Importance of viral and bacterial infections in chronic obstructive pulmonary disease exacerbations

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ABSTRACT

Background: Few studies have evaluated the contribution of both viruses and bacteria in acute exacerbation of chronic obstructive pulmonary disease (AECOPD).
Objectives: This study estimated the burden of both types of pathogens among adults seeking care for an AECOPD during two consecutive winter seasons.
Study design: Patients 50 years or older who consulted within 10 days of AECOPD onset were eligible. Clinical data were collected on a standardized questionnaire, and nasopharyngeal aspirates (NPA), paired sera, and non-induced sputum were collected. Polymerase chain reaction (PRC) assays were used to identify viral, atypical and bacterial pathogens in NPA specimen.
Results: Overall, 108 patients with AECOPD were included, 88% of patients were admitted and 2 patients (2%) received intensive care. A third of patients (31%) had evidence of a viral infection, 9% with influenza A, 7% RSV and 7% with PIV-3. One patient was positive for *Mycoplasma pneumoniae*. Bacterial pathogens were identified in 49% of patients with available sputum, most frequently *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Haemophilus influenzae*. Among virus-infected patients, 14 (58%) also had bacteria in their sputum, but co-infected patients did not present with different symptoms than patients with single infections.
Conclusions: These results suggest that influenza and RSV are frequent contributors of AECOPD, and that coinfection with bacteria does not appear to be more severe among virus-infected patients. Clinicians should be aware that AECOPD may be frequently triggered by viruses, and may consider antivirals and proper infection control measures in appropriate epidemiological setting.

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1. Background

Acute exacerbation of chronic obstructive pulmonary disease (AECOPD) is often triggered by bacterial or viral infection of the airways.[1–4] Bacteria have been implicated in the development of AECOPD but the pathophysiology of this relationship is not fully understood. It is believed that colonization promotes inflammatory response and subsequent airway obstruction, or that infection may directly contribute to the development of AECOPD. Bacteria are isolated in 40–60% of AECOPD.[5] However, 20–40% of patients with stable COPD have positive sputum cul-

tures, suggesting that bacteria may sometimes be an innocent bystander, rather than the actual cause of AECOPD.[6] In addition to bacteria, recent studies using sensitive nucleic acid detection methods have shown that respiratory viruses may be frequent triggers of AECOPD.[7] Viral infections are believed to be responsible for the same proportion of AECOPD[8] than bacterial infections.[6] Viral infections have also been detected in stable COPD patients, suggesting that viruses may cause persistent low-grade infection that could contribute to the pathogenesis of the disease.[9] While influenza appears to be the most frequently detected virus in that condition, respiratory syncytial virus (RSV) is also a leading cause of virus-induced exacerbation.[2–4,10–12] As infected adults shed little virus compared to children, it is only with the advent of sensitive molecular assays capable of detecting few viral copies that the role of RSV in community-acquired adult

lower respiratory tract infections has become more frequently recognized.[13–15]

2. Objectives

The purpose of this study was to estimate the burden of viral and bacterial infections among adults seeking care for an acute exacerbation of COPD by using an array of methods, including bacterial cultures, serology and RT-PCR assays for respiratory viruses and atypical bacteria.

3. Study design

3.1. Subjects and study design

This prospective study was conducted during two consecutive winter seasons and took place in three university-affiliated hospitals, Province of Quebec, Canada. Patients were recruited between 10 January 2003 and 15 May 2003, during the first winter, and between 6 January 2004 and 5 May 2004, during the second winter.

Participants were COPD patients aged ≥50 years, who consulted or were admitted in a participating hospitals for an acute exacerbation of their illness <10 days of onset of symptoms. COPD patients were defined by a baseline forced expiratory volume per second (FEV_1) less than 70% of the predicted value or chronic bronchitis (cough and sputum ≥3 months/year for ≥2 consecutive years). An acute exacerbation was defined as any increase of respiratory symptoms (dyspnea, cough, and sputum) requiring an unscheduled medical visit. Patients with bronchiectasis, cystic fibrosis and those presenting only with an exacerbation of asthma were excluded. During the second year, a control group of stable COPD patients consulting for their regular follow-up was also included. Patients without paired sera were also excluded.

After obtaining informed consent, the research nurse collected clinical data (date of onset and type of symptoms, previous medical conditions and current medication) and medical investigations (chest X-ray, oxygen saturation and FEV_1) on a standardized questionnaire. A nasopharyngeal aspirate (NPA) and a blood test (acute phase sera) were collected as well as non-induced sputum. A second blood sample (convalescent phase sera) was drawn 21–28 days later, and additional data were collected about evolution of the disease. Data about hospital stay and admission to the intensive care unit were extracted from the medical chart after discharge.

