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REVIEW

Chronic bronchial infection in COPD. Is there an infective phenotype?



Zinka Matkovic ^{a,b}, Marc Miravittles ^{a,*}

^a *Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Ciber de Enfermedades Respiratorias (CIBERES), Hospital Clínic, Barcelona, Spain*

^b *University Hospital Dubrava, Zagreb, Croatia*

Received 12 February 2012; accepted 30 October 2012

Available online 4 December 2012

KEYWORDS

Bacteria;
Chronic obstructive
pulmonary disease;
Phenotypes;
Colonisation;
Exacerbation;
Infection;
Microorganisms

Summary

Microorganisms, particularly bacteria, are frequently found in the lower airways of COPD patients, both in stable state and during exacerbations. The host–pathogen relationship in COPD is a complex, dynamic process characterised by frequent changes in pathogens, their strains and loads, and subsequent host immune responses.

Exacerbations are detrimental events in the course of COPD and evidence suggests that 70% may be caused by microorganisms. When considering bacterial exacerbations, recent findings based on molecular typing have demonstrated that the acquisition of new strains of bacteria or antigenic changes in pre-existing strains are the most important triggers for exacerbation onset.

Even in clinically stable COPD patients the presence of microorganisms in their lower airways may cause harmful effects and induce chronic low-grade airway inflammation leading to increased exacerbation frequency, an accelerated decline in lung function and impaired health-related quality of life. Besides intraluminal localisation in the distal airways, bacteria can be found in the bronchial walls and parenchymal lung tissue of COPD patients. Therefore, the isolation of pathogenic bacteria in stable COPD should be considered as a form of chronic infection rather than colonisation. This new approach may have important implications for the management of patients with COPD.

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* Corresponding author. Servei de Pneumologia, Hospital Clínic, Villarroel 170, 08036 Barcelona, Spain.
E-mail address: marcm@separ.es (M. Miravittles).

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Introduction

The role of microorganisms, especially bacteria, in the pathogenesis of chronic obstructive pulmonary disease (COPD) has been the focus of interest for several decades, although many questions remain to be answered. While in the 1950s and 1960s microorganisms were considered among the main aetiologic factors in COPD, and in the 1970s and 1980s their role was minimised, interest in this subject has rekindled in recent years with the introduction of new research techniques which have provided interesting and rather surprising discoveries. The difficulties and limitations of the microbiological assessment of different respiratory samples, the unclear significance of isolation of the same pathogens during both stable COPD and exacerbations and the absence of a standardised definition of COPD exacerbation are only some of the "problems" faced when investigating this issue and will be addressed in depth in this review. Another topic that will be discussed in detail is the significance of the presence of microorganisms, particularly bacterial, in the distal airways during stable COPD, which has recently become of increasing interest due to the emerging evidence that microorganisms may have an active role in the evolution of the disease.

Isolation of bacteria from respiratory specimens during stable COPD

Microorganisms are one of the main aetiologic factors involved in exacerbations of COPD.¹⁻¹³ In contrast, understanding of their role during stable phases of the disease is still incomplete, although some studies have suggested that they actively contribute to chronic airway inflammation leading to the progression of COPD.¹³⁻¹⁹

Our knowledge of the bacterial species that can be found in the lower airways in stable COPD is based on qualitative and quantitative cultures of spontaneous or induced sputum samples, bronchoscopic protected specimen brush (PSB), as well as bronchial lavage (BL) and bronchoalveolar lavage (BAL) samples.¹⁶⁻²⁰ Novel, non-culture detection methods have recently been introduced in respiratory research, although broader use remains limited by their cost. These nonculture-based techniques include polymerase chain reaction (PCR) for the detection

of microbial nucleic acid in respiratory specimens,^{21,22} molecular typing based on polyacrylamide and pulsed-field gel electrophoresis to distinguish different bacterial strains,^{3,22,23} *in situ* hybridisation and immunofluorescence microscopy for detection of intracellular microorganisms in bronchial biopsy specimens.²⁴ Although sputum samples are easier to collect, these samples are mainly obtained from the large airways which are often contaminated by upper airway microbial flora, thereby reducing the reliability of the culture. In addition, PCR studies to detect bacterial nucleic acid have demonstrated false negatives with sputum cultures.^{21,22} In contrast, contamination by upper airway flora is avoided with the use of PSB, BL and BAL culture samples, but the use of fiberoptic bronchoscopy has inherent risks and cost. Additionally, bronchoscopic methods are unsuitable when repeated sampling is required, such as in longitudinal studies.

Another point that should be taken into consideration regarding colonisation is the cut-off value to define a positive culture, which, in most cases, is arbitrary. The thresholds for positive cultures used in most of the recently published studies are as follows: $\geq 10^2$ or $\geq 10^5$ colony-forming units/millilitre (CFU/ml) for sputum,^{14,17,18,25} $\geq 10^2$ CFU/ml for BL,²⁶ and $\geq 10^2$ or $\geq 10^3$ CFU/ml for PSB and BAL samples.^{2,16,19,27-33}

