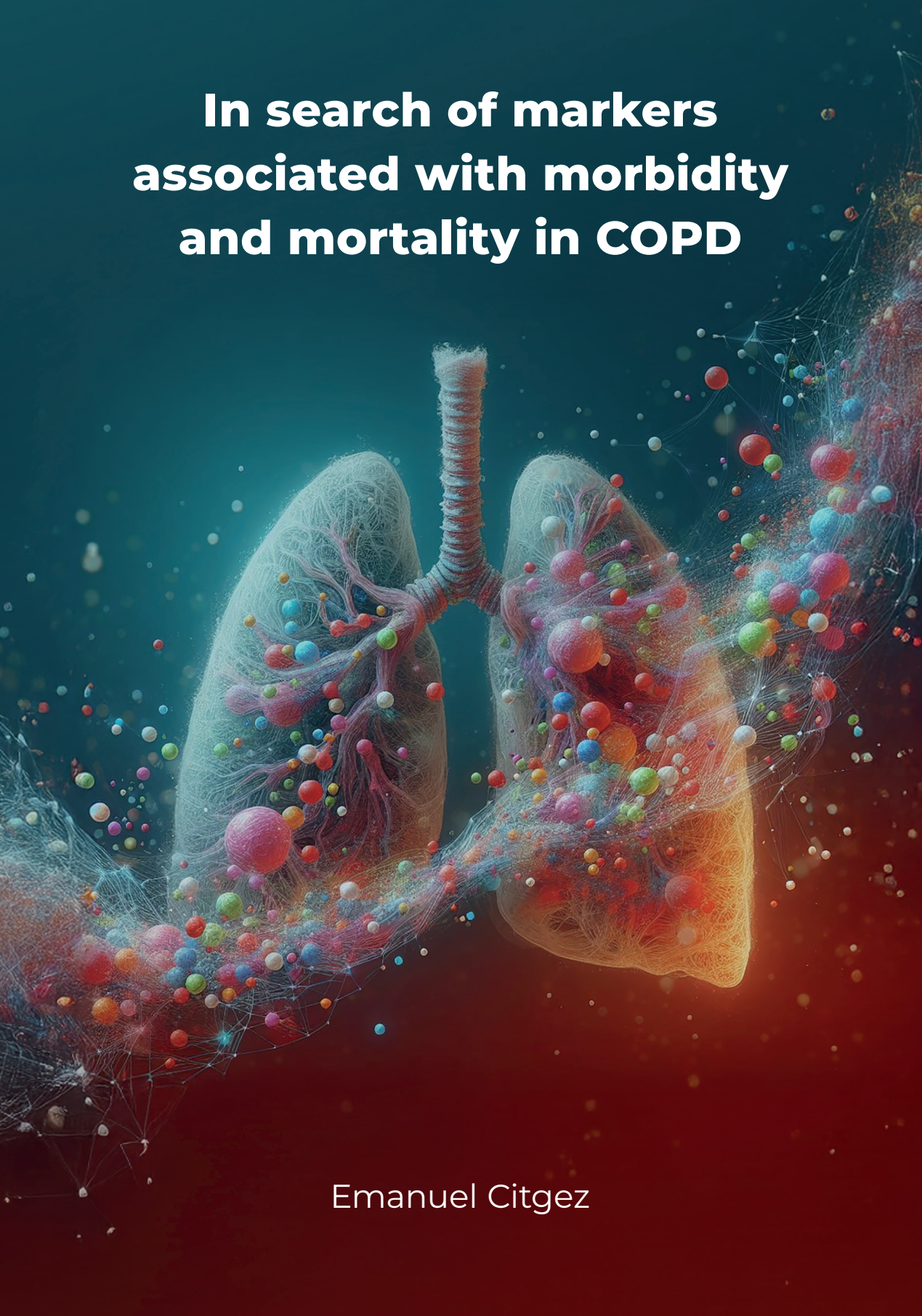
In search of markers associated with morbidity and mortality in COPD



Emanuel Citgez

In search of markers associated with morbidity and mortality in COPD

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IN SEARCH OF MARKERS ASSOCIATED WITH MORBIDITY AND MORTALITY IN COPD

DISSERTATION

to obtain
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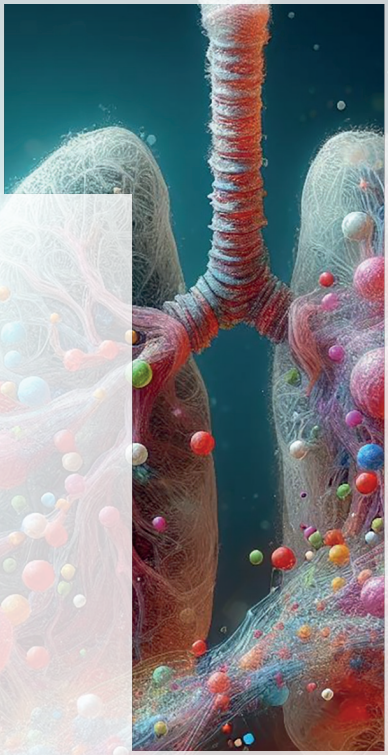
Contents



General introduction

This thesis describes the results of a series of investigations into different (bio-) markers and their associations with morbidity, and mortality in Chronic Obstructive Pulmonary Disease (COPD) that were performed at the department of pulmonary medicine of Medisch Spectrum Twente, Enschede, The Netherlands. The focus with regard to morbidity was on hospitalized (severe) Acute Exacerbations of COPD (AECOPD) and on Community Acquired Pneumonia (CAP). The introduction gives more insight in COPD and its associated morbidity and mortality. Furthermore, the major themes of this thesis will be highlighted.

COPD and Morbidity and Mortality

COPD is a complex and heterogeneous disease accompanied by increased morbidity and mortality.(1,2) Underlying chronic and systemic inflammation play an important role in the pathophysiology of COPD, the progression of COPD, and the associated comorbid conditions and diseases of COPD such as cardiovascular disease, muscle weakness and osteoporosis.(3–6) Smoking has always been one of the most important drivers for this inflammation, leading to structural changes and damage to airways and alveoli, ultimately resulting in lung function decline and irreversible airflow limitation.(7)(figure 1)

Diagnostic criteria for COPD still rely mainly on the presence of spirometric airflow limitation, assessed by the post-bronchodilator ratio of FEV1 to FVC.(8)

This irreversible airflow limitation defining COPD is however not only caused by smoking.(9) Currently available evidence indicates that COPD is the end result of a series of dynamic, interactive, and cumulative gene–environment interactions from conception to death that determine the development, maintenance, and function of the lungs, as well as other systemic organs.(10) This underlies the complexity of COPD in that the disease has several distinct components and is driven by both genetic and environmental factors that dynamically interact over time. COPD is a heterogeneous disease because of the fact that all components are not present in all individuals at any given time point and can vary in severity over time. (11,12)

COPD is associated with a high burden of disease. CAP and severe AECOPD are among the important factors contributing to the morbidity and mortality in COPD and are associated with this high burden of disease and low quality of life.(13,14)

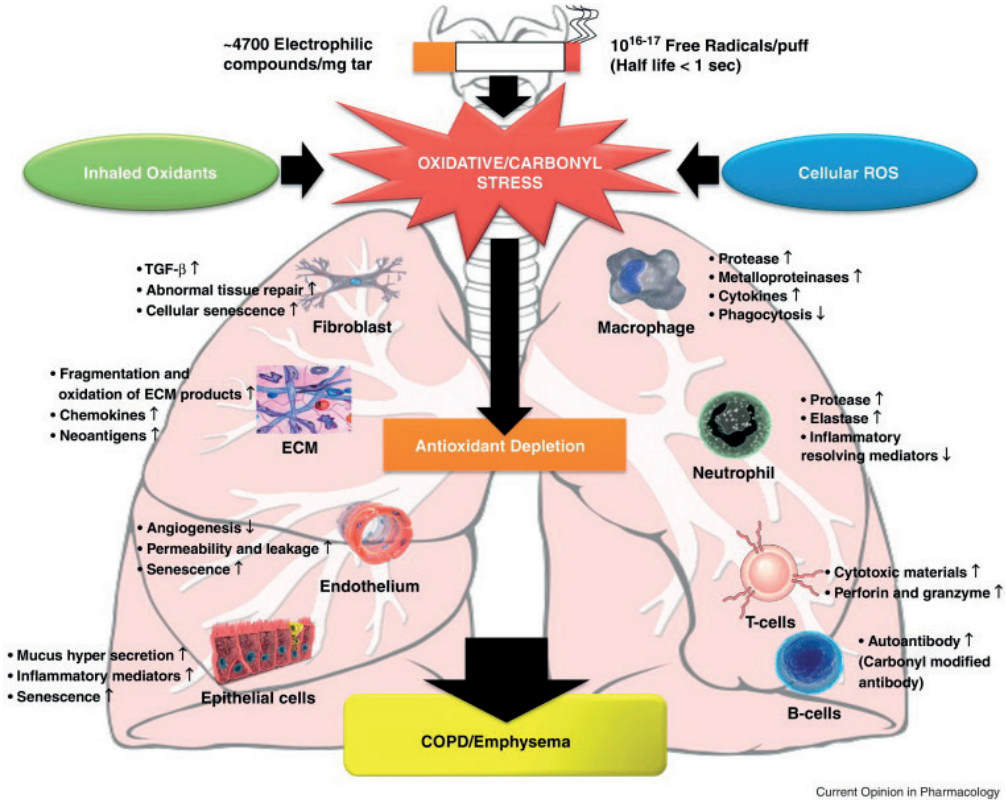


Figure 1: simplified overview of the pathophysiology of COPD.

A COPD exacerbation is clinically defined as an increase in dyspnoea, cough, or sputum purulence with or without symptoms of upper respiratory infection.(15)

Exacerbations of COPD account for more than 1 million hospitalizations annually in Europe and subsequently leading to major healthcare costs.(9) COPD patients also experience a high burden of disease because of pulmonary infections, varying from bacterial colonisation of the airways to CAP. AECOPD are caused in >50% of the cases because of viral or bacterial infections. In fact, pneumonic exacerbations occur in 20–36% of patients with COPD.(16) This highly prevalent morbidity in COPD contributes to the elevated risk for mortality in COPD patients. COPD is the third leading cause of death worldwide and it is estimated that 3 million deaths occur annually because of COPD globally.(17,18) COPD is a risk factor for CAP. (13,16) Three main factors in the pathogenesis of CAP in patients with COPD are a

change in the lung microbiome, abnormal lung immunity and pathogen virulence. Compared with individuals without COPD, those with COPD are estimated to be at a 1.3- to 13.5-fold increased risk of developing CAP. The risk for hospitalization due to CAP is about 50% higher when having COPD.

The heterogeneity in COPD is also illustrated in the differences in experienced morbidity and risk for mortality. Individual patients differ substantially in the frequency of having (recurrent) exacerbations; in having more or fewer pulmonary infections; in having a good or very poor prognosis. Different factors are known to be associated with this morbidity and mortality, such as the severity of airflow limitation. In COPD this is based on the post-bronchodilator value of FEV_1. Worsening of airflow obstruction is associated with an increasing prevalence of exacerbations, hospitalization and risk of death.(8) Other factors associated with survival are for instance the Body Mass Index and exercise tolerance. Subsequently multidimensional indices, such as the BODE index, comprising Body Mass Index, Obstruction of the airflow, Dyspnoea, and Exercise capacity, and the ADO index, comprising Age, Dyspnoea and Obstruction of the airflow, were developed and validated, giving a composite score that is a better predictor of subsequent survival than each single component.(19,20)

It remains difficult however for the individual patient to estimate the risk for subsequent morbidity and mortality. Knowing that your patient is estimated to be at high risk for subsequent early (severe) AECOPD or death is important and may lead to starting advanced care planning in selected patients or starting or adjusting treatment of the COPD. Effective treatment options for COPD are scarce but nowadays, for selected patients, more and more treatment options are being developed.(8)

Traditionally, the backbone of therapy is the use of bronchodilator therapy with or without inhaled corticosteroids. In selected patients, long term oxygen is used and pulmonary rehabilitation programs are available. Nowadays patients are selected for lung volume reduction therapy, Non-Invasive Ventilation for use at night and the use of biologicals as well. Even as important are the preventive strategies. Smoking cessation is of upmost importance to slow down the further decline in lung function. Various vaccination strategies in COPD have been developed to prevent or reduce various infectious diseases such as the seasonal influenza vaccination, the pneumococcus vaccination and more recently the

COVID vaccination. Furthermore, education and COPD self-management are an important backbone in the guidance and treatment of COPD.

All the different (preventive) treatment strategies focus on different treatment goals, such as improving the quality of life, lowering dyspnoea sensation, improvement or preservation of the lung function, improvement of exercise tolerance, prevention of infections and CAP, and lowering the risk of subsequent (severe) exacerbations. These treatment goals will overall lead to a lower burden of disease and in selected cases also to a better survival.

The COMIC study

The COMIC study (Cohort of Mortality and Inflammation in COPD) is a single-centre COPD cohort study from Enschede, the Netherlands. The primary goal of this study was to investigate whether COPD patients with a possible reduced immunity, defined as a low titre after influenza vaccination, had a higher risk for subsequent morbidity and mortality. From December 2005 till April 2010, 795 patients were included with a follow-up period of at least three years. For inclusion in the COMIC study patients had to meet the following criteria; a) a clinical diagnosis of COPD according to the GOLD guidelines; b) current or former smoker; c) age ≥ 40 years; d) no medical condition compromising survival within the follow-up period or serious psychiatric morbidity; e) absence of any other active lung disease (e.g. sarcoidosis); f) no maintenance therapy with antibiotics; g) ability to speak Dutch. Patients were enrolled when visiting the outpatient clinic in stable state (stable state group) or when hospitalized for an acute exacerbation in COPD (AECOPD group). To be included in the AECOPD group (N=172), patients had to be hospitalised for an AECOPD and be able to produce an adequate sputum sample at the day of hospitalisation. To be included in the stable state group (N=623), patients had to meet the following criteria: no use of antibiotic and/or prednisolone 4 weeks prior to enrolment and no exacerbation less than 4 weeks before study entry. All patients were treated according to standard care. In this prospectively followed COPD cohort after 3 years of follow up, approximately 30% of the patients had at least one CAP, 45% had at least one severe AECOPD and more than 25% of the patients died of any cause. This illustrates the severity of this chronic disease and the need to identify the patients at risk for these negative outcomes and the search for markers associated with this morbidity and mortality.

Cardiovascular morbidity

COPD often coexists with other comorbidities such as lung cancer, gastroesophageal reflux disease and osteoporosis. One of the most common and important comorbidity is cardiovascular disease (CVD).(18,21) Cardiovascular complications account for more than 40% of morbidity and mortality in patients with COPD.(22) CVD and COPD share similar risk factors such as ageing, history of cigarette smoking and they frequently coexist. The presence of CVD or cardiovascular conditions (such as heart failure (HF), ischaemic heart disease (IHD) or atrial fibrillation (AF)) increases the risk of frequent exacerbations and mortality.(21,23) On the other hand, COPD exacerbations and lung function decline are associated with increased CV risk and mortality.(23) It is estimated that COPD patients with no history of CVD are 25% more likely to have major CVD events above what their cardiovascular risk profile would predict.(24) In patients with COPD and CVD, the interaction of pathophysiologic processes of the respiratory, cardiac and vascular systems is complex and there is growing evidence for the importance of the underlying local and systemic inflammatory environment in COPD.(25) Systemic low-intensity inflammation, moderate systemic inflammation during exacerbations, and (intermittent) hypoxia, which also increases systemic inflammation, plays a relevant role in the relationship between COPD and cardiovascular disease. Markers of pulmonary inflammation and of systemic inflammation are elevated in patients with stable COPD, and patients with COPD plus CVD have higher blood concentrations of inflammatory markers, such as fibrinogen, interleukin (IL)-6 and IL-8, than those without CVD.(26)

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Besides proven effect on reducing cardiovascular disease by treating hypercholesterolemia, they have other pleiotropic anti-inflammatory effects as well.(27) Human and animal studies indicate that statins have an effect on pulmonary inflammation and remodelling, which is achieved by influencing different pathways such as neutrophil influx, matrix remodelling, apoptosis, oxidant response and mucus production.(28) By lowering the local and systemic inflammation and preventing further remodelling this could be a potential efficacious therapy in patients with COPD, for instance in reducing exacerbation risk, CAP and mortality. In **chapter 2** of this thesis, we will study the association of statin use in COPD patients in the COMIC study with subsequent morbidity and mortality.

Biomarkers

A biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.(29) Biomarkers are increasingly being explored in COPD for various reasons such as patient characterization, predicting treatment response, quantifying severity of disease and for determining the prognosis of the disease. Translation of these biomarkers to clinical practise is challenging because of various reasons such as differences in analytical methodology and difficulty in (external) validation.(30) Various biomarkers have been studied. A well-studied biomarker in COPD is fibrinogen. Fibrinogen is a soluble glycoprotein that is converted to fibrin by thrombin during blood clot formation. Fibrinogen is a major acute-phase reactant and its synthesis is significantly up-regulated in response to inflammatory mediators, with IL-6 being an important cytokine influencing fibrinogen production by the liver. As a result of up-regulation by inflammatory mediators, elevated concentrations of plasma fibrinogen are observed in patients with several chronic diseases that have inflammation as an underlying component. Higher baseline fibrinogen level is associated with a higher incidence of mortality and exacerbations of COPD and for this it is an FDA approved biomarker.(31)

Another important biomarker is MR-proADM, the biologically-inactive midregional fragment of the adrenomedullin prohormone (ADM).(32) The pluripotent regulatory peptide ADM is widely expressed throughout the body and acts as both a hormone and a cytokine.(33) Adrenomedullin mediates pro-angiogenic and pro-inflammatory cytokines in asthma and COPD.(34) ADM can be upregulated by hypoxia, inflammation, bacterial products and shear stress and with its widespread expression in pulmonary tissue, among which endothelial cells (including type 2 pneumocytes), smooth muscle cells, neurons and immune cells, elevated ADM levels can often be seen in (end stage) pulmonary diseases such as COPD and in CAP.(35–37) Furthermore, it is an independent predictor for negative outcome in cardiovascular disease which, as stated before, often coexists with COPD.(38) In **chapter 3** of this thesis we studied in a pooled analysis of two large European prospective observational COPD cohort studies the association of MR-proADM in stable state COPD with subsequent severe AECOPD and CAP. In **chapter 4** we studied for the first time the combination of the biomarkers MR-proADM and

fibrinogen, both measured in stable state, in predicting mortality in COPD. The aim of this study was to confirm in a large well-defined population of COPD patients whether fibrinogen can predict mortality and whether a combination with the biomarker MR-proADM can increase prognostic accuracy.

Maybe the most promising biomarker in COPD at this moment is the blood eosinophil. The blood eosinophil count is correlated to the sputum eosinophil levels and can serve as a surrogate marker for eosinophilic inflammation in the lung.(39) Where initially it was thought that eosinophilic inflammation mainly plays a role in asthma, more and more it has become clear that eosinophilic inflammation is also relevant in a significant subset of the COPD population.(39,40) A relevant subgroup (20-40%) has an eosinophilic phenotype probably associated with underlying type 2 inflammation as can also be seen in patients with asthma.(41) However, the exact pathobiology is not clear. In stable state, elevated blood eosinophil count predicts higher inhaled corticosteroid (ICS) responsiveness in reducing future AECOPD.(40,42) It is suggested that this eosinophil inflammation may be associated with future AECOPD and CAP.(43) If this is also true when measured at AECOPD is not well known. In **chapter 5** we studied the association of the blood eosinophil count measured at severe AECOPD, using various cut-off levels and eosinophil count as a continuous marker, with subsequent morbidity and mortality. This eosinophil inflammation at AECOPD is also associated with steroid responsiveness and different eosinophil-guided strategies for systemic steroid use in AECOPD have been explored.(44,45) This will have major implications for COPD self-management interventions. Furthermore, in stable state COPD, eosinophil levels seem relatively stable over time (46) but on whether this is also true in AECOPD is not known. Therefore first, in **chapter 6** we investigated the stability of the serial measurement of the blood eosinophil count in two subsequent severe AECOPD. Next, in **chapter 7** we propose the potential for the introduction for personalised and biomarker-guided COPD self-treatment approaches and explore the hurdles to be overcome.

Immune status

COPD is associated with a reduced immunity. As mentioned earlier, chronic lung inflammation plays a critical role in COPD leading to extensive lung damage but it also leads to an impaired immunity to respiratory infections. COPD is associated with lung-specific and systemic immune dysfunction that facilitate disease

exacerbations.(47) Approximately 70% of COPD exacerbations are infectious in origin, with respiratory viruses identified in approximately 30% of cases.(48) In severe AECOPD, after rhinovirus, the most common virus detected is the influenza virus.(49) Yearly influenza vaccination is recommended in COPD patients. In general, these vaccinations have proven to be effective in reducing symptoms, exacerbations, hospitalization and even death. In the individual patient, less is known about the immune response to the influenza vaccination, nor whether this immune response can be used to define the immune status. We hypothesized that the degree of immune response to the influenza vaccination is a surrogate marker to indicate the quality of the COPD patient's immune status. In **chapter 8** we studied whether COPD patients with a possible reduced immunity, defined as a low titre after influenza vaccination, was associated with a higher morbidity and mortality risk.

Outline of the thesis

In **chapter 2** we investigated statin use in the COMIC study and its association with morbidity and mortality.

In **chapter 3** in a pooled analysis of two large European prospective observational COPD cohort studies, we studied the association of stable state MR-proADM with subsequent severe AECOPD and with CAP.

In **chapter 4** we evaluated stable state MR-pro-ADM and Fibrinogen as predictors for mortality in COPD. Furthermore, we determined whether combining both biomarkers could increase the accuracy in one-year mortality prediction.

In **chapter 5** we investigated whether the blood eosinophil count measured at severe AECOPD was associated with subsequent morbidity and mortality.

In **chapter 6** we determined the eosinophil categorisation stability at two subsequent severe AECOPD. We used different cut-off levels for the eosinophil count to establish this stability.

In **chapter 7** we propose the potential for the introduction of personalised and biomarker-guided COPD self-treatment approaches.

In **chapter 8** we provided the primary outcomes of the prospective COMIC study. We investigate whether COPD patients with a possible reduced immunity, defined as a low titre after influenza vaccination, had a higher risk for subsequent morbidity and mortality.

In **chapter 9** the general discussion outlines and discusses the findings of the presented research in a broader context and recommendations for further research will be given.

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Statins and morbidity and mortality in COPD in the COMIC study: a prospective COPD cohort study

Emanuel Citgez; Job van der Palen; Kirsten Koehorst-ter Huurne; Kris Movig; Paul van der Valk; Marjolein Brusse-Keizer

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Abstract

Background: Both chronic inflammation and cardiovascular comorbidity play an important role in the morbidity and mortality in patients with chronic obstructive pulmonary disease (COPD). Statins could be a potential adjunct therapy. The additional effects of statins in COPD are however still under discussion. The aim of this study is to further investigate the association of statin use with clinical outcomes in a well-described COPD cohort.

Methods: 795 patients of the Cohort of Mortality and Inflammation in COPD (COMIC) study were divided into statin users or not. Statin use was defined as having a statin for at least 90 consecutive days after inclusion. Outcome parameters were 3-year survival, based on all-cause mortality, time till first hospitalisation for an acute exacerbation of COPD (AECOPD) and time till first community acquired pneumonia (CAP). A sensitivity analysis was performed without patients who started a statin 3 months or more after inclusion to exclude immortal time bias.

Results: Statin use resulted in a better overall survival (corrected hazard ratio 0.70 (95% CI, 0.51 - 0.96) in multivariate analysis), but in the sensitivity analysis this association disappeared. Statin use was not associated with time till first hospitalisation for an AECOPD (cHR 0.95, 95% CI, 0.74 - 1.22) or time till first CAP (cHR 1.1, 95% CI, 0.83 - 1.47).