3.2. Respiratory virus and atypical bacteria detection

NPA specimens were tested for the presence of respiratory viruses (Influenza A and B, RSV, Human metapneumovirus (hMPV), parainfluenza 1–3 (PIV), adenoviruses, rhinoviruses, coronaviruses) and atypical bacteria (*Legionella pneumophila*, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*) by the use of PCR assays.

NPA specimens were first tested using a multiplex real-time RT-PCR assay for influenza A and B, RSV and hMPV. This multiplex assay has been described in details elsewhere.[16] The lower limit of detection is 50 copies for influenza A and B, 100 copies for RSV and 250 copies for hMPV. Using the same methodology, specimens were also tested with a second multiplex RT-PCR assay for the presence of PIV-1, PIV-3, rhinoviruses, and adenoviruses. The amplified genes and lower limits of detection of the second multiplex PCR are the followings: PIV-1 (hemagglutinin-neuraminidase, 500 copies), PIV-3 (nucleocapsid, 500 copies), rhinoviruses (5′ non-coding region, 1000 copies) and adenoviruses (hexon, 5 copies). The third RT-PCR assay detected the presence of coronaviruses OC43, 229E, NL and HKU1. Individual assays were used to test specimens for atypical bacteria, i.e., *M. pneumoniae*, *C. pneumoniae* and *L. pneumophila*.

Acute and convalescent phase sera were tested for the presence of total antibodies directed against influenza A and B, hRSV, hMPV, PIV 1–3, adenoviruses, *L. pneumophila*, *C. pneumoniae* and *M. pneumoniae* by complement fixation test, whereas an ELISA test was used for hMPV.[17] Evidence of acute infection was defined by a ≥4-fold increase in antibody titers or a seroconversion between acute and convalescent sera.

3.3. Sputum

Non-induced sputum was cultured on blood and chocolate agar plates and bacterial pathogens were identified using the Microscan system (Baxter Healthcare Corporation, West Sacramento, California).

3.4. Statistical analyses

Differences in clinical presentation, delay between onset and consultation, and length of stay were evaluated by χ^2 tests between (a) RSV and influenza infections, and (b) viral and non-viral exacerbations.

4. Results

4.1. Patient characteristics

Over two seasons, 108 patients (63 in 2002–2003, and 45 in 2003–2004) with acute exacerbation of COPD met the inclusion criteria (Table 1). Sixty-two patients (57%) were ≥70 years, 29% were 60–69 years old and 14% were 50–59 years old. Overall, 26 patients (24%) had an underlying cardiovascular disease and 9 (8%) had diabetes. Most (83%) cases were vaccinated against influenza. Nearly all (98%) patients had a history of tobacco and 18% were currently smoking. Severe COPD (FEV_1 < 30% of the predicted value) was present in 56%, whereas moderate COPD (FEV_1 between 50% and 70% of the predicted value) was found in 30%. During the course of their exacerbation, 12% were treated as outpatients and 88% of patients were hospitalized. Of those, 2 (2%) were admitted to the intensive care unit.

4.2. Viral and bacterial infections

Among the 108 patients, 34 (31%) had evidence of a viral infection (Table 2): 10 influenza A (9%), 8 RSV (7%), 7 PIV-3 (6%), 4 hMPV (4%) and 3 rhinoviruses (3%). Among the 34 patients with viral infections 29% had influenza A, 24% RSV, and 21% PIV-3. One patient had a mixed viral infection with influenza A and coronavirus OC43. All patients with influenza A had received the influenza vaccine. Excluding coronavirus and rhinovirus infections, for which no serological testing was performed, 40% of viral infections were identified by RT-PCR only, 31% by serology only and 20% by both methods. Among the 8 RSV infected patients, only 2 (25%) were positive by both serology and RT-PCR, 4 were only positive by PCR and 2 were only positive by serology.

One patient had *M. pneumoniae* detected by PCR and serology. Non-induced sputum was available for 72 (67%) patients, of which 35 (49%) had a bacteria identified in culture (Table 3). The most frequent bacteria isolated were *Staphylococcus aureus* (15% of patients), *Pseudomonas aeruginosa* (11%), *Haemophilus influenzae* (7%), *Moraxella catarrhalis* (5%), *Stenotrophomonas maltophilia* (4%) and *Streptococcus pneumoniae* (5%). Eight patients were infected by 2 microorganisms, and 3 patients had 3. Among the 24 patients with viral infections who had results of sputum culture, 14 (58%) patients also had bacteria in their sputum. Viral, bacterial or mixed infection was not found more frequently in patients with severe COPD (defined by FEV_1 < 30% of predicted value) than in those with less severe disease.

