Respiratory infections, exacerbations and the microbiome in COPD

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CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Chronic Obstructive Pulmonary Disease (COPD) is defined as 'a common, preventable and treatable disease that is characterised by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases'[1]. COPD is an umbrella term for small airways disease (obstructive bronchiolitis) and parenchymal destruction (emphysema), conditions resulting in chronic airflow limitation[1]. The most important factor for the development and progression of COPD is tobacco smoke[2]. However, in the last decade growing evidence suggests that risk factors other than smoking also contribute to the pathophysiology of COPD. Outdoor, occupational and indoor air pollution, lung development abnormalities (e.g. respiratory infections during childhood, history of pulmonary tuberculosis), genetics and socioeconomic status account for a substantial proportion of cases worldwide[3, 4].

In subjects with respiratory symptoms, the diagnosis of COPD is confirmed by the presence of persistent airflow limitation assessed by spirometry, with a ratio of post-bronchodilator forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) below 0.70[1]. Recently, it became clear that the burden of COPD is not adequately reflected by the severity of airflow limitation, but also by the impact of the disease on patient's health status, perceived respiratory symptoms and the frequency of exacerbations (defined as an acute worsening of disease symptoms)[1]. Therefore, the updated Global initiative for chronic Obstructive Lung Disease (GOLD) recommends not only to use the degree of airflow obstruction for classifying patients with COPD, but to integrate also the level of patient's symptoms and exacerbation risk[1].

BURDEN OF COPD

COPD is a life-threatening lung disease, expected to be the third leading cause of death worldwide by 2030[5]. The economic burden is significant, including costs directed at the diagnoses of COPD, as well as costs related with the management of COPD, especially exacerbations and the presence of comorbidities[6]. Furthermore, COPD impacts enormously on a patient's life. Characteristic symptoms of COPD are dyspnoea, cough and sputum production, which vary during the day and the week, and are associated with problems in performing basic daily activities[7, 8]. Additionally, patients with COPD are susceptible for respiratory infections and prone for exacerbations, which have a detrimental impact on a patients' wellbeing and the underlying disease. Although many overlap is present, it is important to differentiate between both disease conditions, as they cover a different pathophysiology.

RESPIRATORY INFECTIONS

Respiratory infections include among others community acquired pneumonia (CAP), which is a major health problem worldwide as it affects all age categories and has a high mortality rate[9, 10]. CAP is characterised by an acute infection of the lung parenchyma with onset in the out-of-hospital setting[11]. Several risk prediction scores exist to predict severity and mortality of CAP, to guide CAP management (e.g. determine the level of care and treatment intensity)[12]. CAP in patients with COPD was observed to be more severe as compared to non-COPD patients[13-16]. This was reflected by higher risk prediction scores and more pronounced disturbances in gas exchange. As far as mortality is concerned, there is no clear consensus, as some authors report increased short- and long-term mortality rates in patients with COPD[13, 14, 17], while others observed no differences compared to non-COPD patients[15, 16, 18]. Though, COPD has frequently been observed as underlying cause of death[17, 19].

The mechanisms behind the increased CAP risk in patients with COPD are yet to be revealed. Possibly, smoking-related effects play a role, as smoking is the most important risk factor for developing COPD[2], but has also been associated with an increased risk of CAP in general[11, 20]. In contrast, current smoking status was not associated with CAP incidence in a cohort of patients with COPD[21]. Furthermore, variability of smoking status is common, as many need to undergo several quit attempts before really stopping smoking[22]. The impact of this variability has not been taken into account in former research[23]. Yet, smoking cessation has been related to a significant decreased CAP risk in the general population[24].

In order to optimise the management of CAP it is mandatory to know the pathogens involved. *Streptococcus pneumoniae* is the most frequently identified pathogen in CAP in general[9, 25]. In contrast, *Haemophilus influenzae*, *Pseudomonas aeruginosa* and *Moraxella catarrhalis* are bacterial pathogens frequently detected in patients with COPD[26]. Whether this pattern is also reflected in COPD patients with CAP is currently not sufficiently clear. However, it is necessary to clarify this point, as knowledge of antibiotic susceptibility patterns is needed to guide treatment and avoid inadequate treatment contributing to antibiotic resistance[27]. This is especially true in patients with COPD, as pathogen detection may also represent colonisation. The latter remains a huge challenge, as isolated pathogens in stable condition are comparable to those isolated during infection. This makes it difficult to differentiate between both conditions in clinical practice. Additionally, it is currently unknown whether possible differences in the bacterial aetiology have consequences for short- and long-term mortality.

EXACERBATIONS

Exacerbations are defined as 'an acute worsening of respiratory symptoms that result in additional therapy'[1]. They contribute to the overall severity in individual patients, with a large heterogeneity between patients[28], negatively impacting on patient's wellbeing, disease outcomes and prognosis[29]. Exacerbations occur more frequently than CAP in patients with COPD[30,31]. The presence of exacerbations has been related to the severity of COPD, although patients with mild-to-moderate disease also experience exacerbations[28,32]. The same pattern is seen for hospitalisation, as the proportion of exacerbating patients who are hospitalised increase by severity of COPD[32]. A history of exacerbations is the best predictor for an exacerbation[32], but still relatively little is known about determinants. This is also due to the fact that exacerbations are thought to be underreported.

Treatment of exacerbations is challenging, as exacerbations might involve bacterial or viral infection and environmental pollution[33]. Consequently, prevention of exacerbations is a key component of COPD-management. Prevention and reduction of exacerbations is mostly derived by active immunisation, including influenza and pneumococcal vaccination. Additionally, pulmonary rehabilitation is a promising intervention to prevent exacerbations, especially by increasing self-management skills to early recognize symptoms and react adequately[34]. Furthermore, pulmonary rehabilitation has favourable effects on exercise capacity and health status[35], factors affected by exacerbations. Nevertheless, a subset of patients with COPD does not respond to[36], or complete pulmonary rehabilitation[37]. Exacerbations are often described as cause[38], while evidence is limited.

RESPIRATORY MICROBIOME

Respiratory infections and exacerbations are acute events in patients with COPD. Until now it is unclear whether the pathogen detected by culture is really the infectious trigger. Colonisation might play a role, with pathogens already present in stable state. On the other hand, former research showed that isolation of a new strain of a pathogen was associated with acute exacerbations[39]. Bacterial load seemed not to be related to the presence of exacerbations, when compared to the bacterial load observed in stable state[40]. These results strengthen the use of quantitative assessment of sputum cultures, instead of semi quantitative sputum cultures, which are not useful in determining the etiologic role of the isolated pathogen. Overall, a better understanding of the host-pathogen interaction that underlies exacerbations and infections of COPD is necessary, as this interaction is more complicated than simple changes in concentrations of bacteria[26,40]. As such, it is clear that assessing the microbiology and its relation with disease

status is complex. Clearly, more insight is needed into the pathogen distribution in stable state in patients with COPD and the presence of pathogens in the lungs of healthy individuals, as well as the contribution of infections during disease state. The respiratory microbiome can potentially contribute to these issues, and might overcome limitations of traditional culture methods.

For a long time, the lungs of healthy individuals were considered to be sterile, while the lungs of patients with COPD were believed to be colonised[41,42]. This assumption is revoked, as it was demonstrated that the lungs of healthy individuals are not sterile, but inhabited by communities of microorganisms, also called the respiratory microbiome[41-43]. Research concerning the respiratory microbiome is primarily focussing on the bacteriome (bacteria), while the virome (viruses) and mycobiome (fungi) are also part of it. The precise role of the respiratory microbiome and the possible contribution to, or protection from disease is unknown and needs further assessment.

Until now, microbiome research was mainly performed by using invasive sampling techniques, which are not applicable in daily routine. Sputum wasn't assumed to be representative for the respiratory microbiome, showing an overrepresentation of the oropharyngeal flora, representing a different bronchial compartment than bronchoalveolar lavage samples[44]. In contrast, a lot of respiratory microbiome research has been performed using sputum samples[45-49], with the advantage that it is a non-invasive procedure, allowing multiple sampling periods, to assess the microbiome over time. Insight into different sample types is necessary, in order to determine the optimal sample type for respiratory microbiome analysis in clinical practice.

AIMS OF THIS THESIS

CAP and exacerbations are complex concepts in patients with COPD, which have a tremendous impact on a patient's life. A better understanding of these concepts may contribute to improved disease management. The respiratory microbiome is promising in determining the microbial aetiology of COPD in both stable and acute condition. Therefore, the aims of this thesis were to:

- Study the impact of COPD on CAP severity and mortality.
- Determine the bacterial aetiology of CAP in patients with and without COPD; and to study the association between the bacterial aetiology, empirical antibiotic treatment, serum markers and mortality.
- Evaluate the association between COPD and CAP by smoking status.
- Compare clinical characteristics and health status of spontaneous sputum producers with a positive culture, negative culture and non-sputum producers in a cohort of patients with COPD referred for pulmonary rehabilitation.

- Investigate the impact of acute exacerbations on adherence and outcomes of pulmonary rehabilitation in patients with COPD.
- Determine the optimal sample type for respiratory microbiome analysis by the IS-pro technology.

OUTLINE OF THIS THESIS

Chapter 2 provides more insight in differences between CAP patients with and without COPD concerning severity of CAP and short- and long-term mortality in CAPNETZ, a multi-centre observational study.

Chapter 3 describes the bacterial aetiology of CAP in patients with and without COPD, and possible associations with the empirical antibiotic treatment, serum markers and short- and long-term mortality in CAPNETZ.

Chapter 4 includes data from the Clinical Practice Research Datalink GOLD (CPRD), a population-based cohort study in the UK, exploring the influence of smoking on risk of CAP in patients with and without COPD.

Chapter 5 provides more insight into differences between sputum producers with a positive culture, negative culture and non-sputum producers on clinical characteristics and health status at the start of pulmonary rehabilitation, in the Chance study, an observational mono-center study.

Chapter 6 explores the impact of acute exacerbations on dropout and response of pulmonary rehabilitation, stratified by exacerbation severity, in the Chance study.

Chapter 7 determines which sample type is valid for assessing the respiratory microbiome in patients with COPD in clinical practice.

Chapter 8 discusses the previous chapters and future directions for research and clinical practice.

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2

Increased severity and mortality of CAP in COPD: Results from the German Competence Network, CAPNETZ

Dionne C.W. Braeken, Frits M.E. Franssen, Hartwig Schütte, Mathias W. Pletz, Robert Bals, Peter Martus, Gernot G.U. Rohde, on behalf of the CAPNETZ Study Group.
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ABSTRACT

Background: Mortality of community acquired pneumonia (CAP) remains high despite significant research efforts. Knowledge about comorbidities including Chronic Obstructive Pulmonary Disease (COPD) might help to improve management and ultimately, survival. The impact of COPD on CAP severity and mortality remains a point of discussion.

Objectives: Assess the prevalence and clinical characteristics of COPD in the observational German Competence Network for CAP, CAPNETZ, and to study the impact of COPD on CAP severity and mortality.

Methods: 1307 consecutive patients with CAP (57.0% males, age 59.0±18.5), classified as CAP-only (n=1043; 78.0%) and CAP-COPD (n=264; 20.2%) were followed up for 180 days. Associations between CAP, COPD and mortality were evaluated by univariate/multivariate and Kaplan-Meier survival analyses.

Results: CAP-COPD patients were older, more often males, current/former smokers, with higher confusion-urea-respiratory rate-blood pressure (CURB) scores. Length of hospital stay, urea, glucose and leucocytes plasma levels, and arterial carbon dioxide tension ($PaCO_2$) were significantly increased in CAP-COPD. Thirty, 90- and 180-day mortality rates were significantly increased in CAP-COPD (p=0.046, odds ratio [OR]=2.48, 95% confidence interval [CI] 1.015-6.037; p=0.003, OR=2.80, 95%CI 1.430-5.468; p=0.001, OR=2.57, 95%CI 1.462-4.498; respectively). Intensive care unit (ICU)-admission and age, but not COPD, were identified as independent predictors of short-and long-term mortality.

Conclusion: Severity as well as mortality was significantly higher in COPD patients with CAP. To improve CAP management with the aim to decrease its still-too-high mortality, underlying comorbidities, particularly COPD, need to be assessed.

INTRODUCTION

Community acquired pneumonia (CAP) is a major cause of morbidity and mortality worldwide[1]. Chronic Obstructive Pulmonary Disease (COPD) is a common comorbidity in these patients. Older age, being male and having other comorbidities have been associated with more severe CAP in COPD patients compared with CAP in patients without COPD[2,3].

Conflicting evidence exists regarding mortality rates. Some studies suggested higher mortality rates in COPD patients with CAP[2-4], while others did not confirm this[5-8]. The association between confusion-urea-respiratory rate-blood pressure (CURB) severity scores[9], COPD and mortality, has been studied sparsely. Myint et al.[10] stated that CURB scores were useful in predicting CAP mortality, but did not specify this in COPD patients. The same was reported by others, when comparing different severity scores for CAP[11]. When adding age ≥65 years as a component of the severity score (CURB-65), Snijders et al.[5] did not find any differences between CAP patients with and without COPD in severity scoring and 30-day mortality. Crisafulli et al.[7] reported that CURB-65 scores were significantly higher in CAP-COPD patients, but did not investigate any possible association with mortality. A recent meta-analysis observed no significant association between COPD and increased 30-day mortality in patients with CAP[12]. Less data is available on long-term mortality.

Moreover, information about clinical parameters and their association with CAP and COPD is limited, sometimes contradicting and difficult to compare. The latter may be related to the context, as some investigators described the association between clinical parameters and mortality in CAP-COPD patients[3,6], while others looked at differences in clinical parameters between CAP-COPD patients and CAP-only patients[7,8]. Factors found to be associated with CAP-COPD are respiratory rate ≥30/min and increased arterial carbon dioxide tension ($PaCO_2$)[8]. Others, however, did not confirm this[7]. Mortality in CAP-COPD was found to be related to a $PaCO_2$ ≥45 mmHg[3], an arterial oxygen concentration (PaO_2) ≤60 mmHg[3] and a respiratory rate ≥30/min.[3,6].

The aims of this study were to assess the prevalence of COPD in a well-characterized cohort of CAP patients, represented in the German Competence Network for CAP (CAPNETZ) and to determine clinical characteristics of these patients compared with CAP-only patients. Also, the impact of COPD on CAP severity and mortality and its determinants were prospectively studied.

MATERIALS AND METHODS

Data were derived from a multi-center observational study initiated by CAPNETZ[13]. Methodological details such as sampling techniques, microbiological diagnostics and laboratory processing of this study were previously published[14]. The study design was approved by the local ethics committees of all participating centers. All patients gave written, informed consent.

Study population

CAPNETZ included 1788 CAP patients between December 2009 and June 2012. Patients were eligible to be included in CAPNETZ when age was ≥18 years, having a new pulmonary infiltrate on chest x-ray and 1 or more clinical symptoms consisting of cough, purulent sputum, positive auscultation or fever. Patients were excluded when hospitalized during the previous 28 days, chronically immunosuppressed, HIV-infected or having active tuberculosis. Follow-up included a structured interview on outcome parameters at 30 and 180 days.

The presence of COPD in CAP patients was assessed from the medical record, based on post-bronchodilator forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC) in the previous six months[15]. COPD was defined as FEV_1/FVC ratio <0.7[16]. Study physicians assessed spirometric data, confirmed diagnosis and staged patients according to the Global initiative for chronic Obstructive Lung Disease (GOLD) guidelines. No direct spirometric data was available, only the diagnosis and staging made by study physicians. Patients were excluded from the current study when no data on COPD diagnosis was available. Moreover, cigarette smoke exposure and appraisal of inhalation medication was used in order to identify COPD patients.

Study procedures

CURB scores were calculated based on the sum of points, with 1 point assigned for the presence of each criterion (confusion, urea >7 mmol/l, respiratory rate ≥30/minutes, and blood pressure) on admission to the hospital[9,17-19]. CURB scores were assessed instead of CURB-65 scores, as age was included as a possible confounder.

Date and cause of death was assessed from an autopsy report and/or the medical record. Cause of death was specified as pneumonia including sepsis, cardiac cause, pulmonary embolism, other, or unknown. Overall mortality and groups-specific mortality were analysed, with 30-, 90- and 180-day mortality calculated by subtracting the date of examination from the date of death. COPD severity and comorbidities were taken as additional factors possibly contributing to mortality in the CAP-COPD group. The presence of comorbid diseases was recorded from the medical records of patients.

To avoid possible misdiagnosis of undetected COPD in smoking, non-COPD patients, additional analyses were performed by excluding current and former smokers from the CAP-only group (Supplemental material).

Statistical analysis

Data analyses were performed using SPSS for Windows version 20.0. Variables were tested on normality by the Kolmogorov-Smirnov and Sapiro-Wilk test. Continuous data are presented as mean±standard deviation (SD) or median (interquartile range) and categorical data as counts and percentages. To compare baseline characteristics and clinical parameters of CAP-only and CAP-COPD patients, Mann Whitney U-test, Chi-square test or Fisher's Exact test were performed, as appropriate. Chi-square tests and Kaplan-Meier survival curves were assessed to analyse mortality. A Cox Model was computed, with COPD as covariate and hospitalization as strata, to analyse mortality. Odds ratio (OR) and independent predictors of mortality were assessed by logistic regression analyses. The value of $p<0.05$ was considered to be statistically significant.

RESULTS

In total, 1788 CAP patients were included in the CAPNETZ study. Of these, 1307 CAP patients (73.1%) matched the inclusion criteria of the current study. A total of 264 patients (20.2%) fulfilled the diagnostic criteria for COPD.

Table 1 shows the baseline characteristics of the CAP-only and CAP-COPD patients. CAP-COPD patients were significantly older, more frequently male and had a higher mean pack years of smoking compared to CAP-only patients. The proportion of patients using long-term oxygen therapy (LTOT) was significantly increased in CAP-COPD patients. Comorbidities were more often present in CAP-COPD patients, especially chronic heart failure and other chronic respiratory/lung diseases.

Table 1. Baseline characteristics

	CAP-only (n=1043)	CAP-COPD (n=264)	*p*-value
Age (years)	57.0 (41.0-72.0)	71.0 (63.0-77.0)	<0.001
Gender			
Male	575 (55.1)	170 (64.4)	0.007
Female	468 (44.9)	94 (35.6)	
Smoking			
Never smoked	561 (54.7)	78 (29.8)	<0.001
Stopped smoking	194 (18.9)	104 (39.7)	
Current smoking	270 (26.3)	80 (30.5)	
Pack years	0.0 (0.0-15.0)	25.0 (0.0-40.0)	<0.001
GOLD			
I	-	55 (20.8)	-
II	-	92 (34.8)	
III	-	71 (26.9)	
IV	-	46 (17.4)	
BMI (kg/m^2)	25.4 (22.2-29.4)	24.8 (22.4-28.9)	ns.
LTOT	14 (1.3)	63 (23.9)	<0.001
Vaccination			
Influenza	268 (25.8)	134 (51.0)	<0.001
Influenza A/H_1N_1	53 (9.7)	22 (15.4)	ns.
Pneumococcal	71 (6.9)	63 (24.0)	<0.001
Pertussis	109 (11.1)	11 (4.3)	0.001
Co-morbidities			
0	191 (37.7)	41 (23.0)	<0.001
1	202 (39.8)	76 (42.7)	
2	77 (15.2)	38 (21.3)	
>2	37 (7.3)	23 (12.9)	
Chronic heart failure	121 (11.6)	64 (24.2)	0.002
Chronic renal disease	81 (7.8)	26 (9.8)	ns.
Chronic liver disease	23 (2.2)	12 (4.5)	ns.
Cerebrovascular disease	36 (3.5)	18 (6.8)	ns.
Diabetes mellitus	133 (12.8)	49 (18.6)	ns.
Chronic respiratory/ lung disease	151 (14.5)	74 (28.0)	<0.001
Asthma	97 (9.3)	19 (7.2)	<0.001
Bronchiectasis	9 (0.9)	4 (1.5)	ns.
Lung fibrosis	9 (0.9)	4 (1.5)	ns.
Sarcoidosis	2 (0.2)	1 (0.4)	ns.
Bronchial/lung cancer[a]	5 (0.5)	8 (3.0)	0.028
Sleep apnoea	12 (1.2)	11 (4.2)	ns.
Other	21 (2.0)	31 (11.7)	<0.001
Patient from nursing home	17 (1.6)	9 (3.4)	ns.
Patient hospitalised	825 (79.1)	214 (81.1)	ns.
Length of stay (days)	9.0 (7.0-12.0)	11.0 (8.8-14.0)	<0.001
ICU-admission	65 (7.9)	19 (8.9)	ns.

Notes: Data are presented as median (interquartile range) or n (%). [a] Currently no radiation or chemotherapy.
Abbreviations: CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; BMI, body mass index; LTOT, long-term oxygen therapy; ICU, intensive care unit.

Laboratory data, clinical parameters and CURB-scores are shown in Table 2. Significantly increased plasma levels of urea, glucose and leucocytes were observed in CAP-COPD patients. In addition, higher proportions of CAP-COPD patients were hypercapnic and had acidosis. PaO_2 and O_2 saturations were significantly lower and respiratory rates significantly higher in CAP-COPD patients, compared with CAP-only patients. CAP-COPD patients scored significantly higher on the CURB index, indicating more severe CAP.

Table 2. Laboratory data, clinical parameters and severity scores

	CAP-only (n=1043)	CAP-COPD (n=264)	*p*-value
Urea (mg/dl)	13.6 (10.2-19.3)	16.9 (11.9-25.0)	<0.001
CRP (mg/dl)	100.0 (27.8-200.0)	116.0 (22.0-208.9)	ns.
Glucose (mg/dl)	112.0 (95.0-136.0)	124.0 (102.0-153.9)	<0.001
Leucocytes ($x10^9$/l)	10.7 (7.8-14.8)	12.3 (9.5-16.3)	<0.001
PaO_2 (mmHg)	65.0 (57.0-76.0)	61.0 (55.0-70.0)	0.001
$PaCO_2$ (mmHg)	34.0 (31.0-38.0)	37.0 (33.0-43.0)	<0.001
≤ 45 mmHg	619 (94.5)	153 (78.5)	<0.001
> 45 mmHg	36 (5.5)	42 (21.5)	
pH	7.5 (7.4-7.5)	7.4 (7.4-7.5)	<0.001
≤ 7.35	12 (1.8)	16 (8.2)	<0.001
> 7.35	645 (98.2)	179 (91.8)	
O_2 saturation (%)	94.0 (92.0-96.0)	92.0 (89.3-95.0)	<0.001
Respiratory rate	20.0 (18.0-24.0)	21.0 (18.0-25.0)	<0.001
≥ 30/min	56 (5.6)	27 (10.3)	0.007
CURB			
0	548 (57.3)	114 (44.7)	<0.001
1	320 (33.4)	89 (34.9)	
2	83 (8.7)	48 (18.8)	
3	6 (0.6)	3 (1.2)	
4	-	1 (0.4)	

Notes: Data are presented as median (interquartile range) or n (%). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; CRP, C-reactive protein; CURB, Confusion, Urea, Respiratory rate, and Blood pressure.

Comparisons between CAP-COPD patients and non-smoking, CAP-only patients (to avoid possible misdiagnosis of undetected COPD in smoking, CAP-only patients), are shown in Tables S1 and S2 (Supplemental material). Results of baseline characteristics were comparable. Only bronchial/lung cancer was no longer significant when excluding former and current smokers of the CAP-only group. No differences in results were observed when excluding former and current smokers from the CAP-only group concerning laboratory data, clinical parameters and severity scores.

Mortality

During the whole study period, 55 patients (4.2%) died. Table 3 provides an overview of the mortality rates specified for CAP-only and CAP-COPD patients. A significant difference in 30-, 90- and 180- day mortality (p=0.046, OR=2.48; p=0.003, OR=2.80; p=0.001, OR=2.57; respectively) was observed. Cause of death was not significantly different between CAP-only and CAP-COPD patients at the different time points. Table S3 (Supplemental material) shows the mortality rates and cause of death of non-smoking, CAP-only patients compared with CAP-COPD patients.

Table 3. Mortality rates and cause of death

	Total (n=1307)	CAP-only (n=1043)	CAP-COPD (n=264)	*p*-value	OR (95% CI)
t = 30 days	21 (1.6)	13 (1.2)	8 (3.0)	0.046	2.48 (1.015-6.037)
Cardiac		1 (7.7)	1 (12.5)	ns.	
Pneumonia (incl. sepsis)		7 (53.8)	3 (37.5)		
Other		2 (15.4)	3 (37.5)		
Unknown		3 (23.1)	1 (12.5)		
t = 90 days	37 (2.8)	22 (2.1)	15 (5.7)	0.003	2.80 (1.430-5.468)
Cardiac		3 (13.6)	1 (6.7)	ns.	
Pneumonia (incl. sepsis)		7 (31.8)	4 (26.7)		
Other		8 (36.4)	7 (46.7)		
Unknown		4 (18.2)	3 (20.0)		
t = 180 days	55 (4.2)	34 (3.3)	21 (8.0)	0.001	2.57 (1.462-4.498)
Cardiac		5 (14.7)	1 (4.8)	ns.	
Pneumonia (incl. sepsis)		11 (32.4)	8 (38.1)		
Other		12 (35.3)	8 (38.1)		
Unknown		6 (17.6)	4 (19.0)		

Notes: Data are presented as n (%). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; OR, odds ratio; CI, confidence interval.

In accordance with Table 3, a Kaplan-Meier survival analysis showed an increased 30-, 90- and 180-day mortality in CAP-COPD patients (Figure 1, Log Rank 11.762, p=0.001). A Kaplan-Meier survival analysis of long-term mortality in non-smoking, CAP-only and CAP-COPD patients is shown in Figure S1 (Supplemental material).

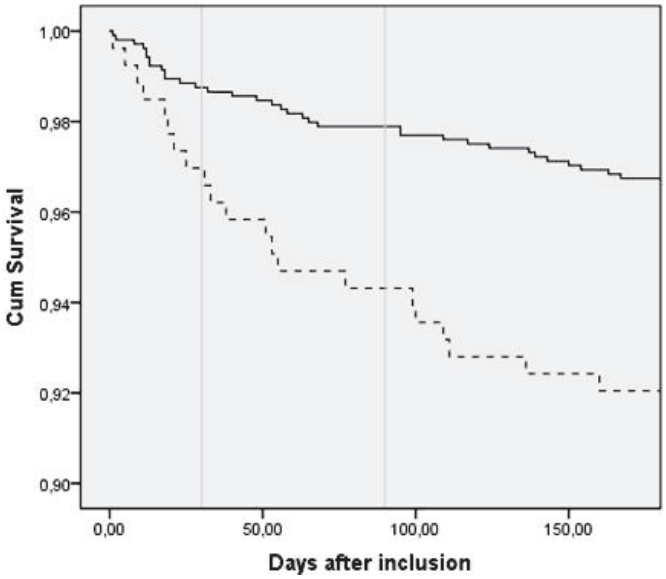

Figure 1. Long-term mortality in CAP patients with and without COPD

Kaplan Meier survival curve (t=180 days) for CAP patients with the presence of COPD (- - - -; n=264) and without (—— ; n=1043) the presence of COPD.

Most patients who died, were hospitalized patients. When stratified for COPD, a significant difference was found for short- and long-term mortality in hospitalized patients, compared to outpatient mortality (Table 4). The Kaplan-Meier survival analysis supports these findings (Figure 2). Moreover, a Cox Model showed a hazard ratio of 2.47 (*p*=0.001; 95%CI 1.432-4.251).

Table 4. Mortality stratified for hospitalisation and COPD

	Outpatient		Hospitalised		*p*-value
	CAP-only (n=214)	CAP-COPD (n=49)	CAP-only (n=825)	CAP-COPD (n=214)	
t = 30 days	-	-	13 (1.6)	8 (3.7)	0.015
t = 90 days	1 (0.5)	1 (2.0)	21 (2.5)	14 (6.5)	0.002
t = 180 days	1 (0.5)	2 (4.1)	33 (4.0)	19 (8.9)	<0.001

Notes: Data are presented as n (%). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease.

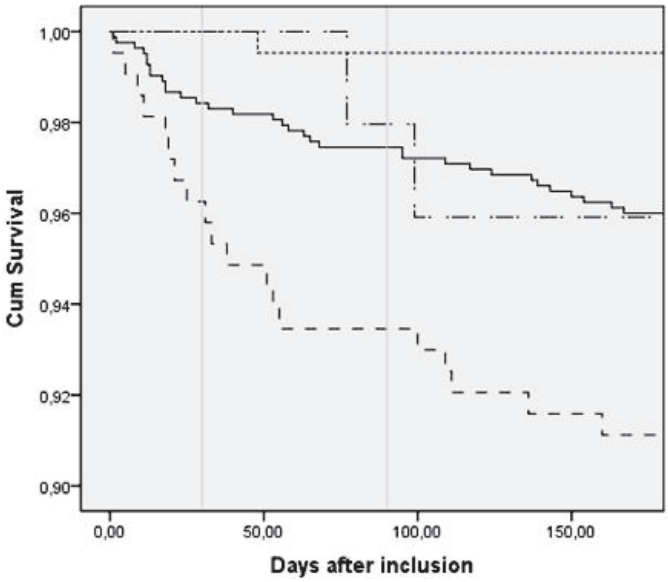

Figure 2. Long-term mortality in outpatient and hospitalised CAP patients with and without COPD

Kaplan Meier survival curve (t=180 days) for outpatient CAP patients without COPD (`····`; n=214), hospitalised CAP patients without COPD (—; n=825), outpatients CAP patients with COPD (-_- -_-; n=49), hospitalised CAP patients with COPD (- - -; n=214) (Log Rank 19.314; p<0.001).

Table 5 shows short- and long-term mortality rates per CURB-score for both CAP-COPD and CAP-only patients. Mortality rates are low, but increase with more severe CAP according to CURB-scores.

Table 5. Short- and long-term mortality per CURB-scores in CAP-only and CAP-COPD patients

	CAP-only				CAP-COPD			
CURB-scores	n	30-day	90-day	180-day	n	30-day	90-day	180-day
0	114	-	2 (1.8)	4 (3.5)	548	3 (0.5)	6 (1.1)	10 (1.8)
1	89	5 (5.6)	6 (6.7)	7 (7.9)	320	5 (1.6)	10 (3.1)	17 (5.3)
2	48	1 (2.1)	5 (10.4)	8 (16.7)	83	2 (2.4)	3 (3.6)	4 (4.8)
3	3	1 (33.3)	1 (33.3)	1 (33.3)	6	1 (16.7)	1 (16.7)	1 (16.7)
4	1	1 (100.0)	1 (100.0)	1 (100.0)	0	-	-	-

Notes: Data are presented as n (%). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; CURB, Confusion, Urea, Respiratory rate, and Blood pressure.

Therapy

Therapy initiated when CAP was diagnosed, could have influenced the outcomes. A significant difference between CAP-only and CAP-COPD patients was found for monotherapy with macrolides, combination therapy of penicillin and macrolides, and combi-

nation therapy of penicillin and fluorchinolone (Table S4, Supplemental material). Mono-therapy with macrolide and combination therapy of penicillin and macrolide was more often initiated in CAP-only patients, while a combination therapy of penicillin and fluorchinolone was more often initiated in CAP-COPD patients. When comparing deceased and survived COPD-patients, survived patients were more frequently treated with fluorchinolone mono-therapy (Table S5, Supplemental material). In CAP-only patients, deceased patients were more often treated with penicillin mono-therapy, while survived patients were more often treated with a combination therapy of penicillin and macrolides (Table S6, Supplemental material). Analysing therapeutic failure, no differences were observed in change of therapy after 14 days, comparing CAP-only with CAP-COPD patients (32.4% versus 26.2%, $p>0.05$).

Predictors of mortality

Logistic regression analyses were conducted to predict short-term mortality in CAP-patients by including age, gender, smoking status, LTOT, vaccination status, comorbidities other than COPD, ICU-admission and COPD as predictors. Test of the full model against a constant-only model was statistically significant (Goodness-of-fit: Chi-square=0.008, Hosmer and Lemeshow-test=0.389). Age was a significant predictor (p=0.008, OR=1.1, 95%CI 1.021-1.149), as well as ICU admission (p=0.047, OR=4.2, 95%CI 1.022-17.669).

For long-term mortality, a logistic regression analysis was conducted by including age, gender, smoking status, LTOT, vaccination status, co-morbidities other than COPD, CURB, COPD, ICU-admission, therapy change and GOLD-stage as possible predictors. Test of the full model against a constant only model was statistically significant for 90- and 180-day mortality (Goodness-of-fit: Chi-square=0.003, Hosmer and Lemeshow-test=0.828; Chi-square=0.002, Hosmer and Lemeshow-test=0.360; respectively). Age was a significant predictor for long-term mortality (p=0.014, OR=1.2, 95%CI 1.035-1.359; p=0.035, OR=1.1, 95%CI 1.008-1.259; respectively). Moreover, ICU-admission was predictive for long-term mortality (90-day mortality p=0.008, OR=16.0, 95%CI 2.087-121.878; 180-day mortality p=0.018, OR=9.6, 95%CI 1.483-62.797).

When excluding current and former smokers from the CAP-only group, results of logistic regression analysis were nearly comparable (Table S7, Supplemental material).