Conclusions: In the COMIC study statin use is not associated with a reduced risk of all-cause mortality, time till first hospitalisation for an AECOPD or time till first CAP in patients with COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic disease accompanied with increased morbidity and mortality. It is estimated that COPD is the third leading cause of death worldwide.(1) COPD is characterised by acute exacerbations (AECOPD) which can accelerate the already existing gradual decline of lung function and increase the risk of death.(2) AECOPD are associated with increased risk of hospitalisations, lower quality of life and increased healthcare costs. Effective therapy or interventions to prevent this morbidity and mortality include for instance inhalation medication, smoking cessation, supplemental oxygen therapy, pulmonary rehabilitation and lung transplantation.

Underlying chronic and systemic inflammation play an important role in the pathophysiology of COPD and its progression.(3;4) The majority of COPD patients have associated comorbidity which is partially the cause of the increased mortality seen in COPD.(5;6) The high concurrence of COPD with cardiovascular morbidity is mainly because of common risk factors.(2) There is a rationale for a treatment that has a positive effect on this ongoing (systemic) inflammation as well as on the, sometimes not yet recognised, cardiovascular morbidity in patients with COPD.

Statins are inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Besides proven effect on reducing cardiovascular disease by treating hypercholesterolemia, they have other pleiotropic anti-inflammatory effects as well.(7;8) In the lung statins have effect on bronchial remodelling, emphysema development and recruitment of inflammatory cells. Systemically effects are observed on various biomarkers and cytokines such as high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumour necrosis factor alpha (TNF-alpha).(9)

Various mainly retrospective studies and reviews have been performed to establish the effect of statin use in patients with COPD.(10-20) These studies reported a reduced mortality(14;20;21), reduced exacerbations with or without hospitalisation(13;15;16), reduced lung function decline(17;19), and lower levels of (Hs)CRP(18) as a marker for systemic inflammation. Even improvement of pulmonary hypertension and dyspnea during exercise by the use of a statin in COPD patients with pulmonary hypertension is observed.(22) Nevertheless the first large randomized trial, the STATCOPE study, showed no beneficial effects of statin use in reducing the frequency or severity of exacerbations in patients with COPD.(23)

The role of statins on outcome in patients with COPD is therefore still not defined.

Differences in study design, immortal time bias, lack of correction for confounders, and unclear definition and measurement of statin use are possible explanations for the difference in outcome. The large COMIC cohort (Cohort of Mortality and Inflammation in COPD) of well-defined COPD patients whom we followed prospectively for more than three years offers a well-documented medication history and therapy adherence during the study including statins. The study is powered for mortality and with available lung function, GOLD stage, smoking status and other predictors such as the ADO score and BOD index the aim of this analysis is to establish the independent association of statin use with morbidity and mortality in these COPD patients.

Methods

Settings and study population

This study was part of the COMIC study, a single-centre cohort study from Enschede, the Netherlands. From December 2005 till April 2010, 795 patients were included with a follow-up period of at least three years. The COMIC study was approved by the hospital's medical ethical committee. All patients provided written informed consent. The COMIC study started before the introduction of trial registries. The study was initiated to investigate the relationship between the immune response after influenza vaccination in COPD patients and mortality. Different papers concerning COPD have been published with this COMIC study. (24-26)

For inclusion in the COMIC study patients had to meet the following criteria; a) a clinical diagnosis of COPD according to the GOLD guidelines; b) current or former smoker; c) age ≥ 40 years; d) no medical condition compromising survival within the follow-up period or serious psychiatric morbidity; e) absence of any other active lung disease (e.g. sarcoidosis); f) no maintenance therapy with antibiotics; g) ability to speak Dutch. Patients were enrolled when visiting the outpatient clinic in stable state (stable state group) or when hospitalised for an acute exacerbation in COPD (AECOPD group). To be included in the AECOPD group, patients had to be hospitalised for an AECOPD and be able to produce an adequate sputum sample at the day of hospitalisation. To be included in the stable state group patients had to meet the following criteria: no use of antibiotic and/or prednisolon 4 weeks

prior to enrolment and no exacerbation less than 4 weeks before study entry. All patients were treated according to standard care.

Definition of statin use

Statin use was recorded from patients' pharmacy records. Since chronic medication in the Netherlands usually is prescribed for a period of three months, statin use was defined as having a statin for at least 90 consecutive days after inclusion in the cohort. When a patient was on a statin at inclusion but follow up was less than 90 days, the patient was defined as a statin user when he or she used the statin for at least 90 consecutive days prior to inclusion. Patients who started a statin after inclusion for a period less than 90 days were not defined as a statin user.

Outcomes

The primary outcome parameter was 3-year survival, based on all-cause mortality. Date of death was verified from the municipal administration.

Morbidity was defined as time till first hospitalisation for an AECOPD and time till first community acquired pneumonia (CAP). AECOPD was defined as an acute negative change from baseline, reported by the patient, in dyspnea and/or sputum volume and/or colour of sputum (yellowish or greenish sputum) and/or cough, which may warrant additional treatment of prednisolone with or without antibiotics by a physician in a patient with underlying COPD. Pneumonia was defined as an acute respiratory tract illness associated with radiographic shadowing on a chest radiograph consistent with infection which was neither pre-existing nor of any other known cause. All X-rays were double read by a radiologist and a chest physician. In case of doubtful shadows in the report, the X-ray was presented to another chest physician for final judgment.

Demographic data including data on common co-morbidities like myocardial infarction, congestive heart failure and diabetes mellitus were collected from medical records. At baseline spirometry was performed according to standardised guidelines(27) and smoking status was determined by the Vlagtwedde questionnaire.(28) Patients completed the modified Medical Research Council dyspnea questionnaire (mMRC).(29) The BOD comprises BODE without exercise capacity measurement.(30) The components were scored according to the same cut-offs as in BODE.(31) The BOD therefore ranges from 0 to 7.(32) The original and updated ADO score ranges, in increasing severity, from respectively 0 to 10 and from 0 to 14 points.(33;34) All measurements were performed in stable state.

Therapy adherence of statin use was defined as follows: theoretical duration of exposure was calculated using information on dispensing date, total supply, and dosage regimen. We computed the total number of days for which patients had collected medication during follow-up and divided this by the total number of days between the first and last medication collection during follow up plus the day's supply of the last refill.(35) This was expressed as a percentage and adherence was deemed good if it was ≥75%.

Statistical analysis

Continuous variables are expressed as mean (± standard deviation (SD)) or as median (interquartile range (IQR)), and categorical variables as counts (percentages). Time from inclusion to event (all-cause mortality, first hospitalisation for an AECOPD, first CAP) was analysed by Kaplan-Meier survival curves and compared between statin and non-statin users with log rank tests. Univariate and multivariate Cox proportional hazard regression models were used to establish the association of statin use with survival, time till first hospitalisation for an AECOPD and time till first CAP. First we studied in univariate analyses the association between statin use and potential confounders such as lung function parameters, GOLD stage, BMI, comorbidity, and sex. Next, we studied in multivariate Cox proportional hazard regression models the association between the outcome parameters (mortality, time till first hospitalisation for an AECOPD, time till first CAP) on the one hand and statin use on the other hand, and added all potential confounders (i.e. variables that were associated with statin use and the outcome parameter of the specific models with a corresponding p-value <0.10). We started the multivariate model with all potential confounders. Variables with the highest p-value were eliminated step by step until the fit of the model decreased significantly, based on -2 log likelihood. All tests were two-sided and a p-value of 0·05 was considered statistically significant. Data were analysed using SPSS, version 22(SPSS Inc. Chicago IL, USA)

Sensitivity analysis

By including patients who started a statin 3 months or more after inclusion for a period of at least 90 consecutive days there is a possibility of introducing immortal time bias. Therefore a sensitivity analysis was performed where these patients were excluded from analysis to prevent the immortal time bias.(36)

Results

Baseline characteristics

Our COMIC cohort included 795 patients. 623 patients (78%) were enrolled when visiting the outpatient clinic in stable state and 172 patients (22%) were enrolled when hospitalized for an acute exacerbation in COPD. 253 patients (32%) were defined as statin users. Of those 62 patients (25%) started a statin ≥ 3 months after inclusion for a period of at least 90 consecutive days. The baseline characteristics of these two groups are displayed in Table 1.

Table 1 Baseline characteristics of 795 individuals with COPD

	Statins N=253	No Statins N=542	p-value
Mean age at enrolment in years (SD)	68.2(8.4)	67.6(10.5)	0.391
Male (number (%))	169(66.8)	317(58.5)	0.025
Current smoker (number (%))	59(23.3)	153(28.2)	0.145
Mean BMI at enrolment (SD)	28.5(5.7)	26.5(5.2)	0.000
GOLD stage (number (%))			0.024
I	28 (11.1)	42 (7.8)	
II	114 (45.2)	209 (38.8)	
III	93 (36.9)	223 (41.4)	
IV	17 (6.7)	65 (12.1)	
Lung function			
Mean FEV_1 in liters (SD)	1.5(0.6)	1.4(0.6)	0.007
Mean FEV_1 % predicted (SD)	54.6(18.7)	50.9(19.6)	0.012
Mean FEV_1/VC % predicted (SD)	46.9(13.7)	43.4(13.4)	0.001
Comorbidities (number (%))			
Congestive heart failure	63 (24.9)	76 (14.0)	0.000
Myocardial infarction	22(8.7)	8 (1.5)	0.000
Hypertension	19(7,5)	21(3,9)	0.029
Diabetes mellitus	37 (14.6)	15 (2.8)	0.000
Mean mMRC score (SD)	1.8(1.2)	1.7(1.3)	0.500
Mean ADO score (SD)	4.2(1.6)	4.1(2.0)	0.745
Mean BOD score (SD)	2.3(1.6)	2.5(1.8)	0.061
Median Packyears	35.4	35.0	0.920

Statin users were more often male and had significantly more cardiovascular morbidity, a higher BMI and a better lung function. Eighty five percent of the statin users had a good therapy adherence to statins defined by a therapy adherence percentage ≥75%.

Survival

Median follow up time was 36 (30 - 36) months. The cumulative survival after 1, 2 and 3 years is displayed in Table 2. Statin users had a significantly better overall survival compared with the non-statin users (log-rank: p=0.028) with a hazard ratio of 0.72 (95% CI, 0.53 - 0.97) in univariate analysis (Figure 1).

Table 2 Cumulative survival at 1, 2 and 3 years

	Statins (Standard Error)	No Statins (Standard Error)
1 y	0.95 (0.01)	0.87 (0.02)
2 y	0.86 (0.02)	0.78 (0.02)
3 y	0.78 (0.02)	0.71 (0.02)

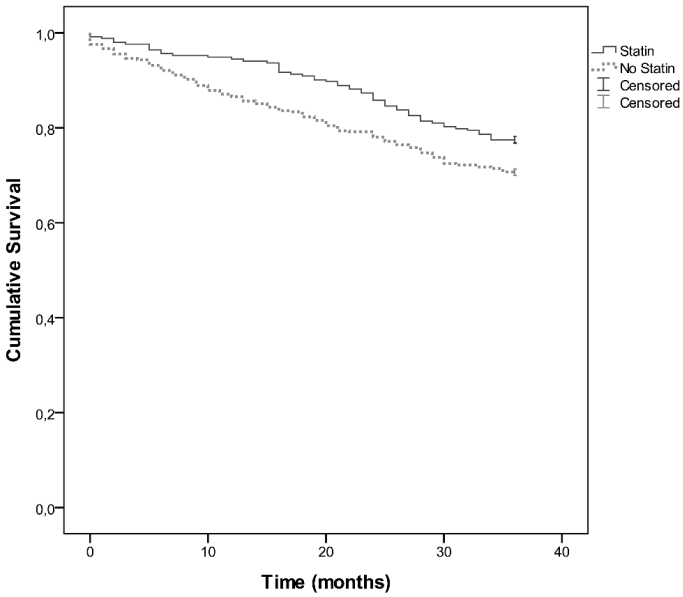

Figure 1 Kaplan–Meier Survival Curve for statin use in patients with COPD.

In a multivariate Cox regression analysis statin use, corrected for the confounders sex, BMI, heart failure and FEV1 (litres), resulted in a better survival with a hazard ratio of 0.70 (95% CI, 0.51 - 0.96).

Morbidity

During the three years of follow up 344 patients (43%) had at least one hospitalisation for an AECOPD and 220 patients (28%) had at least one diagnosed CAP. In a univariate and multivariate Cox regression analysis there were no significant differences between the statin users and non-statin users in time till first hospitalisation for an AECOPD (log-rank: p=0.67) with a cHR of 0.95 (95% CI, 0.74 - 1.22)(Figure 2) and time till first CAP (log-rank: p=0.87) with a cHR of 1.1 (95% CI, 0.83 - 1.47)(Figure 3).

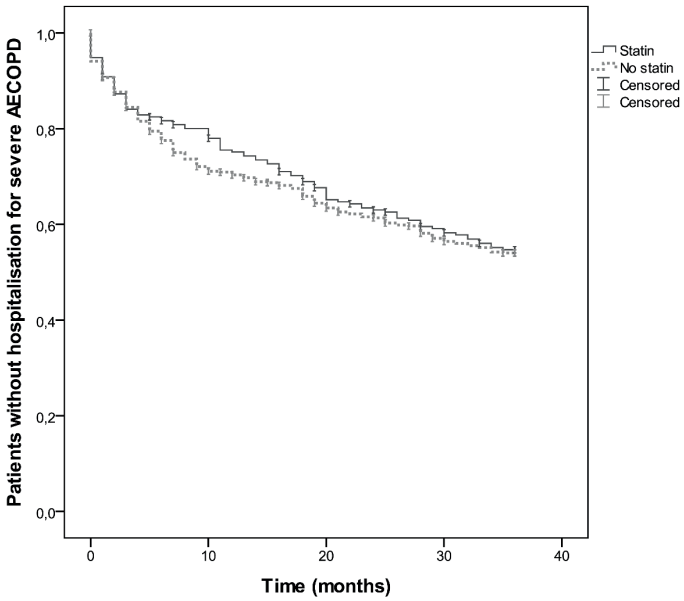

Figure 2 Kaplan–Meier Survival Curve for association of statin use with time till first hospitalization for an AECOPD.

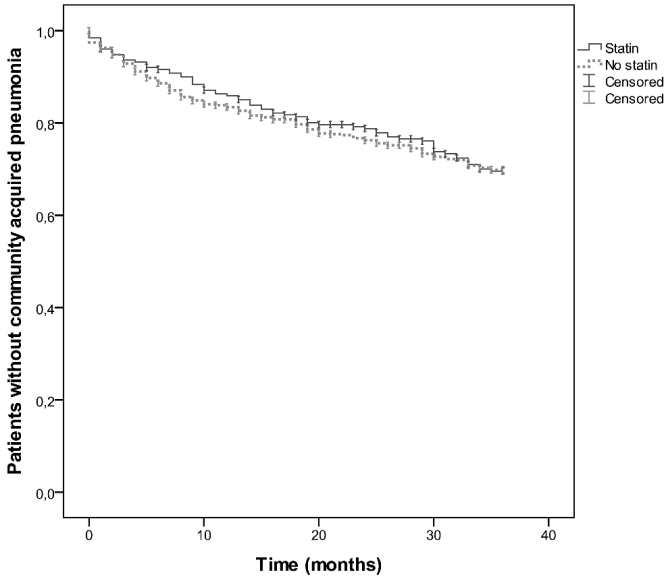

Figure 3 Kaplan–Meier Survival Curve for association of statin use with time till first CAP.

Sensitivity analysis

For the sensitivity analysis the 62 patients who started a statin ≥ 3 months after inclusion for at least 90 consecutive days where excluded from the analysis. With the remaining 733 patients no significant difference was seen in overall survival between the statin and non-statin users. Multivariate Cox regression analysis shows a hazard ratio of 0.82 (95% CI, 0.59 - 1.16, p=0.26).

Discussion

In our large prospective cohort with patients with well-defined COPD, the use of a statin initially seemed to be associated with reduced mortality. However, when corrected for immortal time bias, statin use was not associated with all-cause mortality despite the different anti-inflammatory effects ascribed to statins. Statin use also was not associated with time till first hospitalisation for an AECOPD and time till first CAP.

This is in accordance with the first large randomized controlled trial by Criner et al.(23) They randomly assigned 885 patients with COPD to either daily simvastatin

40mg or placebo. No differences between exacerbation rate or time till first exacerbation were observed during follow up between the two groups and as part of the secondary outcome no difference in mortality was observed as well.

Human and animal studies indicate that statins have an effect on pulmonary inflammation and remodelling.(9) This effect is achieved by influencing different pathways such as neutrophil influx, matrix remodelling, apoptosis, oxidant response and mucus production. They modulate cytokine production such as TNF-alpha, IL-6 and IL-8 which also could reduce serum CRP and subsequently have an effect on systemic inflammation. By lowering the local and systemic inflammation and preventing further remodelling this could be a potential efficacious therapy in patients with COPD.

Subsequent and mainly retrospective and observational studies did show beneficial effects of statins on exacerbations and mortality. In a retrospective nested case-control study Wang et al. observed a 30% decreased risk of COPD exacerbations with hospitalisation in patients with COPD using a statin.(15) In a nested case-control study by Mancini et al. a risk reduction for hospitalization for COPD was seen with use of statin with a RR of 0.72.(14) As part of the Rotterdam study, Lahousse et al. demonstrated a beneficial effect of long term statin use on all-cause mortality in COPD.(18) Soyseth et al. demonstrated in a retrospective cohort design a crude relative mortality ratio among statin users versus nonusers of 0.58 (95%CI 0.39–0.84).(21)

Before the sensitivity analysis, our study demonstrates a positive association with all-cause mortality as well. However, after the sensitivity analysis, this supposed favourable association disappeared. An important explanation for this finding is immortal time bias. The immortal time consists of the time between inclusion and the moment of first starting the statin. The study subject had to survive this time before exposure to the statin could occur. In this period the patient is classified as a statin user while in fact he or she is not (yet). With the immortal time bias a non-existing survival benefit is introduced.(36) Besides this bias a further examination of the 62 patients excluded for the sensitivity analysis shows that for seven of them the reason for starting a statin was a myocardial infarction, which was treated with percutaneous coronary intervention and/or pharmacological treatment. These interventions have a positive effect on mortality and before the sensitivity analysis this positive effect could wrongly be attributed to the use of statins.

So, with our study we have gathered additional evidence that statin use is not associated with clinical outcome in COPD. Other explanations for the difference in outcome between the previous studies and the recent STATCOPE study and our findings could be the following: First, the potential beneficial effects of statins may only be seen in some subsets or phenotypes of COPD patients, such as those with coexisting cardiovascular disease or patients with systemic inflammation. In a nested case control study by Ingebrigtsen et al. statin use was indeed associated with reduced odds of exacerbations in individuals with COPD but not in the most severe COPD patients without cardiovascular comorbidity.(13) These patients were excluded in the STATCOPE study. In our study however, cardiovascular comorbidity was not an exclusion criterion and 27% of the included patients had one or more cardiovascular comorbidities and nevertheless, no favourable association of statin use was observed.

Lahousse et al. demonstrated that when stratified to the level of systemic inflammation, long term statin use was associated with a significantly reduced all-cause mortality if hsCRP level was > 3 mg/l.(18) We have not measured the hsCRP and can therefore not make this stratification.

Second, in observational studies a healthy user effect or non-healthy non user effect are possible as well as the risk of bias other than the immortal time bias, such as confounding bias. Besides in some studies COPD patients are included because of ICD codes in various large registries, while often no lung function data to confirm COPD diagnosis is present. Moreover, different definitions of statin use are used where sometimes having one prescription of a statin is enough to be defined a statin user. This makes it difficult to compare the studies with each other and to make a clear judgement of the clinical effects of statin in COPD.

The strength of our study is that we have a heterogeneous well-defined unselected COPD population with accurate lung function measurements and comorbid conditions, whom we followed prospectively for a minimum of three years. With sufficient events during follow up the power of the study was large enough to identify relevant differences in the outcome parameters between the COPD patients who used a statin or not. In addition we have a well-defined definition for statin use and for the sensitivity analysis we excluded patients who started a statin late during follow up to exclude immortal time bias. Removing the 62 patients to

exclude immortal time bias has reduced our sample size, but, more importantly, reduced the HR in size as well. If we would want to achieve a study power of 80% with this HR, we would need to study 549 patients on statins and 1559 patients without statins. Therefore, we feel that the lack of statistical significance is not so much caused by the lower number of patients, but due to the fact that, when excluding immortal time bias, the protective effect of statins is reduced. With the high percentage of therapy adherence of statins a possible association could be attributed to the actual use of statins. Although pharmacy records give an indication of the amount of dispensed medication and not the actually amount of medication taken we believe that with the three years of follow up there will not be a large difference between them.

A limitation of our study is that statin use was not randomised so there is a risk of selection bias. In the baseline characteristics one can see that the statin group, as expected, has significantly more cardiovascular morbidity. Surprisingly, they have a slightly but significantly better lung function. Nevertheless, corrected for these and other potential confounders statin use in the multivariate Cox-regression model still was not associated with mortality and morbidity.

A healthy user effect implies the fact that using a statin could be a marker for a relatively better health that may result in better overall survival. However in our study because of the significantly more prevalent cardiovascular comorbidity in the statin group there is no indication for a healthy user effect. In the non-statin group there were 8 patients with comorbid myocardial infarction but surprisingly have not been prescribed a statin. The reason therefore is unknown. This could imply a bias of non-healthy non-user effect in the analysis although the number of 8 patients is small.

In conclusion, statin use seemed to be associated with a reduced risk of all-cause mortality in patients with COPD, but in the sensitivity analysis this association disappeared. Statin use also was not associated with time till first hospitalisation for an AECOPD or time till first CAP in patients with COPD. Until more new definitive randomised controlled trials determine otherwise, there is no indication to start a statin in all patients with COPD. Prescribing statins to patients with COPD should therefore be in accordance with nationwide guidelines.

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Stable state MR-proadrenomedullin is associated with severe exacerbations in COPD

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Abstract

Background: Elevated levels of Midrange-proadrenomedullin (MR-proADM) is associated with worse outcome in different diseases among which COPD. The association of stable state MR-proADM with severe exacerbations of COPD requiring hospitalisation (severe AECOPD), or with community acquired pneumonia (CAP) in COPD patients has not been studied yet.

Aims and objectives: The aim of this study was to evaluate the association of stable state MR-proADM with severe AECOPD and CAP in COPD patients.

Methods: This study pooled data of 1285 patients from the COMIC and PROMISE-COPD cohort studies. Time till first severe AECOPD was compared between patients with high (≥0.87nmol/l) or low (<0.87 nmol/l) levels of plasma MR-proADM in stable state as previously defined. For time till first CAP, only COMIC data (n=795) was available.

Results: COPD patients with high level stable state MR-proADM had a significantly higher risk for a severe AECOPD compared with those with low level MR-proADM with a corrected hazard ratio (cHR) of 1.30 (95% CI, 1.01 - 1.68). Patients with high level stable state MR-proADM had a significantly higher risk for a CAP compared with COPD patients with low level MR-proADM in univariate analysis (HR 1.93; 95% CI, 1.24 - 3.01) but, after correction for age, lung function and previous AECOPD, the association was no longer significant (cHR 1.10; 95% CI, 0.68 - 1.80).

Conclusions: Stable state high level MR-proADM in COPD patients is associated with severe AECOPD but not with CAP.

Introduction

Chronic obstructive pulmonary disease (COPD) is a complex and heterogeneous disease accompanied with increased morbidity and mortality.(1;2) Underlying chronic and systemic inflammation play an important role in the pathophysiology of COPD, its progression and its associated comorbidity.(3-6) It is estimated that COPD is the third leading cause of death worldwide.(7) COPD is characterised by acute exacerbations (AECOPD) which can accelerate the already existing gradual decline in lung function and increase the risk of death.(8;9) Furthermore, AECOPD are associated with increased risk of hospitalisation, lower quality of life and increased healthcare costs.(8;10) Effective therapy or interventions to prevent this morbidity and mortality include for instance inhalation medication, smoking cessation, supplemental oxygen therapy, pulmonary rehabilitation and lung transplantation.(11)

For adequate prognostication we need more insight in the pathophysiology of this complex and heterogeneous disease. Different biomarkers have been evaluated for this purpose. One of these biomarkers is MR-proADM, the biologically-inactive midregional fragment of the adrenomedullin prohormone (ADM).(12) The pluripotent regulatory peptide ADM is widely expressed throughout the body and acts as both a hormone and a cytokine.(13) ADM can be upregulated by hypoxia, inflammation, bacterial products and shear stress and with its widespread expression in pulmonary tissue, among which endothelial cells (including type 2 pneumocytes), smooth muscle cells, neurons and immune cells, elevated ADM levels can often be seen in (end stage) pulmonary diseases such as COPD and community acquired pneumonia (CAP).(14-16) ADM potentially has comprehensive effects such as vasodilatory, diuretic, anti-inflammatory and antimicrobial. In preclinical and animal models ADM has been shown to reduce hypoxic pulmonary vascular remodelling, inhibit bronchoconstriction, and downregulate pro-inflammatory factors.(17) Therefore, ADM has compensatory and regulatory capacities to achieve homeostasis.