Bacterial species isolated from respiratory specimens are usually divided into two groups: potentially pathogenic microorganisms (PPMs) and non-potentially pathogenic microorganisms (non-PPMs).^{16,28,34} PPMs are recognised as agents causing respiratory infections and include *Haemophilus* spp. (nontypeable *Haemophilus influenzae* (NTHi), *Haemophilus parainfluenzae* and *Haemophilus haemolyticus*), *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and some members of a large Enterobacteriaceae family (*Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Enterobacter cloacae*).^{16,28,34} Non-PPMs are usually not involved in respiratory infections in immunocompetent individuals; they belong to the normal oropharyngeal or gastrointestinal flora and include bacteria such as *Corynebacterium* spp., *Neisseria* spp., *Enterococcus* spp., coagulase-negative *Staphylococcus* spp. and *Streptococcus viridans* group and fungi such as *Candida* spp.^{28,32} The distribution of bacterial species into PPMs and non-PPMs may change over time in accordance with the

evidence emerging. For example *M. catarrhalis*, previously known as *Neisseria catarrhalis* or *Branhamella catarrhalis*, was first considered as a non-PPM³⁵ but is now deemed as a PPM.^{16,23,28,34} The opposite situation might occur in the near future with *H. haemolyticus* and *H. parainfluenzae*, since recent findings have suggested their pathogenic potential to be much lower in comparison to other PPMs^{4,18,36,37} and some authors have already started to categorise them as non-PPMs.^{33,36} Among PPMs, NTHi, *S. pneumoniae*, *M. catarrhalis* and *H. parainfluenzae* are the most frequently recovered in stable COPD patients,^{2,18,25,27,34,38–40} while *Pseudomonas* spp. predominantly appear in more advanced disease.^{17,26} However, it is not unusual for more than one pathogen to simultaneously be isolated in the same patient.^{2,17,25,40}

It is commonly believed that the lower respiratory tract of healthy non-smoking individuals is sterile, but some investigators have shown that lower airways of healthy subjects can also be colonised,^{2,16,26,28} although the colonisation rate is relatively low (4–40%) and caused mainly by non-PPMs (Tables 1 and 2). The exception is the study by Weinreich et al.²⁶ in which even 90% of healthy subjects (current smoking status unknown) had positive cultures of BL samples, although PPMs were isolated in only 10% (Table 2).

The rates of positive cultures in stable COPD vary depending on the sampling technique and the cut-off points employed. In the studies using sputum samples up to 100% of cultures were positive for any bacteria, while 38–74% were positive for PPMs (Table 3).^{14,17,18,25,39,40} With PSB samples, the colonisation rate was somewhat lower, as

expected, ranging from 22 to 83%, with PPMs isolated in 25–31% (Table 1).^{2,19,27–31} Similarly, BAL and BL cultures were positive in 13–89% of cases, with PPMs isolated in 33–43% (Table 2).^{16,19,26,28}

In most of the studies investigating distal airway colonisation in COPD, patients with previously proven bronchiectasis were excluded from the analysis. After the introduction of high-resolution computed tomography (HRCT) in the routine clinical practice, it has become clear that bronchiectasis of different degrees and extent is a common feature in COPD patients.^{41,42} Therefore, it is expected that the prevalence rate of bronchial colonisation in COPD could be even higher than suggested by the previously mentioned studies. In agreement with this presumption, the colonisation rate with PPMs was of up to 63% in the two studies in which bronchoscopic sampling was preformed in stable patients with bronchiectasis and COPD (Tables 1 and 2).^{26,32} Furthermore, in the study on stable COPD by Patel et al.,⁴¹ after excluding patients with previously diagnosed or clinically evident bronchiectasis, up to 50% of the study population fulfilled radiological criteria for bronchiectasis on HRCT and a positive correlation was found between lower lobe bronchiectasis, distal airway colonisation and bronchial inflammation. In a more recent study by Martinez-Garcia et al.⁴² the prevalence of subclinical bronchiectasis in stable COPD was even higher (58%), and was significantly associated with a higher rate of bronchial colonisation and increased systemic inflammation measured by fibrinogen plasma levels.

The sensitivity of the novel, nonculture-based techniques to detect the presence of microorganisms is even

Table 1 Percentages of positive bacterial cultures using protected specimen brush (PSB) samples in healthy subjects, patients with COPD and bronchiectasis.

Source	Patients, N	FEV ₁ , % pred	Specimen/cut-off value for positive culture, CFU/ml	Culture positive for any bacteria, N (%)	Culture positive for PPM, N (%)	Culture positive for nonPPM, N (%)	Most frequently isolated PPMs
Rosell et al. ²	Healthy, 70 (49% smokers)	92	PSB/≥10 ²	–	3 (4%)	–	Hi
	Stable COPD, 181	56			53 (29%)		Hi, Sp, Mc
	Exacerbated COPD, 86	37			46 (54%)		Hi, Pa, Sp, Mc, En
Monso et al. ²⁷	Stable COPD, 40	51	PSB/≥10 ³	–	10 (25%)	–	Hi, Sp
	Exacerbated COPD, 29	44			15 (52%)		Hi, Sp, Pa, Mc
Cabello et al. ²⁸	Healthy, 16 (12% smokers)	92	PSB/≥10 ²	2 (12%)	–	–	Sa
	Stable COPD, 18	77		15 (83%)			Hi, Sp, Sa
	Stable bronchiectasis, 17	73		14 (82%)			Hi, Pa, Sa
Ruse et al. ²⁹	Stable CB, 42 (45% with COPD)	79	PSB/>10 ³	10 (24%)	–	–	Sp, Hi
Monso et al. ³⁰	Stable CB, 41	75	PSB/≥10 ³	9 (22%)	–	–	Hi, Sp
Zalacain et al. ³¹	Controls, 20 (10% smokers)	88	PSB/≥10 ³	0 (0%)	–	–	–
	Stable COPD, 88	56		36 (41%)	27 (31%)		Hi, Sp, Mc
Angrill et al. ³²	Stable bronchiectasis, 75 (49% with COPD)	75	PSB/≥10 ²	–	46 (61%)	22 (29%)	Hi, Pa, Sp, Mc
Soler et al. ³³	Exacerbated COPD, 40	37	PSB/≥10 ²	27 (68%)	18 (45%)	10 (25%)	Hi, Sp, Mc, Pa

COPD: chronic obstructive pulmonary disease; CB: chronic bronchitis; PSB: protected specimen brush; PPM: potentially pathogenic microorganism; FEV₁: forced expiratory volume in one second; CFU: colony forming units; Hi: nontypeable *Haemophilus influenzae*; Mc: *Moraxella catarrhalis*; Sp: *Streptococcus pneumoniae*; Pa: *Pseudomonas aeruginosa*; En: members of Enterobacteriaceae family; Sa: *Staphylococcus aureus*.