DISCUSSION

The present multi-center prospective study has several important findings. COPD is a common comorbidity of CAP and associated with increased short- and long-term mortality. Age and ICU-admission, and not COPD per se, were independent risk factors for CAP mortality. Additionally, CAP in COPD patients is related to increased severity of pneumonia and prolonged hospitalization. The clinical presentation of CAP in COPD patients is different as compared to CAP-only patients.

In accordance with previous CAPNETZ publications[20,21], overall CAP mortality rates were low in the present population. There are several explanations for this observation. First, it should be noted that the average age of the study population was lower than in most registry studies, which partly explains the lower mortality rates. Moreover, signed informed consent was obligatory for participation in CAPNETZ, while in population-based studies, this is not the case[22]. Thus, possibly less critically ill patients were included in the present study, as more critically ill patients were not able to provide written informed consent.

Despite the low mortality rates, COPD patients had more than twice as high a risk of dying due to CAP than CAP-only patients. Other authors observed no differences in short-term mortality comparing CAP-COPD patients with CAP-only[5-8]. The discrepancies found can possibly be due to inclusion of ICU patients with very severe pneumonia[2], differences in severity of airflow limitation in COPD patients, variability in other patients' characteristics, such as age and comorbidities[3,4,6,8], and the initiated therapy.

To our knowledge, this is the first study prospectively addressing 180-day mortality. Others found contradicting results. Restrepo et al.[4] found a significantly higher 90-day mortality rate for CAP-COPD patients, whereas Crisafulli et al.[7] did not find differences in 90-day, as well as 1-year mortality.

Finding significant differences when comparing CAP-only and CAP-COPD patients in univariate analysis, suggested that COPD could be of influence on mortality rates in CAP patients. However, when adjusting for other characteristics, COPD was no independent predictor of short-and long-term mortality. This is in line with a recent meta-analysis, in which COPD didn't affect mortality in patients with CAP (OR=1.44, 95%CI 0.97-2.16)[12]. By our knowledge, Restrepo et al.[4] were the only ones who assessed the relationship between COPD and long-term mortality in multivariate analyses. They observed an association between COPD and 90-day mortality. It is worth noticing that, all referred studies used different characteristics to adjust for, which could have influenced the results.

Predictors of short- and long-term CAP mortality in the present study were age and ICU-admission. Age as an independent risk factor is not surprising, as older age itself is asso-

ciated with increased mortality. In addition to age, ICU-admission was associated with an increased risk of both short-and long-term mortality. Comparable results of ICU-admission between CAP-COPD and CAP-only patients were observed by others[3,6,8]. ICU-admission was identified as a significant predictor of CAP-mortality by others, which did not sustain when adjusted for other confounders[3,6]. In contrast, Restrepo et al.[4] observed increased ICU-admission in CAP-COPD patients, which did not result in differences in 30- and 90-day mortality rates comparing COPD and non-COPD patients who needed ICU-admission. Rello et al.[2] observed a higher ICU mortality in CAP-COPD patients, compared with non-COPD patients.

The length of hospital stay was increased in CAP-COPD patients. Pifarre et al.[8] showed comparable results in CAP-COPD patients, while others did not find this association[3,5,7]. Differences can partly be explained by the criteria used to discharge patients, which differ between hospitals, the attending physician and different health care systems. However, longer hospital stays could be anticipated[24,25], as CAP-COPD patients suffered from more severe CAP.

The question arose if including former and current smokers in the CAP-only group could have influenced the outcomes, as there is a possibility that smoking, CAP-only patients are in fact CAP-COPD patients. Results of baseline characteristics, laboratory data, clinical parameters, severity scores and mortality rates were almost comparable to the results stated above (Supplemental material). Overall, it could be stated that smoking status of CAP-only patients was not of influence on characteristics of group comparisons and mortality-rates.

A limitation of the present study is that the primary outcome of the initial CAPNETZ-study was not to describe mortality rates in CAP-COPD patients, although mortality in CAP patients was one of the objectives. Assessment of COPD as a comorbidity was added to the study starting in December 2009. Due to the study design, this assessment relied on medical reports because patients were included during the acute phase of their pneumonia. Hereby, there was no structured extensive baseline assessment of COPD severity. However, lung function results, together with cigarette smoke exposure and appraisal of inhalation medication, were used to identify COPD patients. Study physicians assessed the most recent lung function results and defined the appropriate GOLD stage. This resulted in data concerning COPD diagnosis and severity. We chose this concept in order to keep the extensive database manageable with the drawback of lacking specific lung function data.

A strength of the present study is the extensive manner and accurate characterization of CAP patients systematically including chest x-ray. Using a combination of chest x-ray and clinical symptoms to diagnose pneumonia, provides a more objective way of diagnosis, as compared to using only physical examination, clinical characteristics, or chest x-ray alone[2,4]. Moreover, CAPNETZ has a prospective design and is possibly the largest

cohort on CAP worldwide. It is a multi-center study, with centers in 5 different European countries, including both in- and outpatients, which makes the results also relevant for the general population. The cohort is representative of patients with CAP in more economically developed countries[26].

In conclusion, COPD is common in patients with CAP and results in increased severity of pneumonia. Moreover, age and ICU-admission are important risk factors for mortality in CAP. Further study of ICU-admission as a risk factor seems warranted. To us, there is no clear therapeutic pattern which could conclusively explain the differences in mortality discussed above. Although COPD itself was not an independent risk factor for mortality, this group is at higher risk of dying due to CAP, with generally a higher age and more severe CAP. Further research is necessary to identify specific COPD characteristics associated with mortality, which can possibly contribute to better management of CAP-COPD patients.

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of non-smoking CAP-only vs. CAP-COPD patients

	CAP-only (n=561)	CAP-COPD (n=264)	*p*-value
Age (years)	60.0 (41.0-74.0)	71.0 (63.0-77.0)	<0.001
Gender			
Male	253 (45.1)	170 (64.4)	<0.001
Female	308 (54.9)	94 (35.6)	
Smoking			
Never smoked	561 (100)	78 (29.8)	<0.001
Stopped smoking	-	104 (39.7)	
Current smoking	-	80 (30.5)	
Pack years	-	25.0 (0.0-40.0)	-
BMI (kg/m^2)	25.2 (22.3-29.7)	24.8 (22.4-28.9)	ns.
LTOT	11 (2.0)	63 (23.9)	<0.001
Vaccination			
Influenza	152 (27.1)	134 (51.0)	<0.001
Influenza A/H_1N_1	31 (10.4)	22 (15.4)	ns.
Pneumococcal	41 (7.4)	63 (24.0)	<0.001
Pertussis	57 (10.8)	11 (4.3)	0.003
Co-morbidities			
0	99 (37.9)	41 (23.0)	0.007
1	97 (37.2)	76 (42.7)	
2	49 (18.8)	38 (21.3)	
>2	16 (6.1)	23 (12.9)	
Congestive heart failure	66 (25.3)	64 (36.0)	0.016
Chronic renal disease	42 (16.1)	26 (14.6)	ns.
Chronic liver disease	10 (3.8)	12 (6.7)	ns.
Cerebrovascular disease	16 (6.1)	18 (10.1)	ns.
Diabetes mellitus	69 (26.4)	49 (27.5)	ns.
Chronic respiratory/ lung disease	83 (14.8)	74 (28.0)	<0.001
Asthma	55 (67.1)	19 (25.7)	<0.001
Bronchiectasis	8 (9.8)	4 (5.4)	ns.
Lung fibrosis	5 (6.1)	4 (5.4)	ns.
Sarcoidosis	2 (2.4)	1 (1.4)	ns.
Bronchial/lung cancer[a]	4 (4.9)	8 (10.8)	ns.
Sleep apnoea	6 (7.3)	11 (14.9)	ns.
Other	10 (12.2)	31 (41.9)	<0.001
Patient from nursing home	10 (1.8)	9 (3.4)	ns.
Patient hospitalised	435 (77.8)	214 (81.4)	ns.
Length of stay (days)	9.0 (7.0-11.0)	11.0 (8.8-14.0)	<0.001
ICU-admission	24 (4.3)	19 (8.9)	ns.

Notes: Data are presented as median (interquartile range) or n (%). [a] Currently no radiation or chemotherapy.
Abbreviations: CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; BMI, body mass index; LTOT, long-term oxygen therapy; ICU, intensive care unit.

Table S2. Laboratory data, clinical parameters and severity scores of non-smoking CAP-only patients versus CAP-COPD patients

	CAP-only (n=561)	CAP-COPD (n=264)	*p*-value
Urea (mg/dl)	13.5 (10.2-19.5)	16.9 (11.9-25.0)	<0.001
CRP (mg/dl)	98.0 (30.0-183.9)	116.0 (22.0-208.9)	ns.
Glucose (mg/dl)	113.4 (95.0-140.0)	124.0 (102.0-153.9)	0.004
Leucocytes ($x10^9$/l)	10.1 (7.3-14.1)	12.3 (9.5-16.3)	<0.001
PaO_2 (mmHg)	66.0 (58.0-75.0)	61.0 (55.0-70.0)	<0.001
$PaCO_2$ (mmHg)	34.0 (31.0-37.0)	37.0 (33.0-43.0)	<0.001
≤ 43 mmHg	316 (94.3)	153 (78.5)	<0.001
> 43 mmHg	19 (5.7)	42 (21.5)	
pH	7.5 (7.4-7.5)	7.4 (7.4-7.5)	<0.001
≤ 7.35	3 (0.9)	16 (8.2)	<0.001
> 7.35	335 (99.1)	179 (91.8)	
O_2 saturation (%)	95.0 (92.0-96.0)	92.0 (89.3-95.0)	<0.001
Respiratory rate	20.0 (18.0-23.0)	21.0 (18.0-25.0)	0.001
≥ 30/min	26 (4.8)	27 (10.3)	0.004
CURB			
0	299 (58.2)	114 (44.7)	<0.001
1	172 (33.5)	89 (34.9)	
2	42 (8.2)	48 (18.8)	
3	1 (0.2)	3 (1.2)	
4	-	1 (0.4)	

Notes: Data are presented as median (interquartile range) or n (%). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; CRP, C-reactive protein; CURB, Confusion, Urea, Respiratory rate, and Blood pressure.

Table S3. Mortality rates and cause of death of non-smoking CAP-only patients versus CAP-COPD patients

	Total (n=825)	CAP-only (n=561)	CAP-COPD (n=264)	*p*-value	OR (95% CI)
t = 30 days	16 (1.9)	8 (1.4)	8 (3.0)	ns.	2.160 (0.802-5.820)
Cardiac		1 (12.5)	1 (12.5)	ns.	
Pneumonia (incl. sepsis)		3 (37.5)	3 (37.5)		
Other		1 (12.5)	3 (37.5)		
Unknown		3 (37.5)	1 (12.5)		
t = 90 days	27 (3.3)	12 (2.1)	15 (5.7)	0.010	2.756 (1.271-5.975)
Cardiac		2 (16.7)	1 (6.7)	ns.	
Pneumonia (incl. sepsis)		3 (25.0)	4 (26.7)		
Other		3 (25.0)	7 (46.7)		
Unknown		4 (33.3)	3 (20.0)		
t = 180 days	36 (4.4)	15 (2.7)	21 (8.0)	0.001	3.146 (1.594-6.207)
Cardiac		3 (20.0)	1 (4.8)	ns.	
Pneumonia (incl. sepsis)		3 (20.0)	8 (38.1)		
Other		4 (26.7)	8 (38.1)		
Unknown		5 (33.3)	4 (19.0)		

Notes: Data are presented as n (%). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; OR, odds ratio; CI, confidence interval.

Table S4. Initiated therapy when diagnosed with CAP

	CAP-only (n=1043)	CAP-COPD (n=264)	*p*-value
Therapy unknown	34 (3.3)	7 (2.7)	ns.
Penicillin	394 (37.8)	109 (41.3)	ns.
Cephalosporin	155 (14.9)	44 (16.7)	ns.
Macrolide	58 (5.6)	6 (2.3)	0.027
Fluorchinolone	125 (12.0)	40 (15.2)	ns.
Other	12 (1.2)	2 (0.8)	ns.
Penicillin and Cephalosporin	2 (0.2)	-	ns.
Penicillin and Macrolide	145 (13.9)	21 (8.0)	0.010
Penicillin and Fluorchinolone	19 (1.8)	14 (5.3)	0.001
Penicillin and other	1 (0.1)	1 (0.4)	ns.
Cephalosporin and Macrolide	90 (8.6)	16 (6.1)	ns.
Cephalosporin and Fluorchinolone	4 (0.4)	2 (0.8)	ns.
Cephalosporin and other	2 (0.2)	-	ns.
Macrolide and Fluorchinolone	1 (0.1)	-	ns.
Macrolide and other	-	-	-
Fluorchinolone and other	-	1 (0.4)	ns.
Penicillin and Cephalosporin and Macrolide	1 (0.1)	1 (0.4)	ns.

Notes: Data are presented as n (%). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease

Table S5. Initiated therapy of survived versus deceased CAP-COPD patients

	Survived (n=243)	Deceased (n=21)	*p*-value
Therapy unknown	7 (2.9)	-	ns.
Penicillin	97 (39.9)	12 (57.1)	ns.
Cephalosporin	39 (16.0)	5 (23.8)	ns.
Macrolide	6 (2.5)	-	ns.
Fluorchinolone	40 (16.5)	-	0.044
Other	2 (0.8)	-	ns.
Penicillin and Cephalosporin	-	-	-
Penicillin and Macrolide	20 (8.2)	1 (4.8)	ns.
Penicillin and Fluorchinolone	12 (4.9)	2 (9.5)	ns.
Penicillin and other	1 (0.4)	-	ns.
Cephalosporin and Macrolide	15 (6.2)	1 (4.8)	ns.
Cephalosporin and Fluorchinolone	2 (0.8)	-	ns.
Cephalosporin and other	-	-	-
Macrolide and Fluorchinolone	-	-	-
Macrolide and other	-	-	-
Fluorchinolone and other	-	-	-
Penicillin and Cephalosporin and Macrolide	1 (0.4)	-	ns.

Notes: Data are presented as n (%). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease.

Table S6. Initiated therapy of survived versus deceased CAP-only patients

	Survived (n=1009)	Deceased (n=34)	*p*-value
Therapy unknown	34 (3.4)	-	ns.
Penicillin	375 (37.2)	19 (55.9)	0.027
Cephalosporin	146 (14.5)	9 (26.5)	ns.
Macrolide	58 (5.7)	-	ns.
Fluorchinolone	121 (12.0)	4 (11.8)	ns.
Other	12 (1.2)	-	ns.
Penicillin and Cephalosporin	2 (0.2)	-	ns.
Penicillin and Macrolide	145 (14.4)	-	0.017
Penicillin and Fluorchinolone	19 (1.9)	-	ns.
Penicillin and other	1 (0.1)	-	ns.
Cephalosporin and Macrolide	88 (8.7)	2 (5.9)	ns.
Cephalosporin and Fluorchinolone	4 (0.4)	-	ns.
Cephalosporin and other	2 (0.2)	-	ns.
Macrolide and Fluorchinolone	1 (0.1)	-	ns.
Macrolide and other	-	-	-
Fluorchinolone and other	-	-	-
Penicillin and Cephalosporin and Macrolide	1 (0.1)	-	ns.

Notes: Data are presented as n (%). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease.

Table S7. Independent predictors of short- and long-term mortality in CAP-patients (including non-smoking CAP-only patients and CAP-COPD patients)

	OR	*p*-value	95% CI
30-day mortality[a]			
Age	1.1	0.006	1.037-1.235
LTOT	10.5	0.011	1.706-64.442
90-day mortality[b]			
Age	1.1	0.005	1.024-1.144
ICU-admission	14.5	0.006	2.140-98.251
180-day mortality[b]			
Age	1.1	0.004	1.025-1.138
ICU-admission	9.6	0.018	1.483-62.797

Notes: [a] Adjusted for age, gender, LTOT, being vaccinated or not, having co-morbidities besides COPD, CURB and COPD for 30-day mortality (Goodness-of-fit: Chi-square=0.000, Hosmer and Lemeshow-test=0.712). [b] Adjusted for age, gender, LTOT, being vaccinated or not, having co-morbidities besides COPD, CURB, COPD, ICU-admission, therapy change and GOLD stage for 90- and 180-day mortality (Goodness-of-fit: Chi-square=0.003, Hosmer and Lemeshow-test=0.828; Chi-square=0.002, Hosmer and Lemeshow-test=0.360; respectively). **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; OR, odds ratio; CI, confidence interval; LTOT, long-term oxygen therapy; ICU, intensive care unit.

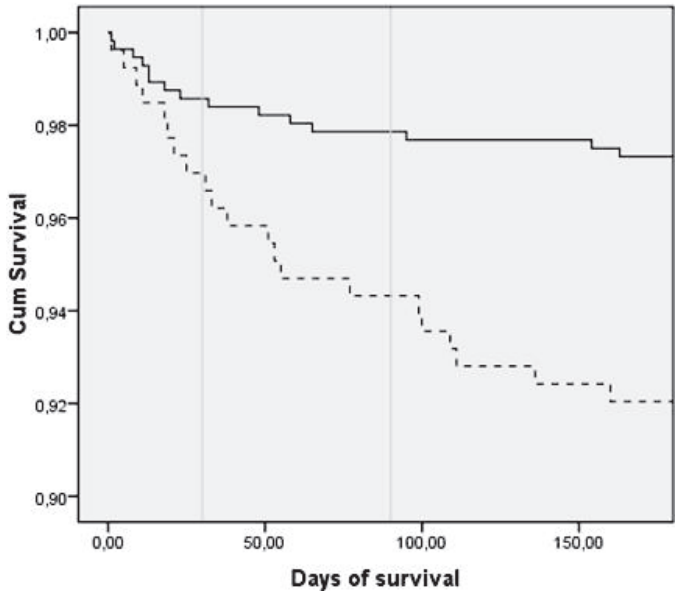

Figure S1. Long-term mortality in non-smoking, CAP-only and CAP-COPD patients

Kaplan Meier survival curve (t=180 days) for CAP patients with the presence of COPD (- - - - ; n=264) and without (—— ; n=1043) the presence of COPD.

3

Bacterial aetiology and mortality in COPD patients with CAP: Results from the German Competence Network, CAPNETZ

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ABSTRACT

Background: Community acquired pneumonia (CAP) is a major cause of morbidity and mortality, and Chronic Obstructive Pulmonary Disease (COPD) is a frequent comorbidity. The bacterial aetiology of CAP-COPD and its possible associations with serum markers and mortality are incompletely understood.

Objectives: 1) To assess the bacterial aetiology of CAP-only and CAP-COPD, and 2) to study the association between bacterial aetiology, empirical antibiotic treatment, serum markers and mortality.

Methods: Of 1288 patients with CAP (57.0% males, age 59.0 years ± 18.5), 262 (20.3%) fulfilled the diagnostic criteria for COPD. Differences between subgroups were investigated using univariate analyses and corrected for multiple comparisons.

Results: *Streptococcus pneumoniae* was the most common pathogen (30.8% CAP-only vs. 26.0% CAP-COPD, not significant). *Haemophilus influenzae* was significantly more frequent in CAP-COPD (5.6% CAP-only vs. 26.0% CAP-COPD, $p<0.001$). The number given adequate empirical antibiotic treatment was comparable (83.3% CAP-only vs. 83.6% CAP-COPD, $p>0.05$). The CAP-COPD group had worse CURB-65 and partial pressure of arterial oxygen levels than the CAP-only group ($p<0.001$). Partial pressure of arterial carbon dioxide levels were increased in CAP-COPD patients without pathogen detection ($p<0.001$). Short- ($p=0.011$) and long-term mortality ($p=0.006$) were highest in CAP-COPD without pathogen detection.

Conclusion: It is important to identify COPD patients with CAP. In particular, those without bacterial pathogen detection have more severe CAP and are at higher risk of dying. Better understanding of the aetiology could contribute to improved management and treatment of CAP in COPD patients.

INTRODUCTION

Community acquired pneumonia (CAP) has an important socio-economic impact, with an incidence ranging from 3 to 10 per 1000 adults per year[1]. Chronic Obstructive Pulmonary Disease (COPD) is a frequent comorbidity of CAP, associated with increased severity and mortality rates[2].

Studies investigating bacterial patterns in CAP with or without COPD have suggested the presence of different pathogens. It is known that *Streptococcus pneumoniae* is the most frequently detected pathogen in CAP[1,3–7], whereas *Haemophilus influenzae* has been reported to be the most common pathogen in COPD patients with CAP[8]. Moreover, *Pseudomonas aeruginosa* and *Moraxella catarrhalis*, which are rarely detected in CAP, are important pathogens in COPD, particularly in more severe disease[9–13].

However, no studies have been specifically designed to study potential differences in the bacterial aetiology of CAP in patients with or without COPD and to investigate whether these differences have consequences for short- and long-term mortality. Moreover, the concordance of empirical antibiotic treatment and pathogens detected has not yet been investigated, but may have significant effects on clinical outcome.

Serum markers of inflammation might be associated with bacterial aetiology. C-reactive protein (CRP) has been proposed to be a helpful tool in the detection of classical bacterial aetiology in CAP, compared to atypical or viral CAP[3]. However, CRP was not predictive for individual differentiation of the bacterial aetiology[14]. A possible relationship between the bacterial aetiology and other serum markers and clinical parameters should be further analysed.

Against this background, we hypothesised that differences in microbiology between CAP subjects without co-existing COPD (CAP-only) and CAP subjects with co-existing COPD (CAP-COPD) are related to mortality. The objectives of the present study were 1) to investigate possible differences in the bacterial aetiology of CAP between subjects with and those without co-existing COPD; 2) to assess empirical antibiotic treatment in relation to bacterial aetiology; 3) to examine the relationship between serum markers and clinical parameters and bacterial aetiology; and 4) to study the relationship between bacterial aetiology, empirical antibiotic treatment, serum markers and mortality in CAP.

MATERIALS AND METHODS

Data were derived from a multicentre observational study initiated by CAPNETZ (CAP network)[15]. The study design has been published elsewhere[15], and was approved by the ethics committees of all local clinical centres. All patients provided written informed consent.

Study population

Between December 2009 and June 2012, CAPNETZ included 1788 CAP patients. Inclusion criteria were age ≥18 years, new pulmonary infiltrate on chest X-ray, clinical symptoms of cough, purulent sputum, positive auscultation or fever. Patients were excluded if they had been hospitalised in the previous 28 days, if they were chronically immunosuppressed, human immunodeficiency virus infected or had active tuberculosis. Follow-up included a structured interview on outcome parameters, including death, at 30 and 180 days.

The presence of COPD was assessed based on post-bronchodilator forced expiratory volume in the first second (FEV_1) and forced vital capacity (FVC) in the previous 6 months[16]. COPD was defined as FEV_1/FVC ratio <0.7[17]. Study physicians interpreted spirometric data, confirmed diagnosis and categorised patients according to GOLD (Global Initiative for Chronic Obstructive Lung Disease) stages[16], as previously published[2]. Patients were excluded from the present study when no data on COPD diagnosis were available (n=481) and when no samples had been collected for microbial analysis (n=19). A cohort of 1288 eligible CAP patients with COPD data was available for the present analyses, divided into two subgroups: 1) CAP patients without COPD (CAP-only); 2) CAP patients with COPD (CAP-COPD).

Study procedures and definitions

The methodological details of the microbiological diagnostic techniques and laboratory processing in this study have been published previously[3,18]. Briefly, serum, sputum, tracheobronchial secretions, bronchoalveolar lavage fluid and punctate samples were obtained for microbiological analysis according to routine local procedures. Urine samples were collected and tested for *Legionella pneumophila* and *S. pneumoniae* using an antigen test. Samples were routinely collected within <72 h of presentation at the emergency department. Respiratory samples were cultured and gram-stained as soon as clinical routine allowed after they had been obtained. Respiratory samples and blood cultures were plated on blood agar, McConkey-agar and chocolate agar. All samples included were adequate for analysis. Inadequate samples were not processed by the local laboratory and were not reported in the database.

Enterobacteriaceae were clustered, including *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *K. oytoca*, *Pantoea agglomerans*, *Proteus mirabilis*, *Rahnella aquatilis* and *Serratia marcescens*. Other bacteria included *Acinetobacter* spp., *Moraxella catarrhalis* and *Stenothrophomonas maltophilia*, among others. Both CAP-only and CAP-COPD patients were stratified according to culture result (positive vs. negative bacterial culture). No data were available on viral pathogens.

The empirical antibiotic treatment given was recorded. A respiratory physician retrospectively assessed the empirical treatment based on typical antibiotic susceptibility patterns to assess whether the treatment was adequate for the bacterial aetiology. Changes in treatment were assessed.

Overall mortality and group-specific mortality rates were analysed, with 30-, 90- and 180-day mortality calculated by subtracting the date of examination from the date of death.

CURB-65 (Confusion of new onset, defined as an abbreviated mental test score of ≤8, blood Urea nitrogen >7 mmol/l [19 mg/dl]), Respiratory rate of ≥30 breaths/min, Blood pressure <90 mmHg systolic or diastolic blood pressure ≤60 mmHg, age ≥65 years) scores were calculated based on the sum of points, with one point assigned for the presence of each criterion[5,19,20].

Statistical analysis

Data analyses were performed using the Statistical Package for the Social Sciences, version 20.0 (IBM Corp, Armonk, NY, USA). Variables were tested for normality using the Kolmogorov-Smirnov and Shapiro-Wilk test. Continuous data are presented as medians (interquartile range) and categorical data as counts (percentages). To compare the bacterial aetiology of CAP-only and CAP-COPD, the χ^2 test was performed. Baseline characteristics, serum markers and clinical parameters assessed between subgroups were performed using Kruskal Wallis test for 2- and κ-independent samples, as well as the χ^2 test. χ^2 tests were also assessed to analyse mortality in relation to bacterial aetiology. $p<0.05$ was considered statistically significant; analyses were corrected for multiple comparisons using Bonferroni correction.

RESULTS

A total of 1288 patients met the inclusion criteria, with 262 classified as COPD (20.3%). Of these, 55 (21.0%) were characterised as mild (GOLD I), 91 (34.7%) as moderate (GOLD II), 70 (26.7%) as severe (GOLD III) and 46 (17.6%) as very severe COPD (GOLD IV).

Bacterial aetiology

Bacterial aetiology could be determined in 271 patients (21.0%). Pathogens were identified significantly more frequently in CAP-COPD than in CAP-only patients: 73 CAP-COPD (27.9% of all CAP-COPD) vs. 198 CAP-only (19.3% of all CAP-only patients). Table 1 gives an overview of the identified pathogens, with *S. pneumoniae* being the most frequent pathogen in CAP-only, while *H. influenzae* and *S. pneumoniae* were identified most frequently in

CAP-COPD. *H. influenzae* detection rates differed significantly between the groups, being more frequent in CAP-COPD. Low rates of *P. aeruginosa* were found in both groups.

Baseline characteristics of CAP-only and CAP-COPD, stratified by culture result, showed that CAP-COPD patients were in general older, more often former smokers and with more pack-years than CAP-only patients (Table S1, Supplemental material). CAP-COPD patients were also more frequently vaccinated against influenza and pneumococci. CAP-COPD patients with negative bacterial culture results were more likely to have underlying chronic heart failure than CAP-only patients with negative bacterial culture, while CAP-only patients had significantly more frequent asthma than CAP-COPD.

Table 1. Bacterial aetiology of CAP-only vs. CAP-COPD patients*

	CAP-only (n=1026) n (%)	CAP-COPD (n=262) n (%)	*p*-value
Bacterial aetiology	198 (19.3)	73 (27.9)	0.002
Typical bacterial pathogens			
Streptococcus pneumoniae	61 (30.8)	19 (26.0)	NS
Staphylococcus aureus	25 (12.6)	6 (8.2)	NS
Other gram-positive cocci (not further specified)	14 (7.1)	2 (2.7)	NS
Haemophilus influenzae	11 (5.6)	19 (26.0)	<0.001
H. parainfluenzae	25 (12.6)	6 (8.2)	NS
Pseudomonas aeruginosa	6 (3.0)	1 (1.4)	NS
Enterobacteriaceae†	33 (16.7)	16 (21.9)	NS
Atypical pathogens			
Legionella pneumophila	9 (4.5)	1 (1.4)	NS
Other bacteria‡	3 (1.5)	3 (4.1)	NS
Mixed infection	11 (5.6)	-	NS

* Corrected for multiple comparisons (p<0.005).
† Including *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella pneumoniae*, *K. oytoca*, *Pantoea agglomerans*, *Proteus mirabilis*, *Rahnella aquatilis* and *Serratia marcescens*.
‡ Including, among others, *Acinetobacter* spp., *Moraxella catarrhalis* and *Stenothrophomonas maltophilia*.
CAP = community acquired pneumonia; COPD = Chronic Obstructive Pulmonary Disease; NS = not significant.

Empirical antibiotic treatment

Empirical antibiotic treatment was started in 19 CAP-only (1.9%) and 3 CAP-COPD (1.2%) patients before sampling. Most patients started treatment on the same day as sampling (40.3% of CAP-only vs. 37.5% of CAP-COPD patients).

Empirical antibiotic treatment was started in almost all patients with positive bacterial culture, (94.9% CAP-only vs. 97.3% CAP-COPD, p>0.05). Figure 1 shows the proportion of patients with adequate treatment, comparable between CAP-only and CAP-COPD patients with positive bacterial culture (83.3% CAP-only vs. 83.6% CAP-COPD, p>0.05).

Antibiotic treatment was changed in 12 CAP-only (33.3%) and 5 CAP-COPD (41.7%) patients. Reasons for the change were inadequate coverage (n=8, 47.1%), resistance (n=4, 23.5%) and de-escalation (n=2, 11.8%). This change in treatment was adequate for two thirds of CAP-only patients (n=8, 66.7%) and for all CAP-COPD patients.

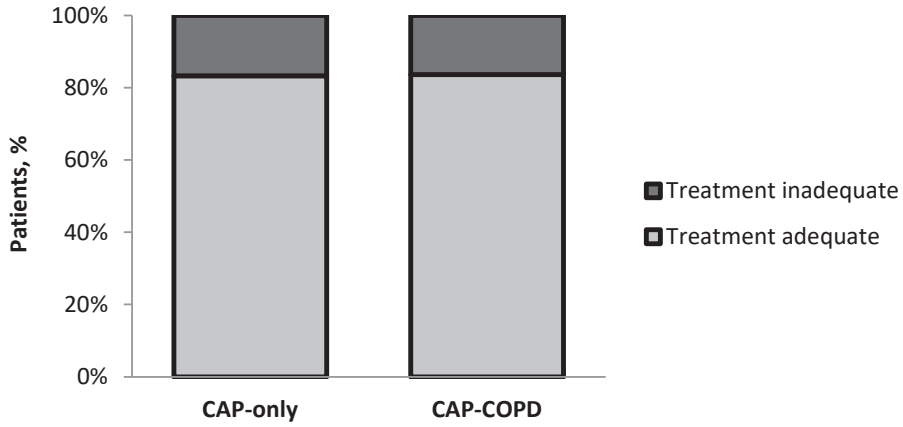

Figure 1. Percentage of patients with adequate/inadequate empirical antibiotic treatment.

CAP = community acquired pneumonia; COPD = Chronic Obstructive Pulmonary Disease.

Serum markers and clinical parameters

Serum markers and clinical parameters of CAP-only and CAP-COPD stratified by culture result are shown in Table 2. No differences were observed between CAP-COPD with negative and positive bacterial culture. CRP levels were statistically significantly different between CAP-only patients, with higher levels in CAP-only patients with positive bacterial culture (CRP 136.0 mg/dl [IQR 53.0–233.5] vs. 89.2 mg/dl [IQR 21.0–189.6], $p<0.001$). Leucocyte levels were statistically significantly higher in CAP-COPD patients with negative bacterial culture than in CAP-only patients with negative bacterial culture (12.2 x 10^9/l [IQR 9.5–16.1] vs. 10.5 x 10^9/l [IQR 7.7–14.6], $p<0.001$). Moreover, CAP-COPD patients with negative bacterial culture had higher levels of urea (16.9 mg/dl [IQR 12.0–25.0]) and partial pressure of arterial carbon dioxide ($PaCO_2$) (38.0 mmHg [IQR 34.0–44.0]) than CAP-only patients, independent of bacterial aetiology. CAP-COPD patients suffered more frequently from acidosis (<pH) than CAP-only patients. Body temperature was highest in CAP-only patients with positive bacterial culture (38.2°C [IQR 37.0–38.8]), indicating fever, than CAP-COPD and CAP-only patients with negative bacterial culture. CAP severity, as measured by CURB-65, was higher in CAP-COPD than in CAP-only patients, with no differences based on culture result (Table 2).