Despite these beneficial and protective effects the upregulation of ADM should be interpreted as a need for these compensatory/regulatory effects of the underlying severity of the illness at that moment.(15) Therefore, although having these beneficial and protective effects, elevated MR-proADM is associated with worse clinical outcome in different diseases such as sepsis, heart failure, myocardial infarction, and CAP.(17;18) Not surprisingly, in COPD elevated MR-proADM is

associated with worse outcome as well and MR-proADM has even been evaluated as being an independent predictor for survival in COPD.(19;20)

CAP and severe AECOPD resulting in a hospitalisation are among the most important factors contributing to the morbidity in COPD and are associated with a high burden of disease and low quality of life.(21) While elevated MR-proADM measured during CAP in the general population and during an exacerbation in COPD are both associated with worse outcome, it would be interesting to study whether already elevated levels in stable state in COPD patients could be a marker for the approach of this morbidity.(18;22) The association of stable state MR-proADM with the occurrence of CAP and AECOPD has however never been studied up till now.

The aim of this study was to evaluate the association of stable state MR-proADM with morbidity in COPD patients, defined as risk of severe AECOPD and CAP. To assess the association we used data of two large European prospective observational cohort studies of patients with COPD in stable state, the COMIC and the PROMISE-COPD study.

Methods

Settings and study population

For this study we performed a pooled analysis of individual patient data of two large European prospective observational COPD cohort studies, the COMIC study (Cohort of Mortality and Inflammation in COPD) and the PROMISE-COPD study (PRedicting Outcome using systemic Markers In Severe Exacerbations of Chronic Obstructive Pulmonary Disease). The COMIC study was approved by the hospital's medical ethical committee (METC Twente, ref: P05-49). The PROMISE-COPD study was approved by the Ethics Committee of Basel (Ethikkommission Beider Basel, ref: EKBB 295/07). Detailed inclusion and exclusion criteria of both studies are included in the supplemental material.

Outcomes

The outcome parameters were time till first hospitalisation for an AECOPD and time till first CAP. For time till first CAP only data from patients included in the COMIC study were available. AECOPD was defined as an acute negative change from baseline, reported by the patient, in dyspnoea and/or sputum volume and/or

colour of sputum (yellowish or greenish sputum) and/or cough, which may warrant additional treatment of prednisolone with or without antibiotics by a physician in a patient with underlying COPD. Pneumonia was defined as an acute respiratory tract illness associated with radiographic shadowing on a chest radiograph consistent with infection which was neither pre-existing nor of any other known cause.(23) All X-rays were double read by a radiologist and a chest physician. In case of doubtful shadows in the report, the X-ray was presented to another chest physician for final judgment.

MR-proADM

Plasma samples of MR-proADM were obtained at stable state and levels were measured with an automated sandwich immunoassay using a time-resolved amplified cryptate emission technology (TRACE).(24) Stable state is defined as the moment in which no exacerbation occurred and no prednisolone or antibiotics were used for a minimum of 4 weeks before the blood sample was taken. MR-proADM level was dichotomised as high ($\geq$0.87nmol/l) or low level (<0.87 nmol/l) as defined in an earlier study with the pooled cohorts.(19)

Statistical analysis

Continuous variables are expressed as mean (± standard deviation (SD)) or as median (interquartile range (IQR)), and categorical variables as counts (percentages). Time from inclusion to event (first hospitalisation for a severe AECOPD, first CAP) was analysed by Kaplan-Meier survival curves and compared between COPD patients with high ($\geq$0.87nmol/l) or low (<0.87 nmol/l) level of MR-proADM with log rank tests. Univariate and multivariate Cox proportional hazard regression models were used to establish the association of MR-proADM with time till first hospitalisation for an AECOPD and time till first CAP. First we studied in univariate analyses the association between MR-proADM and potential confounders within the baseline characteristics such as lung function parameters, previous AECOPD in the year before inclusion, GOLD stage, body mass index (BMI), comorbidity, and sex. Next, we studied in multivariate Cox proportional hazard regression models the association between the outcome parameters (time till first hospitalisation for an AECOPD, time till first CAP) on the one hand and MR-proADM level on the other hand, and added all confounders (i.e. variables that were associated with MR-proADM and the outcome parameter of the specific models with a corresponding p-value <0.10). We started the multivariate model with all confounders. Variables with the highest p-value were eliminated step by step until the fit of the model decreased significantly, based on -2 log likelihood. All tests

were two-sided and a p-value of 0.05 was considered statistically significant. Data were analysed using SPSS, version 22(SPSS Inc. Chicago IL, USA)

Results

Baseline characteristics

In 1285 out of 1433 patients from both studies MR-proADM was measured in stable state and these patients were used for the analyses. The baseline characteristics of these patients are displayed in Table 1, stratified for both studies. Median follow up times of the COMIC and PROMISE-COPD study were respectively 915 (824-1068) and 725 (421-764) days. 321 (25%) patients were defined as having a high level of stable state MR-proADM. The cumulative proportion of patients with at least one severe AECOPD in the pooled analysis and at least one CAP in the COMIC study after 1, 2 and 3 years are displayed in the Tables 2 and 3 respectively, stratified for low and high level of stable state MR-proADM.

Table 1 Baseline characteristics of 1285 individuals with stable COPD included in this analysis

	COMIC N= 671	PROMISE-COPD N= 614	p-value
Mean age at enrolment in years (SD)	67.3 (9.5)	67.0 (9.5)	0.576
Male (number (%))	404 (60.2)	432 (70.4)	<0.001
Current smoker (number (%))	170 (25.3)	204 (33.4)	0.002
Mean Pack-years (SD)[1]	37.7 (22.9)	50.8 (29.8)	<0.001
Mean BMI at enrolment (SD)[2]	27.4 (5.4)	26.0 (5.5)	<0.001
Lung function[3]			
Mean FEV_1 in litres (SD)	1.5 (0.6)	1.3 (0.5)	0.001
Mean FEV_1 % predicted (SD)	53.6 (19.1)	49.4 (16.7)	<0.001
GOLD stage (number (%))[4]			<0.001
I	63 (9.4)	16 (2.6)	
II	299 (44.6)	280 (45.8)	
III	252 (37.6)	214(35.0)	
IV	56 (8.4)	101(16.5)	
Mean mMRC score (SD)[5]	1.8 (1.3)	1.7 (1.2)	0.776
Mean BOD score (SD)[6]	2.4 (1.8)	2.6 (1.8)	0.163
Mean ADO score (SD)[7]	4.1 (1.8)	4.1 (1.7)	0.769
Mean updated ADO score (SD)[7]	7.6 (2.4)	7.8 (2.2)	0.154

Comorbidities (number (%))			
Congestive heart failure[8]	112 (16.7)	93 (15.2)	0.465
Myocardial infarction[9]	27 (4.0)	58 (9.5)	<0.001
Diabetes mellitus[10]	43 (6.4)	74 (12.2)	<0.001
Previous AECOPD (number (%))[11]	265 (39,5)	274 (44.6)	0.063

[1] Pack-years of resp. 626 and 593 patients in the COMIC and PROMISE-COPD study
[2] BMI of resp. 658 and 612 patients
[3] Lung function measures of resp. 670 and 564 patients
[4] GOLD stage of resp. 670 and 611 patients
[5] mMRC score of resp. 654 and 599 patients
[6] BOD score of resp. 642 and 552 patients
[7] ADO and updated ADO score of resp. 653 and 544 patients
[8] Diagnosis of congestive heart failure was recorded of 612 patients in the PROMISE-COPD study
[9] Diagnosis of myocardial infarction was recorded of 611 patients in the PROMISE-COPD study
[10] Diagnosis of diabetes mellitus was recorded of 608 patients in the PROMISE-COPD study
[11] Minimum of 2 AECOPD or 1 severe AECOPD in the year before inclusion

Abbreviations; SD: Standard Deviation; BMI: Body Mass Index; FEV_1: Forced Expiratory Volume in 1 second; GOLD: Global Initiative for Chronic Obstructive Lung disease; mMRC: modified Medical Research Council dyspnoea grade; BOD: Index that combines Body mass, airflow Obstruction (FEV1 % predicted), Dyspnoea (mMRC); ADO: Index that combines Age, Dyspnoea (mMRC) and airflow Obstruction (FEV1 % predicted); AECOPD: Acute exacerbation in chronic obstructive pulmonary disease.

Table 2 Cumulative proportion of patients having at least one severe AECOPD at 1, 2 and 3 years

Follow-up	High MR-proADM (Standard Error) N=321	Low MR-proADM (Standard Error) N=964	Total (Standard Error) N=1285
1 year	0.20 (0.02)	0.13 (0.01)	0.15 (0.01)
2 years	0.33 (0.03)	0.23 (0.01)	0.25 (0.01)
3 years	0.44 (0.04)	0.31 (0.02)	0.34 (0.02)

Table 3 Cumulative proportion of patients having at least one CAP at 1, 2 and 3 years

Follow-up	High MR-proADM (Standard Error) N=197	Low MR-proADM (Standard Error) N=474	Total (Standard Error) N=671
1 year	0.08 (0.02)	0.04 (0.01)	0.05 (0.01)
2 years	0.16 (0.03)	0.09 (0.01)	0.11 (0.01)
3 years	0.24 (0.04)	0.13 (0.02)	0.16 (0.02)

Time till first hospitalisation for an AECOPD

In the pooled analysis COPD patients with high level stable state MR-proADM (N=321) had a significantly higher risk for a severe AECOPD compared with those with low level MR-proADM (N=964) (Figure 1) (log-rank: p<0.001) with a hazard ratio of 1.56 (95% CI, 1.24 – 1.98) in univariate analysis. In the multivariate Cox regression analysis patients with high level stable state MR-proADM, corrected for the confounders FEV1 (liters), BMI, mMRC, heart failure, diabetes mellitus and previous AECOPD still had a significantly higher risk for a severe AECOPD with a corrected hazard ratio of 1.30 (95% CI, 1.01 – 1.68). For more details see Table 4.

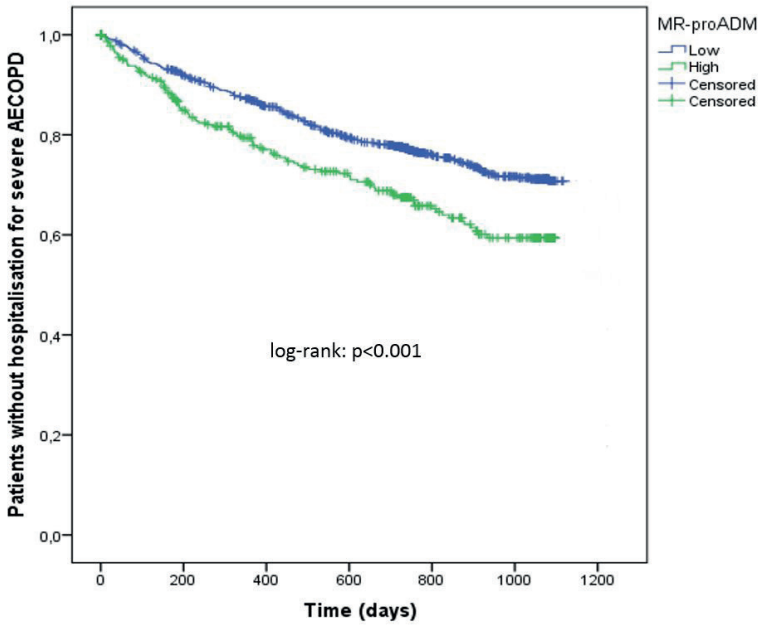

Figure 1 Kaplan-Meier survival curve for association of stable-state MR-proADM with time until first hospitalization for an acute exacerbation of COPD. MR-proADM = midrange proadrenomedullin.

Table 4 Multivariate regression analyses for time till severe COPD and time till CAP

	Severe AECOPD	CAP
	HR (95%CI)	**HR (95%CI)**
MR-proADM		
Low	1.0	1.0
High	1.30 (1.01-1.68)	1.10 (0.68-1.80)
FEV1 (liters)	0.54 (0.41-0.69)	0.43 (0.26-0.72)
Age	-	1.06 (1.03-1.09)
BMI	0.96 (0.94-0.99)	-
mMRC	1.24 (1.12-1.37)	-
Heart failure		-
No	1.0	
Yes	1.43 (1.07-1.90)	
Diabetes mellitus		-
No	1.0	
Yes	1.69 (1.16-2.48)	
Previous AECOPD		
No	1.0	1.0
Yes	1.90 (1.51-2.40)	2.47 (1.55-3.95)

Abbreviations; AECOPD: acute exacerbation of COPD; CAP: community acquired pneumonia; MR-proADM: Midrange-proadrenomedullin; FEV1: Forced Expiratory Volume in 1 second BMI: Body Mass Index; mMRC: modified Medical Research Council dyspnoea grade

Time till first CAP

In the COMIC study COPD patients with high level stable state MR-proADM (N=197) had a significantly higher risk for a CAP compared with COPD patients with low level MR-proADM (N=474) (Figure 2) (log-rank: p=0.003) with a hazard ratio of 1.93 (95%CI: 1.24 – 3.01) in univariate analysis. However, corrected for age, FEV1 (liters) and previous AECOPD in multivariate Cox regression analysis this association was no longer significant (cHR 1.10; 95% CI, 0.68 – 1.80). For more details see Table 4.

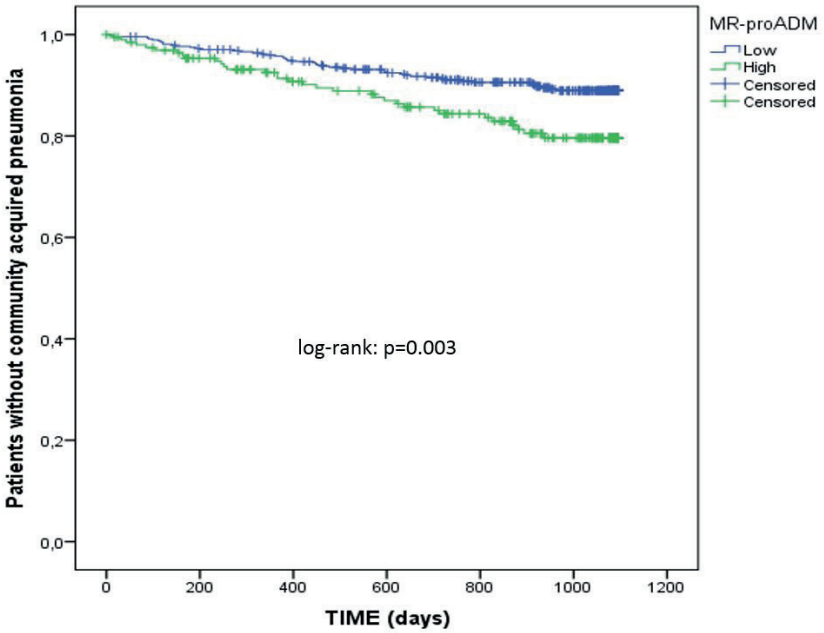

Figure 2 Kaplan-Meier survival curve for association of stable-state MR-proADM with time until first community-acquired pneumonia. MR-proADM = midrange proadrenomedullin.

Discussion

In our pooled analysis of two large European COPD cohort studies MR-proADM, measured in stable state, was shown to be an important biomarker for the occurrence of a future severe AECOPD. A high level of MR-proADM, measured in stable state, was associated with a 30% increased risk of a severe AECOPD when corrected for potential confounders such as lung function, previous AECOPD, age and comorbid status. With 25% of the patients having a high stable state MR-proADM level and with 34% (cumulative proportion) of the patients having at least one severe AECOPD during the 3 years of follow up, our results are not only significant but are clinically relevant as well.

How can we explain this finding? It is known that ADM secretion is stimulated by tumor necrosis factor-alfa, interleukin-Beta**,** and lipopolysaccharide.(17) Besides, hypoxia leads to ADM upregulation through the hypoxia inducible factor-1 pathway.(25) These are all factors accompanying AECOPD and CAP, either causal

or as a result. Elevated ADM in stable state thus probably represents a higher demand on the regulatory mechanisms ascribed to ADM. This higher demand is provoked by a possible higher continuous, or an at that moment active underlying (systemic) inflammation, hypoxia, bacterial exposition or colonization and cardiovascular instability/comorbidity in contrary to patients with non elevated ADM levels. All these processes are associated with disease progression in COPD and promote development of comorbidity such as atherosclerosis and muscle wasting and osteopenia.(26) So in a way stable state is probably not that stable in COPD patients with elevated MR-proADM.

The higher ADM, measured in stable state could implicate that the patient is either at risk for impending disease instability or is already somewhat unstable (although they did not receive any prednisolone or antibiotics for the last four weeks), which in the end could lead to a negative outcome such as a severe exacerbation or infectious disease.

In our study, MR-proADM was not associated with CAP. In the general population MR-proADM measured in patients with CAP was associated with disease severity and was an independent predictor for clinical outcome including mortality and it had additive prognostic value combined with the Pneumonia Severity Index and CURB-65.(24;27-29) As mentioned, there is a rationale that high-levels of stable state MR-proADM could be associated with CAP in patients with COPD. Indeed, there was a positive association between MR-proADM and outcome, albeit not significant after adjustment for age and FEV_1. It cannot be excluded that the smaller sample size could be responsible for this finding, i.e. a β-error.

Evidence accumulates that MR-proADM is associated with morbidity and mortality at different time points during the course of COPD. In stable state high MR-proADM is associated with severe AECOPD, exertional hypoxemia(25) and mortality.(20) During exacerbation, high MR-proADM predicts length of hospital stay and mortality(17) and elevated MR-ProADM at hospital discharge predicts survival as well.(30) It would be interesting to know how the association and predictive value of MR-proADM with morbidity and mortality behaves when serial longitudinal measurements within one patient are made during the course of the disease; are changes in the MR-proADM level followed by changes in disease activity and in which time frame? And does treatment (pharmacological and/or non-pharmacological) of COPD influence MR-proADM levels followed by disease optimisation? Future studies have to address these topics.

There are different causes for exacerbations.(10) It is debatable whether one biomarker would be capable to encompass the whole complexity of exacerbations. Different biomarkers are described as associated with exacerbations (with or without hospitalisation).(31) In a recent large scale biomarker panel study using 2 large and well characterized cohorts by Keene et al. many of these biomarkers were no longer associated with or predictive for exacerbations and their additive value with known predictive clinical parameters was limited as well.(32) The role for these biomarkers in COPD has therefore become uncertain. Stable state MR-proADM has never been evaluated with morbidity in COPD until now. The independent predictive capability of MR-proADM as an independent predictor for AECOPD in COPD patients still has to be established in a prediction model, in which all other known predictors are taken into account. In this way also the additive prognostic value of MR-proADM to other known predictors can be studied.

The cut-off level of MR-proADM used in this study was based on an earlier study.(19) However, that was a study with mortality as the end-point. To establish whether the value of 0.87 nmol/l was the ideal cut-off level for morbidity as well we performed additional analyses with different validated cut-off levels of MR-proADM used in a recent validation study.(33) The different cut-off levels did not result in a stronger association (data not shown), thus the cut-off value of 0.87 nmol/l seems to be reasonable for morbidity as well.

This study has some limitations.

First, the patients in the PROMISE-COPD study had a slightly worse lung function, were more often current smokers and had more comorbidity than the patients in the COMIC study. Conversely, a higher proportion of the patients included in the COMIC study was included during an acute exacerbation of COPD, a fact known to be an important predictor for a new exacerbation.(34) So, a limitation of the study is that the patients included may have a more severe COPD and are mainly controlled and treated in secondary care. Therefore, the results of this study cannot automatically be extrapolated to patients with mild COPD controlled in primary care although pathofysiologically there is no reason why MR-proADM should act different in mild COPD.

Second, the study lacks information about supplemental oxygen use, gastroesophageal reflux disease (GERD), previous pulmonary rehabilitation status and other (inhalation) medication use other than the known ICS used by 80% of the patients.

The strength of this study however includes its large sample size of a well-characterized multicentre cohort from several European countries. The various established and validly assessed covariates made it possible to determine the association of stable state MR-proADM with morbidity. Moreover, this study design with the pooled analysis potentiates the generalizability of stable state MR-proADM being an important biomarker for future severe AECOPD.

In conclusion, plasma MR-proADM measured at stable state in patients with COPD is significantly associated with future severe AECOPD but not CAP in a large, pooled European COPD cohort. Future studies should address the additive value of stable state MR-proADM as an independent predictor with other known (clinical) predictors for severe AECOPD.

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Supplemental Material

The COMIC study is a single-centre cohort study from Enschede, the Netherlands. From December 2005 till April 2010, 795 patients were included with a follow-up period of at least three years. For inclusion in the COMIC study patients had to meet the following criteria; a) a clinical diagnosis of COPD according to the GOLD guidelines; b) current or former smoker; c) age ≥ 40 years; d) no medical condition compromising survival within the follow-up period or serious psychiatric morbidity; e) absence of any other active lung disease (e.g. sarcoidosis); f) no maintenance therapy with antibiotics; g) ability to speak Dutch. Patients were enrolled when visiting the outpatient clinic in stable state (stable state group) or when hospitalised for an acute exacerbation in COPD (AECOPD group). To be included in the AECOPD group, patients had to be hospitalised for an AECOPD and be able to produce an adequate sputum sample at the day of hospitalisation. To be included in the stable state group patients had to meet the following criteria: no use of antibiotic and/or prednisolon 4 weeks prior to enrolment and no exacerbation less than 4 weeks before study entry. All patients were treated according to standard care. Eighty percent of the patients used inhaled corticosteroids during follow-up. (1) The COMIC study was approved by the hospital's medical ethical committee (P05-49). All patients provided written informed consent. The COMIC study started before the introduction of trial registries. The study was initiated to investigate the relationship between the immune response after influenza vaccination in COPD patients and mortality. Various papers concerning MR-proADM in COPD have been published with this COMIC study.(2-4)

The PROMISE-COPD study consecutively recruited and followed 638 patients of 11 pulmonology departments of European hospitals from November 2008-October 2011. Inclusion criteria of the PROMISE-COPD study comprised: a) at baseline, clinically stable, moderate to very severe COPD based on medical history, physical examination, and spirometry ≥ 4 weeks after the latest exacerbation resolved; b) age ≥ 40 years; c) smoking history ≥ 10 pack-years. Exclusion criteria were: a) major respiratory disorder other than COPD; b) rapidly lethal disease; c) immunosuppression, including acquired immunodeficiency syndrome, organ transplantation, or chronic steroids (>20 mg prednisolone equivalent/day); d) musculoskeletal or neuromuscular disorder preventing walking. Enrolees had a baseline examination, and then were followed at least 2 years in scheduled semi-annual visits. Throughout the study, patients were treated as clinically warranted, without restriction. The local ethics committee approved the study. All patients provided written informed consent. The PROMISE-COPD study was registered at www.controlled-trials.com under the identifier ISRCTN99586989.

In both studies spirometry was performed by trained respiratory technicians according to the American Thoracic Society guidelines.(5) Data on common co-morbidities like myocardial infarction, congestive heart failure and diabetes mellitus were obtained from medical records and/or during study visits. Patients completed the modified Medical Research Council dyspnoea questionnaire (mMRC) in local languages, in validated versions when available.(6) The BOD comprises BODE without exercise capacity measurement.(7) The components were scored according to the same cut-offs as in BODE.(8) The BOD therefore ranges from 0 to 7.(9) The original and updated ADO score ranges, in increasing severity, from respectively 0 to 10 and from 0 to 14 points.(10) All measurements were performed in stable state.

