Molecular Diagnosis of Respiratory Tract Infection in Acute Exacerbations of Chronic Obstructive Pulmonary Disease

Sanjay Sethi

Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, University at Buffalo, State University of New York, Veterans Affairs Western New York Health Care System, Buffalo, New York

Acute exacerbations are significant events in the course of chronic obstructive pulmonary disease. Modern diagnostic techniques have revealed an infectious cause for the majority of exacerbations. Common respiratory viruses contribute to 25%–50% of exacerbations. Detection of viral nucleic acids in nasopharyngeal swab or sputum samples has become the preferred method to study viral exacerbations instead of viral cultures and serologic examination. Clinical application of such molecular detection requires additional studies to clarify interpretation of a positive result. Bacteria account for 25%–50% of exacerbations. Studies comparing molecular detection of bacteria in sputum with conventional culture techniques have shown that a substantial proportion of bacteria are not detected by the latter method. However, as with molecular viral detection, clinical application of molecular bacterial diagnosis requires additional studies. Although still faced with several challenges and requiring additional development, it is quite likely that molecular methods will become the preferred methods for determining the etiology of exacerbations of chronic obstructive pulmonary disease.

The course of chronic obstructive pulmonary disease (COPD) is characterized by periodic episodes of increased respiratory (dyspnea, cough, sputum production, and chest discomfort) and systemic (fatigue, sleep disturbance, and low-grade fever) symptoms that are clinically diagnosed as exacerbations [1]. Although poorly characterized in the past, recent investigations have clarified the significance, etiology, and pathogenesis of exacerbations [2]. In a landmark longitudinal study in the 1960s, no relationship was found between loss of lung function and frequency of COPD exacerbation [3]. Recent studies dispute the earlier result [4–5]. Exacerbations are the leading cause of death in advanced COPD, are associated with a more rapid decline in the quality of life of patients with COPD, and as a result of hospitalization, account for 40%–50% of the costs of care of COPD [6–8].

COPD is now recognized as an inflammatory disease of the airways and the parenchyma of the lung [9]. Exacerbations have been characterized as acute inflammatory events superimposed on this background of chronic inflammation [2, 10]. This excess inflammation increases airway obstruction by inducing bronchoconstriction, excess mucus production, and airway edema, which translates to the cardinal clinical symptoms of dyspnea, cough, and sputum production. Many patients with COPD have these symptoms when they are at their stable baseline status; in such patients, an increase in symptoms that are beyond their usual day to day variability defines an exacerbation. Recent work in developing patient-reported symptom tools to measure exacerbations has shown that our focus on these cardinal symptoms is too narrow (NK Leidy, unpublished data). Additional important symptoms of exacerbations described by patients include chest discomfort, fatigue,

and sleep disturbance. Sputum purulence is also common and is often a cue for a patient to seek health care. Of interest, patients seek health care for only half the episodes that meet the definition of exacerbations of COPD (reported exacerbations) [11]. The unreported episodes are milder than the reported exacerbations but still have significant long term-impact on the course of COPD [11].

ETIOLOGY OF ACUTE EXACERBATIONS OF COPD

The typical course of an exacerbation, with a subacute onset over days to a peak, followed by gradual resolution over days, is suggestive of an infectious process. With use of modern diagnostic techniques, it is clear that the majority of exacerbations are of infectious origin (Table 1) [2]. Current estimates are that approximately one-quarter of exacerbations are of viral cause, one-quarter are bacterial in etiology, and one-quarter result from a combination of pathogens [12]. The

cause of the exacerbation in the remaining 25% is unclear. Some episodes appear to be related to environmental or irritant exposures.

The viral and bacterial pathogens associated with exacerbations share several common characteristics. All have tropism for the upper airway in the healthy human host. The primary mode of transmission is human to human. They usually asymptomatically colonize the upper airway (above the glottis) or cause mild infections. In the patient without COPD, the innate defense mechanisms keep the lower airway sterile in spite of repeated inhalation and micro-aspiration of bacteria and viruses [2]. In the context of COPD, the innate lung defense system is damaged, with subsequent increased risk of microbial colonization that extends into the lower (subglottic) airway. Pathogen presence in the lower airway stimulates a host immune-inflammatory response in an attempt to eradicate the pathogens. The inflammatory response causes symptoms of an exacerbation and contributes to progressive airway damage. Furthermore, the effectiveness of the adaptive immune response that follows

Table 1. Microbial Pathogens in Chronic Obstructive Pulmonary Disease (COPD)