Table 2. Serum markers and clinical parameters for CAP-only and CAP-COPD patients stratified by culture result*

	CAP-only (n=1026)		CAP-COPD (n=262)	
	Bacterial aetiology negative culture (n=828) n (%)	Bacterial aetiology positive culture (n=198) n (%)	Bacterial aetiology negative culture (n=189) n (%)	Bacterial aetiology positive culture (n=73) n (%)
Urea, mg/dl, median [IQR]	13.3 [10.2–19.0]	13.6 [10.2–20.7]	16.9 [12.0–25.0]†‡	17.2 [11.1–26.4]
Glucose, mg/dl, median [IQR]	112.0 [95.4–134.8]	114.0 [92.0–142.0]	126.0 [103.0–156.0]	117.0 [98.0–138.6]
CRP, mg/dl, median [IQR]	89.2 [21.0–189.6]	136.0 [53.0–233.5]†	110.0 [19.5–199.0]	126.0 [35.0–226.5]
Leucocytes, $x10^9$/l, median [IQR]	10.5 [7.7–14.6]	11.9 [8.3–15.4]	12.2 [9.5–16.1]†	12.5 [9.4–17.2]
PaO_2, mmHg, median [IQR]	66.0 [58.0–76.0]	61.0 [54.0–71.0]	61.5 [55.8–71.0]	57.0 [49.0–67.0]†
<65.25	258 (48.6)	78 (66.7)	91 (62.3)	33 (70.2)
≥65.25	273 (51.4)	39 (33.3)	55 (37.7)	14 (29.8)
$PaCO_2$, mmHg, median [IQR]	34.0 [31.0–38.0]	34.0 [31.0–38.0]	38.0 [34.0–44.0]†‡	34.0 [30.0–40.0]
≤45	506 (95.3)	112 (95.7)	115 (78.8)†‡	41 (87.2)
>45	25 (4.7)	5 (4.3)	31 (21.2)	6 (12.8)
pH, median [IQR]	7.5 [7.4–7.5]	7.5 [7.4–7.5]	7.4 [7.4–7.5]†‡	7.5 [7.4–7.5]
≤7.35	9 (1.7)	1 (0.9)	12 (8.2)†	4 (8.5)†‡
>7.35	524 (98.3)	116 (99.1)	134 (91.8)	43 (91.5)
O_2 saturation, %, median [IQR]	94.0 [92.0–96.0]	93.0 [90.0–96.0]	93.0 [90.0–95.0]†	92.0 [87.0–95.0]†
Respiratory rate, /min, median [IQR]	20.0 [17.5–23.0]	20.0 [18.0–24.0]	22.0 [18.0–25.0]	20.0 [18.0–24.0]
Body temperature, °C, median [IQR]	37.8 [36.8–38.6]	38.2 [37.0–38.8]	37.3 [36.8–38.3]‡	37.1 [36.7–38.0]‡
<37.8°C	397 (48.6)	79 (40.5)	114 (61.0)‡	50 (69.4)†‡
≥37.8°C	420 (51.4)	116 (59.5)	73 (39.0)	22 (30.6)
CURB-65				
0	326 (43.0)	71 (38.8)	33 (18.2)†‡	13 (18.1)†‡
1	241 (31.8)	62 (33.9)	64 (35.4)	19 (26.4)
2	139 (18.3)	33 (18.0)	52 (28.7)	25 (34.7)
3	49 (6.5)	16 (8.7)	29 (16.0)	15 (20.8)
4	4 (0.5)	1 (0.5)	2 (1.1)	-
5	-	-	1 (0.6)	-

* Approximately one third of blood gas data were missing. Corrected for multiple comparisons ($p \leq 0.001$).
† $p \leq 0.001$ compared to CAP-only negative culture.
‡ $p \leq 0.001$ compared to CAP-only positive culture.
CAP = community acquired pneumonia; COPD = Chronic Obstructive Pulmonary Disease; IQR = interquartile range; PaO_2 = partial pressure of arterial oxygen; $PaCO_2$ = partial pressure of arterial carbon dioxide; CRP = C-reactive protein; CURB-65 = Confusion of new onset (defined as an abbreviated mental test score of ≤8), blood Urea nitrogen >7 mmol/l (19 mg/dl), respiratory rate of ≥30 breaths/min, Blood pressure <90 mmHg systolic or diastolic blood pressure ≤60 mmHg, age ≥65 years.

Mortality

Short- and long-term mortality rates were lowest in CAP-only patients, while COPD patients in general had the highest mortality rates (Table 3; Figure 2). Both short- and long-term mortality rates differed significantly between CAP-only patients with negative bacterial culture and CAP-COPD patients with negative bacterial culture. No differences in mortality rates were observed between CAP-only patients with negative and positive bacterial culture results, and CAP-COPD patients with negative and positive bacterial culture results.

Table 3. Short- and long-term mortality rates for CAP-only and CAP-COPD patients stratified by culture result*

	CAP-only (n=1026)		CAP-COPD (n=262)	
Mortality	Bacterial aetiology negative culture (n=828) n (%)	Bacterial aetiology positive culture (n=198) n (%)	Bacterial aetiology negative culture (n=189) n (%)	Bacterial aetiology positive culture (n=73) n (%)
30-day	10 (1.2)	2 (1.0)	8 (4.2)†	-
90-day	19 (2.3)	2 (1.0)	13 (6.9)†‡	2 (2.7)
180-day	27 (3.3)	6 (3.0)	16 (8.5)†	5 (6.8)

* Corrected for multiple comparisons ($p \leq 0.003$).

† $p \leq 0.003$ compared to CAP-only negative culture.

‡ $p \leq 0.003$ compared to CAP-only positive culture.

CAP = community acquired pneumonia; COPD = Chronic Obstructive Pulmonary Disease.

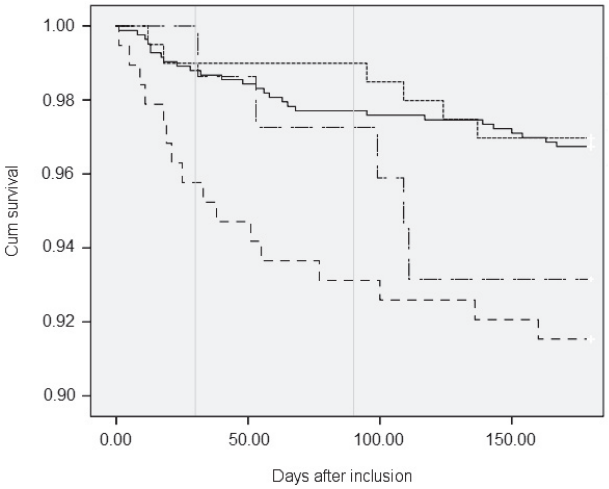

Figure 2. Survival of CAP-only and CAP-COPD patients stratified by culture result.

Kaplan-Meier survival curve (t=180 days) for CAP-only with negative culture (— ; *n*=828), CAP-only patients with positive culture (. . .. . .; *n*=198), CAP-COPD patients with negative culture (- - -; *n*=189) and CAP-COPD patients with positive culture (._. ._.; *n*=73); log-rank 12.691; *p*=0.005. CAP = community acquired pneumonia; COPD = Chronic Obstructive Pulmonary Disease.

Enterobacteriaceae were frequently detected (n=5, 45.5%) in deceased patients. No further analyses could be performed to investigate possible associations of specific bacterial aetiology and serum markers with mortality due to the small number of deceased patients. On comparing the antibiotic treatment administered with the pathogen detected and known antibiotic resistance patterns, three deceased patients (27.8%) had received inadequate empirical antibiotic treatment. However, all of these patients had been prescribed adequate changes to their treatment.

DISCUSSION

The main results of the present study were as follows: *S. pneumoniae* was the most common pathogen in both groups, whereas *H. influenzae* was significantly more frequent in CAP-COPD patients. In general, CAP-COPD patients had more severe CAP than CAP-only patients and, in particular, CAP-COPD patients with negative culture had significantly more frequent signs of respiratory failure and higher mortality rates than CAP-only patients.

Bacterial aetiology

Results of the present study confirm that *S. pneumonia* is the most frequent pathogen identified in CAP-only[9–11,13,21] and CAP-COPD[9–13] patients. *H. influenzae* was observed to be significantly more frequent in CAP-COPD patients. Other studies have also observed that CAP-COPD was often associated with an infection attributable to *P. aeruginosa*[9–13]. In the present study, no difference was observed in the prevalence of *P. aeruginosa* in CAP-only and CAP-COPD patients. This might be due to less severe COPD in our population.

The presence of *H. influenzae* varies in study populations, ranging from approximately 1% to 8%[6]. In the current population, *H. influenzae* was observed in 11.1% of all CAP patients in whom a pathogen was identified. This overall figure is in accordance with Welte et al.[1], who reported that the proportion varied between 2.9% and 14.6% in Europe. The fact that *H. influenzae*, together with *S. pneumoniae*, was most frequently identified in CAP-COPD patients, and significantly more often than in CAP-only patients, is not surprising. A higher proportion of *H. influenzae* pneumonia in COPD patients was also reported by Torres et al.[4], Rello et al.[10] and Restrepo et al.[9] (9.2%, 11.4% and 15.1%, respectively). Respiratory physicians should keep this in mind when choosing their antibiotic treatment, as *H. influenzae* is often not susceptible to standard empirical penicillin treatment. However, it is clinically very difficult to distinguish *H. influenzae* infection from colonisation[22].

Enterobacteriaceae were identified more often in CAP-COPD patients, although this difference was not statistically significant. Conflicting results have been reported, with lower incidence of *Enterobacteriaceae* in both total and CAP-COPD patients[9,10,12,13,21]. This discrepancy can partly be explained by the definition of *Enterobacteriaceae* in the different studies. The more pathogens grouped into *Enterobacteriaceae*, the higher the incidence observed. von Baum et al.[18] also noticed that *Enterobacteriaceae* were associated with older age and comorbid conditions, factors that are more frequently associated with COPD[11,23].

Serum markers and clinical parameters

In general, CAP-COPD patients had more severe CAP than CAP-only patients. CURB-65 scores were higher, a finding in line with previous research[13]. Moreover, CAP-COPD patients were frequently hypoxic, which has been previously associated with COPD in CAP[24,25]. The observed differences in CURB-65 and hypoxaemia were not associated with bacterial aetiology.

In CAP-COPD patients with negative bacterial culture, elevated levels of $PaCO_2$ were observed. Molinos et al. also observed more patients with hypercapnia in COPD than non-COPD CAP, with both hypercapnia and hypoxaemia independently related to 30-day mortality[21]. Hypercapnia has been associated with a poor prognosis even in stable COPD[26].

CRP levels in CAP-only patients were statistically significantly higher in patients with positive bacterial culture. This finding is not surprising, as CRP is a marker for bacterial infection[3]. However, given the high levels in all subgroups, the clinical relevance of the observed differences might be limited.

Mortality

Mortality rates were highest in CAP-COPD patients with negative bacterial culture. As mentioned before, these patients also appeared to have more severe CAP. Respiratory viral infections might be important triggers in these patients. Vaccination status did not differ between CAP-COPD patients with and without positive bacterial culture. The fact that no bacterial aetiology was obtained in these patients does not exclude the possibility of bacterial infection. In COPD patients, this may be of importance, as it is known that such patients are more likely to be infected and colonised with bacteria that are not susceptible to standard antibiotics.

Failure of pathogen detection is common in CAP[27], possibly leading to difficulties in creating treatment strategies, with the antibiotic treatment initiated usually being empirical. In addition, viral infections account for a substantial proportion of CAP cases[7,28],

with a lack of treatment options[29]. It is also well known that viral infections in COPD are associated with worse outcomes, such as prolonged symptom recovery from a COPD exacerbation[30].

Enterobacteriaceae were the most frequently bacterial pathogens detected in deceased patients. Earlier research showed mortality rates of around five times higher in CAP patients identified with *Enterobacteriaceae*[18]. The present results emphasise the importance of using sputum analysis as a possible tool in the assessment of CAP, and advanced culture or sequencing techniques, in particular, might lead to targeted treatment strategies and better outcomes[31].

Strengths and limitations

This study has several limitations. No data were available on viral pathogens. As samples were collected only during disease state, information on colonisation is lacking. Sample sizes for subgroup analysis were small, which may have influenced outcomes. Moreover, treatment adequacy was assessed retrospectively, based on typical antibiotic susceptibility patterns. Ideally, resistance patterns of the specific aetiology should be assessed to guide antibiotic treatment.

A strength of the present study is the systematic use of a new pulmonary infiltrate on chest X-ray to confirm CAP diagnosis[32]. As other studies used clinical symptoms only to diagnose CAP, the current study is therefore able to add important information[33–35]. Moreover, CAPNETZ has a prospective design and is possibly the largest CAP cohort worldwide, including both in- and out-patients. This makes the results also relevant for a more general population.

Conclusion

It is important to identify COPD patients with CAP, especially those without detected bacterial pathogens, as such patients have more severe CAP and are at higher risk of dying. This highlights the role of pathogen testing, which is currently not recommended in standard care, but may be relevant in this patient population due to higher mortality rates. A better understanding of the specific aetiology could contribute to improved management and treatment of CAP in COPD, which might change the outcome.

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of CAP-only and CAP-COPD patients stratified by culture result*

	CAP-only (n=1026)		CAP-COPD (n=262)	
	Bacterial aetiology negative culture (n=828) n (%)	Bacterial aetiology positive culture (n=198) n (%)	Bacterial aetiology negative culture (n=189) n (%)	Bacterial aetiology positive culture (n=73) n (%)
Age, years, median [IQR]	57.0 [41.0–71.0]	59.0 [43.0–75.0]	71.0 [62.5–77.0]†‡	72.0 [65.0–77.5]†‡
Sex				
Male	452 (54.6)	114 (57.6)	125 (66.1)	44 (60.3)
Female	376 (45.4)	84 (42.4)	64 (33.9)	29 (39.7)
Smoking status				
Never smoker	449 (55.3)	102 (52.0)	52 (27.8)†‡	26 (35.6)†
Ex-smoker	159 (19.6)	34 (17.3)	77 (41.2)	26 (35.6)
Current smoker	204 (25.1)	60 (30.6)	58 (31.0)	21 (28.8)
Pack-years, median [IQR	0.0 [0.0–15.0]	0.0 [0.0–18.0]	30.0 [0.0–43.0]†‡	20.0 [0.0–40.0]†‡
GOLD stage				
1	-	-	36 (19.0)	19 (26.0)
2			65 (34.4)	26 (35.6)
3			51 (27.0)	19 (26.0)
4			37 (19.6)	9 (12.3)
Vaccination				
Influenza	216 (26.2)	46 (23.4)	102 (54.0)†‡	32 (43.8)†‡
Influenza A/H_1N_1	43 (10.0)	7 (6.4)	17 (16.3)	5 (13.2)
Pneumococcal	54 (6.6)	15 (7.7)	42 (22.2)†‡	21 (28.8)†‡
Co-morbidities				
Chronic heart failure§	95 (23.7)	25 (26.0)	51 (39.2)*	12 (26.1)
Chronic renal disease§	63 (15.7)	15 (15.6)	18 (13.8)	8 (17.4)
Chronic liver disease§	18 (4.5)	5 (5.2)	5 (3.8)	6 (13.0)
Cerebrovascular disease§	27 (6.7)	8 (8.3)	14 (10.8)	4 (8.7)
Diabetes mellitus§	98 (24.4)	34 (35.4)	40 (30.8)	8 (17.4)
Asthma¶	73 (67.0)	24 (66.7)	13 (25.5)†‡	6 (27.3)†‡
Bronchiectasis¶	6 (5.5)	3 (8.3)	1 (2.0)	3 (13.6)
Lung fibrosis¶	8 (7.3)	1 (2.8)	4 (7.8)	-
Sarcoidosis¶	1 (0.9)	1 (2.8)	1 (2.0)	-
Bronchial/lung cancer¶#	3 (2.8)	1 (2.8)	6 (11.8)	2 (9.1)
Sleep apnoea¶	10 (9.2)	2 (5.6)	8 (15.7)	3 (13.6)
Inhaled corticosteroids	-	-	101 (53.4)	39 (53.4)

* Corrected for multiple comparisons ($p<0.001$).

† $p\leq0.001$ compared to CAP-only negative culture.

‡ $p\leq0.001$ compared to CAP-only positive culture.

§ Missing: CAP-only bacterial aetiology NO: *n*=427; CAP-only bacterial aetiology YES: *n*=102; CAP-COPD bacterial aetiology NO: *n*=59; CAP-COPD bacterial aetiology YES: *n*=27.

¶ Missing: CAP-only bacterial aetiology NO: *n*=719; CAP-only bacterial aetiology YES: *n*=162; CAP-COPD bacterial aetiology NO: *n*=138; CAP-COPD bacterial aetiology YES: *n*=51.

Currently no radiation or chemotherapy.

CAP = community acquired pneumonia; COPD = Chronic Obstructive Pulmonary Disease; IQR = interquartile range; GOLD = Global Initiative for Chronic Obstructive Lung Disease.

4

Risk of community acquired pneumonia in COPD stratified by smoking status: A population-based cohort study in the United Kingdom

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ABSTRACT

Background: Smoking increases the risk of community acquired pneumonia (CAP) and is associated with the development of COPD. Until now, it is unclear whether CAP in COPD is due to smoking-related effects, or due to COPD pathophysiology itself.

Objective: To evaluate the association between COPD and CAP by smoking status.

Methods: In total, 62,621 COPD and 191,654 control subjects, matched by year of birth, gender and primary care practice, were extracted from the Clinical Practice Research Datalink (2005–2014). Incidence rates (IRs) were estimated by dividing the total number of CAP cases by the cumulative person-time at risk. Time-varying Cox proportional hazard models were used to estimate the hazard ratios (HRs) for CAP in COPD patients versus controls. HRs of CAP by smoking status were calculated by stratified analyses in COPD patients versus controls and within both subgroups with never smoking as reference.

Results: IRs of CAP in COPD patients (32.00/1,000 person-years) and controls (6.75/1,000 person-years) increased with age and female gender. The risk of CAP in COPD patients was higher than in controls (HR 4.51, 95% CI: 4.27–4.77). Current smoking COPD patients had comparable CAP risk (HR 0.92, 95% CI: 0.82–1.02) as never smoking COPD patients (reference), whereas current smoking controls had a higher risk (HR 1.23, 95% CI: 1.13–1.34) compared to never smoking controls.

Conclusion: COPD patients have a fourfold increased risk to develop CAP, independent of smoking status. Identification of factors related with the increased risk of CAP in COPD is warranted, in order to improve the management of patients at risk.

INTRODUCTION

Community acquired pneumonia (CAP) is characterized by an acute infection of the pulmonary parenchyma with onset in the out-of-hospital setting[1]. CAP incidence increases with age, smoking and the presence of comorbidities[1,2], varying between 1.5 and 11.0 per thousand adult population[3,4]. In COPD, high incidence rates (IRs) of CAP, up to 22.4 per 1000 person-years, have been reported[5]. Worse outcomes i.e., higher mortality rates[6,7] and longer length of hospital stay[6] were observed, as well as more pronounced hypoxemia[7], hypercapnia[6,7], tachypnea[6] and increased symptoms such as dyspnea and purulent sputum[7].

Smoking individuals have a twofold increased CAP risk[1,4]. Besides, smoking has been associated with increased susceptibility to infections in healthy subjects[8] and COPD patients, triggering exacerbations[9]. Until now, it is unclear whether CAP development in COPD is due to smoking-related increased susceptibility to infections, or due to COPD pathophysiology itself. Müllerova et al[5] observed no association between current smoking and CAP incidence in COPD.

As smoking has been identified as a risk factor for both COPD and CAP, it is important to compare COPD patients to smoking and non-smoking controls, to assess smoking-related effects, and distinguish possibly from additional risks associated with COPD and its pathophysiology. Particularly, it is important to take changes in smoking status over time into account, since smokers are known to undertake several attempts to quit smoking[10,11]. Therefore, the aim of the present study was to evaluate the association between COPD and CAP by smoking status.

METHODS

Source population

A population-based cohort study, with data derived from the world's largest primary care database, Clinical Practice Research Datalink (CPRD), was conducted. CPRD contains computerized medical records of 674 primary care practices in the UK, collected since January 1987, representative for the total population[12]. Coded data are collected on demographics, prescription details, clinical events, preventive care provided, tests, immunizations, specialist referrals, hospital admissions, discharge summaries and details regarding death[12]. The period of data collection for the present study included the period in which the quality and outcomes framework (QOF) was effective (January 2005–January 2014)[13]. CPRD data have been widely used to study CAP[14,15], COPD[16,17] and other respiratory diseases[18,19]. CPRD data have been shown to be accurate and valid[20].

Study population

Two cohorts were extracted. Cohort I: patients aged ≥40 years with a first ever recorded COPD read code, assigned by the general practitioner (Table S1, Supplemental material). COPD diagnosis defined start of follow-up (index date). Cohort II: randomly selected controls, without COPD diagnosis, matched by year of birth, gender and practice, using incidence density sampling. Controls were assigned the index date of their matched COPD patient. Controls with lung medication or lung function with Tiffenau index <0.7 before start of follow-up were excluded[21]. From both cohorts, individuals with history of asthma, history of pneumonia 3 months prior to index date, active tuberculosis or use of tuberculosis medication and unknown smoking status were excluded.

Outcome

The primary outcome was physician-recorded pneumonia diagnosis, identified by read codes (Table S2, Supplemental material). All patients were followed from index date to end of data collection, date of transfer out of the practice, patient's death or outcome of interest, whichever came first. Follow-up time was divided into fixed intervals of 90 days.

Exposure of interest

Smoking status was determined prior to each interval (90-day) and stratified into three subcategories: never, current and former smoking. Smoking was defined by read codes (Table S3, Supplemental material), which have provided valid estimates for the prevalence of current and never smoking[22]. When the most recent smoking status was "never", and the patient had quit smoking, his status was classified as former smoking.

Potential confounders

Potential confounders were time-dependently assessed, except for gender and body mass index (BMI). Time-dependent potential confounders were collected at the start of each time interval (90-day): age, history of pneumonia, cerebrovascular disease, dementia, malignancy (excluding non-melanoma skin cancer), chronic renal disease, diabetes mellitus (use of insulin and/or blood glucose lowering medicines), cardiovascular diseases (heart failure, ischemic heart disease, coronary artery disease, myocardial infarction) and chronic liver disease. Moreover, proxies of the underlying severity of COPD, including number of exacerbations in the year before, and use of the following drugs 6 months before, were collected: short-acting beta-2 agonists, long-acting beta-2 agonists, inhaled corticosteroids, xanthine derivatives, short-acting inhaled anticholinergics, long-acting anticholinergics, cromoglycates, oxygen or systemic glucocorti-

coids[23]. Analyses were adjusted for exposure to antipsychotics, acid suppressants or immunosuppressants in the past 6 months, as well as influenza or pneumococcal vaccination the year before. The most recent forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and Tiffenau index (ratio FEV_1/FVC) were reviewed in the time frame ever before.

Statistical analysis

Data analyses were performed using SAS 9.3. IRs were estimated by dividing the total number of CAP cases by cumulative person-time at risk. Cox proportional hazard models estimated hazard ratios (HRs) of CAP in COPD patients versus controls. Analyses were stratified to gender, age and smoking status during follow-up.

Determinants of CAP within COPD were evaluated during follow-up: age, gender, smoking status and most recently recorded FEV_1. HRs were estimated within each smoking status stratified for most recent level of airflow obstruction. HRs of CAP stratified by time-varying smoking status were assessed within COPD patients and controls separately. Never smoking was used as reference.

All analyses used time-varying Cox regression analysis. HRs were adjusted for gender and time-varying age and potential confounders (specified in previous section). Confounders were entered into the final model when independently changing the beta coefficient for current smoking by at least 5%, or when consensus was reached within the research team, supported by clinical evidence from literature. A test of interaction was performed to compare effects between the defined stratifications[24]. The study protocol was approved by Independent Scientific Advisory Committee, 14_055.

RESULTS

In total, 254,277 subjects were included in the present analysis (Figure 1). Of these, 62,621 had COPD. Table 1 describes baseline characteristics: almost half were female, mean age was 67 years, follow-up time on average was 3.6–4.0 years. At baseline, smoking status differed: COPD patients were frequently former or current smokers, while controls were more often never smokers. FEV_1 data were available for <50% of COPD patients, most classified as mild-to-moderate airflow obstruction.

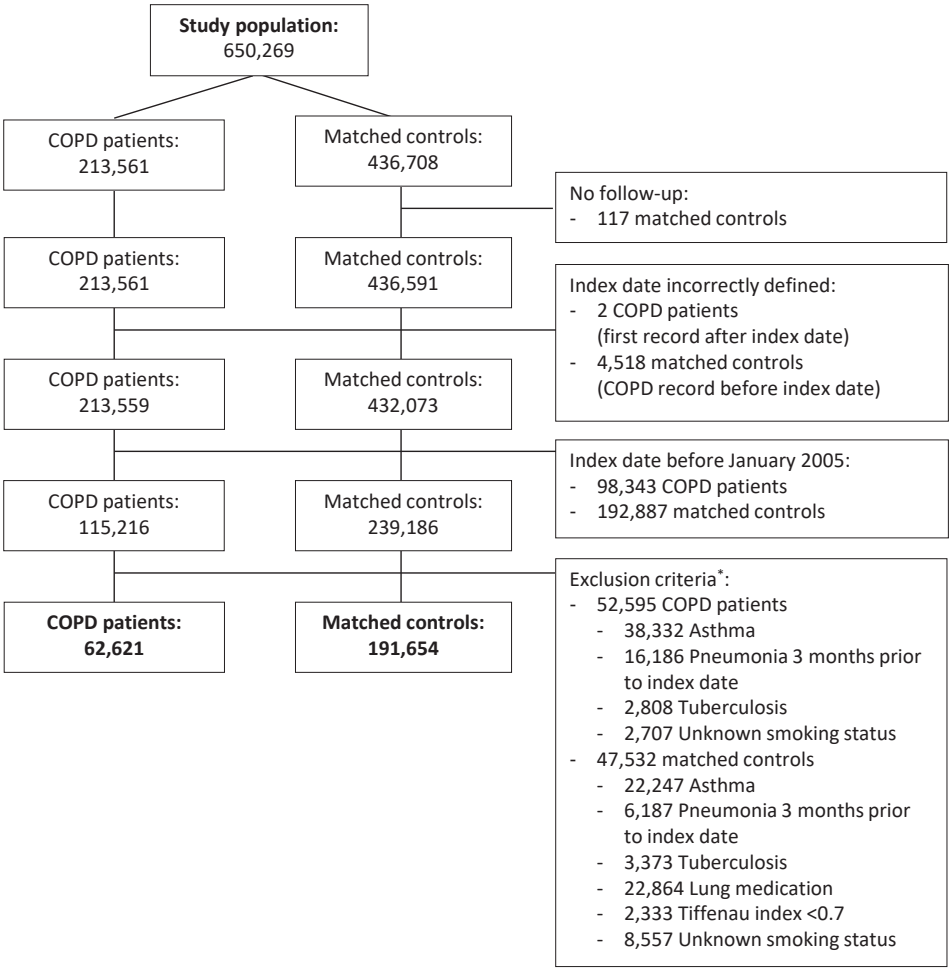

Figure 1. Flow chart.

*Subjects can have more than one exclusion criteria.

Table 1. Baseline characteristics of patients with COPD and matched controls

	COPD patients	Matched controls
Characteristics	n=62 621	n=191 656
Mean follow-up time (years)	3.6±2.5	4.0±2.5
Females	28 044 (44.8)	91 511 (47.8)
Age at index date (years)	67.8±11.2	67.4±11.5
BMI at index date (kg/m^2)	26.8±6.0	27.3±5.2
Underweight (BMI <18.5 kg/m^2)	3 335 (5.3)	2 998 (1.6)
Normal weight (BMI 18.5-<25 kg/m^2)	21 513 (34.4)	59 054 (30.8)
Overweight (BMI 25-<30 kg/m^2)	19 649 (31.4)	71 236 (37.2)
Obese (BMI ≥30 kg/m^2)	15 927 (25.4)	45 481 (23.7)
Unknown	2 197 (3.5)	12 887 (6.7)
Smoking status at index date		
Never	7 033 (11.2)	100 987 (52.7)
Current	30 938 (49.4)	36 989 (19.3)
Former	24 650 (39.4)	53 680 (28.0)
Drug use (<6 months)		
SABAs	27 001 (43.1)	0
LABAs	10 022 (16.0)	0
SAMAs	4 607 (7.4)	0
LAMAs	6 308 (10.1)	0
ICS	13 148 (21.0)	0
Systemic glucocorticoids	8 850 (14.1)	0
Xanthine derivatives	417 (0.7)	0
Oxygen	239 (0.4)	0
Antipsychotics	684 (1.1)	1 368 (0.7)
Acid suppressants	18 631 (29.8)	36 640 (19.1)
Immunosuppressants	264 (0.4)	291 (0.2)
Influenza vaccination	6 093 (9.7)	17 088 (8.9)
Pneumococcal vaccination	1 052 (1.7)	2 707 (1.4)
History of co-morbidities		
Coronary artery disease[a]	11 187 (17.9)	22 779 (11.9)
Heart failure	2 171 (3.5)	2 768 (1.4)
Diabetes Mellitus	5 039 (8.1)	15 908 (8.3)
Cerebrovascular disease	4 113 (6.6)	9 290 (4.9)
Dementia	910 (1.5)	3 355 (1.8)
Malignancy (excl. non-melanoma skin cancer)	9 068 (14.5)	26 350 (13.8)
Chronic renal disease	646 (1.0)	1 522 (0.8)
Chronic liver disease	231 (0.4)	288 (0.2)
Pneumonia[b]	34 519 (55.1)	78 667 (41.1)
FEV_1 %pred.[c] (most recent)	67.8±22.2	93.9±18.0
≥80 %pred.	6 573 (28.6)	3 689 (80.1)
≥50 - <80 %pred.	11 330 (49.3)	867 (18.8)
≥30 - <50 %pred.	4 264 (18.6)	48 (1.0)
<30 %pred.	818 (3.6)	4 (0.1)
FVC %pred. (most recent)	84.6±20.7	94.7±18.6
Tiffenau index (FEV_1/FVC) (most recent)	65.2±14.2	80.9±6.7
Acute exacerbation of COPD (<1 year)	444 (0.7)	0

Notes: Data are presented as mean±SD or n (relative %). [a] Including: ischemic heart disease, myocardial infarction and coronary artery disease. [b] History of pneumonia >3 months prior to index date. [c] COPD n=22,985 (36.7%); matched controls n=4,608 (2.4%). **Abbreviations:** %pred, percentage of predicted; BMI, body mass index; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroids; LABAs, long-acting beta-2 agonists; LAMAs, long-acting muscarinic antagonists; SABAs, short-acting beta-2 agonists; SAMAs, short-acting muscarinic antagonists.

CAP incidence

Around 3.04% (n=7,730) of the total population was diagnosed with CAP during follow-up: 3,819 (6.10%) COPD patients and 3,911 (2.04%) controls. Table S4 shows IRs of CAP in COPD patients (32.00 per 1000 person-years) and controls (6.75 per 1000 person-years). IRs increased with age and female gender. In COPD patients, IR was highest in never smokers (39.51 per 1000 person-years), while former smokers had the lowest IR (28.31 per 1000 person-years). In controls, IR was highest in current smokers (7.82 per 1000 person-years).

CAP risk in COPD patients versus controls

COPD patients had a fourfold increased CAP risk compared to controls (fully adjusted [adj.] HR 4.51 [4.27–4.77]; Figure 2; Table S4, Supplemental material). Stratum-specific CAP risk in COPD was significantly higher (test of interaction HR 1.22 [1.03–1.45]) in younger patients (aged 40–59; fully adj. HR 4.97 [4.40–5.62]), then in elderly patients (aged ≥80; fully adj. HR 4.08 [3.61–4.60]). After stratification to smoking status, the HR of CAP was five times higher in never smoking COPD patients, compared to never smoking controls (fully adj. HR 5.25 [4.75–5.80]). The same for current smoking COPD patients versus current smoking controls (fully adj. HR 4.94 [4.65–5.26]). Former smoking COPD patients had a fourfold increased CAP risk compared to former smoking controls (fully adj. HR 4.26 [4.00–4.55]).

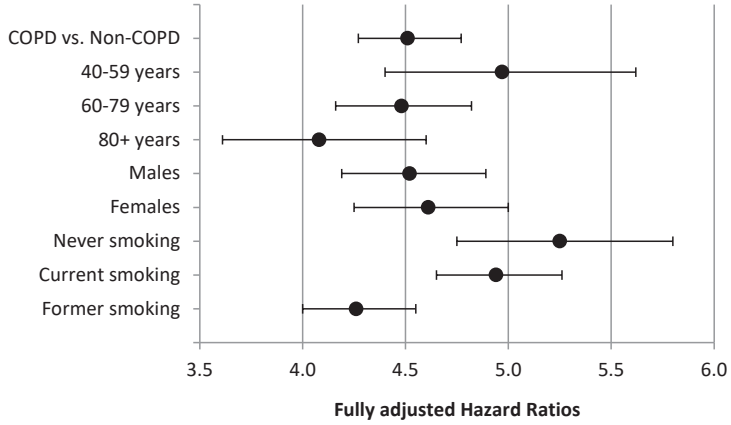

Figure 2. Stratum-specific risk of CAP in patients with COPD compared to matched controls, stratified by age, gender and smoking status.

Notes: Adjusted for age, gender, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease, chronic liver disease and smoking status. **Abbreviations:** BMI, body mass index; CAP, community-acquired pneumonia; ICS, inhaled corticosteroids.