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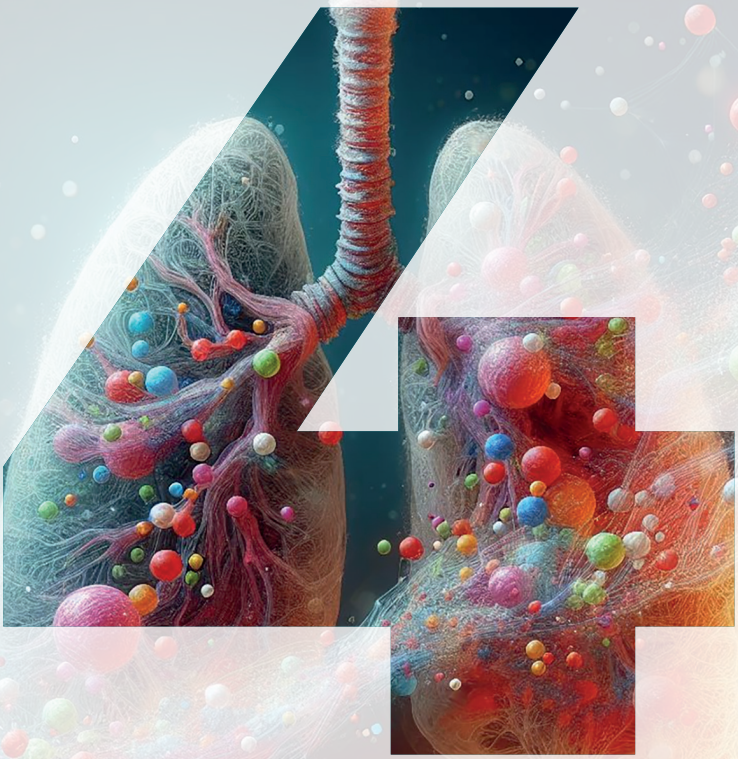

Predicting mortality in COPD with validated and sensitive biomarkers; fibrinogen and Mid-range-proadrenomedullin (MR-proADM)

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Abstract

Although fibrinogen is a FDA qualified prognostic biomarker in COPD, it still lacks sufficient resolution to be clinically useful. Next to replication of findings in different cohorts also the combination with other validated biomarkers should be investigated. Therefore, the aim of this study was to confirm in a large well-defined population of COPD patients whether fibrinogen can predict mortality and whether a combination with the biomarker MR-proADM can increase prognostic accuracy.

From the COMIC cohort study we included COPD patients with a blood sample obtained in stable state (n=640) and/or at hospitalization for an acute exacerbation of COPD (n=262). Risk of death during 3 years of follow up for the separate and combined biomarker models was analysed with Cox regression. Furthermore, logistic regression models for death after one year were constructed.

When both fibrinogen and MR-proADM were included in the survival model, a doubling in fibrinogen and MR-proADM levels gave a 2.2 (95% CI 1.3-3.7) and 2.1 (95% CI 1.5-3.0) fold increased risk of dying, respectively. The prediction model for death after 1 year improved significantly when MR-proADM was added to the model with fibrinogen (AUC increased from 0.78 to 0.83; p=0.02). However, the combined model was not significantly more adequate than the model with solely MR-proADM (AUC 0.83 vs 0.82; p=0.34).

The study suggests that MR-proADM is more promising than fibrinogen in predicting mortality. Adding fibrinogen to a model containing MR-proADM does not significantly increase the predictive capacity of the model.

Introduction

COPD will be the third-leading cause for mortality worldwide by 2020 [1]. Accurate prediction of mortality is important because it helps identify patients in whom the implementation of specific therapeutic measures can improve outcome[1]. Several attempts have been made to identify risk factors for mortality.

Existing models to predict mortality use multiple parameters including lung function, exercise tests, reported dyspnea or patient characteristics like body-mass-index [2] or age. Well known multidimensional tools are the BODE (Body-Mass Index, Obstruction, Dyspnea, Exercise) and ADO (Age, Dyspnea, Obstruction) index. These indices not only have limited predictive value, but may also be cumbersome to regularly determine in a clinical setting. Furthermore, while the models may incorporate parameters reflecting the disease state, none incorporates a direct reflection of the possible ongoing inflammatory process in COPD.

We, among others, have shown that the peptide mid-range proadrenomedullin (MR-proADM) is a valuable predictor for mortality in COPD [3-6]. Adrenomedullin (ADM) has a wide range of actions including immune-modulating, metabolic and vascular actions. It can behave both as a hormone and as a cytokine[7;8]. After an inflammatory stimulus there is also increased expression of this molecule in the lungs[9]. Adding MR-proADM to various multidimensional indices such as the ADO and BODE improved their predictive power significantly and relevantly [3;5;10;11].

Another well studied biomarker in COPD is fibrinogen. Fibrinogen is considered as a prognostic biomarker in COPD by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA)[12] . Besides a blood clothing factor, fibrinogen is also an acute phase reactant. While the liver is the primary source of plasma fibrinogen, it has been shown that lung epithelium may also produce fibrinogen in response to an inflammatory stimulus[13;14]. Normal fibrinogen levels in blood range between 1.5 and 3.5 g/litre [15]. Higher fibrinogen levels in the stable state have been shown to be related with an increased frequency of exacerbations[16;17] and increased mortality[18-21] in COPD, although there are conflicting results[22;23]. The study of Mannino et al. that pooled 5 studies showed that increased fibrinogen is associated with increased risk of death[19].

Replication of data in different cohorts is imperative as underlined by a recent study of Keene [24]. In this study blood biomarkers were significantly associated

with the occurrence of exacerbations but were not robust between the SPIROMICS and COPDGene cohort.

Combining two or more biomarkers for prediction of clinical outcome in COPD, including mortality, improved when two or more biomarkers were combined, but MR-proADM was never included[1;21;25;26]. Therefore, we hypothesized that, in a large well-defined population of COPD patients, the unique combination of fibrinogen and MR-proADM could increase the prognostic accuracy.

Materials and Methods

Setting and study population

In this study we describe the results of a retrospective analysis of prospectively collected data from the COMIC study, a single centre cohort study on the immune status of COPD patients as a determinant for survival. From December 2005 till April 2010, 795 patients were included with a follow-up period of three years. The COMIC study was approved by the medical ethical committee Twente, at Enschede (P05-49). All patients provided written informed consent. The COMIC study was initiated in a phase when registration was not necessary for non-interventional cohort studies.

Patients had to meet the following criteria; 1) a clinical diagnosis of COPD according to the GOLD recommendations[27]; 2) current or former smoker; 3) age ≥ 40 years; 4) no medical condition compromising survival within the follow-up period, or serious psychiatric morbidity, 5) absence of any other active lung disease (e.g. sarcoïdosis), 6) no maintenance therapy with antibiotics, 7) ability to speak Dutch. Patients were enrolled when hospitalized for an acute exacerbation of COPD (AECOPD) or when visiting the outpatient clinic in stable state. To be included in the AECOPD group, patients had to be hospitalized for an AECOPD and be able to produce an adequate sputum sample at the day of hospitalization[28]. An AECOPD was defined as an acute negative change from the baseline, reported by the patient, in dyspnea and/ or sputum volume and/or colour of sputum (yellowish or greenish sputum) and/or cough, which may warrant additional physician-initiated treatment of prednisolone with or without antibiotics. To be included in the stable state group patients had to meet the following criteria; no use of antibiotic and/ or prednisolone 4 weeks prior to enrolment and no exacerbation less than 4 weeks before study entry. Also, data on common co-morbidities like hypertension;

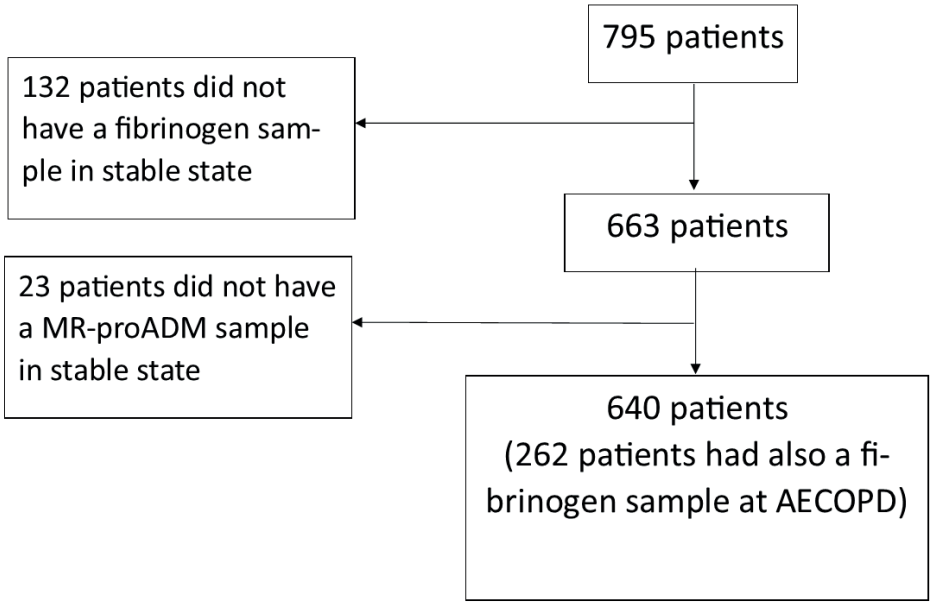

Figure 1. Study design of the COMIC study

myocardial infarction and heart failure were obtained as they probably influence fibrinogen and MR-proADM level. Patients were asked to complete a questionnaire about their dyspnea in stable state (mMRC)[29]. All patients were treated according to the COPD GOLD standard. Approximately eighty percent of the patients used inhaled corticosteroids during follow-up[30]. The study was initiated to investigate the relationship between the immune response after influenza vaccination in COPD patients and mortality.

The study design is depicted in figure 1. Six hundred sixty three of the 795 patients included in the COMIC study had a blood sample in stable state in which fibrinogen was measured. In 640 of these 663 patients, stable state MR-proADM was also measured. From 262 out of 640 patients we also obtained a blood sample at hospitalization for AECOPD in which fibrinogen was determined.

Outcomes

The primary outcome parameter was survival, based on all-cause mortality. Date of death was verified from the municipal administration.

Measurements of fibrinogen

Fibrinogen levels were measured on the STA-R (Stago) by the Clauss fibrinogen assay. This test method involves measuring the rate of fibrinogen to fibrin conversion in diluted sample under the influence of excess thrombin. Since under these conditions the fibrinogen content is rate limiting, the clotting time can be used as a measure of the concentration of the fibrinogen and in fact the clotting time is inversely proportional to the level of fibrinogen in the plasma. The reference value of fibrinogen is 2.0 – 4.0 g/l.

Measurements of MR-proADM

MR-proADM levels were measured in EDTA-plasma (ethylene diamine tetraacetic acid anticoagulant) with an automated sandwich immunoassay using a time-resolved amplified cryptate emission technology [31] (KRYPTOR®; Thermo Scientific Biomarkers, [formerly B.R.A.H.M.S. AG] Hennigsdorf, Germany). The KRYPTOR MR-proADM assay has a measuring range of 0.05-100 nmol/l and a functional sensitivity of 0.25 nmol/l. The reference interval of MR-proADM in a healthy subset is 0.23 - 0.64 nmol/l (median 0.41 nmol/l)(19;40).

Statistical analysis

Continuous variables are expressed as mean (± standard deviation (SD)) in case of parametric data and as median (Interquartile range (IQR)) for non-parametric data. Categorical variables are expressed as counts with percentages. Differences in fibrinogen levels between survivors and non-survivors and between stable state and hospitalization for an AECOPD, were analysed by Mann-Whitney U test. Since the levels of the biomarkers MR-pro-ADM and fibrinogen have a skewed distribution this data was transformed using a logarithm with base two for the other analyses. A Pearson correlation was used to determine the correlation between the transformed fibrinogen and MR-proADM levels. Time from stable state to death, was analysed by Kaplan-Meier survival curves with log rank tests. Hazard ratio's (HR) were determined by Cox regression analysis. All analyses were corrected for age, sex, BMI, and GOLD stage in a multivariate Cox regression analysis (the only confounders from the parameters named in table 1) and the C statistic was calculated[32]. One-year survival after stable state was analysed by logistic regression analyses and corrected for the same confounders. Furthermore, we used the biomarkers fibrinogen and proADM in constructing the prediction models. To test for differences between the AUC we calculated the standard error using a non-parametric test as described by Delong et al.[33]. The confidence intervals of the AUC were calculated as exact binomial confidence intervals.

All tests were two-sided and a p-value of 0.05 was considered statistically significant. The data were analysed using SPSS, version 22 (SPSS Inc. Chicago IL). The standard error of the area under the curve and differences between the curves were analysed using MedCalc 17.2 (Medcalc software, Ostend, Belgium).

Results

Baseline characteristics

The baseline characteristics of the patients in stable state (640) and in a subpopulation during an AECOPD (262) are presented in Table 1. The median follow-up after inclusion at stable state was 34 (IQR 27-35) months. Mortality was 21% during this period.

Table 1. Baseline characteristics

	Stable state N= 640	AECOPD N= 262
Mean age at enrolment in years (SD)	67 (9)	71 (9.6)
Male, N (%)	384 (60)	156 (60)
Smoking status, N (%)		
Current smokers	166 (26)	78 (30)
Lung function (post-bronchodilator)		
Mean FEV1 in liters (SD)	1.46 (0.61)	1.30 (0.54)
Mean FEV1 as % of predicted (SD)	54 (19)	49 (18)
Mean FEV1/VC in % of predicted (SD)	45 (14)	43 (14)
GOLD classification 2005, N (%)		
I	59 (9)	17 (6)
II	288 (45)	94 (36)
III	240 (38)	122 (47)
IV	53 (8)	29 (11)
Mean BMI at enrolment (SD)[a]	27 (5.0)	27 (5.7)
Mean mMRC (0-4) (SD)[b]	1.7 (1.2)	2.1 (1.3)
Mean ADO-score (SD)[c]	4.0 (1.8)	4.9 (1.8)
Comorbidity, N (%)		
Heart failure	109 (17)	49 (19)
Myocardial infarction	26 (4)	17 (7)

[a] BMI is missing of respectively 12 and 0 patients at stable state and at AECOPD
[b] mMRC-score is missing of respectively 17 and 10 patients at stable state and at AECOPD
[c] ADO-score is missing of respectively 17 and 10 patients at stable state and at AECOPD
Abbreviations; AECOPD: Acute exacerbation of COPD; N: Number; SD: Standard Deviation; BMI: Body Mass Index; FEV_1: Forced Expiratory Volume in 1 second; GOLD: Global Initiative for Chronic Obstructive Lung disease; mMRC: modified Medical Research Council dyspnea grade; ADO: Index that combines Age, Dyspnea (mMRC) and airflow Obstruction (FEV1 % predicted).

Fibrinogen levels in survivors and non-survivors

Fibrinogen levels were significantly higher in non-survivors (4.6 ± 3.9-5.4 g/L (median ± IQR)) compared to survivors (4.1 ± 3.6-4.7 g/L) when measured in stable state (p <0.001). There was no significant difference in fibrinogen levels between survivors and non-survivors at hospitalization for AECOPD (p= 0.606) (Table 2). Since this significance in it was lacking, we chose not to further investigate the relation between fibrinogen and mortality in AECOPD. Subsequent analysis are on the biomarkers as measured in stable state

Table 2. Fibrinogen

	Fibrinogen g/l (Median [IQR])
Stable state	
Non-survivors* (N=134)	4.6 [3.9-5.4]
Survivors (N=506)	4.1 [3.6-4.7]
Difference between groups	p <0.001
AECOPD	
Non-survivors* (N=80)	5.4 [4.3-6.4]
Survivors (N=182)	5.2 [4.3-6.4]
Difference between groups	p= 0.606

*Died within follow-up period of three years
Abbreviations; AECOPD: Acute exacerbation of COPD; N: Number; IQR: Inter quartile range

Regression analysis

There was a very weak correlation of 0.2 between fibrinogen and MR-proADM ($p<0.001$; $r^2=0.04$) (see also figure 2).

Prediction of mortality with fibrinogen and MR-proADM in stable state

Table 3 shows univariate (data in table) and multivariate Cox regression. In the multivariate Cox regression with fibrinogen (as a continuous variable) a doubling of fibrinogen levels shows a 2.39 (95% CI 1.41-4.05) fold increased risk of dying, with a C-statistic of 0.73.

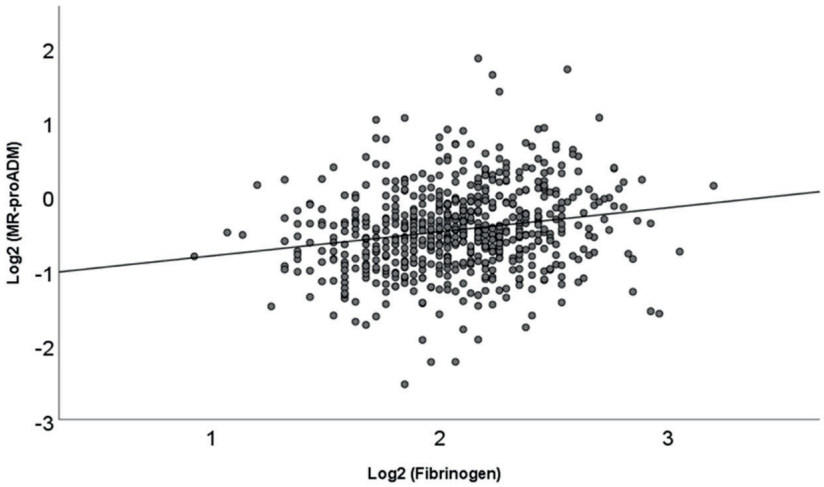

Figure 2. Scatterplot of fibrinogen versus MR-proADM levels in stable state

Multivariate Cox regression with MR-proADM (as a continuous variable) shows a 2.22 (95% CI 1.59-3.10) fold increased risk of dying with a doubling of MR-proADM, with a C-statistic of 0.75.

When both fibrinogen and MR-proADM are included in the fully adjusted Cox regression model, a doubling in absolute fibrinogen levels is associated with a 2.17 (95% CI 1.27-3,7) fold increased risk of dying and a doubling of absolute MR-proADM levels with a 2.12 (95% CI 1.51-2.96) fold increased risk. The C-statistic of the combined model is 0.76 (Table 3).

Table 3. Survival analysis

	HR	95% CI	p-value	cHR*	95% CI	p-value	C-statistic
Fibrinogen *	4.03	2.40-6.76	p<0.001	2.39	1.41-4.05	p<0.001	0.73
MR-proADM *	2.76	2.06-3.69	p<0.001	2.22	1.59-3.10	p<0.001	0.75
Fibrinogen and MR-proADM *							
Fibrinogen *	3.18	1.85-5.45	p<0.001	2.17	1.27-3.70	p<0.01	
MR-proADM *	2.46	1.83-3.32	p<0.001	2.12	1.51-2.96	p<0.001	0.76

*Measured in stable state and corrected for age, sex, GOLD status and BMI (12 missing BMI, total number of patients 628)
Abbreviations; HR: Hazard Ratio; CI: Confidence Interval; cHR: Corrected Hazard Ratio

Table 4. Logistic regression analysis for death after one year

Variable	cOR*	cOR 95% CI	AUC	AUC 95% CI		
Fibrinogen*	4.80	1.80-12.81	0.78	0.74- 0.81	p = 0.18	p = 0.02
MR-proADM*	3.84	2.03-7.27	0.82	0.78- 0.86		
Fibrinogen & MR-proADM*	4.39 3.59	1.60-12.05 1.90-6.78	0.83	0.80- 0.86	p = 0.34	

*Measured in stable state and corrected for age, sex, GOLD status and BMI (12 missing BMI, total number of patients 628)
Abbreviations; cOR: Corrected Odds Ratio; CI: Confidence Interval; AUC: Area Under the Curve

After one year of follow-up, 46 of 640 patients (7.2%) died. When corrected for age, sex, BMI and GOLD, a doubling in absolute fibrinogen levels is associated with a 4.80 (95% CI 1.80-12.81) fold increased risk of dying within the first year of follow-up.

When fibrinogen is combined with MR-proADM, a doubling of fibrinogen shows a significant 4.39 (95% CI 1.60-12.05) fold increased chance of dying within one year, while doubling of MR-proADM shows a significant 3.59 (95%CI 1.90-6.78) fold increased risk of dying within one year (Table 4). Figure 3 presents the ROC curves

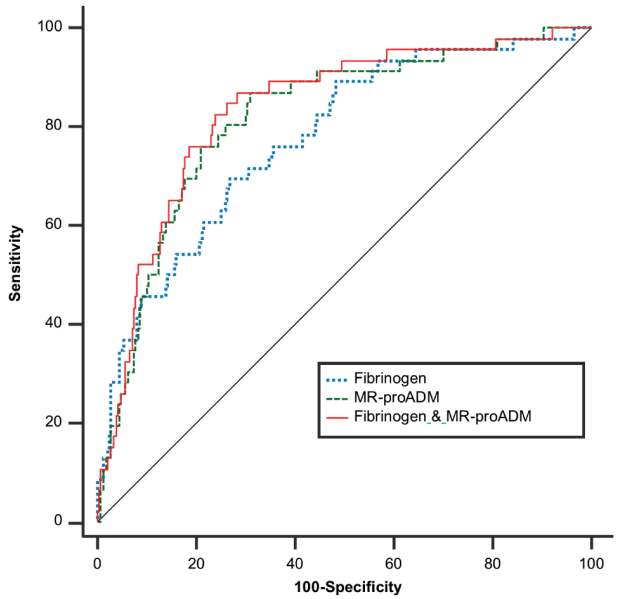

Figure 3. ROC curves for prediction of death after 1 year
Abbreviations; ROC: Receiver Operating Characteristic

of the models as shown in table 4. When MR-proADM is added to the model with fibrinogen, the AUC increased significantly from 0.78 to 0.83 (p=0.02). However, the combined model containing fibrinogen and MR-proADM is not significantly better than the model with solely MR-proADM (AUC 0.83 vs 0.82; p=0.34).

Discussion

This study shows that fibrinogen and MR-proADM, measured in a well-characterised population in stable state, are predictors for mortality in COPD. The study suggests that MR-proADM is more promising than fibrinogen in predicting mortality. Adding fibrinogen, another well-known biomarker, to a model containing MR-proADM does not significantly increase the predictive capacity of the model. On the contrary, adding MR-proADM to fibrinogen increases the accuracy of the short term mortality prediction model (one year follow-up) both relevantly and significantly.

The finding that fibrinogen is a predictor for mortality in stable state is in line with previous studies [18-21]. E.g. a large meta-analysis [18] focused on coronary heart disease and stroke showed people with high levels of fibrinogen had higher risks of dying from COPD. Mannino et al.[19] combined 5 studies containing a total of 6376 individuals with COPD and reports significantly increased mortality (HR 1.94 (95%CI 1.62-2.31)) in individuals with fibrinogen above a level of 3,5 g/L.

Other studies used other cut off values, for example the reference value used by the FDA. In our study fibrinogen showed better results when used as a continuous variable (data not shown).

In the current cohort the median fibrinogen level is well over the upper limit of the normal reference values. This is in accordance with many cross-sectional studies in which blood fibrinogen in patients with COPD are higher than in healthy controls[21;34-36]. We also confirmed high levels of fibrinogen during exacerbations of COPD [34;37-39].

In a previous study we showed that MR-proADM is an accurate predictor for mortality and we validated this in a different population [3;40]. In the current study this predictive capability is reflected in a high C-statistic and AUC. Zeman et al. showed that combining several biomarkers may increase the predictive capability further [25]. And although the model for one-year mortality risk with

solely fibrinogen improved when MR-proADM was added, adding fibrinogen to a model already containing MR-proADM did not significantly improve the predictive power.