Microbe	Role in exacerbations	Role in stable disease
Bacteria		
Haemophilus influenzae	20–30% of exacerbations	Major pathogen
Streptococcus pneumoniae	10–15% of exacerbations	Minor role
Moraxella catarrhalis	10–15% of exacerbations	Minor role
Pseudomonas aeruginosa	5–10% of exacerbations, prevalent in advanced disease	Likely important in advanced disease
Enterobacteriaceae	Isolated in advanced disease, pathogenic significance undefined	Undefined
Haemophilus haemolyticus	Isolated frequently, unlikely cause	Unlikely
Haemophilus parainfluenzae	Isolated frequently, unlikely cause	Unlikely
Staphylococcus aureus	Isolated infrequently, unlikely cause	Unlikely
Viruses		
Rhinovirus	20–25% of exacerbations	Unlikely
Parainfluenza	5–10% of exacerbations	Unlikely
Influenza	5–10% of exacerbations	Unlikely
Respiratory syncytial virus	5–10% of exacerbations	Controversial
Coronavirus	5–10% of exacerbations	Unlikely
Adenovirus	3–5% of exacerbations	Latent infection seen, pathogenic significance undefined
Human metapneumovirus	3–5% of exacerbations	Unlikely
Atypical Bacteria		
Chlamydophila pneumoniae	3–5% of exacerbations	Commonly detected, pathogenic significance undefined
Mycoplasma pneumoniae	1–2% of exacerbations	Unlikely
Fungi		
Pneumocystis jiroveci	Undefined	Commonly detected, pathogenic significance undefined

NOTE. Reproduced with permission from Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med. 2008;359[22]:2355–65. ©Massachusetts Medical Society, 2008

exacerbations is often not helpful because of strain-to-strain heterogeneity [13–14].

MOLECULAR DETECTION OF VIRAL EXACERBATIONS

Several studies done during the 1950s–1970s document a viral etiology of COPD exacerbations. These studies relied on viral culture and serologic findings (Table 2) [15]. With these techniques, ≥1 respiratory virus was associated with ∼30% of exacerbations. In contrast, when the same patients with COPD were studied in stable periods, viral isolation or serologic conversion were found in <1%. A major drawback in these older studies and several recent studies is the reliance on nasopharyngeal (NP) samples for viral culture. There may be a discordance between the presence of virus in the NP and virus in the lower respiratory tract. The presence of virus and a specific 4-fold increase in antibody titer in convalescent serum samples is supportive evidence of infection. A positive serologic response could occur in a viral upper airway infection. Therefore, although earlier studies are supportive of a viral causation of exacerbations, it was presumed that the NP culture results are indicative of infection in the tracheobronchial tree and that an increase in antibody titer reflects lower, and not upper, airway infection.

Introduction of polymerase chain reaction (PCR) methods in the 1990s ushered in a new wave of studies examining the viral causation of exacerbations of COPD [16–21]. These studies report the presence of viral nucleic acid in 30%–60% of exacerbations. Beckham et al [22] found viruses by culture in 23.4% of 194 upper airway samples obtained from 2 studies of adults with respiratory illness, including exacerbations of COPD. When these samples were subjected to reverse-transcriptase PCR (RT-PCR) viral nucleic acid detection, the viral yield increased to 41.8% [22]. In some of the studies with the highest rates of detection, a higher rate of detection in subsequent stable state samples was also seen [17, 19]. The spectrum of viral pathogens detected by the molecular techniques was similar to that seen by culture in earlier studies, with the exception of newly discovered viruses, such as human metapneumovirus [23]. The relative frequency of viruses also changed. Influenza was detected less often, and RSV was detected more often [24]. This redistribution could reflect the change in method of detection or changes in prevalence as a result of influenza vaccination. Of interest, detection of viral nucleic acid was higher using sputum as opposed to NP samples when both sites were simultaneously sampled [16, 25]. Sputum samples are considered to be more representative of lower airway infection than are nasopharyngeal samples, provided the samples meets the quality criteria of >25 leukocytes and <10 epithelial cells per low-power field.

Molecular detection of virus in sputum is sensitive, specific, and less traumatic than that in NP swab specimens. The problem is the interpretation of the results. Should investigators conclude that the presence of viral nucleic acids, detected by PCR of sputum samples, implies (proves) infection by the virus? PCR can detect as few as 10–100 copies of a target respiratory virus. Whether such low viral titers are of pathological significance is unclear. Borg et al [26] quantified respiratory syncytial virus in respiratory secretions with use of real-time RT-PCR in children with acute respiratory tract infection and adults with COPD [26]. The viral quantitation varied by 2000 fold (1.2×10 [7] vs 6.1×10 [3]) [26]. The viral titers were equally low in stable and exacerbated COPD. Is the PCR detecting dead or live virus, invasive disease, or prolonged asymptomatic shedding? Quantitation may help define invasive disease. Another limitation of PCR studies is the lack of immunological assays to determine the presence or absence of an antibody. Other markers of the host response might help. Papi et al [12] report sputum eosinophilia only when viruses are detected by PCR in sputum during an exacerbation. In other studies, increased levels of sputum IL-6 are reported using molecular detection of virus [20].