CAP risk and smoking status

Within COPD, CAP risk in current smokers (fully adj. HR 0.92 [0.82–1.02]) was comparable to the risk in never smokers (reference; Figure 3; Table S5, Supplemental material). Former smoking COPD patients had a lower risk (fully adj. HR 0.81 [0.73–0.90]). Current smokers had a significantly higher CAP risk as compared to former smokers (p<0.001). CAP risk increased by older age (fully adj. HR aged 60–79, 2.26 [2.00–2.57]; aged ≥80, 6.06 [4.86–7.55]) and female gender (fully adj. HR 1.30 [1.22–1.39]). The level of airflow obstruction showed a trend toward increased CAP risk for very severe obstruction (fully adj. HR 1.30 [0.98–1.73]) in comparison to mild obstruction (reference). A sub analysis was performed by smoking status, stratified for level of airflow obstruction (Table S6, Supplemental material). No clear trend of increased risk by severity of airflow obstruction was observed within never, current or former smokers.

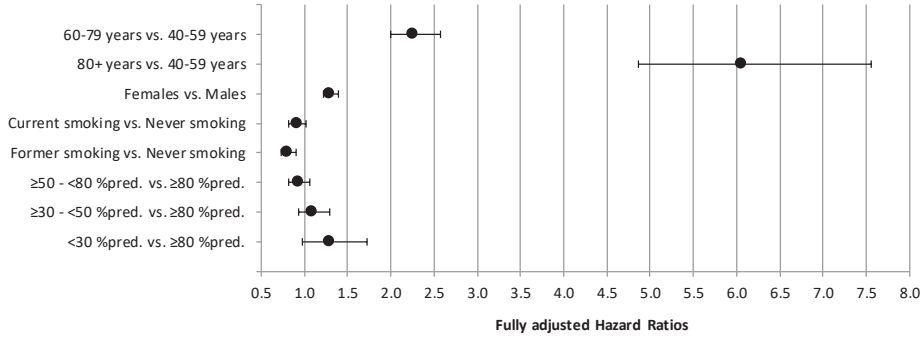

Figure 3. Risk of CAP in patients with COPD, stratified by age, gender, smoking status and the level of airflow obstruction.

Notes: Adjusted for age, gender, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease, chronic liver disease and smoking status. **Abbreviations:** BMI, body mass index; CAP, community-acquired pneumonia; ICS, inhaled corticosteroids.

Within controls, CAP risk was highest in current smokers (fully adj. HR 1.23 [1.13–1.34]), while the risk in former smokers (fully adj. HR 1.07 [0.99–1.16]) was comparable with the risk in never smokers (reference; Table S7, Supplemental material). Older age (fully adj. HR aged 60–79 years, 2.54 [2.23–2.90]; aged ≥80 years, 8.85 [7.10–11.00]) and female gender (fully adj. HR 1.26 [1.18–1.35]) showed increased CAP risk.

DISCUSSION

COPD patients had a fourfold increased CAP risk in comparison with matched controls. Current smoking had no additional impact on CAP risk in COPD. In controls, CAP risk was elevated in current smokers. The risk of CAP increased in both COPD patients and controls with older age and female gender.

CAP risk and IRs

COPD patients had higher CAP IRs and increased CAP risk compared to controls, in line with former research[4,25]. The observed IR in COPD patients was higher than reported by Müllerova et al[5] (22.4/1000 person-years), but in accordance with the incidence reported by DiSantostefano et al[26] (30.9/1000 person-years). In controls, Capelastegui et al[27] observed an IR of 3.1/1000 adults per year, while our observed IR was twice as high (6.8/1000 person-years).

A possible explanation of the higher IRs observed, might be the rising incidence in European countries[28]. A number of factors are associated with this phenomenon: populations grow older, and lifestyle factors and comorbid conditions related to CAP become more prevalent[28]. Older age might also be a potential factor in our study, as the average age was 67 years. This was also shown by Millett et al[29] with an incidence of 8.0/1000 person-years in controls ≥65 years old.

Gender differences appeared, as females had a slightly larger CAP risk than males. This is in contrast with former research[2,30,31]. Reasons for increased risk in females could not be further delineated in this study, but clearly warrant further research. Changes in female lifestyle and risk behaviour have been reported in previous decades and might represent important factors[32,33]. Furthermore, former research showed that respiratory symptoms were more often reported by females than males[34], with higher hospitalization incidences in studies concerning milder CAP cases[35,36].

Smoking status and CAP risk

Current smoking was associated with increased CAP risk in controls, in line with former research[37–39]. Smoking has been related to structural changes in the respiratory tract and a decrease in immune response[40], which might result in microbial invasion of the bronchial tree, triggering CAP.

In COPD patients, CAP risk was comparable between never and current smokers. It was expected that, in accordance with controls, current smokers were at increased CAP risk. However, Müllerova et al[5] also observed no difference in CAP risk between non-smoking and current smoking COPD patients. Moreover, Myint et al[41] observed a larger

proportion of current smokers in COPD without CAP than in CAP–COPD. Maybe, inaccurate recording of smoking status by general practitioners influenced the present results, although smoking is a QOF indicator, rewarding general practitioners to record patients' smoking status every year when diagnosed with COPD. However, smoking is the major risk factor of COPD, but the majority of persistent smokers do not develop COPD. This suggests that the vulnerability to cigarette smoke varies between individuals. The mechanisms behind this are at the moment not completely understood[42]. Never smokers may also develop COPD, but by a different pathway than exposure to smoking[43], for example, by occupational/environmental exposures, alpha-1-antitrypsin deficiency or due to factors early in life which affect the respiratory health in the long-term[44]. Pathophysiological differences between subgroups of COPD might contribute to the observed differences in CAP risk. Besides, it is likely that a combination of factors is responsible for disease development, or not. For example, smoking has been associated with CAP development, but is also associated with a lower socioeconomic status, poor diet, alcohol consumption and reduced physical activity[45], which, in turn, are also risk factors for CAP[4]. Furthermore, there are theoretically three general mechanisms related to the increased CAP risk of smoking: 1) tobacco-induced physiological and structural changes, 2) tobacco-induced increase in bacterial virulence, 3) tobacco-induced dysregulation of immune function[8]. These three mechanisms are also key features in COPD, and probably the smoking-effect related to both the development of CAP and COPD, does not sum-up. Overall, many mechanisms might be associated with the observed results, but further research is warranted to explore exact pathways involved.

We also observed no difference concerning severity of airflow obstruction stratified by smoking status and CAP risk. IRs increased with worse airflow obstruction, but only showing a trend toward increased risk in very severe airflow obstruction. Conflicting results were reported before, some observing increased CAP risk in severe and very severe airflow obstruction[46], while others observed no difference in CAP risk by airflow obstruction[41]. There may be reverse causation underlying this lack of relationship; those with worse airflow obstruction stopped smoking, while others continued their smoking habits. However, this is less likely, as smoking status over time was taken into account, correcting for possible confounding. Furthermore, never and current smoking COPD patients had a comparable risk, which stresses the fact that the observed results are not due to smoking cessation.

Strengths and limitations

The current study includes a large population-based cohort study, providing anonymous longitudinal medical records of primary care patients[12,47]. This study design makes the current results generalizable to a larger population. In addition, by taking smoking status over time into account, the results are representative of real life setting.

In contrast, several methodological issues could have influenced the results. First, primary care databases rely on the quality of information included in records[12]. This depends on the accuracy of individuals responsible for entering data. However, inclusion of a large number of patients will minimize potential bias. Second, we did not use spirometry to confirm COPD diagnosis, as this was available for only one-third of patients. Misclassification of exposure is likely to be non-differential and may have led to a bias toward null[48]. This implies that the fourfold increased risk of CAP in COPD versus non-COPD may have been underestimated. In addition, CAP diagnosis was not confirmed by chest X-ray, but based on clinical features, probably causing CAP overestimation[49]. This may have led to a non-differential misclassification of the outcome and a bias toward the null value. However, it has a small impact on our main findings: the true HR of CAP among smokers versus non-smokers in COPD would have been lower than the non-significant 8% reduced risk, and will still not support our main hypothesis that smoking increases the risk of CAP in COPD. In addition, ICS use was not separately analysed, but only included as a potential confounder, as this would go beyond the primary aim of the objective. However, ICS risk in CAP is of high interest, often showing an increased risk, although probably depending on the specific ICS and dose[50].

Clinical implications

The results of the present analyses highlight the fact that CAP remains a major health issue, impacting both socially and economically[51]. Smoking cessation is an important aspect in the management of CAP and especially in control subjects, this strategy might be beneficial and lead to a decreased risk to develop CAP[38]. In contrast, for patients with COPD, this association seems less clear, but as earlier described, further research is necessary to assess mechanisms associated with the increased risk to develop CAP. For clinical practice it remains important to not underestimate the impact of CAP in patients with COPD. The management of CAP is at the moment focused on treating the disease, and prevention by vaccination[51], while prevention of lifestyle factors, such as smoking, alcohol consumption and bodyweight, is at least as important[4,38,39,52,53].

Conclusion

COPD patients have a fourfold increased risk to develop CAP, independent of smoking status. Identification of factors related with the increased risk of CAP in COPD is warranted, in order to improve the management of patients at risk.

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SUPPLEMENTAL MATERIAL

Table S1. Read codes to define COPD

Read code	Medical code	Clinical event	Read term
66YM.00	11287	382901	Chronic obstructive pulmonary disease annual review
H3...00	1001	338812	Chronic obstructive pulmonary disease
H3...11	998	205995	Chronic obstructive airways disease
66YB.00	9520	161278	Chronic obstructive pulmonary disease monitoring
H312200	1446	153677	Acute exacerbation of chronic obstructive airways disease
66Yf.00	28743	81666	Number of COPD exacerbations in past year
H36..00	10863	52387	Mild chronic obstructive pulmonary disease
H3z..00	5710	50568	Chronic obstructive airways disease NOS
H3y1.00	7884	48220	Chron obstruct pulmonary dis wth acute exacerbation, unspec
H37..00	10802	46111	Moderate chronic obstructive pulmonary disease
66YL.00	18621	45740	Chronic obstructive pulmonary disease follow-up
H32..00	794	44748	Emphysema
66YL.11	18476	30033	COPD follow-up
H31..00	3243	24068	Chronic bronchitis
H38..00	9876	21178	Severe chronic obstructive pulmonary disease
8H2R.00	11019	11033	Admit COPD emergency
66Yd.00	19106	5350	COPD accident and emergency attendance since last visit
66Ye.00	19003	4720	Emergency COPD admission since last appointment
H31z.00	15157	3461	Chronic bronchitis NOS
66YB000	102685	1499	Chronic obstructive pulmonary disease 3 monthly review
66YB100	103007	1470	Chronic obstructive pulmonary disease 6 monthly review
14OX.00	96931	1448	At risk of chronic obstructive pulmonary diseas exacerbation
H39..00	93568	1355	Very severe chronic obstructive pulmonary disease
H312100	14798	1157	Emphysematous bronchitis
H3y..00	12166	1113	Other specified chronic obstructive airways disease
H312.00	27819	1014	Obstructive chronic bronchitis
H32z.00	33450	862	Emphysema NOS
H3z..11	37247	794	Chronic obstructive pulmonary disease NOS
H310.00	25603	560	Simple chronic bronchitis
H320.00	26306	410	Chronic bullous emphysema
66Yi.00	46036	390	Multiple COPD emergency hospital admissions
14B3.12	103494	363	History of chronic obstructive pulmonary disease
H311.00	11150	309	Mucopurulent chronic bronchitis
8BP8.00	100123	152	Antibiotic therapy for acute pulmonary exacerbation
H320z00	23492	77	Chronic bullous emphysema NOS
H312z00	44525	64	Obstructive chronic bronchitis NOS
H32yz00	16410	54	Other emphysema NOS
H32y.00	40788	47	Other emphysema
Hyu3100	65733	47	[X]Other specified chronic obstructive pulmonary disease

Read code	Medical code	Clinical event	Read term
H311000	40159	45	Purulent chronic bronchitis
H310z00	61118	33	Simple chronic bronchitis NOS
H311z00	61513	25	Mucopurulent chronic bronchitis
H313.00	24248	24	Mixed simple and mucopurulent chronic bronchitis
H3A..00	104608	22	End stage chronic obstructive airways disease
H31y.00	66043	18	Other chronic bronchitis
H31yz00	68066	15	Other chronic bronchitis NOS
H320200	60188	14	Giant bullous emphysema
H320000	56860	10	Segmental bullous emphysema
H320100	68662	10	Zonal bullous emphysema
H3y..11	67040	7	Other specified chronic obstructive pulmonary disease

Table S2. Read codes to define pneumonia

Read code	Medical code	Clinical event	Read term
H25..11	16287	1.368	chest infection - unspecified bronchopneumonia
H26..11	19400	517	chest infection - pneumonia due to unspecified organism
H22..11	22795	452	chest infection - other bacterial pneumonia
H20..11	9389	304	chest infection - viral pneumonia
H270.11	29457	131	chest infection - influenza with pneumonia
H21..11	29166	71	chest infection - pneumococcal pneumonia
H23..11	30653	62	chest infection - pneumonia organism OS
H2B..00	104121	1.358	community acquired pneumonia
H26..00	572	152.581	pneumonia due to unspecified organism
H23..00	25694	1.404	pneumonia due to other specified organisms
H231.00	1576	3.638	pneumonia due to mycoplasma pneumoniae
H223.00	12423	781	pneumonia due to streptococcus
H224.00	5612	416	pneumonia due to staphylococcus
H22y200	12061	568	pneumonia - legionella
H23z.00	34251	320	pneumonia due to specified organism NOS
H201.00	31269	240	pneumonia due to respiratory syncytial virus
H220.00	23546	202	pneumonia due to klebsiella pneumoniae
H221.00	30591	170	pneumonia due to pseudomonas
H222.00	37881	97	pneumonia due to haemophilus influenzae
H202.00	36675	36	pneumonia due to parainfluenza virus
H22y.00	50867	79	pneumonia due to other specified bacteria
H200.00	67836	30	pneumonia due to adenovirus
H223000	63858	22	pneumonia due to streptococcus, group B
H222.11	48804	19	pneumonia due to haemophilus influenzae
H22y100	45425	2	pneumonia due to proteus
H22y000	65419	12	pneumonia due to escherichia coli
Hyu0B00	98381	13	{X} pneumonia due to other specified infectious organisms
H22y011	60299	6	e. coli pneumonia
H233.00	17025	107	chlamydial pneumonia
A022200	58896	16	salmonella pneumonia
A54x400	47973	12	herpes cimplex pneumonia
H25..00	886	81.043	bronchopneumonia due to unspecified organism
H21..00	1849	33.743	lobar (pneumococcal) pneumonia
H260.00	9639	9.071	lobar pneumonia due to unspecified organism
H261.00	3683	8.061	basal pneumonia due to unspecified organism
H232.00	73735	3	pneumonia due to pleuropneumonia like organisms
H2z..00	6094	25.862	pneumonia or influenza NOS
H2…00	10086	11.512	pneumonia and influenza
H270.00	15912	369	influenza with pneumonia
H270z00	35745	55	influenza with pneumonia NOS
H270100	62632	7	influenza with pneumonia, influenza virus identified

Read code	Medical code	Clinical event	Read term
H2y..00	11849	4.971	other specified pneumonia or influenza
H28..00	5324	6.049	atypical pneumonia
H20..00	5202	4.455	viral pneumonia
H20z.00	14976	2.153	viral pneumonia NOS
H20y.00	33478	154	viral pneumonia NEC
Hyu0800	52520	12	{X} other viral pneumonia
H22z.00	23095	2.636	bacterial pneumonia NOS
H22yz00	43884	233	pneumonia due to bacteria NOS
H22..00	28634	1.402	other bacterial pneumonia
Hyu0A00	63763	17	{X} other bacterial pneumonia
A3By.00	41721	14	other specified bacterial infection
H56y100	4910	998	interstitial pneumonia
H24y200	27519	137	pneumonia with pneumocystis carinii
H24y700	23726	107	pneumonia with varicella
H243.00	30437	107	pneumonia with whooping cough
H24..00	40498	105	pneumonia with infectious diseases EC
H24y.00	69782	11	pneumonia with other infectious diseases EC
H24yz00	70559	4	pneumonia with other infectious diseases EC NOS
H24z.00	66362	7	pneumonia with infectious diseases EC NOS
H243.11	35082	86	pneumonia with pertussis
H246.00	34274	45	pneumonia with aspergillosis
H24y000	61623	40	pneumonia with actinomycosis
H26000	8318	3.513	Lung consolidation
H263.00	38065	893	Pneumonitis, unspecified
A521.00	25462	417	Varicella pneumonitis
H240.00	41034	135	pneumonia with measles
A551.00	32172	94	postmeasles pneumonia
H530300	35189	67	abscess of lung with pneumonia
H22yx00	52384	3	pneumonia due to other aerobic gram-negative bacteria

Table S3. Read codes to define smoking

Read code	Pegasus code	Clinical event	Read term
1371.00	33	6470929	Never smoked tobacco
137..00	54	2502551	Tobacco consumption
137L.00	60	2304618	Current non-smoker
137S.00	90	3052739	Ex smoker
137P.00	93	2676385	Cigarette smoker
137K.00	776	229539	Stopped smoking
1376.00	1822	21119	Very heavy smoker - 40+cigs/d
137P.11	1823	248966	Smoker
1374.00	1878	306129	Moderate smoker - 10-19 cigs/d
SM7z.11	2758	4192	Smoke inhalation
1375.00	3568	180120	Heavy smoker - 20-39 cigs/day
9OO..12	7130	31688	Stop smoking monitoring admin.
8CAL.00	7622	2065441	Smoking cessation advice
67A3.00	10184	1096	Pregnancy smoking advice
13p..00	10211	66778	Smoking cessation milestones
137R.00	10558	298165	Current smoker
8HTK.00	10742	28366	Referral to stop-smoking clinic
9N2k.00	11356	80957	Seen by smoking cessation advisor
9N4M.00	11527	12018	DNA - Did not attend smoking cessation clinic
1371.11	11788	486937	Non-smoker
137G.00	12240	122363	Trying to give up smoking
137T.00	12878	22138	Date ceased smoking
1372.11	12941	63145	Occasional smoker
137..11	12942	29304	Smoker - amount smoked
137J.00	12943	44369	Cigar smoker
1373.00	12944	192364	Light smoker - 1-9 cigs/day
137M.00	12945	44090	Rolls own cigarettes
137F.00	12946	184203	Ex-smoker - amount unknown
137H.00	12947	42546	Pipe smoker
137Q.11	12951	4825	Smoking restarted
137Q.00	12952	5262	Smoking started
9OO1.00	12953	34338	Attends stop smoking monitor.
ZV4K000	12954	77	[V]Tobacco use
1379.00	12955	102459	Ex-moderate smoker (10-19/day)
137A.00	12956	63329	Ex-heavy smoker (20-39/day)
1378.00	12957	67388	Ex-light smoker (1-9/day)
1372.00	12958	45508	Trivial smoker - < 1 cig/day
137B.00	12959	14127	Ex-very heavy smoker (40+/day)
137Z.00	12960	5648	Tobacco consumption NOS
1377.00	12961	17473	Ex-trivial smoker (<1/day)
137Y.00	12963	3136	Cigar consumption
137C.00	12964	6648	Keeps trying to stop smoking
137X.00	12965	2300	Cigarette consumption

Read code	Pegasus code	Clinical event	Read term
137V.00	12966	2268	Smoking reduced
137a.00	12967	4160	Pipe tobacco consumption
8H7i.00	18573	52913	Referral to smoking cessation advisor
67H1.00	18926	2697	Lifestyle advice regarding smoking
9OOA.00	19485	8032	Stop smoking monitor.chck done
137O.00	19488	2476	Ex cigar smoker
9OOZ.00	21637	6887	Stop smoking monitor admin.NOS
137N.00	26470	4026	Ex pipe smoker
9OO..00	28834	15149	Anti-smoking monitoring admin.
137c.00	30423	2733	Thinking about stopping smoking
137d.00	30762	5336	Not interested in stopping smoking
137b.00	31114	4532	Ready to stop smoking
9OO..11	32083	1482	Stop smoking clinic admin.
E251.00	32687	2802	Tobacco dependence
137W.00	32973	117	Chews tobacco
13p0.00	34126	24577	Negotiated date for cessation of smoking
13p5.00	38112	6814	Smoking cessation programme start date
9OO3.00	40417	725	Stop smoking monitor default
9OO2.00	40418	2940	Refuses stop smoking monitor
8CAg.00	41042	333	Smoking cessation advice provided by community pharmacist
137e.00	41979	358	Smoking restarted
9OO4.00	42722	10208	Stop smoking monitor 1st lettr
137g.00	46300	2262	Cigarette pack-years
137f.00	46321	36	Reason for restarting smoking
13WK.00	52503	230	No smokers in the household
9OO7.00	53101	490	Stop smoking monitor verb.inv.
9OO8.00	58597	395	Stop smoking monitor phone inv
9OO5.00	60720	159	Stop smoking monitor 2nd lettr
137h.00	62686	1277	Minutes from waking to first tobacco consumption
9OO9.00	63901	15	Stop smoking monitoring delete
9OO6.00	66387	17	Stop smoking monitor 3rd lettr
E251z00	68658	242	Tobacco dependence NOS
E251100	70746	12	Tobacco dependence, continuous
E251300	72706	115	Tobacco dependence in remission
745H.00	74907	12882	Smoking cessation therapy
745H000	81440	1883	Nicotine replacement therapy using nicotine patches
745H200	85247	375	Nicotine replacement therapy using nicotine inhalator
745H100	85975	222	Nicotine replacement therapy using nicotine gum
745H300	89464	98	Nicotine replacement therapy using nicotine lozenges
745Hz00	90522	261	Smoking cessation therapy NOS
745Hy00	91708	38	Other specified smoking cessation therapy
745H400	94958	214	Smoking cessation drug therapy
E251000	95610	2	Tobacco dependence, unspecified

Table S4. Stratum-specific risk of CAP in patients with COPD compared to matched controls, stratified by age, gender and smoking status

	CAP	IR[a]		Age and gender adjusted HR (95% CI)	Fully adjusted HR (95% CI)[b]
	n = 7 730	COPD	non-COPD		
Non-COPD	3 911		6.75	reference	reference
COPD	3 819	32.00		4.67 (4.47-4.89)	4.51 (4.27-4.77)
By age (years)[c]					
40-59	817	28.03	5.40	5.39 (4.89-5.94)	4.97 (4.40-5.62)
60-79	2 234	30.06	6.29	4.67 (4.41-4.96)	4.48 (4.16-4.82)
80+	768	48.30	11.75	3.93 (3.56-4.33)	4.08 (3.61-4.60)
By gender[d]					
Males	1 939	29.50	6.18	4.49 (4.21-4.78)[f]	4.52 (4.19-4.89)[g]
Females	1 880	36.69	7.39	4.90 (4.60-5.22)[f]	4.61 (4.25-5.00)[g]
By smoking status[e]					
Never	509	39.51	6.40	5.47 (4.98-6.01)	5.25 (4.75-5.80)[h]
Current	1 758	34.03	7.82	4.86 (4.59-5.14)	4.94 (4.65-5.26)[h]
Former	1 552	28.31	6.78	4.28 (4.03-4.54)	4.26 (4.00-4.55)[h]

[a] IR/1000 person-years. [b] Adjusted for age, gender, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease, chronic liver disease and smoking. [c] Non-COPD of the same age category as reference. [d] Non-COPD of the same gender as reference. [e] Non-COPD with the same smoking status as reference. [f] Adjusted for age. [g] Adjusted for age, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease, chronic liver disease and smoking. [h] Adjusted for age, gender, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease and chronic liver disease. **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; IR, incidence rate; HR, hazard ratio; ICS, inhaled corticosteroids.

Table S5. Risk of CAP in patients with COPD, stratified by age, gender, smoking status and the level of airflow obstruction

	CAP n = 3 819	IR[a]	Age and gender adjusted HR (95% CI)	Fully adjusted HR (95% CI)[b]
By age (years)				
40-59	817	28.03	reference	reference
60-79	2 234	30.06	2.16 (1.91-2.45)	2.26 (2.00-2.57)
80+	768	48.30	6.18 (4.99-7.66)	6.06 (4.86-7.55)
By gender				
Males	1 939	29.50	reference	reference
Females	1 880	36.69	1.31 (1.23-1.39)[c]	1.30 (1.22-1.39)[d]
By smoking status				
Never	509	39.51	reference	reference
Current	1 758	34.03	0.87 (0.78-0.96)	0.92 (0.82-1.02)[e]
Former	1 552	28.31	0.77 (0.70-0.86)	0.81 (0.73-0.90)[e]
By FEV_1				
≥80 %pred.	355	12.68	reference	reference
≥50 - <80 %pred.	627	12.72	0.93 (0.81-1.06)	0.93 (0.82-1.06)
≥30 - <50 %pred.	266	14.33	1.03 (0.87-1.20)	1.09 (0.93-1.29)
<30 %pred.	61	17.70	1.21 (0.92-1.59)	1.30 (0.98-1.73)

[a] IR/1000 person-years. [b] Adjusted for age, gender, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease, chronic liver disease and smoking. [c] Adjusted for age. [d] Adjusted for age, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease, chronic liver disease and smoking. [e] Adjusted for age, gender, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease and chronic liver disease. **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; IR, incidence rate; HR, hazard ratio; FEV_1, forced expiratory volume in 1 second; %pred., percentage of predicted; ICS, inhaled corticosteroids.

Table S6. Risk of CAP in patients with COPD, stratified by the level of airflow obstruction within each smoking category

	CAP	IR[a]	Age and gender adjusted HR (95% CI)	Fully adjusted HR (95% CI)[b]
	n = 3 819			
Never smoking	509			
≥80 %pred.	46	12.15	reference	reference
≥50 - <80 %pred.	73	15.86	1.22 (0.96-1.56)	1.17 (0.81-1.70)
≥30 - <50 %pred.	29	21.87	1.57 (1.08-2.27)	1.61 (1.01-2.56)
<30 %pred.	3	16.21	1.23 (0.40-3.84)	1.40 (0.44-4.51)
Current smoking	1 758			
≥80 %pred.	158	14.58	reference	reference
≥50 - <80 %pred.	267	12.65	0.86 (0.75-0.99)	0.81 (0.67-0.99)
≥30 - <50 %pred.	113	13.45	0.93 (0.77-1.13)	0.91 (0.71-1.16)
<30 %pred.	31	19.65	1.32 (0.92-1.89)	1.30 (0.88-1.93)
Former smoking	1 552			
≥80 %pred.	151	11.29	reference	reference
≥50 - <80 %pred.	287	12.17	0.95 (0.83-1.09)	0.99 (0.81-1.21)
≥30 - <50 %pred.	124	14.04	1.05 (0.87-1.28)	1.16 (0.91-1.48)
<30 %pred.	27	16.04	1.11 (0.76-1.64)	1.21 (0.79-1.85)

[a] IR/1000 person-years. [b] Adjusted for age, gender, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease and chronic liver disease. **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; IR, incidence rate; HR, hazard ratio; %pred., percentage of predicted; ICS, inhaled corticosteroids.

Table S7. Risk of CAP in matched control subjects, stratified by age, gender and smoking status

	CAP	IR[a]	Fully adjusted HR (95% CI)[b]
	N = 3 911		
By age (years)			
40-59	819	5.40	reference
60-79	2221	6.29	2.54 (2.23-2.90)
80+	871	11.75	8.85 (7.10-11.00)
By gender			
Males	1890	6.18	reference
Females	2021	7.39	1.26 (1.18-1.35)[c]
By smoking status			
Never	2003	6.40	reference
Current	1130	7.82	1.07 (0.99-1.16)[d]
Former	778	6.78	1.23 (1.13-1.34)[d]

[a] IR/1000 person-years. [b] Adjusted for age, gender, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease, chronic liver disease and smoking. [c] Adjusted for age, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease, chronic liver disease and smoking. [d] Adjusted for age, gender, BMI, antipsychotics, acid suppressants, immunosuppressants, influenza vaccination, pneumococcal vaccination, ICS, coronary artery disease, heart failure, diabetes mellitus, cerebrovascular disease, dementia, malignancy, chronic renal disease and chronic liver disease. **Abbreviations:** CAP, community acquired pneumonia; COPD, Chronic Obstructive Pulmonary Disease; IR, incidence rate; HR, hazard ratio; ICS, inhaled corticosteroids.

5

Sputum microbiology predicts health status in COPD

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ABSTRACT

Background: Spontaneous sputum production occurs in a subset of COPD patients; however, its clinical relevance has not been established. Differences in health status and clinical outcomes between patients with and without positive sputum cultures are unknown.

Objectives: To compare clinical characteristics and health status of spontaneous sputum producers with a positive culture (SC+) and negative culture (SC-) with non-sputum producers (NP) in a cohort of COPD patients referred for pulmonary rehabilitation.

Methods: In total, 518 clinically stable patients with mild-to-very severe COPD were recruited (mean age 64.1±9.1 years, 55.6% males, forced expiratory volume in 1 second 48.6%±20.0% predicted). Health status was measured using COPD Assessment Test, St. George's Respiratory Questionnaire, and the Clinical COPD Questionnaire. Symptoms of anxiety and depression were assessed using the Hospital Anxiety and Depression Scale. Exercise capacity was measured using the 6-minute walking distance. Spontaneously expectorated sputum was cultured for microbiology.

Results: Almost one-third of patients spontaneously produced sputum (n=164, 31.7%). Despite comparable lung function, SC+ reported more frequent exacerbations than NP (≥2 exacerbations <1 year: 43 [81.1%] vs. 179 [50.6%], $p<0.001$). COPD Assessment Test total score and the Clinical COPD Questionnaire total score were significantly worse in SC+ than NP (23.9±6.1 vs. 21.1±6.7, $p=0.012$; 3.1±1.0 vs. 2.5±1.0, $p=0.002$; respectively). Hospital Anxiety and Depression Scale-D score was significantly higher in SC+ than NP (8.7±4.1 vs. 7.2±4.3, $p=0.046$).

Conclusion: Spontaneous sputum production is common in COPD. Particularly, patients with positive cultures have worse health status and more symptoms of depression. Impact on disease progression and long-term outcomes remain to be established.

INTRODUCTION

COPD is a life-threatening lung disease and is the fourth leading cause of death worldwide[1]. It is known that COPD has a great impact on a patient's life. Symptoms frequently reported by patients with COPD are dyspnoea, wheezing, cough, and sputum production[2].

For many years, the concept chronic bronchitis (CB) has been investigated in patients with COPD. The proportion of patients reporting CB, defined as chronic productive cough, varies from 24.5%[3] to 82%[4]. Research concerning CB focused mainly on disease progression[3,4], with reduced forced expiratory volume in 1 second (FEV_1) and Tiffeneau index (ratio FEV_1/forced vital capacity [FVC]) as end points, in comparison to non-persistent sputum producers[3,4]. Furthermore, different studies observed an increase in the number of exacerbations[3,4] and hospitalizations[4], as well as a higher mortality risk[5]. Subsequently, a reduced quality of life[6] and functional exercise capacity[3] have been previously reported.

Not all patients have symptoms of productive cough; some have only increased mucus production. Mucus hypersecretion (MH) is characterized by an increase in mucin-producing cells, leading to increased mucus secretion[7]. CB and MH can simultaneously exist, although Caramori et al.[8] concluded that there was no link between symptoms of CB and mucus production. MH was related to FEV_1 decline, hospitalization[9], and exacerbation frequency[10]. Contrary results have been published concerning the association with mortality[11].

Although data are available on CB and MH, little is known about the association of spontaneous sputum production at any moment and health status and other clinical outcomes in patients with COPD. Especially, the potential impact of sputum microbiology on these outcomes might be clinically relevant because a substantial proportion of stable patients with COPD are colonized with bacteria[12], which is associated with increased daily symptoms[13] and exacerbation frequency[14]. Banerjee et al.[15] reported a worse health status in stable COPD patients with a positive sputum culture compared to patients without bacterial pathogens in sputum. These last data were limited because the focus of the research was different, and substantial information is lacking (e.g., no mean values, only *p*-values).

Therefore, the aim of the present study was to increase understanding of the impact of spontaneous sputum production on health status and clinical characteristics in patients with COPD, after stratification between those with and without positive sputum cultures.

MATERIALS AND METHODS

The current analyses were based on data collected as part of the COPD, health status, and comorbidities (Chance) study, an observational monocenter study[16]. The rationale and design of the study have been previously published[16]. Chance was approved on April 23, 2012 by the local ethics committee of the Maastricht University Medical Centre+, the Netherlands (MEC 11-3-070). The authors confirm that all on going and related trials are registered (NTR3416, registered at www.trialregister.nl). All patients gave written informed consent.

Study population

Stable patients with COPD referred for pulmonary rehabilitation (PR) to CIRO, a Center of Expertise for Chronic Organ Failure, were consecutively recruited between April 23, 2012 and September 24, 2014 and were included in the current analyses. Patients were classified as COPD based on post-bronchodilator FEV_1/FVC <0.7 according to the Global Initiative for Obstructive Lung Disease (GOLD)[1]. COPD Assessment Test (CAT) total score was used as symptom measure for the combined COPD assessment[1]. When CAT score was missing, the modified Medical Research Council score was used instead[1]. Stable was defined as not having a registered exacerbation in the past 4 weeks. Patients with a history of other respiratory diseases, having undergone lung surgery, or with a malignancy within the last 5 years were excluded from the study[17].