For mortality prediction in a clinical setting it seems therefore most promising to focus on MR-proADM in combination with other scores (ADO, BODE), by which the predictive power can be significantly and relevantly increased[10;11]. Furthermore, the combination of MR-proADM with other biomarkers for example S-RAGE[41] could be investigated. This could increase the predictive power especially when measured longitudinal and in relation to clinical condition. However, fibrinogen may still have a role in prediction in COPD because it has been shown to be a relevant biomarker in many aspect of the disease for example exacerbations[15], but also because this biomarker is already available for use in regular clinical setting. The COPD Foundation Biomarker Qualification Consortium (CBQC) proposes fibrinogen for drug development[12].

Interestingly, the correlation between MR-proADM and fibrinogen was strikingly low (r=0.2). From the lack of correlation, it may be hypothesised that both biomarkers reflect different biological processes. As suggested in the introduction it may be that such processes are directly related to pulmonary condition, but it may also reflect COPD as a systemic disease[42;43].

While fibrinogen and MR-proADM are hardly correlated, there was little benefit when the combined predictive capacity is expressed as AUC or c-statistic. This is explained by the fact that both the AUC and c-statistic are non-linear quantities. Apparently, the prediction-model containing MR-proADM is so superior that adding fibrinogen to the model does not lead to a significant increase in AUC.

The strength of the current study is that the results are based on data obtained from a large cohort study with a well-defined population of COPD patients from a general hospital with three years of follow-up. We recognise that in the current study only two biomarkers are studied. A direct comparison with other biomarkers for mortality prediction in COPD, is lacking. MR-proADM was only measured once in stable state. The next step would be to do longitudinal measurement to study MR-proADM over time, to detect whether changes in MR-proADM are also associated with changes in disease severity, health status and prognosis.

Increased fibrinogen is associated with increased mortality. Adding MR-proADM to fibrinogen increased the prognostic accuracy of the model for short term mortality prediction both relevantly and significantly. But the combination was not superior in predicting short term mortality compared to the model with solely MR-proADM.

We consider MR-proADM superior in predicting mortality in COPD compared to the FDA-approved biomarker fibrinogen. Not only is MR-proADM a strong predictor; it also has been well validated. We therefore advocate that MR-proADM should be included in future biomarker studies in COPD.

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Eosinophilia in severe COPD exacerbations and its association with morbidity and mortality

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To be submitted

Abstract

Background: Elevated blood eosinophil count as a marker for eosinophilic inflammation during an Acute Exacerbation of COPD (AECOPD) is associated with steroid responsiveness. Less is known about its prognostic accuracy to predict future morbidity and mortality, as well as the cut-off point that needs to be used in a severe AECOPD.

Aims and objectives: We aimed to evaluate the association of the blood eosinophil count during a severe AECOPD with morbidity and mortality for different eosinophil cut-off levels and for eosinophil count as a continuous variable.

Methods: 450 COPD patients of the COMIC cohort were assessed when experiencing a hospitalization for a exacerbation of COPD (severe AECOPD). Besides eosinophil count as a continuous variable, 4 different classifications for eosinophils were investigated: counts of eosinophils $\geq 0.2\times10^9$/L (200cells/μL) and $\geq 0.3\times10^9$/L (300cells/μL), and eosinophil percentages of ≥ 2% and ≥ 3% of total leucocyte count. Morbidity was operationalized as subsequent severe AECOPD or community acquired pneumonia (CAP). Time till all-cause mortality, time till first severe AECOPD and time till first CAP were analysed for the different eosinophil classifications.

Results: In total 12%-28% of the severe AECOPD were defined as eosinophilic, dependent on the classification that was used. In multivariate analyses and in sensitivity analyses excluding patients with recent systemic steroid use, none of the four eosinophil classifications nor eosinophil count as continuous variable were associated with time till all-cause mortality, first severe AECOPD or first CAP.

Conclusions: Blood eosinophil count measured during severe AECOPD, either as a continuous variable or with a cut-off level, is not associated with subsequent morbidity and mortality in the COMIC cohort COPD study and cannot be used for prognostication.

Introduction

Chronic obstructive pulmonary disease (COPD) is a complex and heterogeneous disease accompanied by increased morbidity and mortality. Underlying chronic and systemic inflammation plays an important role in the pathophysiology of COPD, its progression and its associated comorbidity.(1–4) It is estimated that COPD is the third leading cause of death worldwide.(5) Community acquired pneumonia (CAP) and severe Acute Exacerbation of COPD (AECOPD) resulting in a hospitalization are among the important factors contributing to the morbidity and mortality in COPD and are associated with a high burden of disease and low quality of life.(6,7)

Various biomarkers measured in stable state or during AECOPD have been established in COPD for prognostication for this morbidity and mortality. However the use of biomarkers in COPD in clinical practice has still been scarce for various reasons such as difficulties in reproducibility, in validation and in data interpretation.(8)

This seems to be different for the eosinophil count as a blood biomarker. The blood eosinophil count is correlated to the sputum eosinophil levels and can serve as a surrogate marker for eosinophilic inflammation in the lung.(9) Where initially it was thought that eosinophilic inflammation mainly plays a role in asthma, more and more it has become clear that eosinophilic inflammation is also relevant in a significant subset of the COPD population.(10,11) This eosinophilic inflammation is relevant for prognostication and has implications for treatment. For instance in stable state, elevated blood eosinophil count predicts higher inhaled corticosteroid (ICS) responsiveness in reducing future AECOPD.(11–13) This led to the introduction of the stable state blood eosinophil count to be used as a guidance for ICS use in the COPD GOLD guideline.(12)

In AECOPD, blood eosinophil counts also predict oral or intravenous steroid responsiveness and various clinical trials evaluated successfully eosinophil guided strategies for the use of steroid therapy during AECOPD.(14–17) This paves the way for a more frequent testing of the blood eosinophil count during AECOPD.

However, whether the blood eosinophil count at AECOPD is associated with future morbidity and mortality and as such can be used for prognostication is still not clear. Knowing if a patient is at high risk for a subsequent new severe AECOPD or

CAP or even death is relevant and can influence patient informing, management and monitoring during and after the AECOPD.

Prior analyses into the prognostic capabilities of this blood eosinophil count measured in AECOPD for future mortality and morbidity are inconsistent.(18–21) Possible explanations are differences in study design and of study population, lack of correction for confounding factors, unknown status of prior steroid use and the definition of eosinophilia since different cut-off levels of the eosinophil count were used.

The aim of this study is to evaluate blood eosinophils during a severe AECOPD for their association with morbidity, defined as risk of a subsequent severe AECOPD and CAP, and with mortality in COPD patients. We want to also establish whether the different expressions of blood eosinophilia are of influence to this association. For this study we will use data of a large prospective observational cohort study of well-defined COPD patients with known prior steroid use, the COMIC study.(22)

Methods

Settings and study population

This analysis is part of the COMIC study, a single-centre prospective cohort study from Enschede, the Netherlands.(22–24) From December 2005 till April 2010, 795 patients with COPD were included with a follow-up period of at least three years. The COMIC study was approved by the hospital's medical ethical committee (P05-49). All patients provided written informed consent. The COMIC study started before the introduction of trial registries. Results from the cohort study have been published previously including detailed inclusion and exclusion criteria.(22) In summary, for inclusion in the COMIC study patients had to meet the following criteria; a) a clinical diagnosis of COPD according to the GOLD guidelines; b) current or former smoker; c) age ≥ 40 years; d) absence of any other active lung disease (e.g. sarcoidosis); e) no maintenance therapy with antibiotics. All patients were treated according to the COPD GOLD standard at that time. For this substudy, all COMIC patients who had at least one severe AECOPD, either at inclusion or during the 3 years of follow up, were included.

Patient and public involvement

Patients and the public were not involved in the design and conduct of this study

Eosinophil counts and cut-off levels

Blood eosinophil counts were determined at admission to the emergency room on the day of hospitalization for a severe AECOPD. The eosinophil counts were determined with increments of $0.1x10^9$ cells/L (100cells/µL). The following four cut-off levels were analysed: absolute count of eosinophils ≥ $0.2x10^9$ cells/L (200cells/µL) or ≥ $0.3x10^9$ cells/L (300cells/µL) and relative eosinophil percentage of ≥ 2% or ≥ 3% of total leukocyte count. Eosinophil count as a continuous variable was analysed as well.

Outcomes

Time till death was based on all-cause mortality. Date of death was verified from the municipal administration.

Morbidity was defined subsequent hospitalization for an AECOPD or CAP, and analysed as time to first occurrence. AECOPD was defined as an acute negative change from baseline, reported by the patient, in dyspnoea and/or sputum volume and/or colour of sputum (yellowish or greenish sputum) and/or cough, which may warrant additional treatment of prednisolone with or without antibiotics by a physician in a patient with underlying COPD. It was defined as severe when necessitating hospitalization. CAP was defined as an acute respiratory tract illness associated with radiographic shadowing on a chest radiograph consistent with infection which was neither pre-existing nor of any other known cause. All X-rays were double read by a radiologist and a chest physician. In case of doubtful shadows in the report, the X-ray was presented to another chest physician for final judgment.

Demographic data including data on common co-morbidities like myocardial infarction, congestive heart failure and diabetes mellitus were collected from medical records. At baseline spirometry was performed according to standardized guidelines(25) and smoking status was determined by the Vlagtwedde questionnaire.(26) Patients completed the modified Medical Research Council dyspnea questionnaire (mMRC).(27) The BOD index comprises the BODE (body-mass index, airflow obstruction, dyspnea, and exercise capacity) index without exercise capacity measurement. The components were scored according to the same cut-offs as in BODE.(28) The BOD therefore ranges from 0 to 7. The original ADO index (age, dyspnea, obstruction) score ranges, in increasing severity, from respectively 0 to 10.(29) All of these measurements were performed in stable state. A history of exacerbations was defined as at least 2 AECOPD, or one severe

AECOPD with hospitalization in the year before inclusion. Inhalation corticosteroid (ICS) use was recorded from patients' pharmacy records.

Statistical analysis

Continuous variables are expressed as mean (± standard deviation (SD)) or as median (interquartile range (IQR)), and categorical variables as counts (percentages). Time from inclusion to event (all-cause mortality, first hospitalization for an AECOPD, first CAP) was analyzed by Kaplan-Meier survival curves and compared between eosinophilic and non-eosinophilic patients with log rank tests. Univariate and multivariate Cox proportional hazard regression models were used to establish the association of eosinophilia during exacerbation with time till all-cause mortality, time till first hospitalisation for an AECOPD and time till first CAP. For each separate multivariate Cox proportional hazard regression model, all baseline characteristics that were associated with both the studied eosinophil count of the model and the outcome of the model (all-cause mortality, first severe AECOPD and first CAP) at $p<0{\cdot}10$, were entered as potential confounders. Variables with the highest p-value were subsequently eliminated step by step until the fit of the model decreased significantly, based on -2 log likelihood. All tests were two-sided and a p-value of 0·05 was considered statistically significant. Data were analysed using SPSS, version 22(SPSS Inc. Chicago IL, USA).

Sensitivity analysis

It is known, primarily from analyses in patients with asthma, that oral steroid use can lower blood eosinophil count significantly, even after one single dose, and that this suppression can last for more than 24 hours.(30,31) To exclude the effect of recent systemic steroid use on the measured blood eosinophil count at AECOPD we performed a sensitivity analysis in which we excluded patients who used any systemic steroids within the last two weeks before the AECOPD.

Results

Baseline characteristics

Our COMIC cohort included 795 patients. 450 patients (57%) had at least one severe AECOPD, either at inclusion or during the 3 years of follow up. Their eosinophil count was established at day one of admission. The baseline characteristics of these 450 patients are displayed in Table 1.

Table 1 Baseline characteristics of 450 individuals with severe AECOPD.

	Severe AECOPD N= 450
Mean age at enrolment in years (SD)	69.7 (10.0)
Male (number (%))	272 (60.4)
Current smoker (number (%))	130 (28.9)
Median Pack-years (IQR)	32.4 (15.5-50.0)
Mean BMI at enrolment (SD)[1]	26.7 (5.7)
Lung function[2]	
Mean FEV_1 in litres (SD)	1.2 (0.5)
Mean FEV_1 % predicted (SD)	47.6 (18.5)
GOLD stage (number (%))[3]	
I	30 (6.7)
II	141 (31.5)
III	217 (48.4)
IV	60 (13.4)
Mean mMRC score (SD)[4]	2.1 (1.3)
Mean BOD score (SD)[5]	3.0 (1.8)
Mean ADO score (SD)[6]	4.7 (1.8)
Comorbidities (number (%))	
Congestive heart failure	82 (18.2)
Myocardial infarction	21 (4.7)
Diabetes mellitus	39 (8.7)
ICS use	361 (80.2)
Previous AECOPD (number (%))[7]	247 (54,9)

[1] BMI of 448 patients
[2] Lung function measures of 448 patients
[3] GOLD stage of 448 patients
[4] mMRC score of 358 patients
[5] BOD score of 358 patients
[6] ADO score of 358 patients
[7] Minimum of 2 AECOPD or 1 severe AECOPD in the year before inclusion

Abbreviations; SD: Standard Deviation; BMI: Body Mass Index; FEV_1: Forced Expiratory Volume in 1 second; GOLD: Global Initiative for Chronic Obstructive Lung disease; mMRC: modified Medical Research Council dyspnoea grade; BOD: Index that combines Body mass, airflow Obstruction (FEV1 % predicted), Dyspnoea (mMRC); ADO: Index that combines Age, Dyspnoea (mMRC) and airflow Obstruction (FEV1 % predicted); AECOPD: Acute exacerbation in chronic obstructive pulmonary disease.

The cumulative survival probability of these 450 patients and the cumulative proportion that had at least one subsequent severe AECOPD or CAP after 1, 2 and 3 years of follow up are displayed in table 2.

Table 2 cumulative survival probability and cumulative proportion of patients that had at least one severe AECOPD or CAP after 1, 2 and 3 years of follow up

Follow up	Survival (SE)	Severe AECOPD (SE)	CAP (SE)
1 y	0.82 (0.02)	0.30(0.02)	0.11(0.01)
2 y	0.70(0.02)	0.41(0.02)	0.17(0.02)
3 y	0.57(0.02)	0.47(0.03)	0.21(0.02)

Eosinophil levels

The median blood eosinophil level at admission to the emergency room was 0.1×10^9cells/L (IQR 0.0 - 0.2). Depending upon which cut-off level was used, 12% to 28% of the AECOPD was classified as eosinophilic (table 3).

Outcomes

In table 3, for the four used eosinophil cut-offs and for eosinophil count as a continuous variable, their association in univariate and multivariate analyses with the outcome parameters (time till death, time till first severe AECOPD and time till first CAP) are displayed.

Mortality

In univariate analyses, eosinophilia was associated with lower all-cause mortality for the cut-off levels ≥ 0.3×109/L and ≥ 2% or ≥ 3% of total leucocyte count (HR 0.72; p=0.045, HR 0.65; p=0.004 and HR 0.70; p=0.050 respectively) (table 3). After correction for the final confounders age and GOLD stage these associations were no longer significantly associated in the multivariate Cox regression analysis.

Morbidity

The blood eosinophil count during severe AECOPD, independent of which cut-off or whether the continuous measure was used, was not significantly associated with time to first subsequent hospitalization for an AECOPD nor time to first CAP in univariate or multivariate analyses although all hazard ratios for hospitalization for severe AECOPD were above 1 indicating direction of increased risk (table 3), and all below 1 for CAP.

Sensitivity analysis

From the 450 patients, 157 had used a systemic steroid in the last two weeks prior to the AECOPD. We performed similar analyses to study the association with the outcome parameters (survival, time till first severe AECOPD and time till first CAP) for the 293 patients without prior systemic steroid use (table 4). None of the HR's were significant and in the univariate analysis all were changed toward the one.

Table 3 univariate and multivariate Cox-regression analyses in 450 patients for morbidity and mortality for the different cut-off levels of eosinophils and as a continuous variable.

Cut-off eosinophils	Mortality Hazard Ratio (95% CI)		Hospitalisation AECOPD Hazard Ratio (95% CI)		CAP Hazard Ratio (95% CI)	
	Uni-variate	Multi-variate	Uni-variate	Multi-variate	Uni-variate	Multi-variate
≥ 0.3×10^9 cells/L. N=69 (15%)	0.72 (0.52-0,99)	0.91 (0.65-1.25)[1]	1.10 (0.75-1.61)	1.12 (0.77-1.64)[3]	0.85 (0.46-1.56)	0.90 (0.48-1.69)[4]
≥ 0.2×10^9 cells/L. N=128 (28%)	0.80 (0.63-1.03)	0.94 (0.73-1.22)[1]	1.09 (0.81-1.47)	1.09 (0.81-1.47)[3]	0.82 (0.51-1.33)	0.87 (0.53-1.43)[4]
≥ 2% of total leucocyte count. N=97 (22%)	0.65 (0.49-0.87)	0.78 (0.59-1.04)[1]	1.15 (0.83-1.59)	1.16 (0.83-1.61)[3]	0.73 (0.42-1.27)	0.74 (0.42-1.32)[4]
≥ 3% of total leucocyte count. N=56 (12%)	0.70 (0.49-1.00)	0.94 (0.65-1.35)[1]	1.06 (0.70-1.61)	1.11 (0.73-1.69)[3]	0.69 (0.33-1.43)	0.70 (0.32-1.52)[4]
Eosinophil as continuous variable per 0.10×10^9 cells/L increase. N=450	0.96 (0.90-1.12)	0.99 (0.94-1.09)[2]	1.01 (0.96-1.06)	NA	0.94 (0.84-1.06)	NA

[1] corrected for age and GOLD stage
[2] corrected for age
[3] corrected for FEV1
[4] corrected for ADO
NA: not applicable, no correction for confounders necessary

Table 4 univariate and multivariate Cox-regression analyses in 293 patients without prior steroid use for morbidity and mortality for the different cut-off levels of eosinophils and as a continuous variable.

Cut-off eosinophils	Mortality Hazard Ratio (95% CI)		Hospitalisation AECOPD Hazard Ratio (95% CI)		CAP Hazard Ratio (95% CI)	
	Uni-variate	Multi-variate	Uni-variate	Multi-variate	Uni-variate	Multi-variate
≥ 0.3×10^9 cells/L. N=53(18%)	0.75 (0.52-1.09)	0.84 (0.57-1.22)[1]	1.06 (0.68-1.67)	1.09 (0.69-1.69)[3]	0.89 (0.45-1.75)	0.88 (0.43-1.79)[4]
≥ 0.2×10^9 cells/L. N=93(32%)	0.84 (0.63-1.14)	0.91 (0.67-1.23)[1]	0.98 (0.67-1.43)	0.97 (0.67-1.43)[3]	0.83 (0.47-1.45)	0.83 (0.47-1.47)[4]
≥ 2% of total leucocyte count. N=73(25%)	0.72 (0.52-1.01)	0.76 (0.54-1.06)[1]	1.03 (0.69-1.56)	1.04 (0.69-1.56)[3]	0.74 (0.39-1.39)	0.72 (0.37-1.39)[4]
≥ 3% of total leucocyte count. N=43(15%)	0.75 (0.49-1.12)	0.88 (0.58-1.33)[1]	1.03 (0.63-1.69)	1.08 (0.65-1.79)[3]	0.77 (0.35-1.69)	0.72 (0.31-1.69)[4]
Eosinophil as continuous variable per 0.10×10^9 cells/L increase. N=293	0.97 (0.91-1.03)	0.99 (0.94-1.04)[2]	1.00 (0.95-1.06)	NA	0.97 (0.86-1.09)	NA

[1] corrected for age and GOLD stage
[2] corrected for age
[3] corrected for FEV1
[4] corrected for ADO
NA: not applicable, no correction for confounders necessary

Discussion

Blood eosinophil count during severe AECOPD in the COMIC study was not associated with morbidity and mortality. In univariate analyses there seemed to be an association with some cut-off levels with survival. However, after correcting for confounders this disappeared. Furthermore, in our sensitivity analyses in which we excluded patients with recent prior steroid use, there was also no association with morbidity and mortality.

COPD is predominantly characterised by neutrophilic inflammation. However a relevant subgroup (20-40%) has an eosinophilic phenotype probably associated with underlying type 2 inflammation as can also be seen in patients with asthma. (19) However, the exact pathobiology is not clear even as the function of this inflammation, since the eosinophil has no or limited actual protective mechanisms or anti-bacterial effect whilst this eosinophil and type 2 inflammation in the lung

is believed to be involved with negative effects in different COPD processes which can be harmful.(10,32,33) Also during AECOPD this eosinophilic inflammation can occur. Bafadhel et al. identified four different biologic clusters for AECOPD of which one is the eosinophilic type of AECOPD.(34) In general the absolute and relative (sputum) eosinophil count increases significantly at AECOPD(35). Having an eosinophilic COPD phenotype does not automatically mean that your AECOPD is mediated by eosinophilic inflammation although it is known to be associated with higher odds of subsequent eosinophilic AECOPD.(36,37) In addition, having an eosinophilic AECOPD does not automatically mean that your next AECOPD is also eosinophilic. We showed in a previous study that of the patients with an eosinophilic AECOPD, only 34%-45% was eosinophilic again during the next AECOPD, depending on which cut-off was used.(38) This lack of stability could limit the prognostic usefulness of this biomarker during AECOPD. Gong et al. studied a group that was persistent eosinophilic during 2 subsequent AECOPD and these had a lower risk of all-cause mortality after discharge.(39) Next to the fact that this finding still needs validation, also the usability in clinical practice is questionable, since you need serial measurements due to the lack of stability of having eosinophilic AECOPDs when predicting longer term outcomes.

A higher risk of future hospitalisations with pneumonia was predicted from a stable state blood eosinophil count of ≥ 0.3×10^9 cells/L in individuals with COPD and FEV1 <50% predicted, compared with individuals with the same degree of airflow limitation but lower eosinophil count by Vedel-Kroch et al.(40) This appeared not to be mediated through ICS use. Pavord et al. reported however a small increased risk of pneumonias in patients with blood eosinophil counts <2%. (41) Martinez-Garcia et al. reported that less than 100 circulating eosinophils/µL combined with the presence of chronic bacterial infection increased the risk of pneumonia in COPD patients treated with ICS.(42) Whether the eosinophil count measured at severe AECOPD is associated with future CAP is not known. Our study now shows that irrespective of the cut off used, the blood eosinophil count was not associated with time till next CAP. Our study lacks knowledge about presence of chronic bacterial infection so this combination with the eosinophil count could not be studied.

Various studies showed various outcomes for the association of elevated eosinophil count at AECOPD with subsequent AECOPD or COPD related re-admissions. Couillard et al. suggested prior steroid use as a confounder for these sometimes conflicting results.(20) In their study patients with corticosteroid use within 48 hours prior to measurement of the blood eosinophil count were excluded. In the

167 remaining patients elevated eosinophil count at AECOPD was associated with a shorter time to first COPD-related readmission. Since the exact duration of the lowering effect of steroids on the blood eosinophil count is not well known we wanted to minimize this potential confounding effect. Therefore, in our study, with a larger sample size, we excluded in the sensitivity analysis patients who used any systemic steroids in the last two weeks before the AECOPD and we found no association with time till next severe AECOPD.

The blood eosinophil count has now extensively been studied in stable state COPD as well as during an AECOPD as a prognostic biomarker for subsequent morbidity and mortality. The results remain inconsistent. This is due to a multitude of factors such as different study designs, different study populations, lack of validation, different length of follow up time, missing correction for confounders etc. In stable state important factors influencing the association of blood eosinophil counts with subsequent morbidity and mortality are ICS use and exacerbation history. In various randomised controlled trials the stable state blood eosinophil count was mainly associated with elevated exacerbation risk in the non-ICS arms and was the clearest in patients with frequent exacerbations. (10,43–45)

The strength of our study in patients with severe AECOPD is that we have a well-defined COPD population with a large sample size and that we do have knowledge of this exacerbation history and ICS use. Furthermore, we analysed the eosinophil count measured at AECOPD as a continuous variable as well as for different cut-off levels and with the sensitivity analysis we could confirm or exclude the confounding effect of recent steroid use on this eosinophil count. Moreover, with our long follow up we could analyse the relation with overall survival. All in all we did not find an association with subsequent morbidity and mortality.