Table 1
Patient characteristics at time of acute exacerbation of chronic obstructive pulmonary disease (COPD).

	2002–2003, N = 63 (58%)		2003–2004, N = 45 (42%)		Total, N = 108	
	n	%	*n*	%	*n*	%
Gender						
Male	35	55.6	24	53.3	59	54.6
Female	28	44.4	21	46.7	49	45.4
Age (years)						
50–59	9	14.3	6	13.3	15	13.9
60–69	20	31.7	11	24.4	31	28.7
70–79	24	38.1	21	46.7	45	41.7
80+	10	15.9	7	15.6	17	15.7
Medical conditions						
Cardiovascular[a]	15	23.8	30	66.7	45	41.7
Immunosupression	6	9.5	3	6.7	9	8.3
Diabetes	6	9.5	9	20.0	15	13.9
Immunization						
Influenza	49	77.8	40	88.9	89	82.4
Pneumococcal	40	63.5	26	57.8	65	60.1
History of smoking						
Past	53	84.1	33	73.3	86	79.6
Current	7	11.1	12	26.7	19	17.6
Severity of disease (FEV$_1$ in % of the predicted value)						
<30%	36	57.1	24	60.0	60	55.6
30–49%	18	28.6	14	35.0	32	29.6
50–69%	6	9.5	2	5.0	8	7.4
Duration of COPD disease (years)						
≤5	18	28.6	14	31.1	32	29.6
6–9	9	14.3	11	24.4	20	18.5
10–19	29	46.0	14	31.1	43	39.8
≥20	3	4.8	2	4.4	5	4.6
Usual COPD treatment before exacerbation						
Bronchodilators	57	90.5	39	86.7	96	88.9
Inh. corticosteroids	40	63.5	32	71.1	72	66.7
Or. corticosteroids	18	28.6	15	33.3	33	30.6
O$_2$ at home	17	27.0	7	15.6	24	22.2

[a] Cardiovascular conditions include: history of myocardial infarction, chronic ischemic heart disease, chronic heart failure, hypertension or other conditions listed as significant by nurse.

4.3. Controls

During the second season 25 patients with stable COPD were tested for viral infections. Only one (4%) was positive compared to 31% patients with AECOPD ($p = 0.002$). This patient had serological evidence of *parainfluenza 3* infection and had a positive RT-PCR for rhinovirus.

5. Discussion

Many studies have attempted to measure the burden of viral or bacterial infections in patients with AECOPD, but few have searched for both types of pathogens. In our sample, 31% of patients admitted for AECOPD had evidence of respiratory viral infections, which supports the idea of their importance in AECOPD. This result is slightly higher than those previously reported in studies relying on culture or serology for identification of respiratory pathogens.[18] It is, however, in agreement with most reports using nucleic acid detection methods such as RT-PCR.[19–22] While the observed prevalence of viral respiratory infections in AECOPD patients is similar to what has been previously reported, the distribution of types of respiratory viruses is different. Influenza and RSV were the most frequently found viruses, and almost equal contributors to AECOPD. While these results are in line with previous studies, we found rhi-

Table 2
Viral infections identified by RT-PCR or serology.

	Any method		RT-PCR only		Sero. only		Both methods	
	n	%	*n*	%	*n*	%	*n*	%
Viral infections[a,b]	35	32.4	17	15.7	11	10.2	7	6.5
Influenza A	10	9.3	3	2.8	4	3.7	3	2.8
RSV	8	7.4	4	3.7	2	1.9	2	1.9
PIV-3	7	6.5	2	1.9	4	3.7	1	0.9
hMPV	4	3.7	2	1.9	1	0.9	1	0.9
Rhinovirus	3	2.8	3	1.9	n/a		n/a	
Coronavirus	3	2.8	3	1.9	n/a		n/a	
Bacterial infections	1	0.9	1	0.9	1	0.9	1	0.9
M. pneumoniae	1	0.9	1	0.9	1	0.9	1	0.9

[a] 35 viral infections occurred in 34 patients, with 1 patient presenting with mixed infection.
[b] Rhinoviruses and coronaviruses identification is made through RT-PCR only.

Table 3
Patients hospitalized for an acute exacerbation of COPD presenting with bacteria in sputum (data in number of patients).