Molecular detection of viral pathogens will likely become the diagnostic method of choice in exacerbations of COPD because of its rapidity, convenience, and sensitivity, compared with culture. Development of new antiviral drugs will spur the use of molecular diagnosis in the clinical setting. Before that happens, we need to ascertain the following in the research setting: is quantitative PCR, rather than qualitative PCR, necessary at the time of exacerbation to distinguish between extremely low titers of likely noninvasive virus and the higher concentrations seen with infection? Technically, are the performance

Table 2. Viral Infection Detected by Culture and/or Serologic Testing in Acute Exacerbations of Chronic Obstructive Pulmonary Disease (COPD) in 8 Studies Published During 1960–1980 [15]

Variable	No. of exacerbations included	Percentage viral	Percentage of total viral isolates					
			Rhinovirus	Influenza	Para influenza	Respiratory syncytial virus	Coronavirus	Adenovirus
Total	1081							
Mean	135	36	38	26	15	11	10	3
Range	42–522	20–61	0–78	0–45	0–39	0–40	6–18	0–10

characteristics of RT-PCR of sputum equivalent to those with NP aspirate samples? Are the immunologic clinical syndromes and inflammatory responses seen in exacerbations with viruses detected molecularly similar to those when the virus is detected by culture methods? How often is the RT-PCR detecting dead virus or nonreplicating virus? Are the results of PCR testing comparable in different laboratories?

MOLECULAR DETECTION OF BACTERIAL EXACERBATIONS

Prior studies that attempted to determine the extent of bacterial causation of exacerbations in COPD were unsuccessful [27]. Bacteria colonize the tracheobronchial tree in stable COPD. Therefore, simply detecting bacteria in sputum samples from patients with COPD does not differentiate between colonization and infection, as was shown in early studies of the bacteriology of sputum in COPD [28–29]. However, by using molecular epidemiology, it is possible to differentiate between newly acquired strains and pre-existing colonizing strains of bacterial pathogens. There is a clear relationship between the acquisition of new strains and exacerbations of COPD [30]. Furthermore, exacerbations with new strains are associated with mucosal and systemic immune response to the infecting strain and are strongly associated with a neutrophilic inflammatory profile in sputum [10, 13, 14, 31]. These studies clearly established non-typeable *Haemophilus influenzae* (NTHI), *Streptococcus pneumoniae*, *Moraxella catarrhalis,* and *Pseudomonas aeruginosa* as causative for a significant proportion of COPD exacerbations. All observations to date are based on the use of sputum cultures to identify the presence of bacterial infection.

Sputum cultures alone do not reflect the scope of bacterial infection in COPD. Evidence for this came from studies of NTHI dynamics in stable COPD. Monthly sputum cultures were performed [32] in the course of a prospective longitudinal study involving a cohort of patients with stable COPD. Strains of NTHI recur in sputum despite prolonged periods of non-detection by sputum culture. It is unclear whether this represents reinfection with the same NTHI strain or the possibility that the strain was never eliminated from sputum and the sputum culture was too insensitive to detect them. Adequacy of lower airway sampling was documented by measuring fibrinogen in culture-negative and -positive sputum samples. When molecular detection for NTHI, based on the PCR sequencing of the P2 gene was performed, NTHI was found in the culture-negative sputum samples in the gaps (Figure 1). Furthermore, the strain of NTHI detected by molecular techniques in the gaps was the same as the strain of NTHI isolated by cultures before and at the end of the gaps.