Limitations of our study are the single centre population design. The patients are mainly controlled and treated for their COPD in secondary care which means they probably have a more severe COPD. So we cannot automatically extrapolate our results to patients with a more mild COPD or patients treated in primary care. Another limitation is that although (recent) steroid use was defined by the dispensing of the medication at the pharmacy we cannot determine with certainty that the patient actually used their medication or if perhaps a patient used steroids that were dispensed longer (>14 days) before the severe AECOPD, which might be the case when patients are enrolled in self-management programs with action plans for AECOPD.

In conclusion, within 3 years of follow up of COPD patients with severe AECOPD in the COMIC cohort, almost a quarter of the patients had at least one subsequent CAP and almost half had at least one subsequent severe AECOPD. Forty-three per cent of the patients died, confirming that a severe AECOPD as such is associated with a poor prognosis. There is an unmet need to better identify these patients at risk for these dismal outcomes. Besides known clinical parameters such as age, lung function and exacerbation history, the use of biomarkers could assist in identifying these patients. Our study shows however that the blood eosinophil count measured at severe AECOPD does not seem suited for this purpose.

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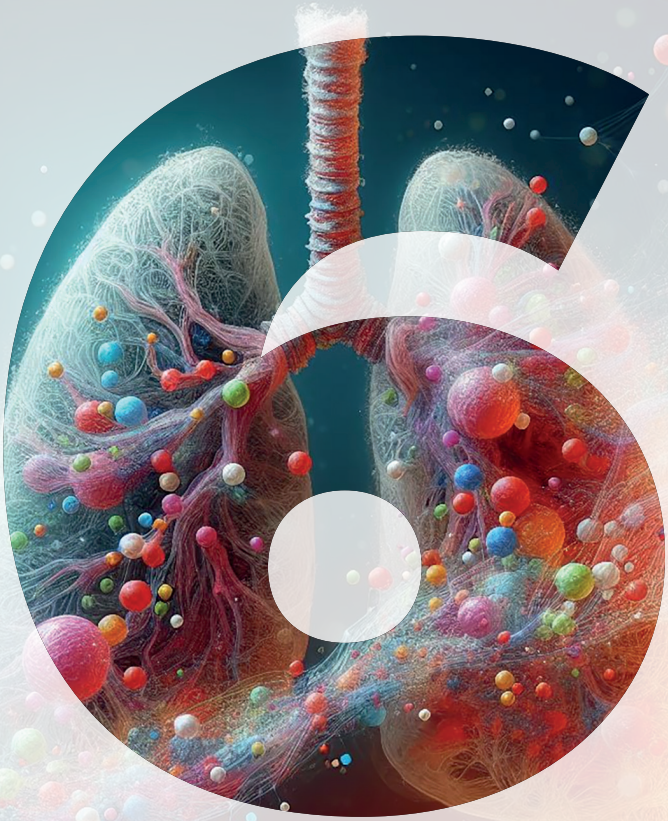

Stability in eosinophil categorisation during subsequent severe exacerbations of COPD

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Abstract

Background: The blood eosinophil count has been shown to be a promising biomarker for establishing personalised treatment strategies to reduce corticosteroid use, either inhaled or systemic, in COPD. Eosinophil levels seem relatively stable over time in stable state, but little is known whether this is also true in subsequent severe acute exacerbations of COPD (AECOPD).

Aims and objectives: To determine the stability in eosinophil categorisation between two subsequent severe AECOPDs employing frequently used cut-off levels.

Methods: During two subsequent severe AECOPDs blood eosinophil counts were determined at admission to the hospital in 237 patients in the COMIC study. The following 4 cut-off levels were analysed: absolute counts of eosinophils $\geq 0.2 \times 10^9$/L (200cells/μL) and $\geq 0.3 \times 10^9$/L (300cells/μL) and relative eosinophil percentage of ≥ 2% and ≥ 3% of total leukocyte count. Categorisations were considered stable if during the second AECOPD their blood eosinophil status led to the same classification: eosinophilic or not.

Results: Depending upon the used cut-off, the overall stability in eosinophil categorisation varied between 70%-85% during two subsequent AECOPDs. From patients who were eosinophilic at the first AECOPD, 34%-45% remained eosinophilic at the subsequent AECOPD while 9%-21% of patients being non-eosinophilic at the first AECOPD became eosinophilic at the subsequent AECOPD.

Conclusions: The eosinophil variability leads to category changes in subsequent AECOPDs, which limits the eosinophil categorisation stability. Therefore, measurement of eosinophils at each new exacerbation seems warranted.

Introduction

The use of corticosteroids, inhaled (ICS) or systemic, plays an important role in the maintenance treatment of COPD and in its associated acute exacerbations (AECOPD).(1) Although beneficial for some, corticosteroids could be detrimental for others because of lack of efficacy combined with potential side effects such as pneumonia, hyperglycaemia, osteoporosis and venous thromboembolism.(1-3) More and more focus is on how to select patients who probably will not benefit from corticosteroid use and in whom exposure to corticosteroids can safely be reduced or withheld.

In recent years, blood eosinophil count has shown to be a promising biomarker for establishing personalised treatment strategies to reduce corticosteroid use in COPD.(2-6) Elevated blood eosinophil count, as a surrogate marker for sputum eosinophilia, is associated with better steroid responsiveness.(7;8) In stable state COPD it has become clear that the blood eosinophil count, in combination with clinical assessment, can be used to determine the treatment effect of ICS use. This has been incorporated in the GOLD 2019 report.(1)

Two studies found evidence for an eosinophil-guided strategy for systemic steroid use also in acute exacerbations.(2;3) Defining an AECOPD to be eosinophilic and defining the stability of an eosinophilic AECOPD phenotype over time are both vitally dependent upon the chosen cut-off level and the expression of the eosinophil as an absolute or a relative count. The study of Bafadhel et al. was in outpatients with a cut-off level of eosinophil count $\geq$ 2% of total leukocytes. The study of Sivapalan et al. in inpatients used the cut-off of an absolute eosinophil count $\geq 0.3 \times 10^9$ cells per L. With their limitations, both studies showed that eosinophil-guided steroid use is non-inferior to standard systemic steroids during AECOPD and leads to a lower cumulative steroid exposure.

In stable state COPD, eosinophil levels seem relatively stable over time (9-11), but on whether this is also true in AECOPD is only knowledge from a small subset of patients (N=67) in one study.(12) Since different aetiologies exist for AECOPD, this stability may not be the case: elevated eosinophil levels may not be present every time. High stability would negate the need to determine blood eosinophils and accelerate the start of treatment. Low stability however implies that the eosinophil count has to be determined at every new AECOPD. This will add an extra barrier in the treatment of AECOPD, especially in the primary care setting and in COPD self-management.

Furthermore, it's possible that in daily practice patients admitted to the hospital for an AECOPD are already on a course of systemic steroids or may have recently been on one. How large this impact is on the eosinophil level at presentation of an AECOPD and whether despite this steroid use patients can still have elevated eosinophil levels, are both not well known.

So the aim of this study is to determine the stability in eosinophil categorisation during two subsequent severe (hospitalised) AECOPD and to study the influence of (recent) use of steroids on this stability. We will analyse this for four different cut-off levels including the two abovementioned ones, and determine to what degree eosinophil variability, prior steroid use, and expression of eosinophilia chosen lead to changes in ensuing treatment choice of systemic steroids.

To assess these questions we used data of the large COMIC (Cohort of Mortality and Inflammation in COPD) study with well-defined COPD patients with a well-documented medication history including steroid use.

Methods

Settings and study population

This study was part of the COMIC study, a single-centre cohort study from Enschede, the Netherlands. From December 2005 until April 2010, 795 patients were included with a follow-up period of at least 3 years. The COMIC study was approved by the hospital's medical ethical committee (METC Twente, No. P05-49). All patients provided written informed consent. The COMIC study started before the introduction of trial registries. Results from the cohort study have been published previously.(13-15) For inclusion in the COMIC study, patients had to meet the following criteria: (A) a clinical diagnosis of COPD according to the GOLD guidelines; (B) current or former smoker; (C) age ≥ 40 years; (D) absence of any other active lung disease (e.g. sarcoidosis); (E) no maintenance therapy with antibiotics. For the current sub-study we performed an analysis in all patients with at least two subsequent severe AECOPDs leading to hospitalisation during follow up.

Patient and public involvement

Patients and the public were not involved in the design and conduct of this study.

Eosinophil counts and cut-off levels

Blood eosinophil counts were determined at admission to the emergency room on the day of hospitalisation for a severe AECOPD. The eosinophil counts were determined with increments of $0.1x10^9$ cells/L (100cells/µL). The following four cut-off levels were analysed: absolute count of eosinophils ≥ $0.2x10^9$ cells/L (200cells/µL) or ≥ $0.3x10^9$ cells/L (300cells/µL) and relative eosinophil percentage of ≥ 2% or ≥ 3% of total leukocyte count. Patients with an eosinophil count above the cut-off were categorised as eosinophilic, and below as non-eosinophilic.

Outcomes

The correlation between the two eosinophil counts during both AECOPDs was determined for both the absolute counts and the relative counts. Subsequently, to determine whether the variability of the two eosinophil counts during both AECOPDs led to a change in eosinophil categorisation, the overall eosinophil categorisation stability was established for all four different cut-off levels. This eosinophil stability was defined as the percentage of patients in whom their blood eosinophil count was categorised the same during the two subsequent AECOPDs.

Role of steroid use on eosinophil categorisation stability

To determine whether (recent) steroid use influenced the stability of eosinophil categorisation in subsequent severe AECOPD, the cohort was subdivided into three groups. Group 1 includes patients without steroid use before both subsequent AECOPDs. Group 2 includes patients with steroid use before both AECOPDs. Group 3 includes patients who used steroids either before the first AECOPD or before the second AECOPD. Recent steroid use was defined as the use of any systemic steroids in the last two weeks before the AECOPD.

Effect steroid use on absolute eosinophil count

To determine whether (recent) systemic steroid use influenced absolute eosinophil counts, the absolute eosinophil counts at the first AECOPD hospitalisation in the total cohort were compared between patients with or without prior steroid use before this hospitalisation. Furthermore, the effect of steroid use on the absolute eosinophil count within individuals was compared in Group 3 in which paired eosinophil assessments were available per patient, one with and one without prior steroid use.

Statistical analyses

Continuous variables are expressed as mean with standard deviation (SD) or median with interquartile range (IQR). Categorical variables are presented as counts with percentages. Baseline differences between the 3 groups of patients with or without prior steroid were tested with either the ANOVA or Krukall Wallis test for continuous variables and with Chi-square or Fisher Exact tests for categorical variables. Correlation of both the absolute and relative eosinophil counts between the two AECOPD hospitalisations were assessed by the Spearman's rank correlation test. The effect of steroids on the absolute eosinophil count between patients with or without prior steroid use in the total cohort were assessed by the Mann Whitney U Test. The effect of steroids on the absolute eosinophil count within patients in group 3 with or without prior steroid was assessed by the Wilcoxon Signed Ranks Test.

Results

Of the 795 patients enrolled in the COMIC study, 237 patients had at least two severe AECOPDs during follow-up. Of these 237 patients, 106 patients (45%) did not use systemic steroids before the first and second severe AECOPD (Group 1), 32 patients (13%) started a course of steroids before the first and second severe AECOPD (Group 2), while 99 patients (42%) started steroids either before the first or the second severe AECOPD (Group 3).The baseline characteristics of these 237 patients and the baseline characteristics of the patients within the three groups are displayed in Table 1. Approximately eighty percent of the patients used inhaled corticosteroids.(16) Patients in group 2 had a worse lung function, the duration between the two subsequent AECOPD was shorter, and they had more previous AECOPD.

Table 1 Baseline characteristics of the patients with two severe AECOPDs for the overall cohort and the 3 groups (patients without steroid use before both AECOPDs (group 1), patients with steroid use before both AECOPDs (group2), patients who used steroids either before the first AECOPD or before the second AECOPD (group 3)).

Patients with at least 2 severe AECOPD	Overall N= 237	Group 1 N=106	Group 2 N=32	Group 3 N=99	p Value
Age: mean in years (SD)	68.2 (10.1)	67.9 (9.6)	68.7 (11.0)	68,3 (10.4)	0.924
Male (number (%))	135 (57.0)	60 (56.6)	15 (46.9)	60 (60.6)	0.397
Current smoker (number (%))	72 (30.4)	37 (34.9)	8 (25.0)	27 (27.3)	0.386
Pack years: median (IQR)	34.5 (20.9 – 51.2)	34,5 (22.0 – 50.3)	34.2 (17.3 – 55.3)	35.5 (23.3 – 53.0)	0.633
BMI: mean (SD)	26.1 (5.3)	25,9 (4,9)	25.5 (4.5)	26.5 (5.9)	0.548
Lung function					
FEV_1 in litres: mean (SD)	1.1 (0.5)	1.1 (0.5)	1.0 (0.3)	1.2 (0.5)	0.009
FEV_1 % predicted: mean (SD)	43.8 (15.8)	43.6 (16.9)	37.9 (11.6)	46.0 (15.4)	0.040
FEV1/FVC in %: mean (SD)	40.2 (12.8)	38.2 (24.3)	37.9 (11.2)	41.5 (13.6)	0.345
GOLD stage (number (%))					0.129
I	5 (2.1)	2 (1,9)	0 (0.0)	3 (3.0)	
II	74 (31.2)	34 (32.1)	5 (15.6)	35 (35.4)	
III	114 (48.1)	46 (43.4)	19 (59.4)	49 (49.5)	
IV	44 (18.6)	24 (22.6)	8 (25.0)	12 (12.1)	
Inhalation corticosteroid use (number (%))	206 (86.9)	94 (88.7)	24 (75.0)	88 (88.9)	0.115
Time between AECOPD: median in days (IQR)	296 (76 – 691)	317 (74 – 802)	120 (44 – 536)	318 (142 – 681)	0.041
Previous AECOPD (number (%))[1]					0.010
0-1 AECOPD	95 (42.2)	51 (50.1)	6 (20.0)	38 (40.4)	
≥ 2 AECOPD	130 (57.8)	50 (49.5)	24 (80.0)	56 (59.6)	
Previous severe AECOPD(number (%))[1]					0.405
≥ 1 severe AECOPD	54 (22.8)	21 (19.8)	10 (31.3)	23 (23.2)	

[1] In year before recruitment (missing data of 12 patients). Abbreviations; SD: Standard Deviation; IQR: Inter Quartile Range; BMI: Body Mass Index; FEV_1: Forced Expiratory Volume in 1 second; GOLD: Global Initiative for Chronic Obstructive Lung disease; AECOPD: Acute exacerbation of chronic obstructive pulmonary disease.

Figure 1 shows the scatter plots for the eosinophil count measured at both severe AECOPD (rho=0.19, p=0.003 for the absolute counts, rho=0.22, p=0.001 for the relative counts).

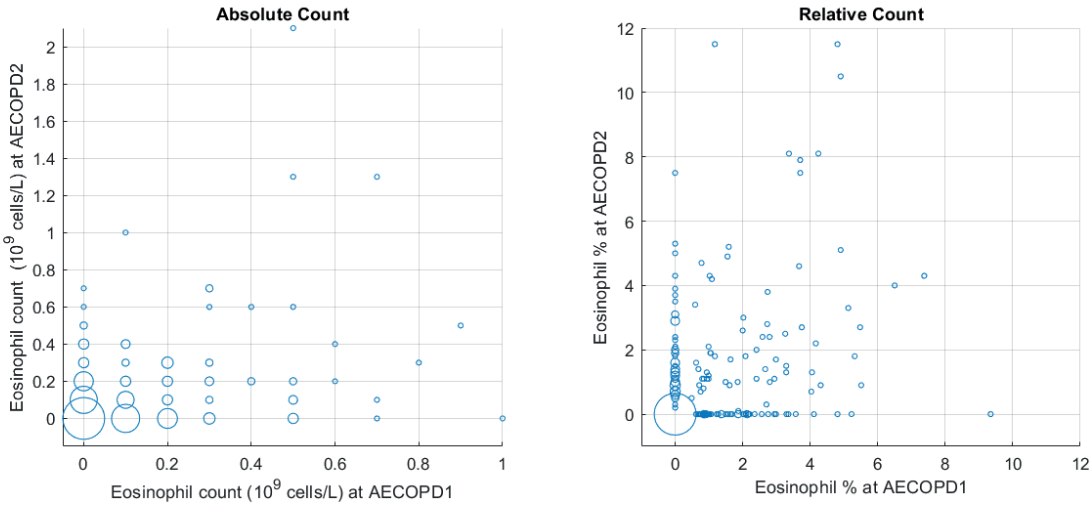

Figure 1: Scatter plot for the absolute and relative eosinophil counts measured at both severe AECOPD.

The size of the circle corresponds to the number of measurements with the smallest circle representing one measurement and the largest 66. Left absolute count. Right relative count.

The median absolute difference in eosinophil count between the two AECOPDs was 0.1x10^9 cells/L (IQR 0.00-0.20). Stability of eosinophil categorization over the two severe AECOPDs is shown in Figure 2 for the overall cohort and the three groups.

In the total cohort, depending upon the used cut-off, 12%-28% of the patients were eosinophilic at the first AECOPD and 34%-45% of those remained eosinophilic at the second AECOPD. In total 5-13% of the patients were eosinophilic at both AECOPD; 57%-80% of the patients were non-eosinophilic at both AECOPD, and 15%-30% changed from category at the second AECOPD. The eosinophil stability during the two AECOPD was 70%-85%.

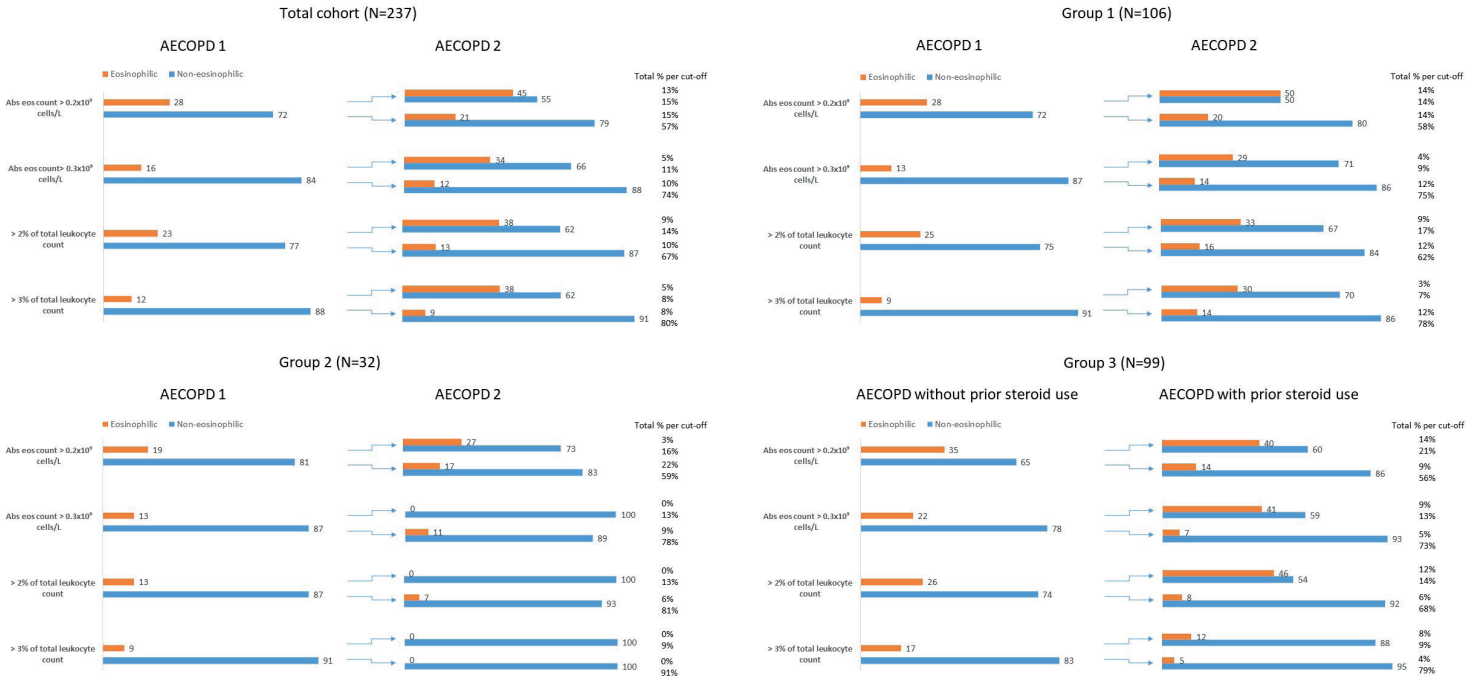

Figure 2. Stability of eosinophil categorization over the two severe AECOPDs

Data presented separately for the total cohort, patients without steroid use before both AECOPDs (group 1), patients with steroid use before both AECOPDs (group2), patients who used steroids either before the first AECOPD or before the second AECOPD (group 3). The bars show the proportions of patients being categorised eosinophilic or non-eosinophilic for the four used cut-offs at the first and second AECOPD.

In Group 1, 9%-28% of the patients were eosinophilic at the first AECOPD, 29%-50% of those remained eosinophilic at the second AECOPD. In total 3%-14% of the patients were eosinophilic at both AECOPD, while 58%-78% were non-eosinophilic at both AECOPD, and 19%-29% changed from category at the second AECOPD. The eosinophil stability was 71%-81%.

In Group 2, 9%-19% of the patients were eosinophilic at the first AECOPD, and 0%-27% of those remained eosinophilic at the second AECOPD. In total 0%-3% of the patients were eosinophilic at both AECOPD, while 59%-91% were non-eosinophilic at both AECOPD, and 9%-38% changed from category at the second AECOPD. The eosinophil stability was 62%-91%.

In Group 3, 17%-35% of the patients were eosinophilic at the AECOPD without prior steroid use, and 12%-46% of them remained eosinophilic at the AECOPD with prior steroid use. In total 8%-14% of the patients were eosinophilic at both AECOPD, 56%-79% of the patients were non-eosinophilic at both AECOPD, and 13%-30% changed from category during both AECOPD. The eosinophil stability was 70%-87%.

Effect of steroid use on absolute eosinophil count

In the total cohort the median eosinophil count in patients (N=145) who did not use steroids in the two weeks prior to the first severe AECOPD was $0.10x10^9$ cells/L (IQR 0.00-0.20), which was significantly higher than the eosinophil count in patients (N=92) who did use steroids in the two weeks prior to the first severe AECOPD (median eosinophil count $0.00x10^9$ cells/L (IQR 0.00-0.10)(p=0.006). Of the 92 patients with systemic steroid use in the two weeks prior to hospitalization for AECOPD 37% still had an eosinophil count ≥ $0.1x10^9$ cells/L and 10-22% were still labelled as eosinophilic depending on criterion used.

In Group 3, including patients that had both an severe AECOPD with and without prior steroid use, the median eosinophil count determined at the AECOPD without prior steroid use was $0.10x10^9$ cells/L (IQR 0.00-0.20), which was significantly higher than the eosinophil count determined at the AECOPD with prior steroid use (median eosinophil count $0.00x10^9$ cells/L (IQR 0.00-0.10)(p=0.016).

Discussion

Our study shows that although a statistically significant correlation existed between eosinophil counts measured at subsequent severe AECOPDs, this correlation was very poor. The eosinophil counts were rather variable and depending upon the used cut-off and expression of the eosinophil, the overall stability in eosinophil categorisation varied between 70%-85%. This means that 15%-30% of the patients changed from category. Furthermore, systemic steroids use immediately prior to AECOPD lowered the eosinophil numbers, but did not normalize them and numerically had no relevant influence on the stability of the eosinophil categorisation.

Our results suggest that independent of which cut-off is used, when making a choice regarding systemic steroid treatment dependent on eosinophil numbers during a severe AECOPD, one cannot rely on the eosinophil count measured during an earlier severe AECOPD. The eosinophil count has to be determined at every new severe AECOPD.