Study procedures

Patients were measured during an inpatient pre-rehabilitation assessment. Clinical characteristics were assessed as described before[16]. Exacerbation history and hospital and ICU admission were based on the medical report and patient recall. CB was defined as the presence of chronic cough and sputum production for at least 3 months a year, for two consecutive years[1]. Disease-specific health status was assessed by CAT[18], the Clinical COPD Questionnaire (CCQ)[19], and the COPD-specific version of the St George's Respiratory Questionnaire (SGRQ-C)[20]. CAT items on cough and phlegm were stratified based on the number of points reported by patients, with a cut off of ≥3 points. Besides, the following minimum clinically important differences (MCID) were taken into account: ≥2 points CAT[21], ≥0.4 points CCQ[22], and ≥4 points SGRQ-C[23]. Symptoms of anxiety and depression were measured using the Hospital Anxiety and Depression Scale (HADS)[24]. Exercise capacity was assessed by the 6-minute walking distance (6MWD)[25]. Moreover, laboratory data including inflammatory parameters and blood gas analysis were collected. All patients were asked to produce spontaneous sputum if possible. Sputum samples were cultured to obtain the microbial aetiology. Patients were categorized into two subgroups: non-sputum producers (NP) and sputum producers (SP), with the last group

stratified by culture result; SP with a negative culture (SC-) and SP with a positive culture (SC+).

Statistical analysis

SPSS version 20.0 (IBM Corporation, Armonk, NY, USA) was used for data analyses. Variables were tested for normality using the skewness and kurtosis statistics. Continuous data are presented as mean ± standard deviation or median (interquartile range), while categorical data are presented as counts and percentages. Subgroups were compared using one-way analysis of variance and post hoc Bonferroni test, Kruskal–Wallis test for *k*- and 2-independent samples, or chi-square test, as appropriate. A *p*-value <0.05 was considered to be statistically significant.

RESULTS

In total, 518 clinically stable patients with COPD were recruited (mean age 64.1±9.1 years, 55.6% males, FEV_1 48.6%±20.0% pred; Table S1). Of these, 164 patients (31.7%) produced a spontaneous sputum sample during their PR assessment. When comparing NP with SP, SP had a higher number of males, were more often classified as COPD GOLD D, were more frequent exacerbators, had more hospitalizations, and the prevalence of CB was higher (Table S1).

To see whether these differences between NP and SP could be explained by the sputum microbiology, Table 1 gives an overview of the baseline characteristics of NP, SC+, and SC-. A positive sputum culture was obtained in 53 patients (32.3%) with a sample. SC+ were more often males compared with NP, and more frequently classified as COPD GOLD D. No discrimination based on culture result could be made for the number of exacerbations in the year prior to assessment and symptoms of CB, although there was a trend toward increased exacerbations (p=0.058) and symptoms of CB (p=0.061) in SC+ vs. SC-. In patients with CB (n=167), 78 patients produced a spontaneous sputum sample (46.7%). No differences were observed in medication use between the defined subgroups (Table S2).

Table 1. Baseline characteristics of NP vs. SC- vs. SC+

	NP (n=354)	SC- (n=111)	SC+ (n=53)	*p*-value
Age (years)	63.8±9.1	64.3±9.2	65.5±8.9	0.466
Male sex	184 (52.0)	68 (61.3)	36 (67.9)[#]	0.037
BMI (kg/m^2)	26.1±5.8	26.2±5.4	27.0±6.4	0.622
COPD GOLD				<0.001
A	13 (3.7)	4 (3.6)	2 (3.8)	
B	162 (45.8)	36 (32.4)	8 (15.1)	
C	7 (2.0)	3 (2.7)	-	
D	172 (48.6)	68 (61.3)	43 (81.1)[#]	
Current smoker	72 (20.4)	32 (28.8)	10 (18.9)	0.362
Pack years*	40.0 (30.0-50.0)	40.0 (25.0-50.0)	41.0 (31.0-51.0)	0.350
FEV_1 (% predicted)	49.0±20.0	48.7±19.2	45.6±21.4	0.525
FEV_1/FVC (Tiffeneau)	37.9±12.1	36.8±11.8	36.5±13.8	0.579
RV (% predicted)	163.0±51.6	155.1±46.3	160.7±53.6	0.372
TLC (% predicted)	117.9±17.1	116.2±17.5	113.7±20.0	0.226
DLCO (% predicted)	49.5±17.5	50.2±16.9	46.2±15.8	0.391
LTOT	82 (23.2)	24 (21.6)	19 (35.8)	0.103
$PaCO_2$ (kPa)*	5.2 (4.7-5.6)	5.1 (4.7-5.6)	5.3 (4.9-5.9)	0.340
PaO_2 (kPa)*	9.6 (8.7-10.4)	9.3 (8.3-10.3)	9.4 (8.4-10.4)	0.397
Exacerbations (<1 year)				<0.001
0	101 (28.5)	23 (20.7)	4 (7.5)	
1	74 (20.9)	17 (15.3)	6 (11.3)	
≥ 2	179 (50.6)	71 (64.0)[#]	43 (81.1)[#]	
Hospitalizations (<1 year)				0.061
0	204 (57.6)	57 (51.4)	23 (43.4)	
1	87 (24.6)	23 (20.7)	14 (26.4)	
≥ 2	63 (17.8)	31 (27.9)	16 (30.2)	
ICU admissions (<1 year)[†]				0.597
0	312 (92.9)	100 (95.2)	51 (98.1)	
1	21 (6.3)	4 (3.8)	1 (1.9)	
≥ 2	3 (0.9)	1 (1.0)	-	
Symptoms of CB	89 (26.1)	46 (45.5)[#]	32 (61.5)[#]	<0.001
mMRC[‡]				0.825
0	7 (2.0)	3 (2.8)	-	
1	62 (17.7)	16 (14.7)	7 (13.5)	
2	134 (38.2)	42 (38.5)	17 (32.7)	
3	82 (23.4)	28 (25.7)	17 (32.7)	
4	66 (18.8)	20 (18.3)	11 (21.2)	

Notes: Data are presented as mean±SD, median (interquartile range), or n (%). [a] $p<0.05$ compared to NP; [b] not normally distributed; [c] missing NP: n=18; SC-: n=6; SC+: n=1; [d] missing NP: n=3; SC-: n=2; SC+: n=1.

Abbreviations: BMI, body mass index; COPD, Chronic Obstructive Pulmonary Disease; GOLD, Global initiative for chronic Obstructive Lung Disease; FEV_1, forced expiratory volume in 1 second; FVC, forced Vital capacity; RV, residual volume; TLC, total lung capacity; DLCO, diffusing capacity for carbon monoxide; LTOT, long-term oxygen therapy; CB, chronic bronchitis; mMRC, modified Medical Research Council; ICU, intensive care unit; SD, standard deviation; NP, non-sputum producers; SC+, sputum producers with a positive culture; SC-, sputum producers with a negative culture.

Health status, depression, and anxiety

Health status using CAT total, cough and phlegm score, SGRQ-C total and symptom score, and CCQ-total score were worse in SP compared to NP (Table S3), although the differences did not exceed the MCID for all total scores. The sputum microbiology was discriminating in CAT total (Figure 1) and CCQ total (Figure 1), with worse scores in SC+ compared with NP (23.9±6.1 vs. 21.1±6.7, p=0.012; 3.1±1.0 vs. 2.5±1.0, p=0.002; respectively). CAT cough and phlegm (Figure 1) and SGRQ-C symptom score (Figure 1) were increased in both SC+ and SC- compared with NP (Table S4). SGRQ-C total score was not significantly different anymore when taking the sputum microbiology into account, although the MCID was reached comparing SC+ with NP (65.4±16.1 vs. 60.0±17.0). Additionally, the MCID was reached for SGRQ-C impact comparing SC+ with NP (53.8±21.6 vs. 48.4±20.7). A significant difference between SC+ and SC- was observed in CAT phlegm (3.1±1.1 vs. 2.5±1.3, p=0.020) and a MCID in SGRQ-C symptom score (71.3±17.5 vs. 66.0±18.2; Table S4).

When stratifying CAT cough and phlegm items based on reported scores, a significantly larger proportion of SC+ and SC- reported scores ≥3 points compared with NP (38 [74.5%] vs. 66 [60.6%] vs. 162 [47%], p<0.001; 38 [74.5%] vs. 55 [50.5%] vs. 134 [38.8%], p<0.001; respectively).

Symptoms of depression by the HADS score (Figure 1F) were more often observed in SC+ compared to NP (8.7±4.1 vs. 7.2±4.3). When comparing groups based on the proportion of patients with an elevated score on health status or symptoms of anxiety and depression, no significant differences appeared (Table S5).

Exercise capacity

Exercise capacity, assessed using the 6MWD, did not differ between NP and SP (424.8±124.7 vs. 422.3±124.1 m).

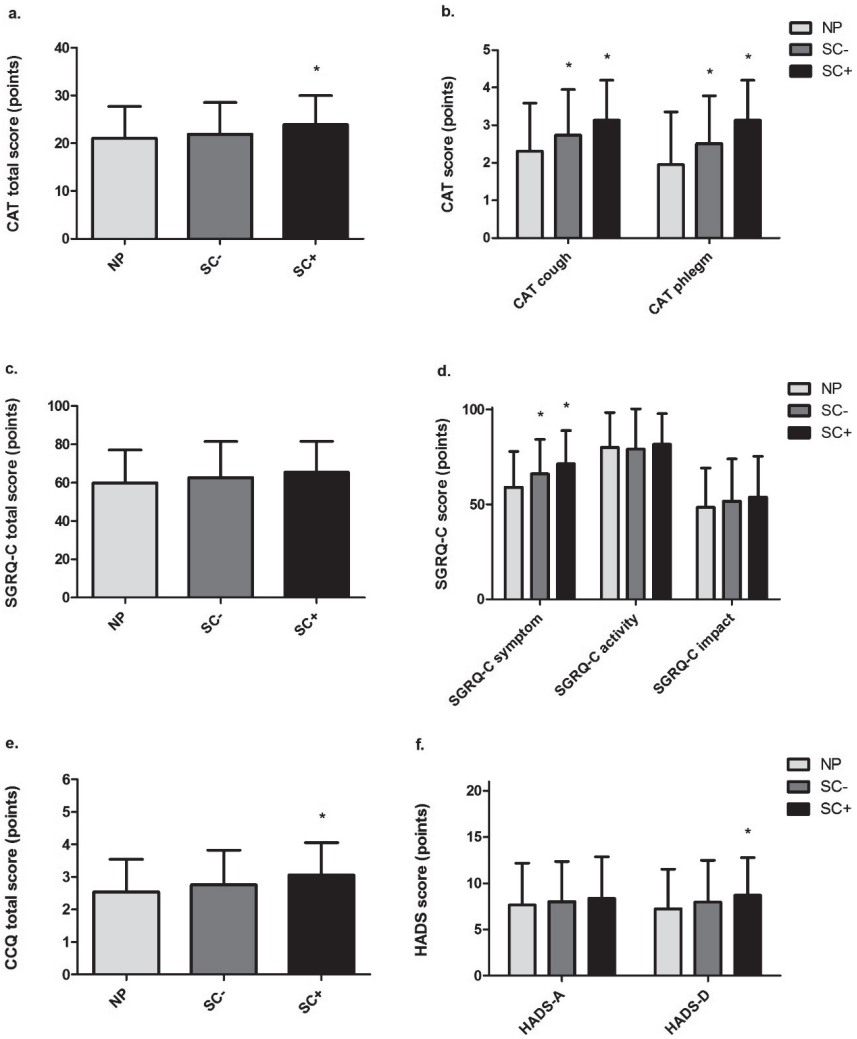

Figure 1. Health status of NP vs. SC- vs. SC+ by CAT total score (A), CAT cough and phlegm (B), SGRQ-C total score (C), SGRQ-C domain scores (D), CCQ total score (E), and HADS score (F).

Notes: Data are presented as mean±SD. One-way ANOVA and post hoc Bonferroni test. *p<0.05 compared to NP. **Abbreviations:** CAT, COPD Assessment Test; SGRQ-C, St. George's Respiratory Questionnaire; CCQ, Clinical COPD Questionnaire; HADS, Hospital Anxiety and Depression scale; SC-, sputum producers with a negative culture; SC+, sputum producers with a positive culture; SD, standard deviation; ANOVA, analysis of variance; NP, non-sputum producers.

Inflammatory parameters and microbial aetiology

Table 2 shows the inflammatory parameters, with no differences between NP and SP.

Table 2. Inflammatory parameters, by peripheral blood collection, of NP vs. SP

	NP (n=354)	SP (n=164)	*p*-value
CRP (mg/l)[a]	2.6 (0.9-7.0)	3.3.0 (1.1-8.0)	0.158
Erythrocytes (10^{E12}/l)	4.7±0.4	4.7±0.5	0.490
Leucocytes (10^{E9}/l)	8.0±2.3	8.3±2.5	0.153
Granulocytes (%)	60.7±9.6	59.5±10.3	0.198
Lymphocytes (%)	28.2±8.1	28.7±8.7	0.538
Monocytes (%)	8.6±3.0	9.0±3.0	0.119
Eosinophilic granulocytes (%)[a,b]	3.0 (2.0-4.0)	3.0 (2.0-5.0)	0.205
≤2%	86 (45.5)	35 (39.3)	0.332
>2%	103 (54.5)	54 (60.7)	

Notes: Data are presented as mean±SD, median (interquartile range), or n (%). [a] Not normally distributed; [b] n=278. **Abbreviations:** CRP, C-reactive protein; NP, non-sputum producers; SP, sputum producers; SD, standard deviation.

The frequency of the detected pathogens in the 53 patients with positive culture is presented in Figure 2. *Haemophilus influenzae* was most frequently present (39.6%), followed by *Pseudomonas aeruginosa* (28.3%). Coinfections existed, as a total of eleven patients were positive for more than one pathogen.

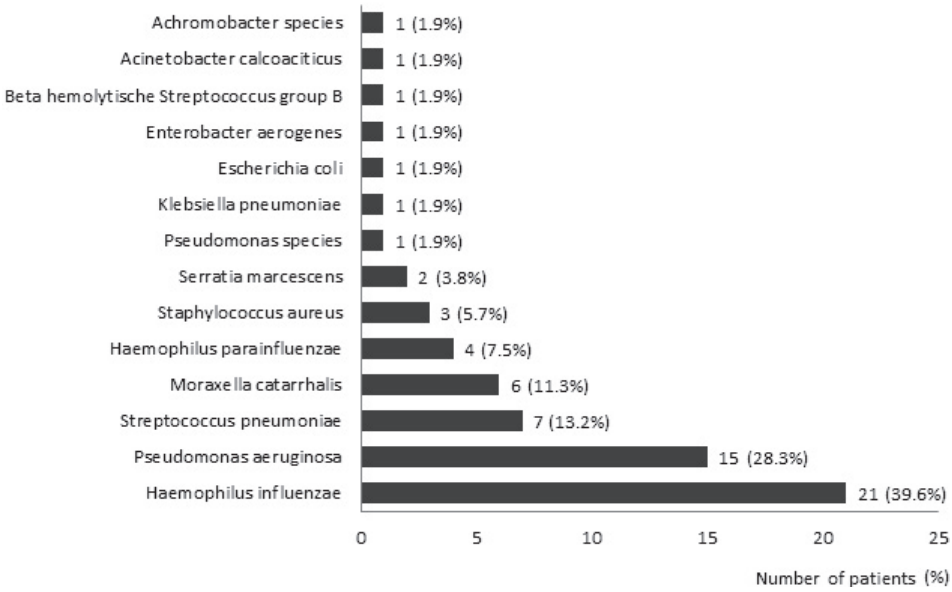

Figure 2. Microbial aetiology of sputum producers with a positive culture.

DISCUSSION

The results of this study can be summarized as follows. Almost one-third of patients with COPD referred for PR were identified as being spontaneous sputum producers. Also, it was shown that patients with COPD who had a sputum sample with a positive culture had worse health status compared with NP. Moreover, these patients had a higher exacerbation frequency and more symptoms of CB. Thus, not the presence of spontaneous sputum production per se, but its microbiological characterization influences outcomes in these patients.

Frequency of spontaneous sputum production

Spontaneous sputum production at any moment was very common in the present study and was associated with increased CAT cough and phlegm scores and an increased frequency of CB. Previously, Putcha et al.[5] observed that sputum alone, or in combination with cough, was reported by 43.1% of 5,887 patients with mild-to-moderate airflow obstruction in the Lung Health Study. A study focusing on symptom variability in 2,441 stable patients with severe COPD showed that sputum was the second most experienced symptom after breathlessness (63.6%)[2]. Although around 30% of these patients reported a low frequency of sputum production in the past 7 days, ~20% reported moderate and ~10% severe or extreme production[2]. Taking these proportions into consideration, it must be concluded that sputum production is common in patients with COPD, highlighting the potential importance of this specific clinical profile.

Impact on outcomes

Health status is an important outcome in the management of COPD. Until now, studies showed that health status is worse in patients with symptoms of CB[3,6,26], mostly based on SGRQ scores. The results support the finding that health status is worse in patients with sputum production, especially in those with a positive culture (CAT total and CCQ total), even when compared with patients with a negative culture (CAT phlegm and SGRQ-C symptom). So, it seems that sputum production influences the symptom domains, while the sputum microbiology influences health status in general. Within the subgroup of patients with CB, however, health status was not affected by sputum culture. This suggests that sputum microbiology may be clinically relevant, especially in COPD patients with incidental sputum production. Although SGRQ-C total score and the domain score impact were not statistically significantly different, the difference observed between patients with a positive sputum culture and those without sputum production exceeded the MCID.

Limited data are available concerning symptoms of anxiety and depression in relation to CB. Corhay et al.[4] reported no difference in symptoms of depression comparing patients with and without CB. The current data showed no difference in anxiety scores, although a significantly higher depression score was observed in patients with a positive sputum culture. Whether treatment of CB can lead to a decrease in symptoms of depression is not known and need further research.

It can be argued whether sputum production affects exercise capacity, as no differences were observed in 6MWD, as well as in SGRQ-C activity. Kim et al.[6] made similar observations in patients with CB. However, this is not supported by others, who observed a reduced 6MWD in patients with CB compared to patients without[3,26]. In the population of this study, sputum production did not seem to affect exercise capacity at baseline, but it would be interesting to see whether sputum production affects long-term exercise capacity. Moreover, the effect of sputum production on physical activities in daily life would be clinically relevant to investigate.

Remarkably, no differences in inflammatory parameters of blood samples were observed between NP and patients with sputum production. Although all patients were in a clinically stable state at time of assessment, a positive sputum culture may indicate microbial colonization. Indeed, worse outcomes were previously described in colonized patients with COPD concerning exacerbation frequency and daily symptoms, when compared with non-colonized patients[13,14]. Although the current study design does not allow to firmly establish colonization, it suggests that sputum microbiology, instead of sputum production per se, affects health status.

The observed difference in frequency of sputum production between sexes might be explained by sex-related differences in reporting respiratory symptoms. Lamprecht et al.[27] observed that females more often reported respiratory symptoms such as dyspnoea and cough compared to males. In contrast, males more often reported sputum production[27]. This in accordance with other studies that observed the same differences between the sexes regarding the presence of CB[3,6].

Microbiological characterization

The sputum microbiology confirms that *H. influenzae* and *P. aeruginosa* are pathogens frequently cultured in patients with COPD[28]. Especially, *H. influenzae* is observed in both stable state and during exacerbations[29]. *P. aeruginosa* is more often found in patients with most severely impaired lung function[30]. The effect of a specific aetiology on the outcomes cannot be studied as the subgroups would become too small.

Strengths and limitations

The current study was based on a rather homogenous population of clinically stable patients with mild-to-severe COPD referred for PR. Therefore, differences in health status between subgroups are not expected to be associated with COPD-specific characteristics. Subgroups had comparable baseline characteristics concerning their level of airflow obstruction, smoking status, and hospital admissions.

Until now, most analyses[3,4,6] were based on patients who reported CB. However, it is difficult to assess this phenotype objectively as this definition relies on patients' perception of the symptoms and might be subject to recall bias. Studies that used sputum samples are few in number and are mainly focused on inflammatory parameters[26]. Moreover, no study differentiated patients with sputum samples based on their microbiological culture result. This is, to the best of our knowledge, the first study investigating differences between subgroups of patients with and without sputum production and with and without positive culture. Therefore, the current study increased the understanding of this specific subgroup of patients.

However, some aspects need to be taken into consideration regarding the current study. Although characterized as a patient with CB, more than half of them could not produce sputum during their 3 days' pre-rehabilitation assessment. No sputum induction was performed as part of the current study, so only patients with a spontaneous sample were included in the producer groups. Besides, SC+ were often classified as GOLD D and were frequent exacerbators, which could act as bias. However, it is known that patients with frequent exacerbations are more often colonized, which explains the present outcomes[14]. Moreover, no high-resolution computed tomography was performed to identify bronchiectasis, which is associated with increased sputum production[31].

Conclusion

In conclusion, health status is worse in patients with COPD who spontaneously produce sputum, especially when having a positive culture. Exercise capacity does not seem to be affected by sputum production. Further research is necessary to investigate the influence of sputum production and microbiology in the long term, especially when looking at positive cultures in relation to disease progression and outcomes of PR. Insight into the clinical profile of this subgroup of patients may contribute to specific recommendations, as not sputum per se seems to influence clinical outcomes, but having a positive culture.

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of the total study population and NP vs. SP

	All (n=518)	NP (n=354)	SP (n=164)	*p*-value
Age (years)	64.1±9.1	63.8±9.1	64.7±9.1	0.321
Male sex	288 (55.6)	184 (52.0)	104 (63.4)	0.015
BMI (kg/m^2)	26.2±5.8	26.1±5.8	26.5±5.7	0.527
COPD GOLD				0.001
A	19 (3.7)	13 (3.7)	6 (3.7)	
B	206 (39.8)	162 (45.8)	44 (26.8)	
C	10 (1.9)	7 (2.0)	3 (1.8)	
D	283 (54.6)	172 (48.6)	111 (67.7)	
Current smoker	114 (22.1)	72 (20.4)	42 (25.6)	0.369
Pack years[a]	40.0 (30.0-50.0)	40.0 (30.0-50.0)	40.0 (28.0-50.0)	0.803
FEV_1 (% predicted)	48.6±20.0	49.0±20.0	47.7±19.9	0.518
FEV_1/FVC (Tiffeneau)	37.5±12.2	37.9±12.1	36.7±12.4	0.300
RV (% predicted)	161.0±50.7	163.0±51.6	156.9±48.6	0.210
TLC (% predicted)	117.1±17.5	117.9±17.1	115.4±18.3	0.133
DLCO (% predicted)	49.3±17.2	49.5±17.5	48.9±16.6	0.713
LTOT	125 (24.1)	82 (23.2)	43 (26.2)	0.450
$PaCO_2$ (kPa)[a]	5.2 (4.7-5.6)	5.2 (4.7-5.6)	5.2 (4.8-5.7)	0.276
PaO_2 (kPa)[a]	9.5 (8.6-10.4)	9.6 (8.7-10.4)	9.3 (8.4-10.3)	0.176
Exacerbations (<1 year)				<0.001
0	128 (24.7)	101 (28.5)	27 (16.5)	
1	97 (18.7)	74 (20.9)	23 (14.0)	
≥ 2	293 (56.6)	179 (50.6)	114 (69.5)	
Hospitalizations (<1 year)				0.018
0	284 (54.8)	204 (57.6)	80 (48.8)	
1	124 (23.9)	87 (24.6)	37 (22.6)	
≥ 2	110 (21.2)	63 (17.8)	47 (28.7)	
ICU admissions (<1 year)[b]				0.347
0	463 (93.9)	312 (92.9)	151 (96.2)	
1	26 (5.3)	21 (6.3)	5 (3.2)	
≥ 2	4 (0.8)	3 (0.9)	1 (0.6)	
Symptoms of CB	167 (33.8)	89 (26.1)	78 (51.0)	<0.001
mMRC[c]				0.775
0	10 (2.0)	7 (2.0)	3 (1.9)	
1	85 (16.6)	62 (17.7)	23 (14.3)	
2	193 (37.7)	134 (38.2)	59 (36.6)	
3	127 (24.8)	82 (23.4)	45 (28.0)	
4	97 (18.9)	66 (18.8)	31 (19.3)	

Notes: Data are presented as mean±SD, median (interquartile range), or n (%). [a] Not normally distributed; [b] missing n= 25 (NP n=18; SP n=7); [c] missing n=6 (NP n=3; SP n=3). *p*<0.05 NP vs. SP. **Abbreviations:** BMI, Body Mass Index; COPD, Chronic Obstructive Pulmonary Disease; GOLD, Global initiative for chronic Obstructive Lung Disease; FEV_1, Forced Expiratory Volume in 1 sec.; FVC, Forced Vital Capacity; RV, residual volume; TLC, total lung capacity; DLCO, diffusing capacity for carbon monoxide; LTOT, long term oxygen therapy; mMRC, modified Medical Research Council; NP, non-sputum producers; SP, sputum producers.

Table S2. Medication use of NP vs. SC- vs. SC+

	NP (n=354)	SC- (n=111)	SC+ (n=53)	*p*-value
SABA	164 (46.3)	41 (36.9)	24 (45.3)	0.218
SAMA	54 (15.3)	15 (13.5)	13 (24.5)	0.170
SABA/SAMA	67 (18.9)	31 (27.9)	11 (20.8)	0.127
LABA	94 (26.6)	28 (25.2)	9 (17.0)	0.327
LAMA	247 (69.8)	84 (75.7)	38 (71.7)	0.486
ICS	69 (19.5)	23 (20.7)	11 (20.8)	0.947
ICS/LABA	238 (67.2)	77 (69.4)	39 (73.6)	0.628
Oral corticosteroids	62 (17.5)	19 (17.1)	13 (24.5)	0.443
Antibiotics	39 (11.0)	6 (5.4)	9 (17.0)	0.062

Notes: Data are presented as n (%). **Abbreviations:** SABA, short-acting beta-agonist; SAMA, short-acting muscarinic antagonist; LABA, long-acting beta-agonist; LAMA, long-acting muscarinic antagonist; ICS, inhaled corticosteroids; NP, non-sputum producers; SC-, sputum producers with a negative culture; SC+, sputum producers with a positive culture.

Table S3. Health status and symptoms of anxiety and depression of NP vs. SP

	NP (n=354)[a]	SP (n=164)[b]	*p*-value
CAT total	21.1±6.7	22.6±6.5	0.020
CAT cough	2.3±1.3	2.9±1.2	<0.001
CAT phlegm	2.0±1.4	2.7±1.2	<0.001
SGRQ-C total	60.0±17.0	63.5±18.0	0.036
SGRQ-C symptom	59.0±18.9	67.7±18.1	<0.001
SGRQ-C impact	48.4±20.7	52.3±22.0	0.055
SGRQ-C activity	80.0±18.4	79.9±19.8	0.929
CCQ total	2.5±1.0	2.9±1.0	0.001
HADS-A	7.6±4.5	8.1±4.4	0.260
HADS-D	7.2±4.3	8.2±4.4	0.022

Notes: Data are presented as mean±SD. [a] Missing CAT n=9; SGRQ-C n=9; CCQ n=9; HADS n=10; [b] missing CAT n=4; SGRQ-C n=5; CCQ n=7; HADS n=8. **Abbreviations:** CAT, COPD Assessment Test; SGRQ-C, St. George's Respiratory Questionnaire; CCQ, Clinical COPD Questionnaire; HADS, Hospital Anxiety and Depression scale; SD, standard deviation; NP, non-sputum producers; SC-, sputum producers with a negative culture; SC+, sputum producers with a positive culture.

Table S4. Health status and symptoms of anxiety and depression of NP vs. SC- vs. SC+

	NP (n=354)[a]	SC- (n=111)[b]	SC+ (n=53)[c]	*p*-value
CAT total	21.1±6.7	21.9±6.6	23.9±6.1[d]	0.013
CAT cough	2.3±1.3	2.7±1.2[d]	3.1±1.1[d]	<0.001
CAT phlegm	2.0±1.4	2.5±1.3[d]	3.1±1.1[d,e]	<0.001
SGRQ-C total	60.0±17.0	62.6±18.8	65.4±16.1	0.070
SGRQ-C symptom	59.0±18.9	66.0±18.2[d]	71.3±17.5[d]	<0.001
SGRQ-C impact	48.4±20.7	51.6±22.3	53.8±21.6	0.133
SGRQ-C activity	80.0±18.4	79.1±21.3	81.6±16.2	0.723
CCQ total	2.5±1.0	2.8±1.1	3.1±1.0[d]	0.001
HADS-A	7.6±4.5	8.0±4.3	8.3±4.5	0.489
HADS-D	7.2±4.3	8.0±4.5	8.7±4.1[d]	0.046

Notes: Data are presented as mean±SD. [a] Missing CAT n=9; SGRQ-C n=9; CCQ n=9; HADS n=10; [b] missing CAT n=2; SGRQ-C n=2; CCQ n=4; HADS n=5; [c] missing CAT n=2; SGRQ-C n=3; CCQ n=3; HADS n=3; [d] $p<0.05$ compared to NP; [e] $p<0.05$ compared to SC-. **Abbreviations:** CAT, COPD Assessment Test; SGRQ-C, St. George's Respiratory Questionnaire; CCQ, Clinical COPD Questionnaire; HADS, Hospital Anxiety and Depression scale; SD, standard deviation; NP, non-sputum producers; SC-, sputum producers with a negative culture; SC+, sputum producers with a positive culture.

Table S5. Number of patients with elevated scores on health status and symptoms of anxiety and depression

	All (n=518)	NP (n=354)	SC- (n=111)	SC+ (n=53)	*p*-value
CAT (≥10)	477 (94.5)	326 (94.5)	102 (93.6)	49 (96.1)	0.812
SGRQ-C (≥25)	489 (97.0)	334 (96.8)	105 (96.3)	50 (100)	0.413
CCQ (≥1)	477 (95.0)	327 (94.8)	101 (94.4)	49 (98.0)	0.586
HADS-A (≥8)	235 (47.0)	154 (44.8)	57 (53.8)	24 (48.0)	0.264
HADS-D (≥8)	238 (47.6)	156 (45.3)	53 (50.0)	29 (58.0)	0.211

Notes: Data are presented as n (%). Missing CAT n=13; SGRQ-C n=14; CCQ n=16; HADS n=18. **Abbreviations:** CAT, COPD Assessment Test; SGRQ-C, St. George's Respiratory Questionnaire; CCQ, Clinical COPD Questionnaire; HADS, Hospital Anxiety and Depression scale; NP, non-sputum producer; SC-, sputum producers with a negative culture; SC+, sputum producers with a positive culture.

6

Impact of exacerbations on adherence and outcomes of pulmonary rehabilitation in patients with COPD

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ABSTRACT

Background and objective: Dropout or lack of response is an important issue in pulmonary rehabilitation (PR), which underlines the need to identify predictors of dropout and response. Acute exacerbations (AEs) of COPD may influence dropout rates and PR response. We aimed to assess differences in dropout and outcomes of PR between COPD with and without AEs.

Methods: Clinically stable patients with moderate-to-very severe COPD (age 64.1±9.1 years, 55.6% males, forced expiratory volume in 1s (FEV_1) 48.6±20.0% predicted) were assessed during PR (inpatient and outpatient). Mild-to-moderate AEs were defined as 'the prescription of systemic glucocorticosteroids and/or antibiotics, following an acute increase in respiratory symptoms'. Severe AEs were defined as 'a hospital admission due to an AE'. Health status was measured by COPD Assessment Test (CAT), COPD-specific version of the St. George's Respiratory Questionnaire (SGRQ-C) and Clinical COPD Questionnaire (CCQ). Symptoms of anxiety and depression were measured by Hospital Anxiety and Depression Scale (HADS). Exercise capacity was measured with the 6-min walking test (6MWT) and constant work rate test (CWRT).

Results: A total of 518 patients were assessed during a pre-rehabilitation assessment. Four hundred and seventy-six patients started PR, of whom 419 (88.0%) completed it. A larger proportion of patients who dropped out had a severe AE during PR (20.0% vs. 3.5%, $p<0.001$). Completers with severe AE showed a deterioration in 6MWT, while completers without AE and with mild-to-moderate AE improved (−24.8 (95% CI: −94.0 to 44.5) vs. 24.2 (95% CI: 16.0 to 32.5) vs. 25.1 (95% CI: 14.0 to 36.3) metres, $p=0.042$). No other significant differences were observed in outcomes comparing completers with and without AE during PR.

Conclusion: Mild-to-moderate AEs do not affect dropout or response of PR, although severe AEs are associated with dropout. AEs should not lead to discontinuation of PR, as response is in general not affected.

INTRODUCTION

COPD is a major health problem and a leading cause of morbidity and mortality. Pulmonary rehabilitation (PR) is targeted at reducing symptoms, improving health status (HS) and increasing physical activity in persistently symptomatic patients[1]. Beneficial effects of PR have been described consistently[2], although a subset of patients does not respond to PR[3,4]. Besides, 10–32% of patients do not complete PR[5].

Identification of factors associated with dropout and response of PR is of broad interest, whilst there is still limited data available. Dropout for medical reasons is frequently reported, mainly due to acute exacerbations (AEs)[5,6]. AEs are common features of the disease; in general, 1.53 episodes per patient per year have been reported (1.20/year for mild-to-moderate AEs; 0.33/year for severe AEs)[7]. Although AEs are recognized as an important factor of dropout in PR, research concerning the impact of AEs on dropout and outcomes is limited. Steele et al.[8] investigated the impact of mild-to-moderate AEs during PR on dropout and PR outcomes, observing that almost one-third of dropout was due to AEs. No differences were observed between completers with and without AEs in outcomes of PR, except for change in 6-min walk distance, with AE patients having a larger improvement following PR compared with patients without AEs[8].