Table 2 Percentages of positive bacterial cultures using bronchoalveolar or bronchial lavage samples in healthy subjects, patients with COPD and bronchiectasis.

Source	Patients, N	FEV ₁ , % pred	Specimen/cut-off value for positive culture, CFU/ml	Culture positive for any bacteria, N (%)	Culture positive for PPM, N (%)	Culture positive for nonPPM, N (%)	Most frequently isolated PPMs
Sethi et al. ¹⁶	Healthy, 15 (non-smokers)	99	BAL/≥10 ²	–	1 (7%)	6 (40%)	<i>Hp</i>
	Healthy, 20 (ex-smokers)	90			0 (0%)	4 (20%)	–
	Stable COPD, 26	60			9 (35%)	6 (23%)	<i>Hi, Hp</i>
Soler et al. ¹⁹	Controls, 12 (100% smokers)	94	BAL/≥10 ³ + PSB/≥10 ²	–	5 (42%)	7 (58%)	<i>Sp, Sa, Hi, Mc</i>
	Stable COPD, 52	28–65			17 (33%)	28 (54%)	<i>Sp, Sa, Hi, Mc</i>
Weinreich et al. ²⁶	Healthy ^a , 48	3.0 L	BL/≥10 ²	43 (90%)	5 (10%)	–	<i>Hi, Sp</i>
	Stable COPD, 53	1.2 L		47 (89)	23 (43%)		<i>Hi, Sp</i>
	Stable bronchiectasis, 32	1.8 L		31 (97%)	20 (63%)		<i>Hi, Sp, En, Pa</i>
Cabello et al. ²⁸	Healthy (12% smokers), 15	92	BAL/≥10 ³	2 (13%)	–	–	–
	Stable COPD, 16	77		2 (13%)			<i>Sp</i>
	Bronchiectasis, 13	73		10 (77%)			<i>Hi, Pa</i>
Angrill et al. ³²	Stable bronchiectasis, 59 (49% with COPD)	75	BAL/≥10 ³	–	33 (56%)	19 (32%)	<i>Hi, Pa, Sp</i>

COPD: chronic obstructive pulmonary disease; CB: chronic bronchitis; BQ: bronchiectasis; PSB: protected specimen brush; BAL: bronchoalveolar lavage; BL: bronchial lavage; PPM: potentially pathogenic microorganism; FEV₁: forced expiratory volume in one second; CFU: colony forming units; *Hi*: nontypeable *Haemophilus influenzae*; *Hp*: *Haemophilus parainfluenzae*; *Mc*: *Moraxella catarrhalis*; *Sp*: *Streptococcus pneumoniae*; *Pa*: *Pseudomonas aeruginosa*; *En*: members of Enterobacteriaceae family; *Sa*: *Staphylococcus aureus*.

^a Current smoking status not provided.

greater. It has recently been demonstrated that with the use of PCR techniques, microbial nucleic acid can be extracted from a certain proportion of culture negative respiratory specimens.^{21,22} Assuming that nucleic acid represents viable bacteria, this would indicate even higher rates of colonisation than culture findings have suggested. Additionally, some microorganisms are able to invade and survive within airway epithelial cells and macrophages.^{43,44} With the use of *in situ* hybridisation and immunofluorescence microscopy for the detection of intracellular NTHi in bronchial biopsies, this microorganism was found in 33% and 87% of stable and severely exacerbated chronic bronchitis patients, respectively, but not in healthy adults.²⁴

The most frequently identified risk factors for colonisation with PPMs are current or previous smoking,^{25,30,31} impaired lung function defined by a reduction in FEV₁ and/or forced vital capacity (FVC),^{2,30,31} comorbid conditions,²⁵ increased rate of previous exacerbations^{25,39} and the presence of bronchiectasis.^{26,42} Purulent sputum and an increased degree of dyspnoea are also recognised as clinical indicators of positive sputum cultures for PPMs in stable COPD.²⁵

Microbial colonisation or chronic bronchial infection?

The host–pathogen interaction is complex but crucial for the establishment and proliferation of microorganisms in

the lower airways. This interaction is best described for NTHi and involves adherence to mucous membranes and extracellular matrix, damage to epithelial cells, ciliotoxic activity, an increase in mucin production, as well as complex mechanisms of evading host immune defences such as invasion into the epithelial cells, production of IgA proteases and antigenic heterogeneity.^{44,45} Some host factors such as reduced mucociliary clearance, mucus hypersecretion and bronchiectasis are also involved,²⁶ although it is not always clear whether some these features are really risk factors for acquiring bacteria or their consequence. Recent findings have stressed the role of innate immunity impairment in COPD, which among others, may manifest as defective phagocytosis and hyporesponsiveness of alveolar macrophages to bacterial antigens, leading to reduced clearance of bacterial pathogens and subsequent predisposition to colonisation and infection.^{46,47}

COPD is characterised by different degrees of chronic inflammation affecting the large and small airways as well as the lung parenchyma and pulmonary vasculature.^{29,48,49} Considering COPD as an inflammatory disorder, several studies have attempted to clarify the impact of bacterial colonisation in mediating this inflammatory process, beyond the effect of cigarette smoking. Bacteria release molecules with potent proinflammatory effects such as endotoxins (e.g. lipooligosaccharide of NTHi), outer membrane lipoproteins (e.g. outer membrane protein

Table 3 Percentages of positive sputum cultures in stable COPD and most frequently isolated pathogens.