An important explanation for the variation in eosinophil count during subsequent severe AECOPDs leading to changes in eosinophil categorisation could lie in the variability in causes of AECOPD, which need not be the same at each exacerbation. Bafadhel et al. described different biologic and clinical COPD phenotypes during outpatient treated AECOPDs.(17) Furthermore, Kolsum et al. showed that patients with a bacterial infection during an AECOPD had a significant decrease in blood eosinophil count compared to stable state, while no changes were observed in patients without bacterial infection.(8) In most cases, the exact pathobiology of eosinophils in blood is unclear. Analyses of RNA expressions levels from sputum or for instance nasal epithelium, and perhaps leukocyte activation markers in blood and or sputum in relation to bacterial and viral load may help elucidate the origins of the eosinophilia.(18;19)

Although this study focuses on severe (hospitalised) AECOPD, we expect the same lack of stability in eosinophil categorisation in subsequent less severe AECOPDs, i.e. without hospitalization. Recently, among others Lenferink et al. reported the

beneficial effects of self-management interventions in COPD, including action plans for exacerbations and they suggested to include this in COPD management. (20) Eosinophil guidance in the treatment of AECOPD to become common practice would have major implications for COPD self-management interventions as this should then lead to measuring eosinophils at the moment of AECOPD. As a consequence, health care providers of patients with COPD and patients with self-management action plans should have easy access to a (point of care) tool also in the outpatient clinic to determine their eosinophil count at AECOPD onset that should not delay treatment. In this study, patients with steroid use before both AECOPDs (group2) had a worse lung function, more previous AECOPD, and a shorter time between the two subsequent AECOPD, possibly suggesting a more serious COPD. Maybe as a consequence these patients possibly have COPD self-management action plans, which could explain why they were already on OCS on the day of laboratory testing for both of their AECOPD.

The use of relative eosinophil percentages and the use of higher cut-off levels were both associated with higher overall stability in eosinophil categorisation. However, fewer AECOPDs will then be defined eosinophilic. This will result in more patients who may be incorrectly withheld from steroids for their AECOPD when an eosinophil-guided steroid strategy is used. The higher cut-off level used in the CORTICO-COP study (3) may therefore have influenced the observed, be it non-significant, higher 30-day readmission and death rates in the eosinophil-guided arm. On the other hand, more patients had the advantage of not being exposed to the potential detrimental effects of steroids. Thus, the chosen cut-off level and the way of classifying eosinophils, relative versus absolute, are important factors to be considered when using eosinophil-guided strategies.

Another finding of this study is that also in COPD (recent) steroid use leads to significantly lower eosinophil counts measured at admission for their AECOPD, as expected. It is known, primarily from analyses in patients with asthma, that oral steroid use can lower blood eosinophil count significantly, even after one single dose, and that this suppression can last more than 24 hours.(21;22) Despite this lowering effect, significant eosinophilia persisted in many patients; steroid use did not have major influence on the eosinophil categorisation stability during subsequent AECOPD in our study and still about 10-22% of the patients were categorized eosinophilic when admitted to the hospital for AECOPD. This may contain important information. When patients need to be hospitalized for AECOPD, even though they had prior systemic steroid use, routine care often leads

to higher and /or longer steroids. Where that may be justified in patients with high eosinophil counts, it might be less useful or even detrimental in patients without eosinophilia.(3) In this latter CORTICO-COP study a strategy quite similar to this suggestion was used, resulting in a reduced duration of systemic corticosteroid exposure without significant difference in number of days alive and out of hospital.

In a study by Schumann et al. blood eosinophil counts were longitudinal collected in COPD patients in stable state and in AECOPD.(12) During severe exacerbations, up to one-half of the patients had discordant or variable blood eosinophil levels, dependent on which cut-off was used, which is even worse than our results. These analyses were however in a small subset of patients (N=67) and no information about previous steroid use was available. This in contrary to our study in which being able to study the influence of (recent) steroid use in a large group of COPD patients is the major strength of our analysis.

A limitation of our study is however that although (recent) steroid use was defined by the dispensing of the medication at the pharmacy we cannot determine with certainty that the patient actually used the medication or if perhaps a patient used steroids that were dispensed longer (>14 days) before the severe AECOPD.

Since differences in steroid use prior to the two included severe AECOPD could have influenced the stability of eosinophil categorisation, we also performed sensitivity analyses in three subgroups of the cohort (based on prior steroid use). These sensitivity analyses showed that (recent) steroid use (within two weeks before the AECOPD) or not in fact has no relevant influence on this stability of eosinophil categorisation. Additionally, we studied recent steroid use defined as the start of OCS within one week before the AECOPD (see supplementary index figure 1) indicating definite OCS use during laboratory testing. Numerically this had no effect on stability of eosinophil categorisation, although groups 2 and 3 became much smaller now.

So far, there is no consensus on an acceptable degree of stability of eosinophil categorisation, so the results of our study are open for debate. We strongly believe, as our results show, that if ≥ 55% of the patients categorised as eosinophilic were not categorised as eosinophilic at the subsequent AECOPD an eosinophil guided strategy for steroid use for AECOPD can only be incorporated if the eosinophil count will be determined at every new AECOPD. As with any binary categorisation, small changes around the threshold have major impact on the category. Maybe,

just as in stable state, one should not use just one cut-off but see the eosinophil count more as a continuous variable and use for instance 2 cut-off levels. This results in 3 categories in which the high (eosinophilic group) level should receive steroids, the low (non-eosinophilic group) level should not receive steroids and for the intermediate group steroid use should be individualised using other (clinical or historical) parameters. Of course this strategy should be prospectively studied as well.

Finally, the eosinophil count was established with increments of $0.1x10^9$ cells/L. Therefore another frequently used cut-off, i.e. $0.15x10^9$ cells/L, could not be used in our analyses. However, this cut-off has mainly been used in stable state and was not one of the cut-offs used for steroid use in AECOPD. We have performed additional analyses for the $\geq 0.10x10^9$ cells/L cut-off (see supplementary index figure 2) which is used in the GOLD guideline for assessing ICS use in stable state. This resulted in lower eosinophil categorisation stability which is in line with our findings that lower cut-offs are associated with lower overall eosinophil categorisation stability.

Time between the two AECOPD (using the median) was not associated with the categorisation stability in the overall cohort tested for all the cut-offs (data not shown). It would be interesting for further research to study factors that could predict this eosinophil categorisation stability. Perhaps in subgroups of patients (i.e. persistent smokers, elderly patients) this stability may be better or worse. A relevant factor should then also be the aetiology of the AECOPD.

In summary, eosinophil categorisation varies in subsequent severe AECOPDs. This variability leads to category changes in subsequent AECOPDs which limits the eosinophil categorisation stability. Although (recent) steroid use was associated with lower baseline eosinophil count, numerically it has no relevant influence on the categorisation stability. Until we understand more about the causes of eosinophilia, quick access, point of care measurements at each new exacerbation seem warranted.

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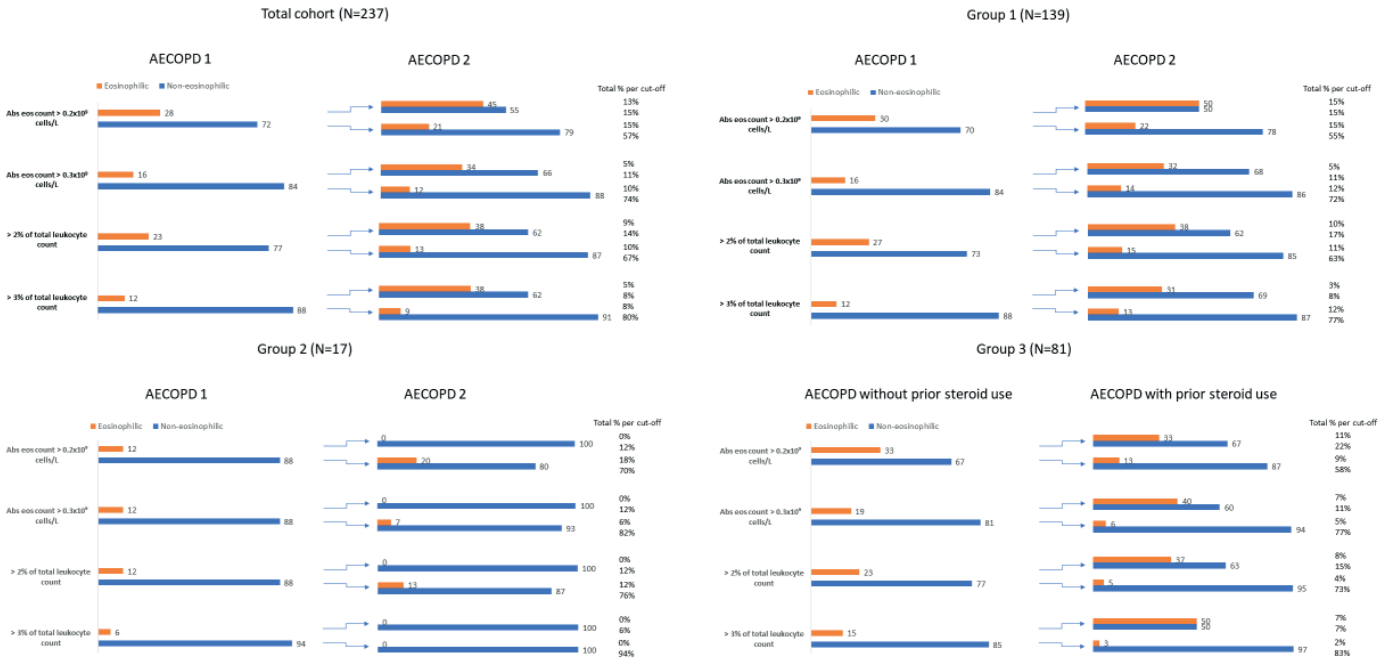

Figure 1. Stability of eosinophil categorization over the two severe AECOPDs

Data presented separately for the total cohort, patients without steroid use in the week before both AECOPDs (group 1), patients with steroid use in the week before both AECOPDs (group2), patients who used steroids either in the week before the first AECOPD or the week before the second AECOPD (group 3). The bars show the proportions of patients being categorised eosinophilic or non-eosinophilic for the four used cut-offs at the first and second AECOPD.

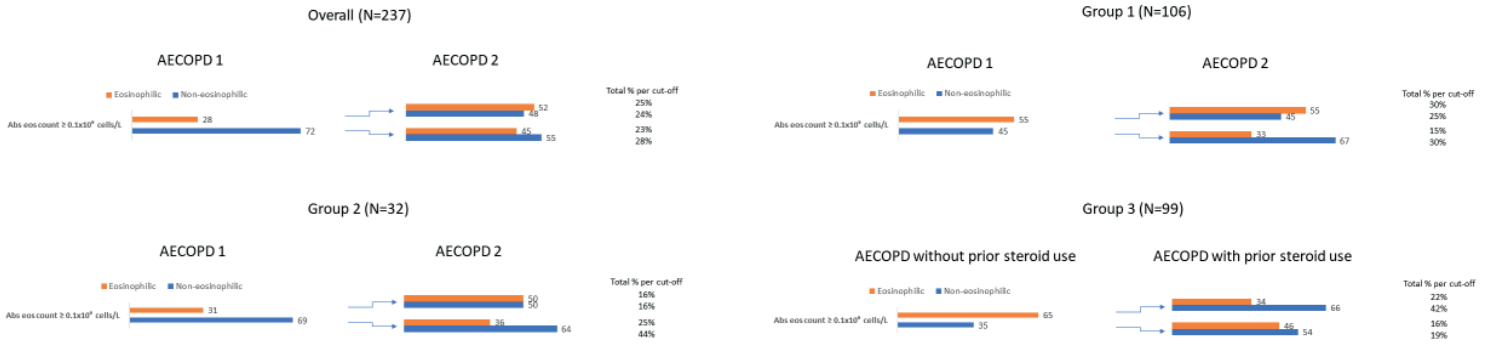

Figure 2. Stability of eosinophil categorization over the two severe AECOPDs for the ≥ 0.1x10^9 cells/L cut-off

Data presented separately for the total cohort, patients without steroid use before both AECOPDs (group 1), patients with steroid use before both AECOPDs (group2), patients who used steroids either before the first AECOPD or before the second AECOPD (group 3). The bars show the proportions of patients being categorised eosinophilic or non-eosinophilic for the ≥ 0.1x10^9 cells/L cut-off at the first and second AECOPD.

Potential for personalised and biomarker-guided COPD self-treatment approaches

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In this Correspondence, we use the paper of Sanjay Ramakrishnan and colleagues[1] as a starting point to discuss the potential use of blood eosinophil-guided exacerbation action plans integrated in personalised chronic obstructive pulmonary disease (COPD) self-treatment approaches.

Ramakrishnan and colleagues conclude that blood eosinophil- guided prednisolone therapy at the time of an acute exacerbation of COPD is non-inferior to standard care and can be used to safely reduce systemic glucocorticoid use in clinical practice.[1] They briefly discuss their results in the light of current practices of COPD rescue pack use, and conclude that underlying inflammatory endotypes should be taken into account when considering the use of these packs. It is important to acknowledge that, whereas there is a growing body of evidence regarding the effectiveness of using self-treatment exacerbation action plans combined with the provision of patient training in formalised self-management interventions,[2] there is no evidence regarding effectiveness and safety to support handing out rescue packs without any training and support.

The paper of Ramakrishnan and colleagues states that no other field of medicine advocates for self-treatment of potential life-threatening events.[1] Self-treatment is, however, common in the fields of chronic diseases, such as diabetes and asthma, ideally in combination with ongoing patient training, support, and regular review, embedded in a personalised self- management intervention, rather than as a standalone approach.[2] In addition to tailoring COPD self-treatment approaches to the patient's individual symptom levels, exacerbation risk, needs of support in self-treatment, health beliefs, capabilities, medication use, smoking cessation, literacy level, and comorbidities,[3] we believe that the integration of a self-treatment approach guided by blood eosinophil count should be considered to safely reduce the systemic exposure and toxicity of universal prednisolone therapy. However, there are still some important features that need to be defined before blood eosinophil- guided exacerbation action plans can be integrated in clinical practice.

Defining an acute exacerbation of COPD to be eosinophilic is crucially dependent on the chosen cutoff values and the eosinophil expression as an absolute or relative count. There is, however, still no consensus regarding the optimal cutoff value to guide steroid treatment in general, let alone in a self-treatment approach.[4] Ramakrishnan and colleagues used a 2% blood eosinophil count as a threshold to guide steroid treatment, and reported that in their COPD population 65% of the acute exacerbations of COPD were eosinophilic.[1] However, in other studies

the percentage of acute exacerbations of COPD that were eosinophilic was much lower (10–30%).[4,5]

Although different phenotypes and various biological clusters for acute exacerbation of COPD in different populations have been identified previously,[5] both the phenotypes and clusters can differ in subsequent acute exacerbation of COPD[4] and influence personalised treatment decisions. A large prospective COPD cohort study showed that, when using the 2% cutoff for defining an eosinophilic acute exacerbation of COPD in patients with severe acute exacerbation of COPD, only 38% of subsequent severe acute exacerbation of COPD were defined as eosinophilic.[6] This variability in eosinophil count (ie, absolute vs relative counts for different cutoff values) necessitates establishment of the eosinophil count at every new acute exacerbation of COPD. For patients with acute exacerbation of COPD who are visiting the hospital or primary care (as in the study by Ramakrishnan and colleagues)[1], it will be relatively easy to establish eosinophil count with point-of-care testing during each acute exacerbation of COPD. We recognise that for integration of blood eosinophil measurements within self-treatment approaches, patients should have easy access to a point-of-care tool establishing their eosinophil count at acute exacerbation of COPD onset, without delaying treatment. Since the benefits from the use of self-treatment exacerbation action plans arise mainly from earlier initiation of appropriate treatment, it must be ensured that adding a tool will not become a hurdle for timely treatment of an acute exacerbation of COPD. Additionally, as the majority of the acute exacerbations of COPD are expected to have an eosinophil count below the steroid treatment threshold, alternative treatment strategies need to be defined for these acute exacerbations of COPD. Moreover, developments in biomarker-guided antibiotic treatment in acute exacerbation of COPD might require even more adjustments in acute exacerbation of COPD self-treatment approaches (eg, integration of rapid point-of-care tests to guide antimicrobial therapy of acute exacerbations of COPD with a bacterial origin and reduce antimicrobial resistance). The use of patient-friendly digital technology will probably be crucial to enable the use of complicated underlying decision models, and optimise the quality, accessibility, and efficiency of self- treatment of acute exacerbation of COPD.

The integration of necessary refinements and tailoring of self- treatment approaches will be an ongoing and evolving process, with practical and logistical challenges to overcome. We believe that the study by Ramakrishnan and colleagues[1] will not be the end of self-treatment for COPD, it will rather be the beginning of more personalised and biomarker-guided COPD self-treatment approaches that are embedded in self-management interventions.

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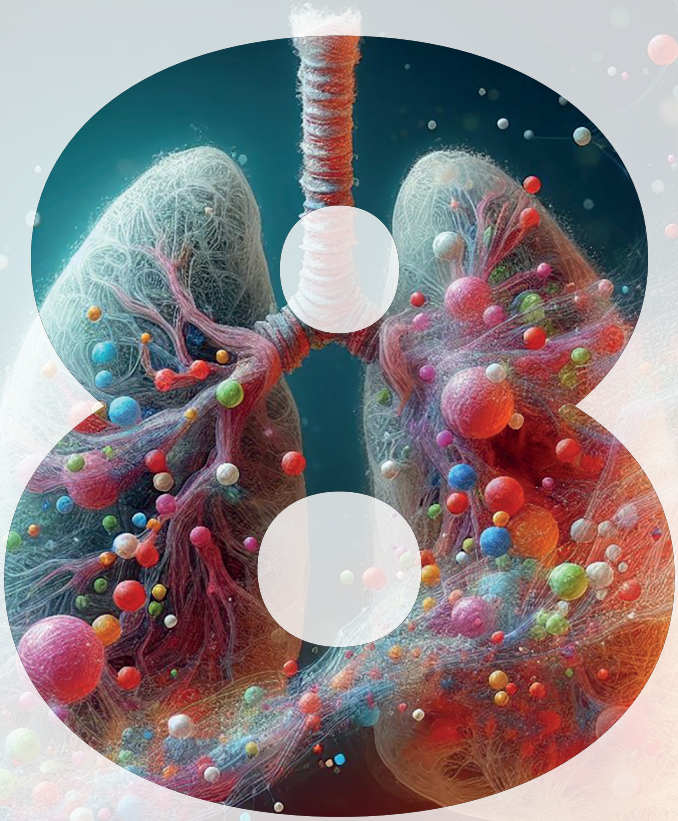

Difference in survival between COPD patients with an impaired immune reaction versus an adequate immune reaction to seasonal influenza vaccination: the COMIC study

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Abstract

Aim: To study the hypothesis that COPD patients who do not achieve seroprotective levels after influenza vaccination, are a less immune-competent group with a higher risk of morbidity and mortality.

Methods: 578 patients included in the COMIC cohort had pre- and post-vaccination stable state blood samples in which influenza-vaccine specific antibodies were measured. Post-vaccination titers of ≥40 were considered protective and indicative of being immuno-competent. Primary outcome was all-cause mortality. Morbidity was defined as time till first severe acute exacerbation in COPD (severe AECOPD) and time till first community acquired pneumonia (CAP).

Results: 42% of the patients achieved seroprotective levels to *both* H1N1 and H3N2 after vaccination. Seroprotective levels to H3N2 were markedly higher (96%) than to H1N1(43%). Having seroprotective levels to *both* H1N1 and H3N2 was not associated with less morbidity (severe AECOPD HR 0.91 (95% 0.66-1.25; p=0.564) (CAP HR 1.23 (95% 0.75-2.00; p=0.412)) or lower mortality (HR 1.10(95% 0.87-1.38; p=0.433)).

Conclusion: In a large well-characterized COPD cohort only the minority of patients achieved seroprotective titers to H1N1 and H3N1 after the yearly influenza vaccination. While achieving seroprotection after vaccination can be considered a surrogate marker of being immunocompetent, this was not associated with lower morbidity and mortality. Whether this means that the immune status is not a relevant pheno/endotype in COPD patients for the course of their disease or that seroprotection is not an adequate (surrogate) marker to define the immune status in COPD needs to be further studied.

Introduction

Since COPD is a major burden of morbidity and mortality, the assessment of prognostic factors to determine the probability of (the time-to) death and other clinically relevant outcomes such as risk of exacerbations, pneumonia and accelerated lung function decline is a topic of major interest. Our understanding of COPD is shifting to a personalized approach in which we have a better appreciation of the multiple factors involved in its course.

Although several multi-component prognostic indices are available, they still lack in accuracy. One of the indices is the 2019 revised GOLD classification that assesses COPD patients using three different domains: severity of airflow limitation (spirometric grade), its impact on dyspnea (mMRC) or symptoms (CAT), and their history of moderate and severe exacerbations (including prior hospitalization).(1) Well known multidimensional tools are the BODE-index (based on the body mass index (BMI), airflow Obstruction (FEV_1), Dyspnea (mMRC), and 6-minute-walk distance(6MWD),(2) and the ADO index (which combines age, mMRC and FEV_1). (3) Both are internationally validated and updated.(3)

None of these multi-component prognostic indices, however, addresses the systemic aspect of COPD. Although multiple biomarkers have shown some promise in predicting risk of morbidity and mortality, (e.g. CC16, SP-D, IL-6,(4) hsCRP,(5) sRAGE,(6), fibrinogen(7) and MR-proADM(8)), not all biomarkers remain prognostic when studied in validation cohorts.(9) More importantly, their prediction of major outcomes (morbidity, mortality) remains rather poor.

An aspect that is under-exposed in COPD is the status of the immune system. The immune system, both the humoral and cellular response, is vitally important to protect against pathogens, without overshoot or immune deviation. However, COPD is often associated with bacterial colonization of the airways and severe bacterial and viral infections.(10) This suggests an impairment of the immune system, either innate, acquired, or both. Specific immune deviations in COPD have been suggested previously.(11) Since mortality in COPD is related to acute and severe exacerbations, when patients are hospitalized, and since roughly one third of these exacerbations is associated with bacterial and one third with viral infections,(12) it is attractive to postulate a direct link between increased morbidity and mortality and impaired immune responses. However, it is difficult to assess these immune responses in individual patients and over repeated

exacerbations due to the variety of triggers that cause these exacerbations. A more standardized trigger to assess immune-competence is the annual influenza vaccination as recommended to COPD patients in the international guidelines. Influenza vaccinations can reduce the incidence and severity of lower respiratory tract infections and is associated with reduced later AECOPD risk including hospitalizations.(13–15) However, it is unclear whether it leads to a survival benefit. (13,14) Parpaleix et al. showed that both the humoral and the cellular responses to influenza vaccination were impaired in patients with COPD.(16) Also Nath et al. observed that the humoral immune response to the 2010 influenza vaccine was lower in persons with COPD compared to non-COPD controls.(17) Both studies were very small (n=15 and n=34) and did not report an association with disease outcome.

Seroconversion to the seasonal influenza vaccination is difficult to interpret in COPD patients since pre-vaccination antibody titers can be elevated because of previous vaccinations and/or influenza infections.(17,18) Seroprotection is another method to determine vaccination effectiveness. Eagan et al. showed varying percentages of COPD patients with protective titers (>40). Furthermore, they showed that having high titers at baseline did not impact later risk for exacerbations, but seemed to be associated with higher all-cause mortality, even after adjustment for COPD disease characteristics. The authors themselves already indicated some methodological issues, one being the use of self-reported vaccination status as a proxy for actual vaccination. Furthermore, accurate data on the time of vaccination were lacking, while it is preferred to measure antibody titers before and after vaccination at fixed time intervals. (18)

In the COMIC cohort, a large well-characterized COPD cohort (8,19–25) in which patients received the influenza vaccination yearly through their GP, we were able to check the actual date of vaccination. By this we could measure antibody titers before and after vaccination at fixed time intervals. We investigated the humoral response to the influenza vaccination, to study our hypothesis that COPD patients who do not achieve seroprotective levels after influenza vaccination reflect a less immune-competent group and have a higher risk of morbidity, defined as time to first severe AECOPD and time to first CAP, and mortality.

Methods

Setting and study population

The COMIC study (Cohort of Mortality and Inflammation in COPD) is a single center cohort study from Enschede, the Netherlands. From December 2005 till April 2010, 795 patients were included. All patients were followed-up for at least three years.