Although former research[8] did not look at severe AEs, AEs resulting in hospitalization might play a major role in dropout and response. Besides, research is needed in larger samples to clarify the role of AEs in dropout and outcomes of PR, whilst it is expected that AEs, especially severe AEs, have a negative impact on completion and outcomes of PR. Therefore, we aimed to assess the impact of both mild-to-moderate as well as severe AEs on dropout and outcomes of PR.

METHODS

Data were derived from the COPD, Health Status and Co-morbidities (Chance) Study, a longitudinal observational single-centre study[9]. The rationale and design of the study were published before[9], as well as data concerning the responsiveness and minimum clinically important difference (MCID) estimates for COPD Assessment Test (CAT), Clinical COPD Questionnaire (CCQ) and Hospital Anxiety and Depression Scale (HADS) in patients with COPD undergoing PR[10]. The Chance study was approved by the local ethics committee of Maastricht University Medical Centre+, The Netherlands (MEC11-3-070/trialregister.nl NTR3416). All patients gave written informed consent.

Study population

Stable COPD patients referred for PR to CIRO, a centre of expertise for patients with chronic organ failure in Horn, The Netherlands, were recruited between April 2012 and September 2014. Patients were classified as COPD based on post-bronchodilator forced expiratory volume in 1s (FEV_1)/forced vital capacity (FVC) <0.7 according to the Global Initiative for Obstructive Lung Disease (GOLD)[11]. Stable was defined as not having a registered AE 4 weeks before pre-rehabilitation assessment. Patients with a history of other respiratory diseases, having undergone lung surgery or with a malignancy within the last 5 years were excluded[9].

Study procedures

Patients were screened and included during an inpatient pre-rehabilitation assessment. Patients eligible for PR followed an inpatient rehabilitation programme of 8 weeks or an outpatient rehabilitation programme of 16 weeks[4], both 40 sessions. A multidisciplinary team evaluated patients during the pre-rehabilitation assessment, in combination with the severity of the disease and co-morbid conditions, as well as the access to facilities nearby, and made a decision for inpatient or outpatient rehabilitation. After PR, patients were evaluated at end-assessment.

Clinical characteristics were assessed as described before[9]. Disease-specific HS was assessed by CAT[12], COPD-specific version of the St. George's Respiratory Questionnaire (SGRQ-C)[13] and CCQ[14]. MCIDs were taken into account: ≥2 points CAT[15], ≥4 points SGRQ-C[16] and ≥0.4 points CCQ[15]. Symptoms of anxiety and depression were measured with HADS[17], MCID ≥1.5 points[18]. Exercise capacity was assessed by 6-min walking test (6MWT)[19], MCID ≥30 m[20], and constant work rate test (CWRT)[21], MCID ≥100 s.[22] Patients performed the CWRT at 75% of the predetermined peak work rate assessed at pre-rehabilitation assessment, for a maximum of 20 min.

Mild-to-moderate AEs were defined as 'prescription of systemic glucocorticosteroids and/or antibiotics following acute increase in respiratory symptoms'[23]. Severe AEs were defined as 'admission to a hospital due to AE for (non)invasive ventilatory support and/or parenteral administration of medications'. When patients had both mild-to-moderate and severe AE, patients were classified as having severe AE. AEs in the year prior to PR were assessed based on patient recall, following the same definitions.

Patients were categorized into three subgroups: not started (not started PR), dropouts (dropped out PR) and completers (completed PR).

Statistical analysis

SPSS version 22.0 (IBM Corp. Armonk, NY, USA) was used for data analyses. Continuous variables were tested for normality by skewness and kurtosis, presented as mean±SD or median (interquartile range), as appropriate. Categorical data are presented as counts (%). Mean change was expressed as mean (95% CI). Subgroups were compared by one-way analysis of variance (ANOVA) and post hoc Bonferroni test, Kruskal-Wallis-test or chi-square test, as appropriate. PR response was analysed by paired samples t-test. A *p*-value of <0.05 was considered to be statistically significant, with analyses corrected for multiple comparisons by Bonferroni.

RESULTS

A total of 518 stable COPD patients were assessed during pre-rehabilitation assessment and included into the study (Figure 1) (mean age: 64.1±9.1 years, 55.6% male, mean FEV_1: 48.6±20.0% predicted, most classified as COPD GOLD B or D (Table S1, Supplemental material)).

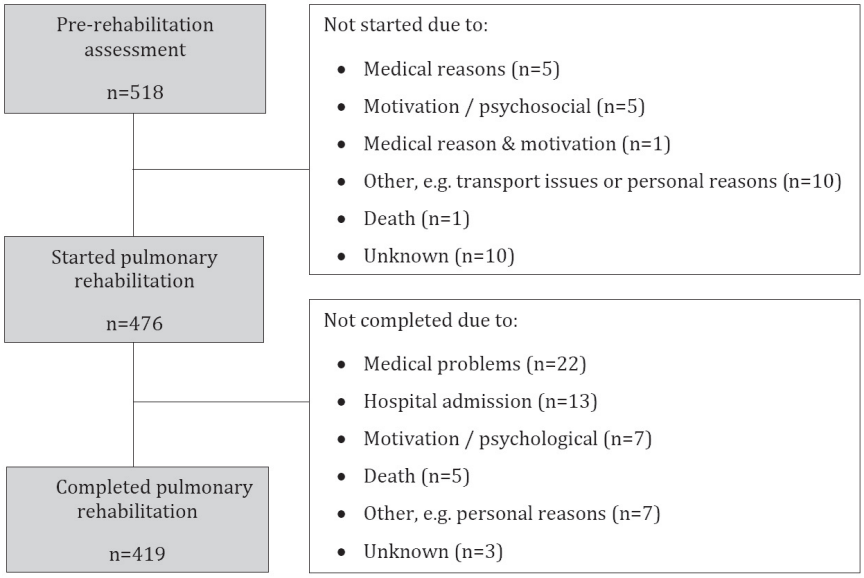

Figure 1. Flow diagram of the study time points.

Notes: Reasons for dropout due to medical problems: musculoskeletal problems n = 5, 22.7%; cardiovascular problems n = 1, 4.5%; planned operation (i.e. rectum and cancer) n = 2, 9.1%; cancer diagnosis n = 1, 4.5%; physical problems not further specified n = 2, 9.1%; disease/medical problems not further specified n = 11, 50.0%.

Dropout

Forty-two patients (8.1%) underwent baseline assessment, but did not start PR due to various reasons (Figure 1). Consequently, no post-rehabilitation data were available. Of those who started PR, 419 patients (88.0%) completed PR. Main reasons to dropout were medical problems (n = 22, 38.6%) and hospital admissions (n = 13, 22.8%; Figure 1).

Table 1 presents baseline characteristics of patients who completed (n = 419), dropped out (n = 57) and not started PR (n = 42). No significant differences were observed between subgroups, only a trend towards more current smokers in dropouts (n = 21, 36.8%) and patients not started (n = 14, 34.1%), compared with completers (n = 79, 18.9%).

Subgroups did not differ in the proportion of patients with one or more AEs in the year prior to pre-rehabilitation assessment, regardless AE severity (Table 2). However, dropouts had more often severe AE during PR compared with completers (n = 8, 20.0% vs. n = 14, 3.5%, respectively), while the other way around for mild-to-moderate AE (n = 6, 15.0% vs. n = 154, 38.4%, respectively; $p < 0.001$). The setting of PR did not influence dropout.

Table 1. Baseline characteristics of completers, dropouts and not started patients

	Completers (n = 419)	Dropouts (n = 57)	Not started (n = 42)
Age (years)	64.3±8.8	64.7±9.7	61.3±10.8
Male gender (%)	232 (55.4)	34 (59.6)	22 (52.4)
BMI (kg/m^2)	26.2±5.7	26.7±6.6	25.5±5.8
COPD GOLD (%)			
A	15 (3.6)	1 (1.8)	3 (7.1)
B	174 (41.5)	17 (29.8)	15 (35.7)
C	8 (1.9)	-	2 (4.8)
D	222 (53.0)	39 (68.4)	22 (52.4)
Current smoker (%)	79 (18.9)	21 (36.8)	14 (34.1)
Pack years† (number)	40.0 (30.0–50.0)	40.0 (35.0–52.5)	39.0 (26.3–50.0)
FEV_1 (% predicted)	48.9±20.0	46.2±20.1	48.9±20.3
FEV_1/FVC (%)	37.3±12.1	38.2±13.8	38.7±11.7
Charlson co-morbidity index (%)			
1	257 (61.3)	27 (47.4)	25 (59.5)
2	98 (23.4)	15 (26.3)	13 (31.0)
3	45 (10.7)	10 (17.5)	3 (7.1)
4	13 (3.1)	3 (5.3)	1 (2.4)
5	4 (1.0)	-	-
6	2 (0.5)	1 (1.8)	-
10	-	1 (1.8)	-

Notes: Data are presented as mean±SD, median (interquartile range) or n (%). Corrected for multiple comparisons by Bonferroni correction, $p<0.002$. †Not normally distributed. **Abbreviations:** BMI, body mass index; COPD, Chronic Obstructive Pulmonary Disease; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Obstructive Lung Disease; SD, standard deviation.

Table 2. Mild-to-moderate and severe exacerbations in the year prior to pre-rehabilitation assessment and during PR of completers, dropouts and not started patients

	Completers (n = 419)	Dropouts (n = 57)	Not started (n = 42)	*p*-value
AE 1 year prior to PR				
0	96 (22.9)	10 (17.5)	7 (16.7)	Ns.
≥1 mild-to-moderate	144 (34.4)	13 (22.8)	14 (33.3)	
≥1 severe	179 (42.7)	34 (59.6)	21 (50.0)	
AE during PR				
0	233 (58.1)	26 (65.0)	0	<0.001
≥1 mild-to-moderate	154 (38.4)	6 (15.0)	0	
≥1 severe	14 (3.5)	8 (20.0)		
Setting of PR				
Inpatient	261 (62.3)	40 (70.2)	0	Ns.
Outpatient	158 (37.7)	17 (29.8)	0	

Notes: Data are presented as n (%). Corrected for multiple comparisons by Bonferroni correction, $p<0.006$. Data on exacerbations during PR were missing for 17 dropouts. For 18 completers, it was known they had at least 1 exacerbation during PR, but not the exact amount and severity. **Abbreviations:** AE, acute exacerbation; PR, pulmonary rehabilitation.

Response to PR

Health status

Table 3 displays HS scores of completers at pre-rehabilitation assessment, end-assessment and change in scores between pre-rehabilitation and end-assessment. On average, HS improved in completers following PR (ΔCAT: −3.0 (95% CI−3.7 to −2.4) points; ΔSGRQ-C: −9.1 (95% CI: −10.5 to −7.7) points; ΔCCQ: −0.6 (95% CI: −0.7 to −0.5) points). This improvement was statistically significant and clinically relevant, as for all questionnaires the mean improvement exceeded the MCID (Table 3). When comparing inpatient and outpatient PR completers (Table S2, Supplemental material), outpatients had a significantly better HS, less symptoms of anxiety and depression and a significantly better performance on the 6MWT and CWRT at pre-rehabilitation assessment. On average, this difference was exceeding the MCID for all outcomes, except for the CWRT. Response to PR was significantly lower in outpatients compared with inpatients on HS scores, symptoms of anxiety and depression and 6MWT.

Table 3. Health status, symptoms of anxiety and depression and exercise capacity of completers

	Pre-rehabilitation†	End-assessment‡	Δ	*p*-value
CAT total score	21.5±6.6	18.5±6.9	−3.0 (−3.7 to −2.4)	<0.001
SGRQ-C total score	60.1±17.1	51.0±17.4	−9.1 (−10.5 to −7.7)	<0.001
SGRQ-C symptom score	61.2±19.0	54.5±19.0	−6.6 (−8.4 to −4.7)	<0.001
SGRQ-C activity score	79.2±19.0	72.2±21.0	−7.2 (−8.8 to −5.5)	<0.001
SGRQ-C impact score	48.4±20.4	37.1±20.9	−11.2 (−13.0 to −9.4)	<0.001
CCQ total score	2.6±1.0	2.0±1.0	−0.6 (−0.7 to −0.5)	<0.001
HADS-A score	7.5±4.4	5.8±4.2	−1.7 (−2.1 to −1.3)	<0.001
HADS-D score	7.4±4.2	5.3±3.9	−2.1 (−2.5 to −1.8)	<0.001
6MWT (m)	431.1±123.7	457.4±122.1	22.9 (16.4 to 29.5)	<0.001
CWRT (s)	234.5 (174.25–338.0)	376.5 (210.3–708.5)	206.4 (175.5–237.3)	<0.001

Notes: Data of pre-rehabilitation and end-assessment are presented as mean±SD or median (interquartile range). Change is presented as mean (95% CI). Corrected for multiple comparisons by Bonferroni correction, $p < 0.005$.

†Missing: CAT n = 9; SGRQ-C n = 10; CCQ n = 10; HADS n = 12; 6MWT n = 2; CWRT n = 27.

‡Missing: CAT n = 23; SGRQ-C n = 26; CCQ n = 34; HADS n = 38; 6MWT n = 16; CWRT n = 37.

Abbreviations: 6MWT, 6-min walking test; CAT, COPD Assessment Test; CCQ, Clinical COPD Questionnaire; CI, confidence interval; CWRT, constant work rate test; HADS, Hospital Anxiety and Depression Scale; SD, standard deviation; SGRQ-C, COPD-specific version of the St. George's Respiratory Questionnaire.

Completers with mild-to-moderate and severe AE during PR had a mean improvement of at least the MCID on all HS scores, except for CCQ in patients with severe AE (Table 4). Mean change in HS between completers with AE, regardless of severity, and completers without AE, was not statistically different or clinically relevant. The proportion of patients exceeding the MCID for CAT and SGRQ did not differ between completers with and without AE (Figure 2). The proportion of patients exceeding the MCID of CCQ was significantly lower in completers with severe AE (n = 3, 23.1%) than completers without AE (n = 125, 59.8%, $p = 0.009$) and completers with mild-to-moderate AE (n = 77, 56.2%, $p=0.022$; Figure 2).

Table 4. Change in health status, symptoms of anxiety and depression and exercise capacity of completers with and without AE during PR

	Completers without AE[†] (n = 233)	Completers with mild-to-moderate AE[‡] (n = 154)	Completers with severe AE[§] (n = 14)	*p*-value
Δ CAT total score	−3.4 (−4.2 to −2.5)	−2.9 (−4.0 to −1.8)	−2.2 (−4.9 to 0.6)	Ns.
Δ SGRQ-C total score	−9.9 (−11.9 to −7.8)	−8.5 (−10.6 to −6.5)	−4.5 (−11.4 to 2.5)	Ns.
Δ SGRQ-C symptom score	−7.8 (−10.4 to −5.2)	−5.4 (−8.2 to −2.5)	−4.0 (−12.6 to 4.6)	Ns.
Δ SGRQ-C activity score	−7.9 (−10.3 to −5.5)	−6.1 (−8.6 to −3.5)	−2.3 (−8.3 to 3.8)	Ns.
Δ SGRQ-C impact score	−11.8 (−14.3 to −9.3)	−11.1 (−13.9 to −8.2)	−5.8 (−16.1 to 4.4)	Ns.
Δ CCQ total score	−0.7 (−0.8 to −0.5)	−0.6 (−0.7 to −0.4)	−0.1 (−0.4 to 0.3)	Ns.
Δ HADS-A score	−1.7 (−2.2 to −1.2)	−1.7 (−2.3 to −1.1)	−0.6 (−2.4 to 1.2)	Ns.
Δ HADS-D score	−2.1 (−2.7 to −1.6)	−2.0 (−2.6 to −1.4)	−2.0 (−4.1 to 0.1)	Ns.
Δ 6MWT (m)	24.2 (16.0 to 32.5)	25.1 (14.0 to 36.3)	−24.8 (−94.0 to 44.5)	0.042[¶]
Δ CWRT (s)	229.2(186.2 to 272.1)	167.3 (120.7 to 213.9)	174.8 (−91.0 to 440.6)	Ns.

Notes: Change is presented as mean (95% CI). Corrected for multiple comparisons by Bonferroni correction.
[†]Missing: CAT n = 18; SGRQ-C n = 20; CCQ n = 24; HADS n = 26; 6MWT n = 6; CWRT n = 16.
[‡]Missing: CAT n = 12; SGRQ-C n = 14; CCQ n = 18; HADS n = 20; 6MWT n = 9; CWRT n = 19.
[§]Missing: CAT n = 1; SGRQ-C n = 1; CCQ n = 1; HADS n = 1; 6MWT n = 2; CWRT n = 4.
[¶]Completers with severe AE versus completers without AE $p = 0.041$; completers with severe AE versus completers with mild-to-moderate AE $p = 0.040$.
Abbreviations: 6MWT, 6-min walking test; AE, acute exacerbation; CAT, COPD Assessment Test; CCQ, Clinical COPD Questionnaire; CI, confidence interval; CWRT, constant work rate test; HADS, Hospital Anxiety and Depression Scale; PR, pulmonary rehabilitation; SGRQ-C, COPD specific version of the St. George's Respiratory Questionnaire.

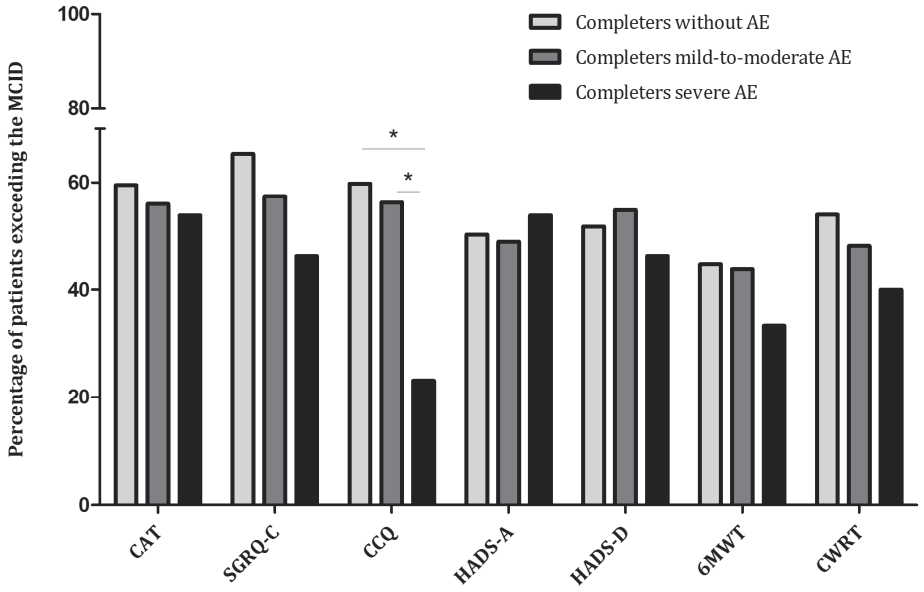

Figure 2. Percentage of patients exceeding the minimum clinically important difference (MCID) stratified by acute exacerbation (AE) severity.

Notes: *p=0.009; p=0.022. COPD Assessment Test (CAT): n = 128 completers without AE (59.5%), n = 80 completers with mild-to-moderate AE (55.9%), n = 7 completers with severe AE (53.8%). COPD-specific version of the St. George's Respiratory Questionnaire (SGRQ-C): n = 139 completers without AE (65.3%), n = 81 completers with mild-to-moderate AE (57.4%), n = 6 completers with severe AE (46.2%). Clinical COPD Questionnaire (CCQ): n = 125 completers without AE (59.8%), n = 77 completers with mild-to-moderate AE (56.2%), n = 3 completers with severe AE (23.1%). Hospital Anxiety and Depression Scale (HADS)-A: n = 104 completers without AE (50.2%), n = 66 completers with mild-to-moderate AE (48.9%), n = 7 completers with severe AE (53.8%). HADS-D: n = 107 completers without AE (51.7%), n = 74 completers with mild-to-moderate AE (54.8%), n = 6 completers with severe AE (46.2%). 6-Min walk test (6MWT): n = 102 completers without AE (44.7%), n = 64 completers with mild-to-moderate AE (43.8%), n = 4 completers with severe AE (33.3%). Constant work rate test (CWRT): n = 117 completers without AE (53.9%), n = 65 completers with mild-to-moderate AE (48.1%), n = 4 completers with severe AE (40.0%). ▭, completers without AE; ▬, completers with mild-to-moderate AE; ■, completers with severe AE.

Serum markers and clinical parameters

HADS scores on average decreased in completers following PR (HADS-A Δ: −1.7 (95% CI: −2.1 to −1.3) points and HADS-D Δ: −2.1 (95% CI: −2.5 to −1.8) points, respectively; Table 3), exceeding the MCID.

No significant difference was observed in mean change of HADS when comparing completers with and without mild-to-moderate or severe AE (Table 4). The mean improvement of anxiety symptoms in completers with severe AE did not exceed the MCID (Δ: −0.6 (95% CI: −2.4 to 1.2) points). There was no significant difference in the proportion of completers exceeding the MCID comparing completers with and without AE

regardless of severity (Figure 2; HADS-A n = 104 (50.2%), n = 66 (48.9%), n = 7 (53.8%), *p*>0.05; HADS-D n = 107 (51.7%), n = 74 (54.8%), n = 6 (46.2%), *p*>0.05, respectively).

Exercise capacity

On average, completers significantly increased the distance walked at 6MWT and seconds performed on CWRT, comparing pre-rehabilitation with end-assessment (Δ: 22.9 (95% CI: 16.4–29.5) metres; Δ: 206.4 (95% CI: 175.5–237.3) seconds, respectively; Table 3). However, this mean change was not clinically relevant for 6MWT.

Completers with severe AE on average decreased on 6MWT (Δ: −24.8 (95% CI: −94.0 to 44.5) metres), while completers without and with a mild-to-moderate AE showed a mean improvement (Δ: 24.2 (95% CI: 16.0 to 32.5) metres; Δ: 25.1 (95% CI: 14.0 to 36.3) metres, respectively; Table 4). For CWRT, all subgroups showed a mean improvement, exceeding the MCID (Table 4), observing no significant difference in the proportion of completers with and without AE exceeding the MCID (Figure 2).

DISCUSSION

The current findings show that mild-to-moderate exacerbations do not affect dropout and response of PR, although severe exacerbations are associated with increased dropout. However, when completing PR after a severe exacerbation, patients generally respond positively, although less pronounced. These results emphasize that disease instability during PR should not discourage patients and healthcare professionals from PR continuation.

Around 8.0% of the study population did not start PR, a smaller number than observed by others[5,24]. Of the patients who started, 12.0% dropped out, in between the range (9.7–31.8%) as described by Keating et al.[5] Reasons of dropout in our population were mainly medical problems and hospital admissions. These reasons are, besides motivational and psychological factors[6], known factors accounting for a substantial proportion of dropouts[6,24]. Nevertheless, the present study observed no association between mild-to-moderate exacerbations prior to and during PR, and dropout. The same for severe exacerbations prior to PR. However, the proportion of patients with severe exacerbations during PR was significantly larger in dropouts compared with completers. This difference might even be larger as we consider that dropouts completed less sessions. Besides, data on exacerbations were missing for 17 dropouts. However, it is expected that these patients had no exacerbation, as assessment of exacerbations is a standard procedure during PR. So, exacerbations in the year prior to PR do not influence dropout, although severe exacerbations during PR are associated with dropout. These results are in line with Steele et al.[8]

Smoking status has been related to dropout[3,24,25]. Smoking status or pack-years was not different between subgroups, although there was a trend towards more current smokers in not started patients and dropouts, compared with completers. Some clinicians have doubts of including smokers in PR. However, smoking cessation is an important aspect in COPD management, and often offered during PR[1]. On the other hand, smoking is related to skeletal muscle dysfunction, affecting the oxygen delivery and the ability of the mitochondria to use oxygen[26]. This might have negative consequences on PR outcomes, although comparable outcomes have been reported in smokers and non-smokers[25,27]. Based on the latter, current smokers should not be withheld from PR.

In general, patients made progress following PR, with a mean improvement exceeding the MCID for HS, HADS and CWRT. These results are not surprising, as the benefits of PR have been described frequently[2]. Moreover, these results confirm that especially patients who are limited and/or having recent and frequent exacerbations are admitted to PR. The setting of PR appeared to influence the outcomes. However, a lower response in outpatients was expected, as these patients had less room for improvement following the significantly better HS and better performance on exercise capacity at pre-rehabilitation assessment.

Completers with AE during PR, regardless of severity, performed comparable on CAT, SGRQ-C, HADS and CWRT as completers without AE. This was also seen in the percentage of patients exceeding the MCID for these measures. These results are in line with Steele et al.[8] Probably, patients are monitored closely when following PR, resulting in fast and adequate treatment[28].

Conversely, mean change in distance walked at 6MWT significantly differed between completers with severe AE and completers without or with mild-to-moderate AE. Completers with severe AE on average deteriorated on the 6MWT, while others showed a mean improvement. On the other hand, the amount of patients exceeding the MCID of the 6MWT was comparable between completers with and without AE, regardless of severity. The amount of patients exceeding the MCID of CCQ was significantly lower in completers with severe AE than in completers without or with mild-to-moderate AE. Exacerbations, especially those resulting in hospitalizations, are known to have a detrimental impact on patients' outcomes, partly supporting these findings[29,30].

What is now the consequence for clinical practice following the current results? As mentioned before, severe exacerbations are associated with dropout. However, the current analysis and former research showed beneficial effects of PR in these patients, if they are able to restart PR[31,32]. Given these data, we believe that patients who are hospitalized for an exacerbation during PR, should be motivated to return and complete PR. Probably, the programme should be adjusted, depending on the specific patient, for example extension of programme duration, providing a less or more intensive programme. Besides, continuing rehabilitation during the admission is recommended[31].

Neuromuscular electrical stimulation (NMES) is a feasible and effective intervention to prevent skeletal muscle function deterioration in patients with severe exacerbations[33]. Early start of NMES during admission, and continuation during PR are probably good options to prevent deterioration after initial gain during PR. Troosters et al.[31] showed that resistance training applied during an admission has favourable effects on quadriceps muscle force. Another favourable effect of both interventions might be that patients stay motivated to finish their initial PR. So, in-hospital rehabilitation during severe exacerbations may be an effective bridge between hospitalization and PR continuation. Furthermore, facilitating care for acute respiratory failure in an inpatient setting might be a way to avoid hospitalizations and offer the possibility to continue PR during exacerbations. Training with non-invasive ventilation is promising, and might also be beneficial in patients with severe exacerbations, but well-designed studies are needed[34].

Furthermore, it is important to offer 'strategies' to prevent hospitalizations. Besides optimal medication treatment, a possible 'strategy' can be education of exacerbation management. Self-management has found to be effective in reducing emergency department visits and hospital admissions[35], by teaching patients to monitor signs and symptoms, and provide them with action plans and skills to improve health-enhancing behaviours. Eventually, patients will be his own expert in identifying and managing exacerbations[36].

There are some methodological considerations that should be taken into account. The study included a population of well-characterized moderate-to-very severe COPD patients. A subset of outcome measures was studied independently, while Spruit et al.[4] suggests a multidimensional response measure, allowing more insight into the multidimensional effects of exacerbations on PR. Besides, data on exacerbations were underreported or missing for a small proportion of patients, especially from patients rehabilitating in an outpatient setting. We expect that this small proportion of lacking data did not influence results. Additionally, exact data on the amount of completed session were not available, but would be interesting to study. This also in light of programme extension, and the effect on outcomes. Furthermore, it would be interesting to follow-up dropouts to study the amount of exacerbations over the same time period. Overall, precaution is necessary when interpreting the results, as the subgroup of severe exacerbators is quite small. Further research in a larger cohort is recommended.

In conclusion, while mild-to-moderate AEs do not affect dropout or outcomes of PR, severe exacerbations are associated with increased dropout. However, AEs should not lead to discontinuation of PR, as response is generally not affected.

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SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of the total population

	All (n=518)
Age (years)	64.1±9.1
Male gender	288 (55.6)
BMI (kg/m^2)	26.2±5.8
COPD GOLD	
A	19 (3.7)
B	206 (39.8)
C	10 (1.9)
D	283 (54.6)
Current smoker	114 (22.1)
Pack years*	40.0 (30.0-50.0)
FEV_1 (% pred.)	48.6±20.0
FEV_1/FVC	37.5±12.2
Charlson comorbidity index	
1	309 (59.7)
2	126 (24.3)
3	58 (11.2)
4	17 (3.3)
5	4 (0.8)
6	3 (0.6)
10	1 (0.2)

Notes: Data are presented as mean±SD, median (interquartile range) or N (%).*Not normally distributed. **Abbreviations**: BMI, Body Mass Index; COPD, Chronic Obstructive Pulmonary Disease; GOLD, Global initiative for chronic Obstructive Lung Disease; FEV1, Forced Expiratory Volume in 1 sec.; FVC, Forced Vital Capacity.

Table S2. Health status, symptoms of anxiety and depression and exercise capacity of inpatient completers versus outpatient completers

	Pre-rehabilitation			Δ Pre-rehabilitation – end-assessment		
	Inpatient	Outpatient	*p*-value	Inpatient	Outpatient	*p*-value
CAT total score	23.3±6.0	18.7±6.5	<0.001	-3.8 (-4.7— -2.9)	-1.7 (-2.7— -0.9)	0.002
SGRQ-C total score	66.7±13.5	49.5±16.9	<0.001	-11.0 (-12.7— -9.3)	-6.1 (-8.5— -3.7)	0.001
SGRQ-C symptom score	66.0±16.6	53.6±20.1	<0.001	-8.4 (-10.8— -6.1)	-3.7 (-6.7— -0.7)	ns
SGRQ-C activity score	85.7±14.6	68.7±20.5	<0.001	-8.5 (-10.3— -6.6)	-5.1 (-8.3— -1.9)	ns
SGRQ-C impact score	55.7±17.5	36.6±19.4	<0.001	-13.4 (-15.7— -11.1)	-7.6 (-10.4— -4.8)	0.002
CCQ total score	3.0±0.9	2.0±0.9	<0.001	-0.8 (-1.0— -0.7)	-0.2 (-0.35— -0.1)	<0.001
HADS-A score	8.35±4.4	6.2±3.9	<0.001	-2.1 (-2.6— -1.6)	-1.1 (-1.6— -0.6)	0.005
HADS-D score	8.4±4.0	5.8±4.0	<0.001	-2.6 (-3.1— -2.1)	-1.3 (-1.9— -0.8)	<0.001
6MWT (m)	388.2±120.2	502.2±93.2	<0.001	36.5 (27.5—45.5)	0.7 (-7.3—8.7)	<0.001
CWRT (s)	216.0 (162.0-304.0)	285.0 (193.5-390.5)	<0.001	196.4 (158.6—234.1)	221.8 (168.5—275.0)	ns

Notes: Data of pre-rehabilitation are presented as mean±SD or median (interquartile range). Change is presented as mean (95%CI). Corrected for multiple comparisons by Bonferroni correction, $p<0.005$. **Abbreviations:** CAT, COPD Assessment Test; SGRQ-C, COPD specific version of the St. George's Respiratory Questionnaire; CCQ, Clinical COPD Questionnaire; HADS, Hospital Anxiety and Depression Scale; 6MWT, 6-minute walking test; CWRT, Constant Work Rate Test.

7

Validation of respiratory specimens for respiratory microbiome analysis in patients with COPD by the IS-pro technology

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Submitted

ABSTRACT

Background: Different types of specimens have been used to assess the respiratory microbiome in COPD. However, no consensus on suitable specimens to use in clinical practice has been reached, while the use of the respiratory microbiome as diagnostic tool is of great interest.

Objective: To determine the patient-specific and specimen-specific signatures of the respiratory microbiome, using the IS-pro technology, in specimens from different anatomical locations in the respiratory tract, in order to asses similarities/differences between the different sampling locations as well as between different patients.

Methods: 11 patients with mild-to-moderate COPD (mean age 72.3±8.0 years, 81.8% males, forced expiratory volume in 1 second 74.5±17.9 %pred.) were assessed. A throat swab, spontaneous sputum sample, bronchial aspirate and bronchoalveolar lavage (BAL) were collected. Specimens were analysed by the IS-pro technology, a 16S-23S rDNA interspace(IS)-region-based profiling method. Alpha-diversity was measured to assess the diversity within a specific specimen. Beta-diversity was measured to assess similarities between IS-profiles of the defined specimens within and between patients.

Results: BAL specimens had a significantly lower alpha-diversity than sputum and throat (2.9±0.5 vs. 3.4±0.4 vs. 3.6±0.3, respectively; $p<0.05$). A principal coordinate analysis revealed a segregation of upper and lower respiratory specimens, while sputum and throat had a significantly lower beta-diversity compared to BAL and bronchial aspirate ($p<0.05$). Interestingly, we observed a higher level of microbiome similarity within different respiratory specimens of an individual (0.4±0.2), than within a particular type of respiratory specimen between individuals (0.5±0.1, $p<0.001$).

