia. Curr Med Chem 2006; 13:2267–2290.

17. Hurst JR, Perera WR, Wilkinson TM, Donaldson GC, Wedzicha JA. Systemic and upper and lower airway inflammation at exacerbation of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006; 173:71–78.

18. Johnston S, Papi A, Bates P, Mastronarde J, Monick M, Huntinghake G. Low grade rhinovirus infection induces a prolonged release of IL-8 in Pulmonary Epithelium. J Immunol 1998; 160:6172–6181.

19. Papi A, Bellettato M, Bracchioni F et al. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. Am J Respir Crit Care Med 2006; 173:1114–1121.

20. Hurst JR, Donaldson GC, Perera WR et al. Use of plasma biomarkers at exacerbation of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006; 174:867–874.

21. Pinto-Plata V, Tosó J, Lee K et al. Profiling serum biomarkers in patients with COPD: associations with clinical parameters. Thorax 2007; 62:595–601.

22. 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; 164:1618–1623.

23. Steel D, Donoghue FC, O’Neill RM, Uhlar CM, Whitehead AS. Expression and regulation of constitutive and acute phase serum amyloid A mRNAs in hepatic and non-hepatic cell lines. Scand J Immunol 1996; 44:493–500.

24. Yamada T. Serum Amyloid A (SAA): a concise review of biology, assay methods and clinical usefulness. Clin Chem Lab Med 1999; 37:381–388.

25. Bozinovski S, Hutchinson AF, Thompson MA et al. Serum Amyloid A is a biomarker of acute exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008; 177:269–278.

26. Monto A, Bramley T, Barnes M. Development of a predictive index for picornavirus infections. Clin Infect Dis 2003; 36:253–258.

27. Pepe MS. The Statistical Evaluation of Medical Tests for Classification and Prediction. Oxford: Oxford University Press, 2003.

28. Awad M. An introduction to ROC analysis. Pattern Recognit Lett 2006; 27:861–874.

29. Hanley JA, McNeil BJ. A method of comparing the areas under receiver operating characteristics curves derived from the same cases. Radiology 1983; 148:839–843.

30. Walsh E, Cox C, Falsey A. Clinical features of influenza A virus infection in older hospitalized persons. J Am Geriatr Soc 2002; 50:1498–1503.

31. Raghmann M, Warner J, Mackowiak P. The relationship between age and fever magnitude. Am J Med Sci 2001; 322:68–70.

32. Norman D. Fever in the elderly. Clin Infect Dis 2000; 31:148–151.

33. Zhu J, Qiu Y, Majumdar S et al. Exacerbations of Chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006; 173:71–78.

34. Papi A, Johnston S. Rhinovirus infection induces expression of its own receptor intercellular adhesion molecule 1 (ICAM-1) via increased NF-kB-mediated transcription. J Biol Chem 1999; 274:9707–9720.

35. Falsey A, Walsh EE, Looney RJ, Francis CW, Hall WJ, Abraham GN. Response of C-reactive protein (CRP) and serum amyloid A (SAA) to influenza infection in older persons. J Infect Dis 2001; 183:995–999.