The *P. aeruginosa* dynamics of colonization were examined in the same study. In the culture-negative sputum samples,
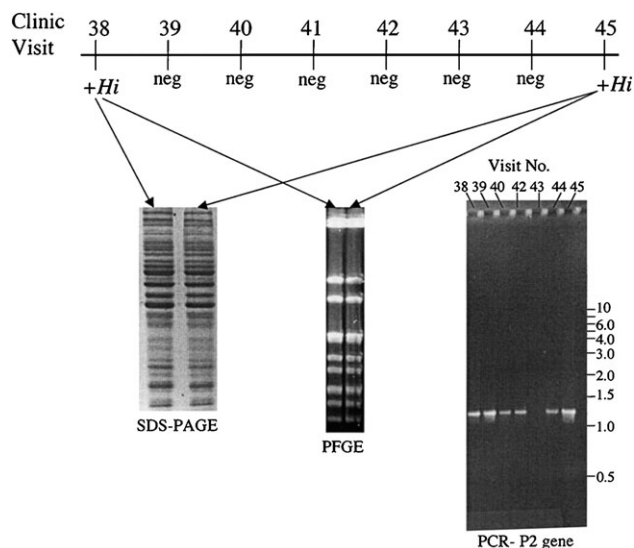

Figure 1. Time line from a study clinic patient depicting clinic visits 38–45, which occurred ∼1 month apart. Results of sputum cultures are noted below each clinic visit. ``+*Hi*'' indicates culture positive for *H. influenzae*. ``Neg'' indicates culture negative for *H. influenzae*. Identical results of typing by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and pulsed-field gel electrophoresis (PFGE) are shown for the strains isolated at visits 38 and 45. An agarose gel at the right shows the results of PCR of the P2 gene (about 1.2 kb) of the sputum pellets from clinic visits as noted at the top of the gel. Molecular mass markers are noted in kilobases on the right. Reproduced with permission from Murphy TF, Brauer AL, Schiffmacher AT, Sethi S. Persistent colonization by Haemophilus influenzae in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2004;170(3):266–72. ©American Thoracic Society, 2004.

molecular detection of the Opa gene of *P. aeruginosa* was present [31]. Therefore, sputum cultures are limited in their ability to detect bacterial pathogens in stable COPD. Whether this limitation extends to exacerbations of COPD has not been systematically examined. These observations are consistent with data in other respiratory infections, such as otitis media and pneumonia; detection of bacteria, such as NTHI, *M. catarrhalis,* and *S. pneumonia,* is enhanced by the use of molecular detection for the same bacteria.

Curran et al [33] evaluated 30 induced sputum samples obtained from patients with COPD and control patients. Nested real-time PCR was more sensitive than culture for the detection of a variety of typical and atypical pathogens. Their methods included the use of nested rather than 1-step PCR, and thus, there was a greater risk of contamination and an inability to quantitate. They also used the *ply* gene for *S. pneumoniae* and the *p*6 gene for NTHI, both of which are known to lack specificity for these pathogens.

Antibiotics are widely used for acute exacerbations of COPD. If rapid, inexpensive, validated techniques for molecular detection of bacteria were available, they would be clinically embraced with enhanced stewardship of antibiotic use. However, as

with viral detection, several questions arise. How can a positive PCR result separate colonization from infection? Is there a quantitative threshold that defines infection? Is the airway and systemic inflammatory profile of exacerbations in which pathogens are detected by molecular techniques only similar to that seen when bacteria are detected by culture? Is detection by molecular methods associated with immune responses similar to those seen with bacteria isolated by culture?

There are practical limitations to molecular detection. There is no isolate for molecular epidemiology. This difficulty can be surmounted by PCR sequencing of selected genes that are heterogeneous between strains of a species, such as the P2 gene of NTHI [32]. However, that would increase the complexity and turn-around time of molecular detection. Determination of specific antibody response after exacerbation is best done with the homologous strain. This can be circumvented by the use of pooled heterologous strains as the test antigen. Finally, there is no isolate for antibiotic susceptibility testing. For some organisms, it is possible to test for the gene responsible for antibiotic resistance.

THE FUTURE

The use of 16s rRNA–based microarray and terminal restriction fragment-length polymorphism techniques is an emerging application to better characterize the airway microbiota of COPD exacerbations [34–35]. Further studies in this exciting new field would enable us to get an even better understanding of the microbiology of COPD and its exacerbations.