	Total $N = 108^a$	Flu A $N = 10^a$	PIV-3 $N = 7^a$	RSV $N = 8^a$	Coronavirus $N = 10^a$	Any virus $N = 34^a$	No virus $N = 74^a$
Number of patients with culture	72 (67%)	8 (80%)	5 (71%)	5 (63%)	2 (20%)	24 (71%)	48 (65%)
Number of patients w/bacteria	35 (32%)	5 (50%)	5 (71%)	3 (38%)	1 (10%)	14 (42%)	21 (28%)
Number of organisms	49	6	6	3	1	16	33
Bacteria in sputum culture	35 (49%)	5 (63%)	5 (100%)	3 (60%)	1 (50%)	14 (58%)	21 (44%)
Staphylococcus aureus	11 (15%)	1 (13%)	1 (20%)	1 (20%)	1 (50%)	4 (17%)	7 (15%)
Pseudomonas aeruginosa	8 (11%)	2 (25%)	1 (20%)	–	–	3 (13%)	5 (10%)
Haemophilus influenzae	5 (7%)	1 (13%)	–	1 (20%)	–	2 (8%)	3 (6%)
Moraxella catarrhalis	4 (5%)	1 (13%)	–	–	–	1 (5%)	3 (6%)
Streptococcus pneumoniae	4 (5%)	–	1 (20%)	1 (20%)	–	2 (8%)	4 (8%)
Stenotrophomonas maltophilia	3 (4%)	–	–	–	–	–	3 (6%)
Other nonfermenting bacilli	2 (3%)	–	–	–	–	–	2 (4%)
Klebsiella oxytoca	1 (1%)	–	–	–	–	–	1 (2%)
Legionella spp.	1 (1%)	–	–	–	–	–	1 (2%)
Klebsiella pneumoniae	1 (1%)	–	1 (20%)	–	–	1 (5%)	–
Citrobacter freundii	1 (1%)	–	1 (20%)	–	–	1 (5%)	–
Serratia marcescens	1 (1%)	–	–	–	–	–	1 (2%)

[a] Total number of patients.

noviruses in only 4 cases (9% of all viral exacerbations) which is much less than previously observed. Reasons for our lower detection rate for rhinoviruses may include the low sensitivity of our multiplex RT-PCR assay for these pathogens (1000 copies), a different epidemiological season, and a more selected population (88% of patients were hospitalized).

A pathogenic bacteria was found in the sputum of 49% of patients with AECOPD. This proportion is consistent with other reports and explains why clinical trials found antibiotics to clinically improve patients with AECOPD.[23] The most frequently identified pathogen was *S. aureus* (15%), and *P. aeruginosa* (11%). Soler et al., found that more severe COPD cases infected with influenza and RSV were also coinfected by bacterial pathogens.[1] We did not find a higher frequency of mixed infections in patients with severe disease but this may reflect the small number of patients with severe COPD and influenza or RSV infection in our study. Bacterial analysis of sputum was only conducted on patients who were able to submit an adequate sample (67%). The failure rate in obtaining a valid sputum sample that we have observed in this study is consistent with what has been reported in the litterature.[24] Because patients with sputum samples were not more likely to have a viral infection or to have a more severe disease, we believe that our results were not affected even if sputum cultures were not available from all patients.

Methodological limitations may have led to bias in the estimation of the true impact of respiratory viruses on AECOPD patients. Despite the exclusion of patients who consulted >10 days after disease onset, viral shedding in adults may last shorter than that and we may have missed some viral cases. Also, we did not seek for all viruses associated with respiratory tract infections, such as parainfluenza 2 (PIV-2), PIV-4 and enteroviruses. This may explain the fact that no virus or bacteria were found in 38% (27/72) of patients with sputum. Seasons also differ epidemiologically but data obtained from the province's reference laboratory showed a moderate to high respiratory virus activity during both study years.

The increased sensitivity of RT-PCR comes with the inability to distinguish acute infection from prolonged shedding. Neither the detection of a virus by PCR nor a seroconversion mean unequivocally that these viruses have been causing the actual disease. Recently, researchers have identified RSV from large percentages of stable COPD patients, which raised the possibility of persistent low-grade RSV infection.[20,21,25–30] Despite the fact that RSV detection has been associated with elevated markers of inflammation and a more rapid decline in lung function suggesting a role in pathogenesis and evolution of the disease, the presence of a high proportion of RSV-infected stable CPOD patients raises the possibility that col-

onization could have been detected in some patients.[31] While our group of controls was small and limited to only one season, we found none with RSV infection.

In conclusion, viral infections are frequent in patients with AECOPD, and RSV appears to be as frequent as influenza A in these patients. Bacterial pathogens were not more frequent among virus-infected patients and were not associated with more severe disease. Because influenza was detected in 10% of AECOPD hospitalizations, testing for this virus during its epidemic period can be considered along with the need for antiviral therapy.

Competing interests

Acknowledgements

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