Source	Patients, N	FEV ₁ , % pred.	Specimen, N/cut-off value for positive culture, CFU/ml	Culture positive for any bacteria, N (%)	Culture positive for PPM, N (%)	Most frequently isolated PPMs
Papi et al. ¹	Stable, convalescent COPD (8–10 weeks after an exacerbation), 64	50	I sputum, 64/≥10 ⁶	–	24 (38%)	<i>Hi, Sp, Mc, Sa</i>
Wilkinson et al. ¹⁴	Stable COPD, 30	35	S or I sputum, 30/>10 ⁵	30 (100%)	At baseline: 16 (53%) After 1 year: 17 (57%)	<i>Hi, Mc, Hp</i>
Hill et al. ¹⁷	Stable CB, 160: - COPD, 55 - COPD with A1AT def, 62 - bronchiectasis, 43	28 21 62	S sputum, 336/≥10 ⁵	–	247 (74%)	<i>Hi, Hp, Mc, Pa</i>
Marin et al. ¹⁸	Stable COPD, 40	58	I sputum, 79/≥10 ²	–	At baseline: 58 (73%) After 9 months: 56 (71%)	<i>Hi, Pa, En, Hp</i>
Miravittles et al. ²⁵	Stable COPD, 119	46	S or I sputum, 119/≥10 ²	–	58 (49%)	<i>Hi, Hp, Pa</i>
Patel et al. ³⁹	Stable COPD, 29	39	I sputum, 29/cut-off not provided	–	15 (52%)	<i>Hi, Sp</i>
Banerjee et al. ⁴⁰	Stable COPD, 67	~40	I sputum, 67/cut-off not provided	67 (100%)	27 (40%)	<i>Hi, Mc, Sp</i>

COPD: chronic obstructive pulmonary disease; CB: chronic bronchitis; A1AT def: alpha 1-antitrypsin deficiency; PPM: potentially pathogenic microorganism; FEV₁: forced expiratory volume in one second; CFU: colony forming units; S: spontaneously expectorated; I: induced; *Hi*: nontypeable *Haemophilus influenzae*; *Hp*: *Haemophilus parainfluenzae*; *Mc*: *Moraxella catarrhalis*; *Sp*: *Streptococcus pneumoniae*; *Pa*: *Pseudomonas aeruginosa*; *En*: members of Enterobacteriaceae family; *Sa*: *Staphylococcus aureus*.

[OMP] P2 and OMP P6 of NTHi), peptidoglycan fragments, lipoteichoic acid, toxins (e.g. pneumolysin from *S. pneumoniae*), and others.^{20,50–52} In response to these bacterial molecules, airway epithelial cells and macrophages produce a variety of different inflammatory mediators (e.g. interleukin [IL]-6, IL-8, IL-10, tumour necrosis factor [TNF]- α , macrophage chemotactic protein [MCP]-1, macrophage inflammatory protein [MIP]-1 α), which further lead to recruitment and activation of neutrophils, macrophages and other inflammatory cells.^{51–54} The neutrophils, which prevail in the bronchial secretions of COPD patients and healthy smokers,¹⁹ are recruited from the circulation and activated in response to chemoattractants, particularly IL-8 and leukotriene B4 (LTB4) via adhesion molecules, such as E-selectin and intercellular adhesion molecule (ICAM)-1, expressed on the vascular endothelium. The activated neutrophils degranulate releasing myeloperoxidase and different types of proteinases (elastase, cathepsin G, proteinase 3, matrix metalloproteinase), which together participate in various aspects of tissue damage.³⁸

Colonised COPD patients have higher concentrations of inflammatory cytokines, neutrophils and their products in

respiratory secretions in comparison with non-colonised patients with a similar degree of airflow obstruction, which would indicate a higher level of airway inflammation induced by bacteria.^{15,16,40} Different bacterial species and even different strains of the same species differ in virulence and inflammatory potential.^{4,17,55} In the study by Hill et al.,¹⁷ *P. aeruginosa* was recognised as the most potent inducer of inflammation followed by NTHi, whereas *M. catarrhalis* provoked a significantly milder reaction. Additionally, Sethi et al.⁴ demonstrated the significant inflammatory potential of NTHi and *M. catarrhalis*, while *H. parainfluenzae*-associated exacerbations had an inflammatory profile similar to PPM-negative exacerbations. Furthermore, there is an interspecies diversity in the duration of colonisation, e.g. *M. catarrhalis* is usually cleared from the respiratory tract efficiently, even without antibiotics, within a period of only one month,²³ while the carriage of NTHi and *P. aeruginosa*, particularly the mucoid strains, may last much longer.^{18,21,22}