Conclusion: Both, the alpha and beta diversity differ between different anatomical locations of the respiratory tract. However, the respiratory microbiome contains a patient-specific signature in all respiratory specimens, which is more pronounced than the specimen-specific signature.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of death and morbidity worldwide[1]. Patients with COPD are prone to exacerbations most often triggered by respiratory infections, and their airways are often colonised with microorganisms[2]. Up to now, laboratory analysis of bacterial pathogens in respiratory specimens was based on culture-dependent techniques. However this excludes unculturable micro-organisms which form a large proportion of the respiratory microbiome[3]. Shifts in this microbiome have already been associated with disease; it was shown that Rhinovirus infection in COPD patients was associated with increased bacterial load and a change in microbiome composition[4]. Moreover, differences in the respiratory microbiome in relation to disease severity have been observed, as well as changes in the respiratory microbiome during COPD exacerbations[5-7]. However, it is still unclear whether a core microbiome exists across individuals, certain disease (pheno)types, or within single individuals. In addition, the possible influence of the clinical specimen, thus the anatomical location, is largely unknown. The use of the respiratory microbiome in clinical practice is of increasing interest, and could develop into an important diagnostic tool, although comprehensive evidence in this field is still largely lacking.

The IS-pro technology seems to be suitable for a clinical laboratory in analysing the respiratory microbiome and microbial shifts based on a predefined algorithm. In order to use this technique in clinical practice, it is important to define suitable types of specimens. Until now, different sampling techniques have been used to assess the respiratory microbiome: (induced) sputum[8], bronchial aspirate[8], bronchoalveolar lavage (BAL)[8,9], bronchial mucosa[8] and surgical specimens from lung transplants[9]. Specimens of the upper respiratory tract are expected to be contaminated with oropharyngeal flora, and to be composed of a different respiratory microbiome than specimens of the lower respiratory tract[8]. On the other hand, these specimens have the advantage of being non-invasive which allows longitudinal follow-up[10,11]. For clinical practice, it is important to have patient friendly techniques providing sufficient information for adequate management. Therefore, we aimed to determine the patient-specific and specimen-specific signatures of the respiratory microbiome, using the IS-pro technology, in specimens from different anatomical locations in the respiratory tract, in order to asses similarities or differences between the different sampling methods as well as between different patients.

MATERIALS AND M.ETHODS

The Maastricht Respiratory Microbiome Study (MRMS-study) is a study performed at the Maastricht University Medical Centre (MUMC+), Maastricht, the Netherlands. The study was approved by the medical ethical committee of Maastricht (MEC141117), and registered at www.trialregister.nl (NTR5369). All patients gave written informed consent before study entry.

Study population

COPD patients with all grades of severity could be included in the MRMS-study. They were eligible to participate if they had a respiratory physician-based diagnosis of COPD, as defined by the Global initiative for chronic Obstructive Lung Disease (GOLD)[12], and were planned to undergo a bronchoscopy for a non-infectious indication. Patients with chronic use of oral corticosteroids >10mg/day were excluded.

Study procedures

Patients were screened and included into the study at the outpatient clinic for respiratory medicine of the Maastricht University Medical Centre, the Netherlands. Patient characteristics were collected including demographic variables, Charlson comorbidity index[13], clinical data (e.g. vaccination status and saturation) and smoking status. Furthermore, information regarding the number of acute exacerbations (AEs) during the last year, as well as the amount of hospitalisations due to AEs in the last year were recorded (patient recall). Disease-specific health status was assessed with the COPD Assessment Test (CAT)[14] and the level of dyspnoea according to the modified Medical Research Council (mMRC)[15]. The most recent lung function assessment was recorded (including post-bronchodilator forced expiratory volume in 1 second (FEV_1) and forced vital capacity (FVC)).

All patients were planned to undergo bronchoscopy. Before bronchoscopy, a throat swab (BD ESwab 220245, Sparks USA) and a spontaneous sputum sample were collected. During bronchoscopy, first a bronchial aspirate was collected, followed by a BAL sample. Bronchoscopy was performed by an experienced lung physician. Both, bronchial aspirate and BAL were taken at the medial segment of the middle lobe (10cc NaCl 0.9%; 50cc NaCl 0.9%; respectively). If the primary abnormality was located at the middle lobe, specimens were collected at the lingula instead. Only patients who had all different types of specimens collected, were included in the current analysis.

IS-pro procedure

The throat swab was collected in standard transport medium. Upon arrival in the lab the swab was mixed during 5', then the swab was discarded and the remaining

transport medium was directly stored at -80°C. Bronchial aspirates and BAL were mixed with sputolysin (0.1xtotal volume) till homogeneity and immediately stored at -80°C. Sputum samples were diluted with sterile PBS (1:1), sputolysin (0.1xtotal volume (sputum+PBS)) was added and homogenized, and immediately stored at -80°C. For DNA isolation: 100 µl of throat swab medium, 250 µl of bronchial aspirate or BAL solution and 200 µl of sputum solution was used. DNA isolation of all specimens was performed by routine DNA isolation in the MagNA Pure 96 automated DNA isolation instrument using the MagNA Pure MP 96 DNA and viral NA Large volume kit following manufacturer's instructions (Roche Molecular Diagnostics ltd.). The IS-pro technology was used on a phylum specific profiling level with a standardized protocol, as previously described by Budding *et al.*[16] for the phyla Firmicutes, Actinobacteria, Fusobacteria, Verrucomicrobia (FAFV), Bacteriodetes and Proteobacteria following standard manufacturer's recommendations (IS-Diagnostics, Amsterdam, The Netherlands). In brief, DNA was amplified on a thermal cycler T3000 (Biometra GmbH, Göttingen, Germany) using fluorescently labelled phylum-specific primers, after which the PCR products were further analysed on a ABI Prism 3500 Genetic Analyzer (Applied Biosystems Carlsbad, California, USA). All data were pre-processed with the IS-pro proprietary software suite (IS-Diagnostics, Amsterdam, The Netherlands) and were subsequently visualized with the Spotfire software package (TIBCO, Palo Alto, CA, USA). Hierarchical clustering of IS-profiles, visualized in a heat map Plot, was performed using the Unweighted Pair Group Method *with* Arithmetic Mean (*UPGMA*).

Statistical analysis

Baseline characteristics of patients were assessed with SPSS version 23.0. Continuous variables were tested for normality by Skewness and Kurtosis, presented as mean±standard deviation or median (interquartile range), as appropriate. Categorical data are presented as counts (%). Statistical analyses were performed on the detected fragments as determined by the proprietary software developed for IS-pro analysis. One-way ANOVA and post-hoc Tukey HSD test were performed to assess the alpha-diversity between types of specimens, using the Shannon wiener index. The alpha-diversity measures the diversity within a specific specimen, with other words, how many different types of sequences are present in one specimen. A principal coordinate analysis (PCoA) was used to explore variation and patterns in the microbiome composition between the types of specimens. The beta-diversity was analysed using cosine distance. It is a term for the comparison of types of specimens to each other, and provides a measure of the distance or dissimilarity between types of specimens. To assess similarities between IS-profiles of the defined specimens within and between patients, a hierarchical clustering heat map plot was performed. Further statistical analyses were performed using two-sided student t-test, additional correction was applied using Bonferroni in the analysis of within- and between-groups using QIIME 1.8.0.

RESULTS

In total, 11 patients with mild-to-moderate COPD were included into the current analysis. Clinical characteristics are presented in Table 1. Patients were on average 72 years old, half were former and half current smokers, and nearly half of the patients were classified as COPD GOLD IIB. Disease-specific health status, assessed by the CAT, was on average 14.4 points, indicating a moderate impact of respiratory symptoms. The level of dyspnoea measured by mMRC was low in most patients.

Table 1. Baseline characteristics

	COPD patients (n=11)
Age (years)	72.3±8.0
Male gender	9 (81.8)
BMI (kg/m^2)	24.2 (23.3-27.4)
Smoking status	
Former	5 (45.5)
Current	6 (54.5)
Pack years	33.8 (21.6-60.0)
Years stop smoking	9.0 (1.6-25.0)
Vaccination status	
Influenza	7 (63.6)
Pneumococcal	1 (9.1)
Charlson comorbidity index	3.1±2.0
FEV_1 %pred.	74.5±17.9
FEV_1/FVC	52.6±15.0
COPD assessment	
IA	3 (27.3)
IB	2 (18.2)
IIB	5 (45.5)
IIC	1 (9.1)
Number of patients with	
AE in last 3 months	4 (36.4)
AE in last year	5 (45.5)
Hospitalisation due to AE in last year	1 (9.1)
CAT total score	14.4±7.9
mMRC score	
0	3 (27.3)
1	5 (45.5)
2	1 (9.1)
3	1 (9.1)
4	1 (9.1)

Notes: data are presented as mean±SD, median (interquartile range) or n (%). **Abbreviations:** AE, acute exacerbation; BMI, body mass index; CAT, COPD Assessment Test; COPD, Chronic Obstructive Pulmonary Disease; FEV_1, Forced Expiratory Volume in 1 second; FVC, Forced Vital Capacity; mMRC, modified Medical Research Council.

FAFV are most abundant in respiratory specimens

In total, 44 microbiome profiles from the respiratory samples of 11 patients were generated by IS-pro. At the phylum level, 49% of the detected bacteria species belonged to the FAFV, whereas the Bacteriodetes comprised 26% and the Proteobacteria 25% of the bacteria found amongst all respiratory isolates. The two most abundant IS-fragments, with a fragment size of 321 and 297 bp (both belonging to the group of FAFV), were found in respectively 66 and 61% of all clinical specimens. The three most abundant fragments (third fragment with a size of 289 bp) all belonged to bacteria of the family of Streptococcaceae and were identified in respectively 9, 11 and 10 out of the 11 patients.

Alpha-diversity

The alpha-diversity, as assessed by the one-way ANOVA, showed a statistically significant effect of specimens of all phyla (Figure 1; p=0.005), with 3 degrees of freedom for the error term and an F statistic of 5.0. As the corresponding p-value of the F statistic is lower than 0.05, this suggests that one or more specimens are significantly different with regard to their respiratory microbiome composition. Post-hoc analyses (Tukey HSD test, 40 degrees of freedom for the error term) showed that BAL and throat specimens (2.9±0.5 vs. 3.6±0.3, p=0.004), as well as BAL and sputum specimens (2.9±0.5 vs. 3.4±0.4, p=0.044) significantly differed from each other. On the phylum level, there were no statistical significant differences in alpha-diversity between Bacteriodetes and FAFV. However, the one-way ANOVA showed a statistically significant difference amongst the Proteobacteria in clinical specimens (p<0.001). Further post-hoc Tukey HSD analyses showed that only bronchial aspirate compared to sputum (1.5±0.7 vs. 2.1±0.4), and sputum compared to throat (2.1±0.4 vs. 2.5±0.4) did not show any significant difference. All other combinations were significantly different, in which bronchial aspirate and BAL had a p-value of <0.05 (1.5±0.7 vs. 0.6±0.8) and all other combinations had a p-value of <0.01.

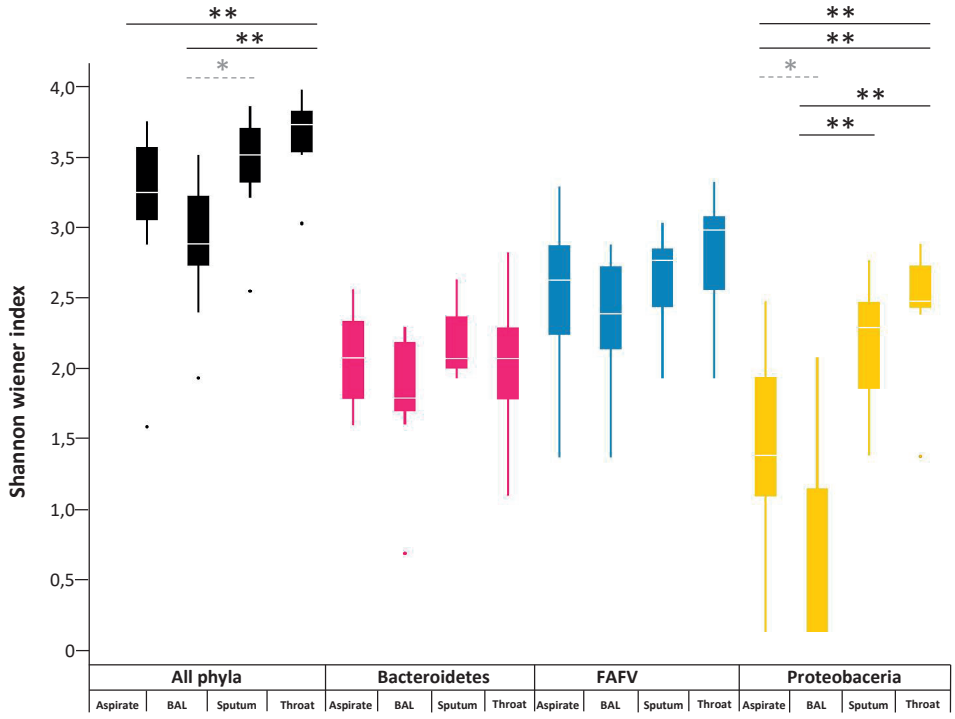

Figure 1. Shannon wiener index of all phyla, Bacteriodetes, FAFV and Proteobacteria, stratified by respiratory specimen.

Notes: ** $p<0.001$; * $p<0.05$.

Beta-diversity

A PCoA analysis showed that, in general, the most distal respiratory tract specimens (BAL and bronchial aspirate) clustered apart from the more proximal respiratory tract specimens (sputum and throat swabs; Figure 2). This indicates that the beta-diversity is different between the more proximal and more distal respiratory tract specimens.

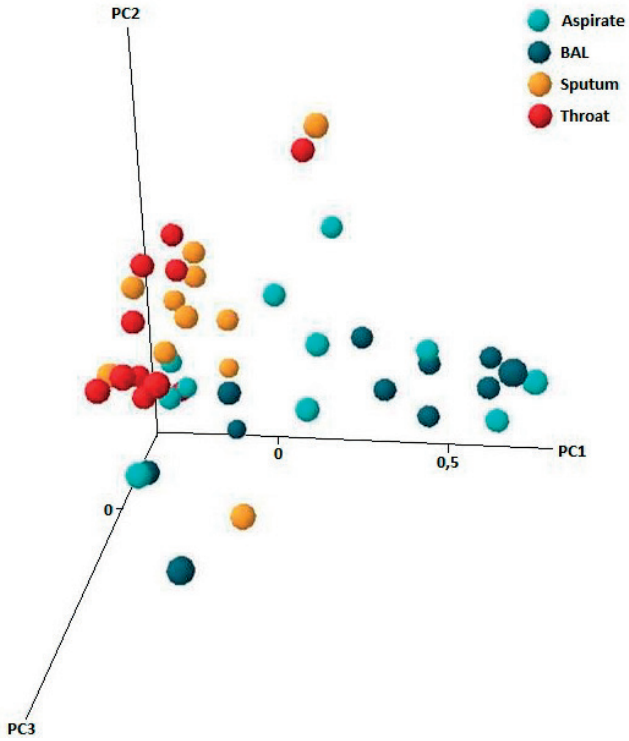

Figure 2. Principal Coordinate Analysis

In order to further examine the differences between the four respiratory specimens, the beta-diversity distances of each of the types of specimens were calculated and plotted (Figure 3). As expected, throat specimens were most homogeneous followed by sputum, bronchial aspirate and BAL specimens. Samples from the same location (within-types of specimens) were significantly more similar to each other, than samples from different locations (between-types of specimen; p=0.001; Figure 3A). Independent analysis of the different types of specimens showed that throat differed significantly from BAL (p=0.003) and bronchial aspirate (p<0.001) but not from sputum (p=0.210), whereas sputum in itself was also significantly different from BAL (p=0.047) and bronchial aspirate (p=0.009; Figure 3B). This confirmed the finding of the PCoA analysis that showed that the lower respiratory tract specimens (BAL and bronchial aspirate) were significantly different from those of the relatively higher respiratory tract specimens (sputum and throat swabs).

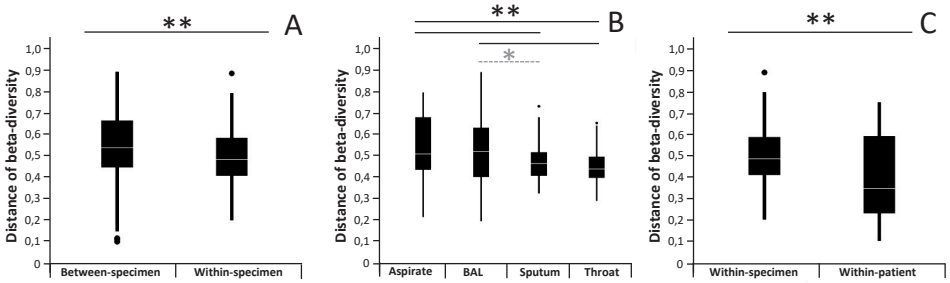

Figure 3. Distance of beta-diversity

Notes: A. Between-specimen vs. Within-specimen; B. Bronchial aspirate vs. BAL vs. Sputum vs. Throat; C. Within-specimen vs. Within-patient. ** $p<0.001$; * $p<0.05$.

Patient specific clustering

The hierarchical clustering heat map plot revealed a patient-specific clustering (Figure 4). As expected, the upper (sputum and throat) and lower (BAL and bronchial aspirate) respiratory tract specimens also clustered. This finding was confirmed on the raw data set, indicating the strength of this finding.

In three out of the eleven patients, all types of respiratory specimens clustered together (Figure 4), whereas there were three patients in which 75% of the types of specimens clustered and five patients in which only two types of specimens clustered. Sputum and throat specimens clustered in 100% of the patients. To further analyse whether this patients-specific signature is more important than the specimen-specific signature that we identified earlier, we performed a student t-test between the within-types of specimens and within-patients beta-diversity distances (Figure 3C). Interestingly, the similarity of specimens within individual patients (0.4±0.2) was significantly higher from that of the specific types of specimens between patients (0.5±0.1; $p<0.001$).

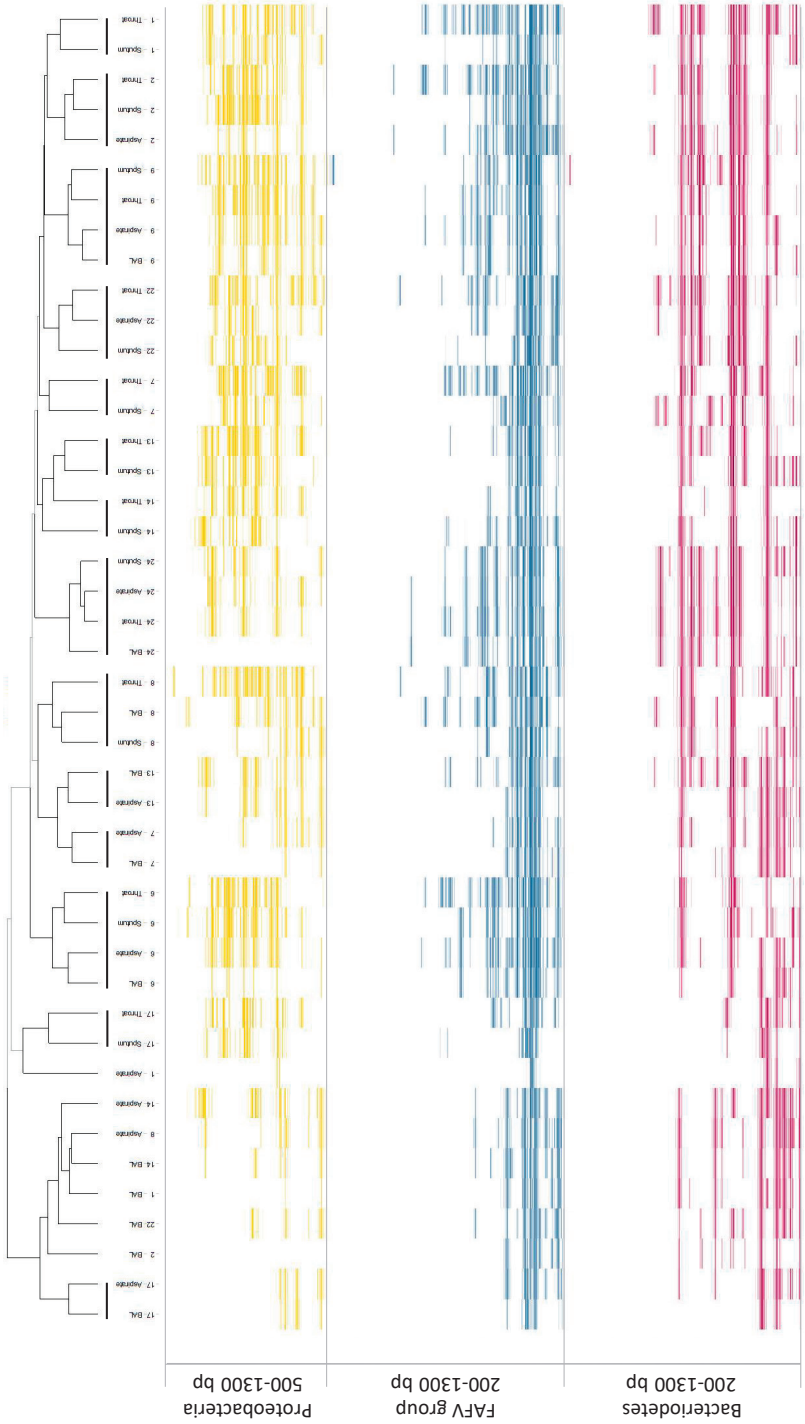

Figure 4. Heat map plot
Notes: Stratified to Bacteroidetes (purple), FAFV (blue) and Proteobacteria (yellow).

DISCUSSION

In this study, throat swabs, spontaneous sputum samples, bronchial aspirates and BAL fluids of 11 patients with COPD were analysed for microbiome composition. Our findings indicate that there are differences in the microbiome composition between the lower and upper respiratory tract, but also that all types of respiratory specimens contain a patient-specific signature.

Abundance and alpha-diversity

In alignment with previous research[8,17-19], we observed that approximately half of the detected fragments belonged to FAFV, with the Streptococcaceae family being the most abundant. Streptococcaceae is a well-known bacterial family of the respiratory tract and often observed in both patients with COPD as well as 'healthy' controls[17,20,21].

When focussing on the alpha-diversity, a significant difference in Shannon wiener index was observed between the different types of specimens analysed. Especially BAL had a significantly lower diversity compared to sputum and throat specimens. This pattern was probably due to the microbial diversity observed between types of specimens in the Proteobacteria. As expected, the lower respiratory specimens harboured a lower diversity of Proteobacteria. However, previous research also showed colonisation by Proteobacteria of the lower respiratory tract in patients with COPD[2,22]. Colonization of the lower airways in patients with COPD implies a vicious cycle of among others epithelial cell damage, mucus hypersecretion and inflammatory cell infiltration, promoting further dysfunction of host defences and bacterial adherence and growth[23]. Nevertheless, still much remains unknown about colonization and associated mechanisms, as well as the relationship with disease severity. Overall, our findings are supported by Carbera-Rubio and colleagues[8], who also observed a distinct alpha-diversity in upper and lower respiratory samples, although they observed a larger diversity in the lower respiratory samples.

Beta-diversity and patient-specific clustering

The current study confirms distinct clustering of lower and upper respiratory specimens[8], as shown by the PCoA analysis and significant differences observed in the beta-diversity. It is expected that specimens of the same region of the respiratory tract are more alike to each other, than specimens of different regions. This was also demonstrated in lung explants; there seemed to be marked regional differences in the respiratory microbiome within a patient[9], which may be very important for our understanding of respiratory health and disease.

When hierarchically clustering the respiratory microbiome in a heat map plot, we observed patient-specific clustering. A higher level of microbiome similarity (expressed as lower beta-diversity) was observed within an individual, compared to a specific type of respiratory specimen between individuals. This finding suggests that upper respiratory specimens may be representative for microbiome analysis, which would make collection of samples considerably easier. However, we should interpret this result with caution, as longitudinal research is necessary in order to assess shifts in the respiratory microbiome. At the moment, we assessed the respiratory microbiome in stable state, while changes in the respiratory microbiome are expected to occur during exacerbation. How this affects types of specimens and the level of microbiome similarity is currently unknown.

Charlson and coworkers[24] described in healthy subjects that different types of respiratory specimens within an individual were more similar to each other than they were to a particular specimen type between individuals. In other words, types of specimens within one subject were more closely related to their own specimen, than to those of any other subject[24]. Comparable results were observed by Dickson et al.[25], who also looked at different respiratory specimens in healthy subjects. Overall, these results support the patient-specific signature of the respiratory microbiome. By our knowledge, this is the first study to investigate the patient-specific signature in patients with COPD. Cabrera-Rubio and colleagues[8] assessed different respiratory specimens, but only focussed on the alpha-diversity and the specimen-specific signature, while it would be interesting to also investigate the patient-specific signature.

Clinical implications

This study provides promising results for respiratory microbiome analysis in clinical practice. Sputum samples and especially throat swabs are non-invasive procedures, easy to collect from patients. This way of sampling may increase the use of respiratory microbiome analysis in clinical practice. However, we should interpret these results with caution, as longitudinal studies and sampling during exacerbations are required to support our recent findings. Until now, a limited number of studies were conducted in patients with COPD exacerbation[5,10,26]. Overall, these studies showed a significant heterogeneity of the microbial composition at both baseline and during exacerbation in patients with COPD[26]. Moreover, they observed changes in the microbial composition during and after an exacerbation, with diverse effects of antibiotic versus corticosteroid treatment[5,10]. At the moment, usage of the respiratory microbiome in clinical practice is challenging, but insights into the microbial patterns of patients, and change of these patterns over time, might play a role in a better understanding of the pathophysiology of COPD exacerbations in the near future.

Methodological considerations

There are some methodological issues that should be taken into consideration when interpreting the current analyses. First, the population of patients with COPD is relatively small. Analyses need to be expanded to a larger population. Moreover, the current population existed of mild-to-moderate patients with COPD. It would be interesting to perform comparable analyses in severe-to-very severe patients with COPD, and to possibly assess patients based on clinical characteristics as frequent exacerbators, and patients with a high burden of respiratory symptoms. Second, the current study included patients who were planned to undergo a bronchoscopy for a non-infectious indication. However, the influence of the different indications to undergo bronchoscopy on the respiratory microbiome are currently unknown. Although patients were clinically stable at the time of sampling, one third of our patients had an exacerbation in the previous three months and the possible effects of previous use of antibiotics and/or oral corticosteroids on microbiome diversity remain unknown in the current study. Finally we used the IS-pro technology for our study[16]; the discriminatory power of this method is high, even below the species level for some species. This technology can be used in different ways. We used this method for pattern recognition and phylum level analysis for putative use in routine diagnostics. Microbial pattern analysis might be an easy way to follow up patients in clinical practice and sufficient to predict or monitor exacerbations of COPD.

Conclusion

In conclusion, the respiratory microbiome in patients with COPD contains a patient-specific signature in all types of respiratory specimens, which is more pronounced than the specimen-specific signature. In future, respiratory microbiome analysis might be possible on upper respiratory samples, which are much easier to collect compared to lower respiratory tract samples. Longitudinal studies also covering COPD exacerbations are required, in order to advance the understanding of the respiratory microbiome. Our study provides a new approach to such much-needed studies.

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8

General discussion

GENERAL DISCUSSION

Chronic Obstructive Pulmonary Disease (COPD) is a highly prevalent disease, causing significant morbidity and mortality worldwide[1]. Symptoms such as dyspnoea, cough and sputum production are often experienced by patients with COPD, limiting them in their daily functioning[2]. These symptoms are also related to acute respiratory events, including infections and exacerbations[2]. Although, respiratory infections such as community acquired pneumonia (CAP) have been studied frequently in patients with COPD[3], the impact of COPD on outcomes and clinical parameters of CAP remains uncertain. Furthermore, limited evidence is available concerning the influence of airway microbiology and exacerbations in patients with COPD on clinical outcomes and the effects of (non-) pharmacologic interventions, including pulmonary rehabilitation. Meanwhile, new techniques for microbiome analysis have been introduced[4], but the clinical implication of the choice of respiratory samples in patients with COPD is insufficiently addressed. This thesis investigated the impact of respiratory infections and exacerbations on COPD outcomes and the clinical perspective of respiratory samples used for respiratory microbiome analysis. In this chapter, the main findings of this thesis will be discussed.

RESPIRATORY INFECTIONS AND COPD

Respiratory infections are ranked the greatest single contributor to the overall burden of disease in the world[5]; this relates to a high disease as well as economic burden[6]. In patients with COPD, a higher prevalence is observed, as these patients appear to be more susceptible for respiratory infections[7]. This was confirmed by our research performed in **chapter 4**, where we observed a substantial higher incidence of CAP in patients with COPD compared to matched control subjects, resulting in a fourfold increased risk of developing CAP. These numbers emphasize the importance of research in this field, to improve disease management focussed on prevention and treatment.

Although many studies have been performed in the field of CAP and COPD, results concerning the impact of COPD on outcomes in patients with CAP remain controversial. **Chapter 2** discussed the differences in outcomes of CAP patients with and without concurrent COPD in the CAPNETZ population. Patients with COPD appeared to have more severe CAP, as expressed by the CURB-score. Furthermore, they had a longer length of stay, and disturbances in gas exchange were more pronounced in patients with COPD. These results are comparable with earlier findings[8,9]. Interestingly, conflicting results have been observed for short- and long-term mortality[10]. We demonstrated in the CAPNETZ population increased short- and long-term mortality rates in patients with COPD, when compared to non-COPD patients with CAP. However, when adjusted for other possible confounders, COPD was not related to CAP mortality. Possibly, patients

with COPD may seek medical care earlier, be admitted to the hospital sooner and receive more attention from physicians. Overall, intensive care unit (ICU)-admission and older age were independently related to both short- and long-term mortality. Older age as risk factor for all-cause mortality in patients with COPD is not surprising[11], and frequently reported in COPD patients identified with CAP[7,12]. COPD patients admitted to the ICU have been observed to present with more severe CAP and frequently comorbidities are present, which has been related to increased mortality rates[13]. Probably, a combination of factors influences the disease outcome. Close follow-up of, for example, comorbid conditions is important[14]. Other possible mechanisms described to be associated with increased mortality rates in patients with COPD, included the presence of resistant bacteria and reduced pulmonary reserve[15]. Moreover, there is much debate about the role of inhaled corticosteroids (ICS) in relation to an increased CAP risk in COPD[16]. However, evidence is controversial concerning the impact of ICS on outcomes in CAP, as some showed a protective effect[17], while others observed no impact[18,19]. Possibly, the specific kind of ICS and the dosage play a role in this[20]. Though, the European Medicines Agency completed a review and observed no difference in the risk of pneumonia between different products[21]. Well-designed research is necessary to identify the precise relationship of ICS with CAP and its role in outcomes within patients with COPD.

To target treatment of CAP in patients with COPD, it is important to focus on the microbiology of CAP. In chapter 3 we observed a distinct microbiological pattern in CAP patients with COPD, compared to CAP patients without COPD. As confirmed by our study, *Haemophilus influenzae* is frequently detected in patients with COPD[22-25], both in stable and acute phase. Normally, *H. influenzae* colonises the nasopharynx, and is related to upper respiratory tract infections. Infection of the lower respiratory tract is less common, because host immune responses prevent the spread to the lower respiratory tract[20]. This implies, that in patients with COPD, certain host immune mechanisms are impaired. Another pathogenic bacterium often detected in both patients with COPD and CAP is *Streptococcus pneumoniae*[26,27]. In the CAPNETZ population, this was the most frequently detected pathogen in CAP patients without COPD and together with *H. influenzae*, the most identified pathogen in COPD patients with CAP. We aimed to investigate the impact of specific pathogens on outcomes of CAP, but the detection rate was unfortunately too low to draw conclusions. Forstner and colleagues[22] observed in CAP-cases, not specifically specified to patients with COPD, that 30- and 90-day mortality rates between CAP patients with *H. influenzae* detection and patients with other or unknown aetiology, were comparable. Similar results were observed by Capelastegui et al.[28], showing no difference in 30-day mortality between patients with conventional bacteria, atypical bacteria, viral infection and mixed infection. On the other hand, patients infected with conventional bacteria frequently had severe sepsis and septic shock, and were significantly more often hospitalised and admitted to an ICU than the

other subgroups[28]. Large, well-designed studies are needed to investigate the impact of specific pathogens on outcomes of clinical infection. Insight in the latter might optimise CAP management in general, but also for specific subgroups of patients with concomitant comorbidities as COPD.

Besides the patients with a detected pathogen, there is a large subset of patients who had clinical signs of CAP, but culture techniques failed to obtain a bacterial aetiology. Unfortunately, identification of pathogens is still challenging. Culture-dependent techniques are at the moment the golden standard for microbiological analysis, but lack the detection of many pathogens[25,29]. Additionally, this technique is not helpful when antibiotic treatment is already started. Often, the treatment is empirical, which might induce treatment failure as well as antibiotic resistance. Besides, there are some clinical consequences: patients without pathogen detection are difficult to treat, have the highest mortality rate and also appear to have more severe CAP (**chapter 3**)[30]. Possibly, these pneumonias were caused by viral infections, which are expected to cause up to 25% of CAP cases and are often accompanied by a secondary bacterial infection, especially *S. pneumoniae*[31,32]. Influenza and rhinovirus are the viruses most frequently detected[33]. Mortality rates of patients with a viral infection were not significantly higher, but bacterial co-infection has been associated with an increased short-term mortality risk[32]. In the last decades, vaccination is recommended for vulnerable patient groups, in order to prevent influenza and pneumococcal infection[34]. Vaccination aims to reduce the risk of adverse outcomes, as risk of CAP, hospitalisation and mortality. Clearly, the incidence of these pathogens decreased, and prevention can be as high as 60-80% if the vaccine matches the circulating strain[35,36]. In patients with COPD, there is an increased risk of inadequate immune response following vaccination, showing lower efficacy percentages[36].