References

1. Anzueto A, Sethi S, Martinez FJ. Exacerbations of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2007; 4:554–64.
2. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med 2008; 359:2355–65.
3. Fletcher F, Peto R. The natural history of chronic airflow obstruction. Br Med J 1977; 1:1645–8.
4. Kanner R, Anthonisen NR, Connett JE. The Lung Health Study Research G. Lower respiratory illnesses promote FEV(1) decline in current smokers but not ex-smokers with mild chronic obstructive pulmonary disease: results from the lung health study. Am J Respir Crit Care Med 2001; 164:358–64.
5. Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. Thorax 2002; 57:847–52.
6. Calverley PM, Anderson JA, Celli B, et al. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. N Engl J Med 2007; 356:775–89.
7. Spencer S, Calverley PMA, Burge S, Jones PW. Health status deterioration in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2001; 163:122–8.
8. Andersson F, Borg S, Jansson SA, et al. The costs of exacerbations in chronic obstructive pulmonary disease (COPD). Respir Med 2002; 96:700–8.
9. Hogg JC, Chu F, Utokaparch S, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med 2004; 350:2645–53.
10. Sethi S, Wrona C, Eschberger K, Lobbins P, Cai X, Murphy TF. Inflammatory profile of new bacterial strain exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2008; 177:491–7.
11. Langsetmo L, Platt RW, Ernst P, Bourbeau J. Underreporting exacerbation of chronic obstructive pulmonary disease in a longitudinal cohort. Am J Respir Crit Care Med 2008; 177:396–401.
12. 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.
13. Murphy TF, Brauer AL, Grant BJ, Sethi S. Moraxella catarrhalis in chronic obstructive pulmonary disease: burden of disease and immune response. Am J Respir Crit Care Med 2005; 172:195–9.
14. Sethi S, Wrona C, Grant BJ, Murphy TF. Strain-specific immune response to Haemophilus influenzae in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2004; 169:448–53.
15. Murphy TF, Sethi S. Bacterial infection in chronic obstructive pulmonary disease. Am Rev Respir Dis 1992; 146:1067–83.
16. Seemungal TA, Harper-Owen R, Bhowmik A, Jeffries DJ, Wedzicha JA. Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. Eur Respir J 2000; 16:677–83.
17. 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.
18. Falsey AR, Formica MA, Hennessey PA, Criddle MM, Sullender WM, Walsh EE. Detection of respiratory syncytial virus in adults with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2006; 173:639–43.
19. 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.
20. 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.
21. Cameron RJ, de Wit D, Welsh TN, Ferguson J, Grissell TV, Rye PJ. Virus infection in exacerbations of chronic obstructive pulmonary disease requiring ventilation. Intensive Care Med 2006; 32:1022–9.
22. Beckham JD, Cadena A, Lin J, et al. Respiratory viral infections in patients with chronic, obstructive pulmonary disease. J Infect 2005; 50:322–30.
23. Martinello RA, Esper F, Weibel C, Ferguson D, Landry ML, Kahn JS. Human metapneumovirus and exacerbations of chronic obstructive pulmonary disease. J Infect 2006; 53:248–54.
24. Falsey AR, Hennessey PA, Formica MA, Cox C, Walsh EE. Respiratory syncytial virus infection in elderly and high-risk adults. N Engl J Med 2005; 352(17):1749–59.
25. McManus TE, Marley AM, Baxter N, et al. Respiratory viral infection in exacerbations of COPD. Respir Med 2008; 102:1575–80.
26. Borg I, Rohde G, Loseke S, et al. Evaluation of a quantitative real-time PCR for the detection of respiratory syncytial virus in pulmonary diseases. Eur Respir J 2003; 21:944–51.

27. Tager I, Speizer FE. Role of infection in chronic bronchitis. N Engl J Med **1975**; 292:563–71.
28. Gump DW, Phillips CA, Forsyth BR, McIntosh FK, Lamborn KR, Stouch WH. Role of infection in chronic bronchitis. Am Rev Respir Dis **1976**; 113:465–73.
29. McHardy VU, Inglis JM, Calder MA, Crofton JW. A study of infective and other factors in exacerbations of chronic bronchitis. Br J Dis Chest **1980**; 74:228–38.
30. Sethi S, Evans N, Grant BJ, Murphy TF. New strains of bacteria and exacerbations of chronic obstructive pulmonary disease. N Engl J Med **2002**; 347:465–71.
31. Murphy TF, Brauer AL, Eschberger K, et al. Pseudomonas aeruginosa in chronic obstructive pulmonary disease. Am J Respir Crit Care Med **2008**; 177:853–60.
32. Murphy TF, Brauer AL, Schiffmacher AT, Sethi S. Persistent colonization by Haemophilus influenzae in chronic obstructive pulmonary disease. Am J Respir Crit Care Med **2004**; 170:266–72.
33. Curran T, Coyle PV, McManus TE, Kidney J, Coulter WA. Evaluation of real-time PCR for the detection and quantification of bacteria in chronic obstructive pulmonary disease. FEMS Immunol Med Microbiol **2007**; 50:112–8.
34. Rogers GB, Daniels TW, Tuck A, et al. Studying bacteria in respiratory specimens by using conventional and molecular microbiological approaches. BMC Pulm Med **2009**; 9:14. PMCID: 2678980.
35. Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. PLoS One **2010**; 5:e8578. PMCID: 2798952.