Bacterial colonisation is a dynamic process with changes in pathogens, their strains and loads occurring over time.^{3,14,18,21,23} It has been suggested that a change in

bacterial strain and/or a rise in bacterial load can increase the degree of airway and systemic inflammation and trigger the onset of an exacerbation,^{3,10,12,17,56,57} which will be discussed in more detail later. Most adults who acquire bacteria in their respiratory tract develop an immune response, as has been demonstrated in the study by Murphy et al.²³ in which 72% of episodes of acquisition of *M. catarrhalis* were followed by a systemic (serum IgG) and/or mucosal (sputum IgA) antibody response to the homologous strain. It is of clinical importance that colonised patients have worse health status,⁴⁰ increased exacerbation frequency as well as more symptoms during exacerbations.³⁹ Bacteria may also contribute to an accelerated decline in lung function and, consequently, to progression of COPD. This is supported by the study of Wilkinson et al.,¹⁴ in which a decline in FEV₁ was related to a change in the colonising bacterial type and a rise in airway bacterial load. This observation was confirmed by a more recent study in patients with moderate COPD, in which neutrophilic airway inflammation, mainly found in patients colonised by PPMs, was associated with more than a two-fold increased risk of FEV₁ decline over the median decline during follow-up.¹⁸ Bacteria may accelerate FEV₁ decline by both, directly increasing basal airway inflammation in the stable state, as well as by indirectly inducing more frequent and severe exacerbations.^{14,18,39}

In view of the above, it is clear that the isolation of PPMs from respiratory samples does not fulfil the definition of "colonisation", because it is associated with an inflammatory response and significant damage to the target organ. This is why it has recently been suggested that the term chronic bronchial infection would be more appropriate when addressing the presence of significant concentrations of PPMs in the lower airways of stable COPD patients.^{13,58} Chronic bronchial infection in COPD can be defined as the presence of PPMs in respiratory secretions that cause an inflammatory reaction manifested by the chronic production of coloured/purulent sputum. This syndrome can be accompanied by recurrent infective exacerbations and systemic manifestations in the form of malaise, febricula, asthenia or weight loss, similar to what has been described in patients with bronchiectasis.⁵⁹ In fact, most of these patients have significant bronchiectasis when studied by HRCT scan. In the study by Martínez-García et al.⁴² the isolation of a PPM in sputum in a patient with stable COPD, together with a FEV₁ <50% predicted and the history of at least one hospital admission for an exacerbation the previous year were associated with a 99% probability of having bronchiectasis on CT scan. Conversely, the term colonisation should only be used to describe the isolation of non-PPMs from the distal respiratory tract, either in healthy subjects or in patients with a chronic airway disease.

Besides common bacteria, respiratory viruses and atypical bacteria in the form of chronic infection may also have an impact on the course of COPD.^{8,48,60} Seemungal et al.⁸ analysed nasal aspirates and blood samples of stable COPD patients by PCR and detected low-grade viral infection with respiratory syncytial virus (RSV) in 24% of patients, as well as infection with viruses other than RSV (dominantly rhinoviruses and coronaviruses) in 16% of patients. Patients in whom virus was detected were more

likely to have increased exacerbation frequency as well as higher concentrations of serum IL-6 and plasma fibrinogen (potential predisposition to thrombotic events) when stable.⁸ Furthermore, examining lung tissue of smokers with different degrees of emphysema, Retamales et al.⁴⁹ determined a 5- to 40-fold increase in the number of alveolar epithelial cells expressing adenoviral E1A protein in mild and severe emphysema, respectively, and suggested the impact of latent adenoviral infection on the amplification of cigarette smoke-induced lung inflammation which is present in severe emphysema. In addition to promoting an inflammatory process, lower respiratory viral or atypical bacterial infection may enhance the susceptibility of the airways to other pathogens.^{60,61} One example is chronic *Chlamydomphila pneumoniae* infection in COPD patients, which has been associated with a higher rate of airway colonisation with common bacteria, frequent exacerbations and more severe functional impairment.⁶⁰

From chronic bronchial infection to exacerbation of COPD

Exacerbations are relatively frequent events in the course of COPD, especially in moderate-to-severe disease, with a significant impact on health status, quality of life,^{62,63} disease progression,⁶⁴ mortality,^{65,66} health-care utilisation and costs.^{67,68} Before discussing the role of microorganisms in the exacerbation of COPD (ECOPD), it should be mentioned that there is still no standardised definition of ECOPD.^{69,70} The reason for this probably lies in the complex pathophysiology and the different aetiological factors involved, as well as in the variety of clinical manifestations presented by these patients. The American Thoracic Society/European Respiratory Society (ATS/ERS) Task Force on COPD⁷¹ defines ECOPD as an increase in respiratory symptoms over baseline that usually requires a change in therapy. Nonetheless, the most widely used clinical criteria for ECOPD are increased dyspnoea, sputum volume and sputum purulence as defined by Anthonisen et al.⁷² based on the probability of response to antibiotic therapy.

Despite all the limitations in defining ECOPD, investigations performed in recent years have sought to establish the underlying pathophysiological process of these events. In comparison to the stable state, there is an acute increase in the level of airway inflammation during periods of exacerbations, assessed by the rise in sputum inflammatory cells (neutrophils, eosinophils and lymphocytes) and different inflammatory mediators including IL-6, IL-8, LTB₄, TNF α , neutrophil proteases, myeloperoxidase, eosinophil cationic protein (ECP) and RANTES (Regulated upon Activation, Normal T-cell Expressed and Secreted).^{1,4,50,54,57,58,73–75} Intensified inflammation may induce airway mucosal oedema, bronchospasm and mucus hypersecretion, which further lead to increased respiratory symptoms.¹² The association between neutrophilic inflammation, sputum purulence and bacterial exacerbations is well established and provides a rationale for using sputum purulence as a clinical indicator of bacterial infection and the need for antibiotic therapy.^{4,10,33,74,76,77} Additionally, sputum neutrophilia positively correlates with the severity of an

exacerbation assessed by the impairment in lung function and the level of hypoxia.¹ Inflammation in ECOPD is not only limited to the lower airways. There is also a certain degree of upper airway and systemic inflammation, which is proportional to the lower airway inflammation and is higher in bacterial exacerbations.^{58,73,74,76} Furthermore, the clinical severity of exacerbations is significantly correlated with the level of local and systemic inflammation.⁵⁷