Furthermore, there is at the moment limited understanding of the impact of bacterial pathogens in the airways in the absence of an acute infection. Worse outcomes have been reported for *H. influenzae* colonisation: increased airway and systemic inflammation, frequent exacerbations and a poorer health status[20,37]. Additionally, pathogenic colonisation in general in patients with COPD has been associated with a clinically significant increase in daily symptoms[38]. This strengthens the need for research on daily symptoms and its impact on disease features. **Chapter 5** partly focussed on this aspect, and will be discussed in the next paragraph.

The current insights emphasize that physicians and other health care professionals should not underestimate the impact of CAP in patients with COPD. A shift towards prevention of respiratory infections is needed, as treatment of these infections becomes increasingly harder due to antibiotic resistance. Besides vaccination as prevention method (as mentioned above)[34], there are some modifiable lifestyle factors related to an increased CAP risk. The most well-known preventable risk factor associated with

CAP is smoking[39], which in turn is one of the main risk factors for COPD. It is often difficult to determine the specific influence of smoking in the development of CAP in patients with COPD, due to this dual pathway. We examined in **chapter 4** the effect of smoking in a large population of patients with COPD in comparison with matched control subjects. As described earlier in this paragraph, patients with COPD had an increased CAP risk compared to matched controlled subjects. Current smoking was associated with an increased risk of CAP in healthy subjects, as expected. Mechanisms such as tobacco-induced physiological and structural changes, and increase in bacterial virulence and dysregulation of immune function are related to this observed association[40]. In patients with COPD, we expected to see a comparable association. However, the risk of CAP was comparable between never and current smoking COPD. While this finding was unexpected, it was confirmed by other research in this field[12,41]. The mechanisms behind this observation are not yet clear. Maybe, smoking does not induce the risk of CAP in patients with COPD, as this risk is already augmented due to the underlying disease itself. The smoking effect does not sum up. On the other hand, pathophysiological aspects might be related to the comparable risk in never smokers. The development of COPD in these patients is not smoking-related, but for example by occupational or environmental exposures, alpha1-antitrypsin deficiency or factors early in life which effect the respiratory health in long-term[42,43].

Other lifestyle factors associated with CAP risk are alcohol consumption and body weight[39]. It appeared that consumption of ≤40 g alcohol daily protects against CAP compared to no alcohol consumption. On the other hand, consumption of >40 g alcohol daily was associated with an increased risk. Hence, moderate alcohol consumption seems to have some beneficial effects. The same applies for body weight, showing an increased risk in underweight patients, but a reduced risk in overweight patients. These results underline that a balance in lifestyle factors, which can be effected by patients themselves, is still a good way to reduce CAP risk. For patients with COPD, the relationship with CAP is complex and is not as straight forward as in healthy subjects. Still, these factors are also important to take into account for patients with COPD, as these lifestyle factors are not only related to CAP risk, but many health problems. Probably, patients with COPD are more vulnerable to changes, and pursuing a healthy life style is important for their overall disease burden.

EXACERBATIONS IN COPD

Respiratory symptoms play an important role in the life of patients with COPD. Therefore, assessment of symptoms is part of the international disease stratification for COPD[34]. Symptoms are increased in patients with bacterial airway colonisation[38] and have a major impact on the daily functioning[44]. Moreover, they are the key feature of

exacerbations, as these events are defined as 'an acute worsening of respiratory symptoms that result in additional therapy'[34]. In addition to pharmacotherapy, pulmonary rehabilitation (PR), defined as 'a comprehensive intervention based on thorough patient assessment followed by patient-tailored therapies that include, but are not limited to, exercise training, education, self-management intervention aiming at behaviour change, designed to improve the physical and psychological condition of people with chronic respiratory disease and to promote the long-term adherence to health-enhancing behaviours', is targeted to reduce respiratory symptoms in patients with COPD[34]. Besides reduction of respiratory symptoms, PR has been shown to improve quality-of-life and exercise tolerance of patients with COPD[45].

The second most experienced symptom reported by patients with COPD was sputum production[44]. In **chapter 5** we were interested whether patients with sputum production, and especially a positive culture result, indicative for bacterial colonisation, are clinically different from those without sputum production and/or a negative culture result before start of PR. We observed that a substantial proportion of patients with COPD produced sputum during a pre-rehabilitation assessment. Of these, almost one third had a positive culture. It appeared that patients with a positive sputum culture had a worse health status based on the CAT total score and CCQ total score, and had more symptoms of depression than patients who did not produce sputum. Our results are an addition to research performed in patients with chronic bronchitis[46,47]. Chronic bronchitis is defined as 'the presence of chronic cough and sputum production for at least 3 months a year, for two consecutive years'[34]. Objectively diagnosing chronic bronchitis is complex and subject to patient recall. The definition covers no incidental cough and sputum, while many patients experience those symptoms without meeting the criteria. A recent study of Deslee and colleagues[48] assessed cough and sputum in the past 7 days. They showed that current cough was associated with health related quality of life impairment[48]. It appeared that cough alone was a stronger predictor than chronic bronchitis, or current sputum production. Hence, we should be aware of the fact that incidental cough and sputum production in patients with COPD also impacts on health related outcomes, especially health status. Providing patient centred care through a holistic and dynamic approach is needed, to focus on patients' needs and values[49].

Exacerbations are important events in the course of COPD[34]. They negatively impact health status and exercise capacity, and are associated with disease progression, hospital admission and mortality[50-52]. Key features of exacerbations include increased airway inflammation, increased mucous production and marked gas trapping, resulting in symptoms as dyspnoea, sputum, cough and wheezing. While there is currently a paucity of data on the effects of PR on exacerbation risk, post-exacerbation PR has been shown to reduce the risk of exacerbation readmissions[53,54]. Additionally, exacerbations are reported to be one of the most common reasons of drop out during PR[55]. We did a similar observation in **chapter 6**: having at least one severe exacerbation during PR was

associated with increased dropout. Mild-to-moderate exacerbations were more often identified in completers. By our knowledge, we were the first to stratify exacerbations by severity, highlighting the fact that especially exacerbations resulting in a hospitalisation, had a negative effect on completion of PR. Nevertheless, it remains a challenge to foresee the impact of exacerbations on dropout in the long-term. The effect of an exacerbation might last longer, even though dropout was reported by a different cause.

When we focussed on outcomes of PR, stratified by exacerbation severity, we observed a mean improvement on health status scores, symptoms of anxiety and depression and exercise capacity in completers without an exacerbation and those with at least one mild-to-moderate exacerbation. These mean improvements exceeded the minimal clinically important difference, except for the 6-minute walking test. The beneficial effects of PR have been described more often[56], and our results confirmed the valuable addition of PR in patients with COPD, even when having a mild-to-moderate exacerbation. Completers with at least one severe exacerbation, had in general comparable results, but the improvement was not significant. Still, these patients should be encouraged to complete PR. It would be very interesting to compare completers with a severe exacerbation, with those who dropped out after a severe exacerbation. Staying stable over time, and not showing a large deterioration following PR, is in these patients a result that should be fostered.

We believe, the shift from treatment of exacerbations towards prevention is desired. Prevention involves different aspects, including non-pharmacological and pharmacological options. Next to PR, one of the key concepts is smoking cessation. Former smokers appeared to have a reduced risk of exacerbations, when compared with current smokers[57]. Furthermore, as discussed before, vaccination is recommended in patients with COPD, as the majority of exacerbations are triggered by bacterial and viral infections[58]. Vaccination has been shown to reduce the rate of exacerbations[59,60]. Moreover, there is a legion of pharmacological options associated with reduced exacerbation risk, but more evidence in this field is necessary[61,62]. Another aspect, which is involved in all of the before mentioned interventions, is self-management. Self-management training aims to 'help patients acquire and practise the skills they need to carry out disease-specific medical regimens, to guide changes in health behaviour and to provide emotional support to enable patients to adjust their roles for optimal function and control of their disease'[63]. An international expert group stated that 'besides developing skills, enhancing the patient's motivation to actually execute these skills on a daily basis is an essential component of a COPD self-management intervention'[64]. Multi-component COPD self-management programs are promising, but the effect on outcomes varies[63,65,66]. Overall, compliance remains a huge challenge in all proposed interventions. Likewise, it is important to concentrate on the individual patient, as COPD is a very heterogeneous disease, and interventions should be adapted to specific needs of a patient.

RESPIRATORY MICROBIOME

Following the impact of acute respiratory events in patients with COPD, and the shortcomings of research in the field of microbiology in patients with COPD, new techniques are desired to understand mechanisms and overcome pressing issues related to respiratory infections and exacerbations. Microbiome analysis is promising in gaining insight in patterns of the microbial environment and its role in the development and prevention of respiratory infections and exacerbations.

First, it is important to identify which respiratory specimens are sufficient to assess the respiratory microbiome for clinical practice in patients with COPD. By now, only one study compared specimens from different regions of the respiratory tract[67]. Cabrera-Rubio and colleagues observed a distinct microbial environment in the upper respiratory tract as compared to the lower respiratory tract in patients with COPD[67]. Lower respiratory tract specimens were more diverse, and clustered together concerning their microbial composition. Contrary, upper respiratory specimens were less diverse and expected to be contaminated by the oropharynx[67]. Hence, the implementation of respiratory microbiome analysis in clinical practice is depending the accessibility to obtain respiratory specimens. Molyneaux and co-workers suggested to use sputum for microbiome analysis, as it allows prolonged sampling over time to assess changes in the respiratory microbiome, and avoids invasive sampling[68]. Because there is some discussion regarding the different sample types, we performed a research to compare the clinical implication of specimens from different regions in the lung (chapter 7). We specifically focussed on the patient-specific as well as the specimen-specific signatures of the respiratory microbiome, to assess similarities/differences between different sampling locations as well as between different patients. In general, the alpha-diversity (specimen-specific signature – diversity within a specific specimen) significantly differed between upper and lower respiratory specimens, as a result of Proteobacteria. This pattern was also observed by former research[67], concluding that upper respiratory specimens were not representative for the respiratory microbiome. Therefore, we also assessed the beta-diversity between and within specimens (distance or dissimilarity between types of specimens). The beta-diversity of the upper respiratory specimens was significantly lower than that of lower respiratory specimens, suggesting that upper respiratory specimens were more alike each other between patients. However, the patient-specific signature in relation to the different types of specimens remained unclear. Thus, a hierarchical clustering heat map plot was performed and showed a patient-specific clustering, which was also confirmed by a significantly lower within-patients beta-diversity in comparison with the within-types of specimens beta-diversity. A higher level of microbiome similarity was observed within an individual. Unfortunately, no comparable research had been performed within patients with COPD, only within healthy individuals, showing comparable results[69,70]. Overall, our findings indicated that there are differ-

ences in the microbiome composition between upper and lower respiratory tract specimens, but also suggests that the patient-specific signature in all respiratory specimens was more pronounced than the specimen-specific signature. The latter must be interpreted with caution, as this is the first research performed including the beta-diversity and assessing the patient-specific signature in patients with COPD. Clearly, more well-designed research, in larger study populations, is necessary to confirm our findings. Moreover, we should take into mind that COPD is a highly heterogeneous disease, which makes it difficult to draw firm conclusions across the research performed[71].

Additionally, it would be very interesting to perform longitudinal research, in order to assess the respiratory microbiome over time, taking into account possible shifts in the respiratory microbiome due to disease alterations. As discussed before, respiratory infections and exacerbations are highly prevalent in patients with COPD and have a tremendous impact on a patients wellbeing. Molyneaux and colleagues[68] investigated the effect of rhinovirus infection on the respiratory microbiome. They observed an increase of bacterial burden in patients with COPD, driven by a significant outgrowth of *Haemophilus influenzae*, suggesting that rhinovirus infection altered the respiratory microbiome and may precipitate secondary bacterial infections[68]. Others observed a reduction in alpha-diversity following COPD exacerbation[72]. Furthermore, medication might influence the respiratory microbiome, especially antibiotic and corticosteroid use are of interest. Evidence in this field is conflicting, as some observed[72] a decreased alpha-diversity in subjects treated with corticosteroids alone and an increasing alpha-diversity trend in patients treated with antibiotics alone or in combination with steroids. While an opposite trend was observed by others[73]. Generally, before we can oversee the precise role of the respiratory microbiome in patients with COPD more research is necessary, although results are promising.

CLINICAL IMPLICATIONS AND FUTURE DIRECTIONS

This thesis described different aspects of respiratory infections and exacerbations in patients with COPD. Both conditions negatively impact on disease outcomes and thus have important clinical implications. First, it is important to distinguish between respiratory infections in patients with COPD and COPD exacerbations[74]. Although both events present with comparable symptoms, they result in different treatment options. Misuse of antibiotics has consequences for future treatment options[75]. Besides, prevention should play a key role, including among others vaccination and pursuing a healthy lifestyle. Second, assessing the microbiology in stable and disease state provides insight in airways colonisation, which is often present and possibly drives physicians into the wrong direction. Third, assessment of comorbidities is essential, not only in stable state, but also in disease state. It has been observed that patients with COPD present with

many comorbidities[76,77], highlighting the need for a personalised approach in the management of the disease. Fourth, pulmonary rehabilitation might play a substantial role in the latter. This intervention is multi-component and patient-centred, showing in general positive effects, even when interrupted by a COPD exacerbation. Nevertheless, COPD is a complex disease with a large heterogeneity between patients, which makes research and development of interventions necessary, but also challenging. Microbiome analysis is promising in understanding the role of the respiratory microbiome in health and disease, but further research is clearly needed.

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Summary

SUMMARY

Chronic Obstructive Pulmonary Disease (COPD), characterised by persistent respiratory symptoms and airflow limitation, is one of the leading causes of death worldwide with a high economic and societal burden. COPD is a very heterogeneous disease, which makes the management complex. The disease is featured by acute events, impacting on health related quality of life, exercise capacity and activities of daily living. These acute events can be discriminated in respiratory infections, including community acquired pneumonia (CAP), and exacerbations (AECOPD). Although both disease conditions present with comparable symptoms, discrimination is important for prevention strategies, treatment options and outcomes. Therefore the current thesis aimed to better understand both disease conditions as well as to provide recommendations for which specimens to use for respiratory microbiome analysis (**Chapter 1**).

To optimise disease management, it is important to gain insight into both disease conditions. **Chapter 2** and **3** aimed to assess the prevalence of COPD in CAP patients, as well as to determine characteristics and the bacterial aetiology of patients with COPD compared to non-COPD patients. A large observational study revealed that CAP patients with COPD were significantly older, more often current or former smokers, with more severe CAP, compared to non-COPD patients with CAP. Different bacterial pathogens were observed in patients with and patients without COPD, which advocates the use of different treatment options. Besides, worse outcomes were observed in patients with COPD; higher mortality rates and longer length of hospital stay. Older age and admission to an intensive care unit were observed to be related to short- and long-term mortality for patients with CAP, independent of COPD. Bacterial pathogen detection seemed to be important, as patients without pathogen detection had more severe CAP and higher mortality rates. Overall, these chapters suggest that insight into characteristics of patients with COPD and the aetiology underlying CAP might contribute to better management and treatment of patients.

Since smoking is the most important risk factor to develop COPD, and is also related to an increased risk of developing CAP, **chapter 4** studied the impact of smoking on the risk to develop CAP in patients with COPD compared to patients without COPD. This study showed that current smoking is not related to an increased risk to develop CAP in patients with COPD, while this is the case in patients without COPD. Interestingly, current smoking patients with COPD had a comparable risk to develop CAP as never smoking patients with COPD. Which mechanisms underlie this observation is currently unknown and needs further investigation.

One of the main symptoms related to COPD is sputum production. This symptom is also part of the definition of AECOPD, and frequently reported by patients. In **chapter 5** the influence of sputum production and sputum aetiology on patient characteristics, health status and

exercise capacity was assessed in a population of patients with stable COPD who were referred for pulmonary rehabilitation (PR). Almost one-third of the patients with stable COPD produced a spontaneous sputum sample, with a positive culture related to a worse health status compared to patients who did not produce spontaneous sputum. Besides, patients with a positive sputum culture had a higher exacerbation frequency before PR and reported more often symptoms of chronic bronchitis. This study makes us aware of specific clinical profiles of patients with COPD, which is important in order to develop specific recommendations following the general management of patients with COPD.

Next to CAP, patients with COPD are susceptible to acute exacerbations of the disease. AECOPD are often subject of research, but still much remains unclear due to the heterogenic character of this event. In chapter 6 the impact of AECOPD, stratified by severity, on pulmonary rehabilitation (PR) was determined. This research observed that even though patients were having a mild-to-moderate AECOPD during PR, positive results were achieved at the end of PR. Unfortunately, patients with severe AECOPD during PR had on average no improvement of health related quality of life, symptoms of anxiety and depression and exercise capacity. However, these results also showed no overall decline in outcomes, which might be considered as a benefit. This should however, be investigated by comparing a group of patients with severe AECOPD during PR who finished PR, with a comparable group who did not finish PR. Still, it is important to motivate and encourage patients to complete PR even though exacerbating, as the benefits of PR have been convincingly demonstrated in the past.

Overall, the before mentioned results highlight the fact that a lot more research is necessary in the field of respiratory infections and exacerbations in patients with COPD. In the last few years, respiratory microbiome analysis is positioned as a promising technique to overcome pressing questions. Chapter 7 aims to provide a recommendation on which respiratory specimens to use for respiratory microbiome analysis in patients with COPD. Different respiratory specimens were assessed, of both the upper and lower respiratory tract. In general, the alpha and beta diversity differed between the respiratory specimens, but the patient-specific signature in the respiratory specimens was more pronounced than the specimen-specific signature. So, different respiratory specimens of one subject are more alike to each other than similar respiratory specimens of other subjects. This observation makes respiratory microbiome research in the future easier, as less invasive sampling techniques might be sufficient enough for clinical practice.

In conclusion, this thesis discussed various concepts of respiratory infections, exacerbations and the respiratory microbiome in patients with COPD (Chapter 8). Many steps are needed to understand the different disease conditions and to optimize treatment and overall management. Respiratory microbiome analysis is challenging, as well as promising to understand the development of COPD. Possibly non-invasive sampling techniques can be used to assess the respiratory microbiome for clinical practice.

Samenvatting

SAMENVATTING

Chronisch obstructieve longziekten (COPD), gekarakteriseerd door persisterende respiratoire symptomen en luchtwegobstructie, is één van de belangrijkste doodsoorzaken wereldwijd, met een hoge economische en sociale last. COPD is een heterogene ziekte, wat het management rondom de ziekte complex maakt. De ziekte wordt gekenmerkt door acute gebeurtenissen, welke van invloed zijn op de gezondheidsgerelateerde kwaliteit van leven, de inspanningscapaciteit en activiteiten van het dagelijkse leven. Deze acute gebeurtenissen kunnen worden onderscheiden in respiratoire infecties, onder andere een longontsteking opgelopen buiten het ziekenhuis (CAP), en longaanvallen (AECOPD). Ondanks dat beide ziektecondities gepaard gaan met vergelijkbare symptomen, is het belangrijk om onderscheid te maken tussen beide condities in het kader van preventiestrategieën, behandelingsmogelijkheden en uitkomsten. Daarom had het huidige proefschrift tot doel om beide ziektecondities beter te begrijpen en aanbevelingen te geven voor welk respiratoir monster het best gebruikt kan worden voor respiratoir microbioom analyse (Hoofdstuk 1).

Voor het optimaliseren van het management rondom COPD, is het belangrijk om inzicht te krijgen in beide ziektecondities. Hoofdstuk 2 en 3 hadden tot doel de prevalentie van COPD in CAP patiënten te bepalen, alsook het vaststellen van karakteristieken en de bacteriële etiologie van patiënten met COPD vergeleken met niet-COPD patiënten. Uit een grote observationele studie bleek dat CAP patiënten met COPD significant ouder waren, vaker huidige of voormalige rokers, met ernstiger CAP, in vergelijking met niet-COPD patiënten met CAP. Verschillende bacteriële pathogenen werden gevonden in patiënten met en zonder COPD, wat het belang aangeeft van verschillende behandelopties. Bovendien bleken patiënten met COPD slechtere uitkomsten te hebben; hogere sterftecijfers en een langer verblijf in het ziekenhuis. Oudere leeftijd en opname op een intensive care afdeling werden gerelateerd aan korte- en lange termijn mortaliteit in CAP patiënten, onafhankelijk van COPD. Detectie van bacteriële pathogenen lijkt belangrijk, omdat patiënten zonder detectie van pathogenen ernstiger CAP en hoger sterftecijfers hadden. Over het algemeen suggereren deze hoofdstukken dat inzicht in karakteristieken van patiënten met COPD en de etiologie van CAP mogelijk bijdragen aan een beter management en behandeling van patiënten.

Omdat roken de belangrijkste risicofactor is voor het ontwikkelen van COPD, en ook verband houdt met een verhoogd risico op het ontwikkelen van CAP, werd in hoofdstuk 4 de invloed van roken op het risico om CAP te ontwikkelen bij COPD-patiënten in vergelijking met patiënten zonder COPD onderzocht. Dit onderzoek toonde aan dat huidig roken niet gerelateerd is aan een verhoogd risico op CAP bij patiënten met COPD, terwijl dit wel het geval is bij patiënten zonder COPD. Interessant is dat de huidige rokers met COPD een vergelijkbaar risico op het ontwikkelen van CAP hadden ten opzichte van

nooit-rokende patiënten met COPD. Welke mechanismen ten grondslag liggen aan deze observatie is momenteel onbekend en zal verder onderzocht moeten worden.

Een van de belangrijkste symptomen gerelateerd aan COPD is sputumproductie. Dit symptoom maakt ook deel uit van de definitie van AECOPD en wordt vaak gemeld door patiënten. In hoofdstuk 5 werd de invloed van sputumproductie en de etiologie van het sputum op patiëntkarakteristieken, gezondheidsstatus en inspanningscapaciteit onderzocht in een populatie van patiënten met stabiel COPD die werden verwezen voor longrevalidatie (PR). Bijna een derde van de patiënten met stabiel COPD produceerde spontaan sputum, met een positieve kweek gerelateerd aan een slechtere gezondheidsstatus vergeleken met patiënten die geen spontaan sputum produceerde. Bovendien hadden patiënten met een positieve sputumkweek een hogere AECOPD frequentie voor PR en meldden ze vaker symptomen van chronische bronchitis. Deze studie maakt ons bewust van specifieke klinische profielen van patiënten met COPD, wat belangrijk is om specifieke aanbevelingen te ontwikkelen volgend op het algemene management van patiënten met COPD.

Naast CAP zijn patiënten met COPD ook gevoelig voor longaanvallen. AECOPD zijn vaak onderwerp van onderzoek, maar er is nog steeds veel onduidelijkheid vanwege het heterogene karakter van deze ziekteconditie. In hoofdstuk 6 werd de impact van AECOPD, gestratificeerd naar ernst, op longrevalidatie (PR) onderzocht. Uit dit onderzoek bleek dat hoewel patiënten een mild tot matige AECOPD hadden tijdens PR, er aan het einde van de PR positieve resultaten werden behaald. Helaas hadden patiënten met ernstige AECOPD tijdens PR gemiddeld geen verbetering van de gezondheidsgerelateerde kwaliteit van leven, symptomen van angst en depressie, en inspanningscapaciteit. Deze resultaten lieten echter ook geen algemene daling van resultaten zien, wat als een vooruitgang kan worden beschouwd. Dit moet echter worden onderzocht in een groep patiënten met ernstig AECOPD die PR afronden in vergelijking met een vergelijkbare groep patiënten die PR beëindigden. Toch is het belangrijk om patiënten met een AECOPD te blijven motiveren en aan te moedigen PR te voltooien, gezien de voordelen van PR in het verleden dikwijls overtuigend zijn aangetoond.

Al met al wijzen de eerder genoemde resultaten erop dat veel meer onderzoek nodig is op het gebied van luchtweginfecties en longaanvallen bij patiënten met COPD. In de laatste paar jaar is respiratoire microbioom analyse gepositioneerd als een veelbelovende techniek om prangende vragen te beantwoorden. Hoofdstuk 7 heeft tot doel een aanbeveling te geven over welk respiratoir monster gebruikt kan worden voor respiratoire microbioom analyse bij patiënten met COPD. Verschillende monsters werden onderzocht, zowel van de bovenste als de onderste luchtwegen. Over het algemeen verschilden de alfa- en betadiversiteit tussen de respiratoire monsters, maar het patient-specifieke signatuur in de respiratoire monsters was meer uitgesproken dan het monster-specifieke signatuur. Verschillende respiratoire monsters van één persoon

lijken dus meer op elkaar dan vergelijkbare respiratoire monsters van verschillende personen. Deze observatie maakt onderzoek naar het respiratoire microbioom in de toekomst eenvoudiger, omdat minder invasieve technieken voldoende kunnen zijn voor de klinische praktijk.

Concluderend besprak dit proefschrift verschillende concepten van luchtweginfecties, longaanvallen en het respiratoire microbioom bij patiënten met COPD (Hoofdstuk 8). Er zijn veel stappen nodig om de verschillende ziektecondities te begrijpen en de behandeling en het algemene management te optimaliseren. Analyse van het respiratoire microbioom is een uitdaging, evenals dat het een veelbelovende techniek is om het ontstaan van COPD te begrijpen. Het is mogelijk dat niet-invasieve technieken gebruikt kunnen worden om het respiratoire microbioom in de klinische praktijk toe te passen.

Valorisation

VALORISATION

This thesis includes a series of studies to broaden our knowledge of respiratory infections, exacerbations and the respiratory microbiome in patients with Chronic Obstructive Pulmonary Disease (COPD). Practical implications are described in various chapters of this thesis, in order to make this knowledge suitable and available for clinical practice. In the current chapter, these studies and their outcomes are further positioned in a broader economical and societal framework, in order to transfer the scientific knowledge described into clinical practice, as well as to put the findings into future perspective.

RELEVANCE

COPD is a chronic disease, with a high morbidity and mortality, which affects many people. Research in the field of COPD is necessary, in order to gain insight into disease development and management. It is known that the prevalence of community acquired pneumonia (CAP) in patients with COPD is remarkably higher compared to controls, and that subgroups of patients with COPD are susceptible to COPD exacerbations. Both disease conditions influence socially and economically, which warrants the fact that a lot of obstacles need to be taken.

The first part of this thesis focussed mainly on the impact of COPD with concomitant CAP on various health related outcomes. Adverse outcomes are more frequent and more severe in patients with COPD, for example a longer length of hospital stay and higher mortality rates. Targeted diagnostics of patients during hospitalisation play an important role, including microbiological sampling. Especially, the last should be a key component of the management of hospitalised CAP-COPD patients, as therapy can be better targeted to the spectrum of pathogens, which has the potential to reduce unnecessary coverage, increase antimicrobial efficiency and also reduce the economic burden.

Next to CAP, patients with COPD are vulnerable to COPD exacerbations. The concept of COPD exacerbations is still incompletely understood, since there is no clear definition yet. In the second part of this thesis, the impact of exacerbations on disease related outcomes following pulmonary rehabilitation was assessed. The results made clear that pulmonary rehabilitation has a positive effect on health status and exercise capacity, even when exacerbating mildly-to-moderately during the course of treatment. These results emphasise that completing pulmonary rehabilitation is essential, even for patients with a severe exacerbation.

Following the research questions and outcomes of the first two parts of this thesis, it became clear, that many steps should be taken to broaden our knowledge, in order to really understand what are the particular pathophysiologic features of the respiratory system of patients with COPD. Therefore, the last part of this thesis is especially relevant for the future. Respiratory microbiome analysis is a central component in this and is expected to contribute to finding answers to health-related questions. Until now, the focus is on gaining insight into the microbial composition of the lungs in both health and disease. But, for the future, it has the potential to provide guidance in prevention of the disease and acute disease conditions, as well as treatment of the latter.

TARGET GROUPS

Health care professionals

The main target group of this thesis are respiratory physicians, who are involved in the care of patients with COPD. Besides, for other health care providers, such as physician assistants, nurse practitioners and respiratory nurses, this thesis provides important clinical implications. Insight into respiratory infections is important to guide the management of patients with COPD. Therefore, microbiological sampling is an essential part of disease management. Moreover, insight into the microbiology involved in acute conditions of the disease can trigger pharmaceutical companies to develop new treatment options.

On the other hand, we also suggest a shift from treatment towards prevention. Paramedics such as occupational therapists, physiotherapists and dieticians are expected to motivate and stimulate patients to maintain or gain a healthy lifestyle. Even when chronically ill, delaying disease progression can often be accomplished by healthy lifestyle. Furthermore, health care professionals need to create a supportive environment and are encouraged to improve a patient's performance.

Patients with COPD

Unfortunately, patients with COPD do not directly benefit from the research performed for this thesis. However, patients are always the main target, as research questions arise from problems patients encounter. By performing this research, our knowledge is extended, and might result in further research and recommendations for patients with COPD. One direct key message for patients with COPD is the fact that patients should not be withheld from completing pulmonary rehabilitation when exacerbating. For both, patients and health care professionals, this should result in motivation and faith that even after an acute event, encouraging results can be accomplished. Second, it is

worth mentioning, that we observed promising results for microbiome analysis with non-invasive sampling. Non-invasive sampling makes it easier to perform microbiome analysis in clinical practice, but also makes it easier to perform research in this field. We hope our research provides the basis for future research projects more directly targeted to clinical implementation and application.

ACTIVITIES AND PRODUCTS

The findings of this thesis have led to several activities in the field of expertise. The results of chapter 2 to 6 have been presented during the European Respiratory Society (ERS) Congress in 2013 (Barcelona, Spain), 2014 (Munich, Germany), 2015 (Amsterdam, the Netherlands) and 2016 (London, United Kingdom). Furthermore, the findings have been translated into original manuscripts, published in different scientific international journals. Besides, results have been presented at different meetings and courses organized by CIRO, the Maastricht University Medical Centre and other institutions. Following these activities, the current findings have been distributed and may hopefully inspire future research.

FUTURE PERSPECTIVE

For the near future, it is important to distinguish between CAP and exacerbations in patients with COPD. Both acute events present with comparable symptoms and substantially impact on the disease, although treatment options differ. The disease conditions are often mixed in clinical practice, while it is expected that clear definitions would positively contribute to both the societal and economical field, as disease management can be optimised.

Second, respiratory microbiome analysis has a lot of potential, but is still in its infancy, and comprises many aspects which remain to be studied. Especially the technique used for microbiome analysis in this thesis needs to be further explored. Until now, respiratory microbiome analysis changed our perspective concerning the microbial composition of the lungs. In future, microbiome analysis has the potential to shed light on the development of disease and its relationship with health and disease alterations by advanced microbiology. This hopefully leads to new products such as medication, and techniques to prevent disease and/or disease alterations.

Dankwoord

DANKWOORD

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Curriculum Vitea

CURRICULUM VITEA

Dionne Braeken is geboren op 13 juni 1987 in Houthem St.-Gerlach. In 2004 behaalde zij haar HAVO-diploma aan het Stella Maris college te Meerssen. In hetzelfde jaar startte zij met de studie HBO-verpleegkunde aan de Hogeschool Zuyd Heerlen, welke ze in 2008 met goed gevolg afrondde. Vervolgens heeft ze een jaar gewerkt als verpleegkundige in Zorgcentrum Vroenhof, gecombineerd met een cursus statistiek en methodiek als voorbereiding op een master opleiding. In 2009 startte ze met de master Gezondheidswetenschappen aan de Universiteit Maastricht. Na haar afstuderen in 2010 heeft ze twee jaar gewerkt als onderzoeksmedewerkster bij GALA, een onderdeel van het Hart- en Vaat Centrum in het MUMC+ te Maastricht. Het laatste half jaar heeft ze dit werk gecombineerd met een baan als ARBO-verpleegkundige bij EASE travel clinic. Vanaf oktober 2012 is ze gestart met haar promotieonderzoek bij CIRO te Horn op het gebied van respiratoire infecties, exacerbaties en het respiratoire microbioom bij patiënten met COPD onder leiding van Prof. Dr. Emiel F.M. Wouters, Dr. Frits Franssen en Prof. Dr. Gernot Rohde. De resultaten van dit promotieonderzoek werden gepresenteerd op verschillende internationale congressen en resulteerde in 2013 in een Young Scientist Award van de European Respiratory Society (ERS). In 2017 is Dionne werkzaam geweest als wetenschappelijk onderzoekster in het MUMC+ voor de afdeling Longziekten en heeft ze binnen CIRO het project ‘Beslist Samen’ geleid. Sinds 2018 is Dionne werkzaam als trombosevigilantie functionaris in het MUMC+. Dionne is getrouwd met Michael Limpens en samen zijn ze trotse ouders van Funs.

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