Published data suggest that up to 70% of exacerbations are caused by respiratory infections including aerobic bacteria (40–60%), respiratory viruses (about 30%) and atypical bacteria (5–10%).^{11,78} Some authors have demonstrated polymicrobial aetiology in as much as 33% of exacerbations, being particularly important in the most severe cases.^{1,79,80} Environmental factors including low temperature and air pollution (exposure to higher levels of black smoke particulate matter, sulphur dioxide, nitrogen dioxide and ozone) are considered as the cause of approximately 10% of ECOPD, depending on the season and geographical setting.^{78,81–83} Furthermore, non-compliance with therapy and/or abrupt withdrawal of therapy are provocation factors in some exacerbations.⁸⁴ Nonetheless, in a certain proportion of ECOPD the aetiology remains unknown.

Considering bacterial exacerbations, NTHi, *S. pneumoniae* and *M. catarrhalis* are the most frequently isolated PPMs from respiratory secretions during exacerbations, similar to what occurs in the stable state.^{1,2,27,85} Other Gram-negative bacteria such as *P. aeruginosa*, *Stenotrophomonas maltophilia* and members of the *Enterobacteriaceae* family are more often present in patients with a greater degree of functional impairment, recent antibiotic or systemic steroid therapy, and in those with severe exacerbations.^{85–87}

Interestingly, PPMs are isolated more frequently and in higher loads during exacerbations compared to the stable state. They can be found in about 50% of exacerbated patients when using PSB samples (Table 1), usually with $>10^7$ CFU/ml in sputum cultures and $>10^3$ CFU/ml in PSB cultures.^{1,2,10,27,79} As mentioned previously, when searching for the presence of NTHi intracellularly in the bronchial mucosa in exacerbated chronic bronchitis, NTHi was detected more frequently in comparison with the stable state.²⁴ All these findings and the fact that the severity of bronchial inflammation is directly correlated with bacterial load¹⁷ led to the development of the “fall and rise” or quantitative hypothesis of bacterial ECOPD.¹⁰ Based on this hypothesis, symptoms of exacerbation appear when the inflammatory reaction caused by the increasing bacterial load in the airways exceeds a certain threshold, which is determined by a combination of bacterial and host factors.¹⁰ On the other hand, recent findings based on molecular typing of bacterial isolates have suggested that the acquisition of new strains of bacteria or antigenic change in pre-existing strains are crucial in the pathogenesis of bacterial exacerbations, and that the change in the bacterial load with subsequent enhancement of inflammation are just secondary phenomena.^{3,12,22,34,57} Following a cohort of ambulatory COPD patients during more than four years, Sethi et al.³ demonstrated that the acquisition of a new strain of NTHi, *S. pneumoniae* and *M. catarrhalis* was associated with more than a two-fold higher risk for an exacerbation. In a more recent study of a similar design but

with a longer follow-up, Murphy et al.²² have shown that the same association between change in a strain and occurrence of exacerbations also exists for *P. aeruginosa*. It is suggested that after a new strain is acquired due to the absence of an effective host immune response, bacteria may intensively proliferate in the airways resulting in a higher bacterial load, more severe local and systemic inflammation and the development of symptoms of exacerbation.^{12,57} The immune response to the infecting strain, which develops after an ECOPD, is strain-specific and not protective against acquisition of new strains,^{5,23,88,89} which may explain recurrent exacerbations even by the same species.⁵⁶ Moreover, this specific immune response provides indirect evidence that the particular microorganism was actually the cause of the exacerbation.^{5,23,88,89} However, a change in strain may not be the only mechanism of exacerbations. In the previously mentioned studies,^{3,22} only 33% and 43%, respectively, of the visits involving isolation of a new bacterial strain were associated with the onset of an exacerbation. Additionally, in both studies a certain proportion of exacerbations were diagnosed at visits in which the isolated bacteria did not belong to a new strain, and also some exacerbations occurred at visits without isolation of any bacterial pathogen. It seems that the increase in airway inflammation and clinical manifestations of exacerbation are the result of a complex host–pathogen interaction in which a change in a bacterial strain plays a central role, but other factors may influence as well, e.g. host defences, virulence capacity of different strains or bacterial species, the presence of microorganisms other than bacteria and non-microbial provocation factors, among others.^{3,12,13,36,56,90}

Role of viruses and atypical bacteria in exacerbations of COPD

The role of viruses and atypical bacteria in ECOPD has recently received increasing attention thanks to novel diagnostic techniques such as PCR and reverse transcription PCR (RT-PCR), which have a higher sensitivity in detecting these pathogens compared with previously established methods (cell cultures, serology). In a systematic review on the prevalence of viral infection in ECOPD⁷ including eight studies using PCR and/or RT-PCR in sputum and nasal lavage fluid samples, the weighted mean prevalence of respiratory viral infection was 34%, being as high as 56% in one of these studies.⁹¹ Picornavirus (especially rhinovirus) was most commonly detected, followed by influenza (prevalence depended on the vaccination status of the study population) and respiratory syncytial virus. Coronavirus, parainfluenza, adenovirus and human metapneumovirus were detected in significantly smaller percentages.^{7,8} Considering the clinical presentation, viral exacerbations have been associated with a higher total symptom count at presentation, a higher frequency of additional symptoms (cold, sore throat, increased dyspnoea, fever), slower recovery and frequent previous exacerbations.⁸

Direct evidence of the causal relationship between viral infections and ECOPD has recently been obtained in the study by Mallia et al.⁹ who demonstrated that experimental rhinovirus infection in subjects with COPD induces clinical

and inflammatory changes similar to those seen in naturally occurring exacerbations. After infection with rhinovirus, COPD patients developed more severe and prolonged respiratory symptoms, greater lung function impairment and increased airway inflammation in comparison with healthy controls. These authors suggested that experimental rhinovirus infection may be used in the future as a human model of ECOPD.⁹

Atypical bacteria such as *C. pneumoniae*, *Mycoplasma pneumoniae* and *Legionella* spp. are intracellular pathogens that share some characteristics of viruses.⁷⁸ Their contribution in the emergence of exacerbations is not completely clear because the data on their prevalence significantly differ depending on the diagnostic methods and study populations involved.^{6,92–95} It is commonly accepted that atypical bacteria cause 5–10% of ECOPD,^{11,78} either as independent pathogens or, more frequently, as co-pathogens.^{80,93,94} However, in several serologic studies, much higher proportions of exacerbated patients had evidence of a recent infection with *C. pneumoniae*, *M. pneumoniae* or *Legionella* spp. – up to 34%, 14% and 17%, respectively.^{6,92–94}

Polymicrobial aetiology is frequently found in the most severe ECOPD and manifests either as simultaneous co-infection, secondary bacterial after a viral infection or vice versa.^{5,79,80} One of the proposed mechanisms of interaction between viruses and bacteria includes changes in the receptor molecules on the respiratory epithelial cells induced by one microorganism leading to increased susceptibility to the other microorganism.^{13,61} There is evidence that patients with polymicrobial exacerbations have more severe deterioration in lung function, a higher symptom count and longer hospitalisation.^{1,79}

Is COPD a chronic infective disease?

Although smoking cessation is effective in slowing the accelerated decline in lung function, it has been observed that the inflammatory process in the lungs of patients with COPD continues after smoking cessation, and in some aspects may even increase, contrary to the case in healthy ex-smokers whose airway inflammation usually decreases.^{96,97} Furthermore, inflammation in COPD patients is not limited to their lungs; namely, they have persistent systemic inflammation as well.⁹⁸ Some of the suggested promoters of this on-going inflammation in ex-smoking COPD patients are microorganisms, autoimmunity, inflammation as part of a repair process, recovery of the proinflammatory capacity of epithelial cells after smoking cessation or disappearance of anti-inflammatory factors such as carbon monoxide from cigarette smoke.⁹⁶ However, after review of the evidence, microorganisms emerge as the most likely “candidates” to perpetuate bronchial inflammation. In favour of this hypothesis, Moller et al.⁹⁹ detected *H. influenzae* more frequently in tissue sections of lungs from patients with COPD and cystic fibrosis than from others when analysing lung explants from transplant recipients with different end-stage pulmonary diseases. Their results indicate a generalised distribution of *H. influenzae* in the lungs of these patients, with the presence of bacteria in the epithelium and submucosa of the bronchi and bronchioles,

parenchymal lung tissue (alveolar epithelium, interstitium, alveoli), as well as in the visceral pleura.⁹⁹ Other *in vitro* and *in vivo* studies have confirmed the ability of bacteria to invade lung tissue and persist therein.^{24,43} Apart from the frequent intraluminal localisation of bacteria in the airways of COPD patients, it has been suggested that such “tissue reservoir” protects these patients from the circulating antibiotics and effective host immune defences may serve as an endogenous source for recurrent infections as well as a chronic inflammatory stimulus.^{43,52} The hypothesis of chronic microbial stimulation is supported by the histological changes observed in the lungs of COPD patients. Namely, besides tissue remodelling, progression of the disease leads to increased infiltration of the small airways by the lymphocytes and their organisation into lymphoid follicles, probably as an adaptive immune response to persistent microbial stimulation.¹⁰⁰

Evidence suggests that, together with smoking, microorganisms play a significant role in the development and progression of COPD. The sequence of events in the pathogenesis of COPD with microbial involvement is summarised in Fig. 1. The most important difference with other chronic infective disease is that COPD does not involve only one causative pathogen but rather there is a change in the pathogens, their strains and loads occurring over time. Whether this influence of infection exists for all COPD patients or only for those with an “infective phenotype” still needs to be established since most of the previous longitudinal studies investigating this issue have only included COPD patients with chronic bronchitis. In fact, even exacerbations themselves may have different phenotypes. Bacterial exacerbations present with a particular inflammatory profile that is different from the viral, the so-called eosinophilic-predominant and the paucinflamatory.¹⁰¹ Interestingly, the bacterial exacerbation phenotype can be identified from the stable state in patients with an “infective phenotype” and these patients will develop the same type (or phenotype) of bacterial exacerbation in successive episodes.¹⁰¹ This infective phenotype of COPD could be described as patients with chronic production of coloured sputum during a stable state, recurrent bacterial exacerbations and frequently associated bronchiectasis. In other words, the infective phenotype in COPD refers to patients with the syndrome of chronic bronchial infection.

However, many aspects of the relationship between infection (acute and chronic) and COPD require further research, e.g. the significance of simultaneous isolation of multiple bacterial strains, possible cooperation of PPMs and non-PPMs in disease progression and the development of antibiotic resistance, mechanisms of interactions of different pathogens in polymicrobial infections as well as the relation between microbial and environmental factors in the pathogenesis of exacerbations. The use of novel, molecular, cellular and immunologic techniques is crucial for progress in this research. In addition, efforts should be made to seek new, reliable, non-invasive methods for detection of lower airway infection/inflammation in order to avoid limitations of sputum cultures and the inconvenience of bronchoscopic sampling. Further evidence on the impact of bacteria in COPD can be expected from the results of the currently ongoing clinical trials on antibiotics in stable COPD.^{102,103} Finally, the relevance of the described

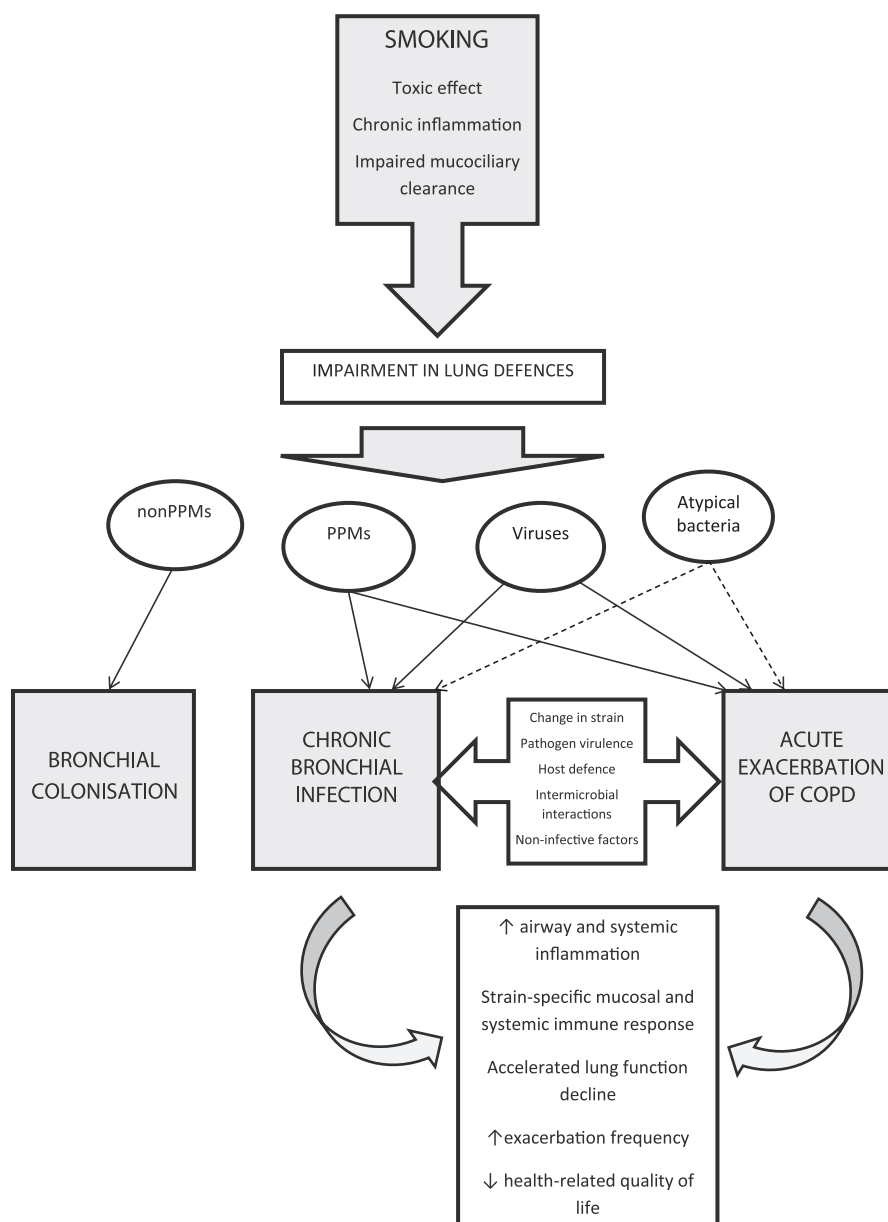


Figure 1 The impact of microbial infection on the pathogenesis of COPD, both in the stable state and in exacerbations. Since the current evidence for the role of atypical bacteria is incomplete, they are presented with a dashed line. COPD: chronic obstructive pulmonary disease; PPM: potentially pathogenic microorganism.

microbiota on lung health must be established. Molecular culture-independent techniques have identified bacteria previously not amenable to culture. Analysis of the highly conserved 16S rRNA gene has been used to assign phylogeny and allows a picture of the complete microbial community in an environment (the bronchial tree) to be constructed, offering a more comprehensive analysis than classical culture-based techniques.¹⁰⁴ The number of studies examining the lower airways microbiome is limited and there is some overlap between bacteria seen in COPD and healthy individuals¹⁰⁵; however, a recent study has reported a significantly different bacterial community (microbiome) in patients with very severe COPD compared with nonsmokers, smokers and patients with cystic fibrosis.¹⁰⁶ Further studies are clearly needed to understand the role of these microbiomes in healthy individuals and patients with COPD.

Acknowledgements

Dr. Zinka Matkovic from the University Hospital Dubrava, Zagreb, Croatia, is the recipient of a joint European Respiratory Society/Spanish Society of Pneumology and Thoracic Surgery (ERS/SEPAR) LTRF fellowship 47-2010.

Conflict of interest

Marc Miravittles has received speaker fees from Boehringer Ingelheim, Pfizer, AstraZeneca, Bayer Schering, Novartis, Talecris, Nycomed, Merck, Sharp & Dohme and Novartis, consulting fees from Boehringer Ingelheim, Pfizer, GSK, AstraZeneca, Bayer Schering, Novartis, Almirall, Merck,

Sharp & Dohme and Nycomed and grant support from Talecris.

Zinka Matkovic has no conflict of interest to disclose.

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