Patients included in the COMIC study had to meet the following criteria; a) a clinical diagnosis of COPD according to the GOLD guidelines; b) current or former smoker; c) age ≥ 40 years; d) no medical condition compromising survival within the follow-up period or serious psychiatric morbidity; e) absence of any other active lung disease (e.g. sarcoidosis); f) no maintenance therapy with antibiotics; g) ability to communicate in Dutch. Patients were enrolled when hospitalized for an acute exacerbation of COPD (AECOPD group) or when visiting the outpatient clinic in stable state (stable state group). To be included in the AECOPD group, patients had to be hospitalized for an AECOPD and be able to produce an adequate sputum sample at the day of hospitalization. To be included in the stable state group patients had to meet the following criteria: no use of an antibiotic and/or prednisolone 4 weeks prior to enrolment and no exacerbation less than 4 weeks before study entry.

The study was approved by the Medical Ethics Committee Twente in 2005 (study number P05-49) and was conducted according to the principles of the Declaration of Helsinki. All patients provided written informed consent.

Influenza vaccination

Patients received their annual influenza vaccination through their GP. The compositions of the influenza vaccination were all based on the WHO recommendation (26) and are shown in Table 1.

Table 1. Composition of the seasonal influenza vaccines 2006-2011*

Year	viruses
2006-2007	- an A/New Caledonia/20/99(H1N1)-like virus - an A/Wisconsin/67/2005 (H3N2)-like virus - a B/Malaysia/2506/2004-like virus
2007-2008	- an A/Solomon Islands/3/2006 (H1N1)-like virus - an A/Wisconsin/67/2005 (H3N2)-like virus - a B/Malaysia/2506/2004-like virus
2008-2009	- an A/Brisbane/59/2007 (H1N1)-like virus - an A/Brisbane/10/2007 (H3N2)-like virus - a B/Florida/4/2006-like virus
2009-2010	- an A/Brisbane/59/2007 (H1N1)-like virus - an A/Brisbane/10/2007 (H3N2)-like virus - a B/Brisbane/60/2008-like virus
2010-2011	- an A/California/7/2009 (H1N1)-like virus - an A/Perth/16/2009 (H3N2)-like virus - a B/Brisbane/60/2008-like virus

*As recommended by the World Health Organization

Antibody response

Influenza-vaccine specific antibodies were measured with the hemagglutination inhibition (HAI) assay,(27–29) performed on blood samples collected in a stable state pre-vaccination serum sample in the preceding month(s) or week(s) and a post-vaccination serum sample obtained 4 to 6 weeks after vaccination. Post-vaccination titers reaching an antibody titer of 40 or above were considered indicative of being immunized, either by vaccination or infection (i.e. protective titers).(30) Patients were divided into two groups based on their post-vaccination titers. Patients were considered to be more immune-competent if they had a protective titer ($\geq$ 40) to both H1N1 and H3N2 and less immune-competent if they had no or only one protective titer to H1N1 and H3N2.

Outcomes

The primary outcome parameter was survival, based on all-cause mortality. Date of death was verified from public registries.

Morbidity was defined as both time till first hospitalization for an acute exacerbation in COPD (severe AECOPD) and as time till first community acquired pneumonia (CAP).

AECOPD was defined as an acute negative change from baseline, reported by the patient, in dyspnea and/or sputum volume and/or color of sputum (yellowish or greenish sputum) and/or cough, which warranted additional treatment with prednisolone with or without antibiotics by a physician in a patient with underlying COPD.(31) Pneumonia was defined as an acute respiratory tract illness associated with radiographic shadowing on a chest radiograph consistent with infection which was neither pre-existing nor of any other known cause.(32) All X-rays were double read by a radiologist and a pulmonary physician. In case of doubtful shadows in the report, the X-ray was presented to another independent pulmonary physician for final judgment.

Demographic data was collected from medical records. Spirometry was performed by trained lung function technicians according to the American Thoracic Society guidelines.(33) Smoking status was determined by the Vlagtwedde questionnaire and pack-years were calculated.(34) Data on common co-morbidities like myocardial infarction, congestive heart failure and diabetes mellitus were obtained from medical records and/or during study visits. Number of previous moderate AECOPD in the year preceding inclusion was determined based on prescribed prednisolone courses as retrieved from pharmacy data. Previous severe AECOPD was defined as an hospitalization in the year preceding inclusion and was retrieved from hospital records. Patients completed the modified Medical Research Council dyspnea questionnaire (mMRC)(35) and the Clinical COPD Questionnaire (CCQ) (36) The BOD comprises BODE without the exercise capacity measurement. The components were scored according to the same cut-offs as in BODE.(2) The BOD therefore ranges from 0 to 7. The ADO score ranges, in increasing severity, from 0 to 10.(37) All measurements were performed in stable state.

Sample size calculation

A predefined hazard ratio of 2.0 was assumed and a median survival time of the less immune-competent group of 60 months was estimated based on mortality data of Groenewegen et al. (38) and Almagro et al.(39) Groenewegen et al. showed that 1 year after hospital admission approximately 22% of patients had died. According to Almagro et al., 36% had died after 24 months of follow-up. Since a reasonable amount of patients in the COMIC cohort will be included at admission to the hospital, we assumed a conservative median survival time of 60 months. We also estimated the proportion of less immune-competent patients to be 10%. With an inclusion period of 60 months, a minimal follow up of 24 months, an alpha of 0.05 and a power of 0.8, in total 600 patients needed to be included.

Statistical analyses

Continuous variables are expressed as mean with standard deviation (SD) or median with interquartile range (IQR); categorical variables as counts with corresponding percentages.

Time to death, time till first hospitalization for AECOPD and time till first pneumonia were analyzed by Kaplan-Meier survival curves. Univariate and multivariate Cox proportional hazard regression models were used to establish the relationship between the humoral response to the influenza vaccination on the one hand, and time to all-cause death, time till first hospitalization for AECOPD, and time till first pneumonia on the other hand. In the multivariate Cox proportional hazard regression models potential confounders were taken into account based on a stepwise backward method. Potential confounders were baseline characteristic that were associated with both the humoral response and the outcome variable of interest (both $p<0.10$).

All tests were two-sided and a p-value of 0·05 or lower was considered statistically significant. Data were analyzed using SPSS, version 25 (SPSS Inc. Chicago IL).

Results

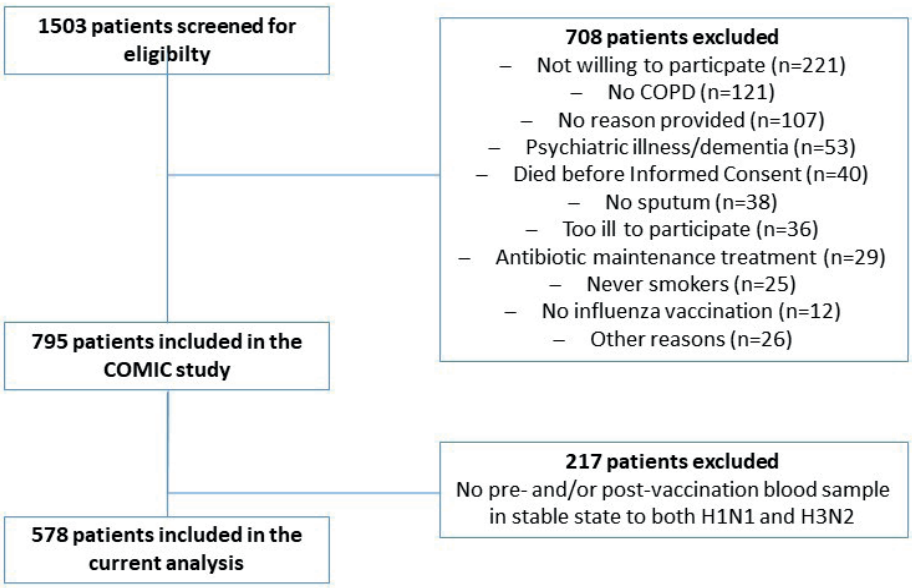

Fig 1. Flowchart patient inclusion

Table 2: Baseline characteristics of the 578 patients, including the differences in baseline characteristics in patients with and without a protective antibody titer to both H1N1 and H3N2.

Characteristic		Post-vaccination titer		
	Total group N=578	<40 to both H1N1 and H3N2 or to one of them (n=334)	>40 to both H1N1 and H3N2 (n=244)	p
Mean age (SD)	67.1 (9.1)	66.3 (9.2)	68.1 (8.9)	.019
Sex, male, N (%)	348 (60.2)	193(57.8)	155 (63.5)	.164
Smoker, N (%)				.814
Current smoker	144 (24.9)	82(24.6)	62(25.4)	
Ex-smoker	434 (75.1)	252 (75.4)	182(74.6)	
Mean BMI (SD)[1]	27.6 (5.4)	27.5 (5.0)	27.8 (5.8)	.429
Median Pack-years (IQR) [2]	35.0 (22.0-50.0)	34.1(21.9-48.4)	37.0 (22.0-53.1)	.282
Mean lung function (SD)[3]				
FEV_1 in l	1.5 (0.6)	1.52 (0.65)	1.40 (0.56)	.018
FEV_1 % predicted	54.0 (18.9)	55.0 (19.3)	52.6 (18.4)	.127
FEV_1/VC ratio	44.9 (13.4)	45.4(13.4)	44.2 (13.3)	.297
GOLD (2007), N (%)[3]				.823
I-II	320 (55.5)	186 (55.9)	134 (54.9)	
III-IV	257 (44.5)	147 (44.1)	110 (45.1)	
Previous moderate AECOPD, N(%)[4]				.694
0-1 AECOPD	365 (63.1)	213 (68.3)	152 (66.7)	
≥ 2 AECOPD	175 (30.3)	99 (31.7)	76 (33.3)	
Previous severe AECOPD, N (%)				.414
≥ 1 severe AECOPD	74 (12.8)	46 (13.8)	28(11.5)	
ICS use, yes, N (%)	492 (85.1)	279 (83.5)	213 (87.3)	.209
Mean mMRC (SD)[5]	1.7 (1.3)	1.60 (1.21)	1.81 (1.31)	.045
Comorbidities, N (%)				
Heart failure	98 (17.0)	53 (15.9)	45 (18.4)	.415
Diabetes Mellitus	38 (6.6)	25(7.5)	13(5.3)	.301
Myocardial Infarction	24 (4.2)	9 (2.7)	15(6.1)	.040
Mean CCQ score (SD)[6]	1.7 (1.0)	1.67 (0.98)	1.82 (0.99)	.083
Mean ADO score (SD)[7]	4.0 (1.8)	3.85 (1.75)	4.32 (1.76)	.002
Mean BOD score (SD)[7]	2.4 (1.7)	2.24 (1.67)	2.46 (1.78)	.135

[1] BMI missing of resp. 11, 9 and 2 patients in the total, <40 and ≥40 group; [2] Pack-years is missing of resp. 35, 25 and 10 patients in the total, <40 and ≥40 group; [3] Lung function and GOLD is missing of resp.1 patient in the total and <40 group;[4] Previous AECOPD is missing of resp. 38, 22 and 16 patients in the total, <40 and ≥40 group; [5] mMRC is missing of resp. 12, 6 and 6 patients in the total, <40 and ≥40 group; [6] CCQ is missing of resp. 4, 2 and 2 patients in the total, <40 and ≥40 group; [7] ADO is missing of resp. 13 , 7 and 6 patients in the total, <40 and ≥40 group; [8] BOD score is missing of resp. 23, 15 and 8 patients in the total, <40 and ≥40 group. Abbreviations: SD: standard deviation, N: number, IQR: interquartile range, BMI: body mass index, FEV_1: Forced expiratory volume in 1 second, mMRC: modified Medical Research Council dyspnea questionnaire, CCQ: Clinical COPD Questionnaire, ADO: age dyspnea, airflow obstruction, BOD: BMI, airflow obstruction, dyspnea.

Of the 795 included patients in the COMIC cohort, 578 patients had both a pre- and post-vaccination blood sample and could be included in the current analysis on the humoral response to the influenza vaccination (see flowchart in fig 1). Table 2 shows the baseline characteristics of the 578 patients.

The percentage of patients with a protective antibody titer to H1N1 and H3N2 itself, to either H1N1 or H3N2, and to both H1N1 and H3N2 is displayed in Table 3. Most patients had a protective titer to H3N2, while more than half of the patients did not have a protective titer to H1N1.

Table 3: Percentage of patients with a protective antibody titer to H1N1 and H3N2.

Antibody titer	**H1N1**	**H3N2**	
≥40, n (%)	250 (43.3)	555 (96.0)	244 (42.2)

Comparison of the pre- and post-vaccination antibody titers to H1N1 (Table 4a) and H3N2 (Table 4b), shows that also prevaccination titers to H3N2 (in 94.5% of patients) were more often ≥ 40 than prevaccination titers to H1N1 (in 40.1 % of patients).

Table 4a: the change in absolute pre- and post-vaccination antibody titers to H1N1 per patient

		Post-vaccination titer									
		1	**8**	**16**	**32**	**64**	**128**	**256**	**512**	**1024**	**Total**
Pre-vaccination titer	**1**	30	13	5	6	-	-	-	-	-	54
	8	2	11	18	4	2	1	-	-	-	38
	16	5	5	50	31	11	2	-	-	-	104
	32	2	1	12	93	37	2	1	-	-	148
	64	2	1	5	26	82	14	2	-	-	132
	128	-	-	2	4	12	32	6	1	-	57
	256	-	-	-	-	1	2	30	2	-	35
	512	-	-	-	-	-	-	3	6	-	9
	1024	-	-	-	-	-	-	-	1	-	1
	Total	41	31	92	164	145	53	42	10	0	578

Table 4b: the change in absolute pre- and post-vaccination antibody titers to H3N2 per patient

		Post-vaccination titer									
		1	8	16	32	64	128	256	512	1024	Total
Pre-vaccination titer	1	-	-	-	-	-	-	-	-	-	0
	8	-	1	-	-	-	-	-	-	-	1
	16	-	-	-	-	1	-	-	-	-	1
	32	-	-	-	20	4	1	4	1	-	30
	64	-	-	-	2	100	4	4	2	1	113
	128	-	-	-	-	14	77	18	6	8	123
	256	-	-	-	-	-	13	50	43	10	116
	512	-	-	-	-	-	1	16	58	38	113
	1024	-	-	-	-	1	-	3	18	59	81
	Total	0	1	0	22	120	96	95	129	116	578

Baseline differences in patients with and without a protective antibody titer to both H1N1 and H3N2

Patients with protective titers to both H1N1 and H3N2 were somewhat older, had a worse lung function and a higher mMRC and ADO score (see Table 2).

Mortality

Patients with a protective response to both H1N1 and H3N2 had 1.26 times higher risk of dying than patients with either one or no protective titer to H1N1 and H3N2 (HR 1.26; 95%CI 1.01-1.58;p=0.043), see also Fig 2. After correction for the confounders BOD, CCQ total score and age the corrected HR was no longer significant (HR 1.10(95% 0.87-1.38; p=0.433).

Morbidity

There was no difference in time till first hospitalization between patients with a protective titer to both H1N1 and H3N2 vs patient with no or only one protective titer to H1N1 and H3N2(Fig 3;p=0.53, HR 1.11 (95% 0.81-1.52)). After correction for the confounders age, FEV_1 in liters, myocardial infarction, CCQ total score the corrected HR was 0.91 (95% 0.66-1.25; p=0.564).

Neither was there a difference in time to first pneumonia between patients with a protective titer to both H1N1 and H3N2 vs patient with no or only one protective titer to H1N1 and H3N2(Fig 4; HR 1.45 (95% 0.89-2.35) p=0.135). After correction for the confounders age, FEV_1 in liters, myocardial infarction, mMRC and CCQ total score the corrected HR was 1.23 (95% 0.75-2.00; p=0.412).

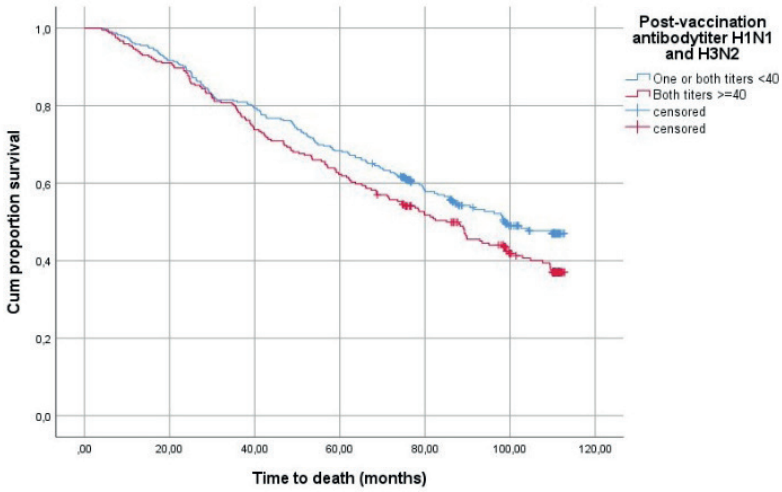

Fig 2. Kaplan-Meier survival curve for patients with a protective titer to both H1N1 and H3N2 vs patient with no or only one protective titer to H1N1 and H3N2.

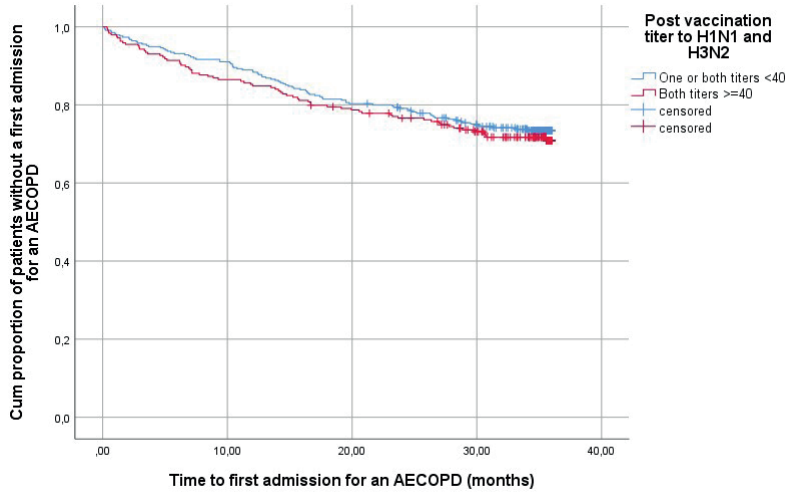

Fig 3. Kaplan-Meier curve for a)time till first hospitalization for an AECOPD for patients with a protective titer to both H1N1 and H3N2 vs patient with no or only one protective titer to H1N1 and H3N2 .

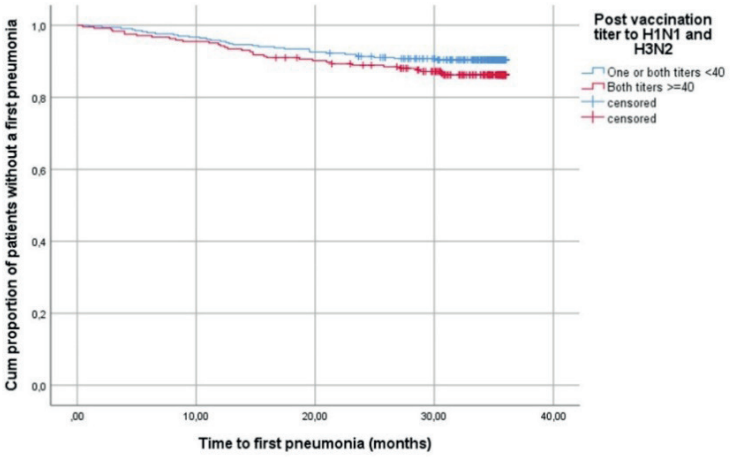

Fig 4. Kaplan-Meier curve for time till first pneumonia for patients with either an protective titer to both H1N1 and H3N2 vs patient with no or only one protective titer to H1N1 and H3N2 .

Discussion

The main outcome of our study is that 42% of the patients achieved seroprotective levels to both H1N1 and H3N2 after the influenza vaccination. The seroprotective levels to H3N2 were markedly higher (96%) than the seroprotective levels to H1N1(43%). Having seroprotective levels to both H1N1 and H3N2 was not associated with less morbidity or lower mortality compared to having no seroprotective levels or only seroprotective levels to either H1N1 or H3N2.

The yearly influenza vaccination has been recommended for a long time in the annual GOLD-report and different international guidelines. As concluded in the Cochrane review by Kosaftis et al. (40) and recently in a retrospective analyses (15) influenza vaccination in COPD patients lead to lower risk of AECOPD. Acquiring an adequate immune-response to vaccinations is essential for achieving this favorable outcome.(41) Our study however reveals that for COPD patients having seroprotective levels, as a surrogate marker for an adequate acquired immune-response after this annual vaccination, or not is apparently not a major factor to be taken into account for achieving this favorable outcome. Furthermore, in the ongoing discussion whether the annual vaccination leads to better survival as well, our hypothesis that this may be true for the patients being able to achieve seroprotective levels could not be demonstrated with this study. These are surprising but relevant outcomes.

Because COPD patients often experience recurrent bacterial and viral infections, it has been proposed that they may be relatively immune-deficient compared to healthy persons, and as such may be less able to mount an effective immune response to vaccination. It is well established that the immunogenicity of influenza vaccine is lower in healthy elderly people than in healthy younger people. (42) However, only little information is available on the extent to which the influenza vaccination can induce an adequate adaptive immune response in COPD. There are indications that the immune response to influenza vaccination in COPD patients is impaired and therefore previous studies suggested adaptation of the influenza vaccination formulations for (subgroups) of patients with chronic (pulmonary) diseases because of this impaired immune response.(16–18) Although the COPD population is a somewhat older population in which the percentage of people with seroprotective titers is already somewhat lower (around 60%),(27) this percentage is still not comparable to the observed percentage of patients with seroprotective titers in the current cohort. Since almost 60% of our patients did not achieve seroprotective titers, adaptation or boosting of this vaccination might seem obvious for further studies.(43) However, our results show that the achieved levels of antibody post vaccination is not associated with relevant outcome parameters in COPD. Also in the Bergen COPD Cohort Study, seroprotective titers did not impact later risk for AECOPD, but this study was in patients with self-reported influenza vaccination. Furthermore, those researchers used a baseline influenza titer that could be determined at any moment in the year. (18) In our study, the antibody titers were determined in patients at fixed time intervals in which actual date of vaccination was checked at their GP, and yet we observed similar results.

With our study we used seroprotection as a marker to distinguish within our COPD population a more immune-competent group vs. a less immune-competent group. Indeed we found different baseline patient characteristics that were associated with achieving seroprotection. Patients with protective titers to both H1N1 and H3N2 were somewhat older, had a worse lung function and a higher mMRC and ADO score. Seroprotection status, as determined by the international accepted cut-off values, was however not associated with outcome. We have to keep in mind that there is no gold standard test to define a person's immune status, and achieving seroprotection is only a surrogate marker, which may only inform us for a small part of the complexity of our immune system as a whole. Besides, various other factors are known to be associated with the immune system and its competence such as nutritional status, use of immunosuppressive

medication and (hemato-) oncological disease. Unfortunately, data on nutritional status are lacking in our study, patients were however not allowed to use additional immunosuppressive medication and have another medical condition compromising survival. For many vaccines and diseases the true correlates of protection have not been established, despite the fact that threshold antibody levels have been defined. For influenza, as for COVID-19 and other infections, next to attained antibody levels, T cell immunity and B cell memory do ultimately contribute to protection against the disease.

Another possible explanation why higher seroprotective levels did not lead to better outcome could be the 'original antigenic sin', whereby immunological memory from prior (influenza) vaccinations prevents the immune system from mounting an effective response to subsequent vaccine strains of the influenza virus.(44) Imprinting of the specific molecular image of a given protein antigen into immunological memory is one of the hallmarks of immunity and the underlying principle for vaccination. A later contact with the same, for example in the form of a second contact with the virus, would trigger specific memory B- and T-lymphocytes and would result in a faster, higher and better immune response. In case the virus is mutated and one or more surface protein are changed, the memory cells would not be triggered. In case the specific memory cells would be triggered, resulting antibodies may be able to bind to the surface proteins, but not lead to virus neutralization. In that case those antibodies could block and render the response ineffective, a phenomenon termed the 'original antigenic sin'.(45). It is possible that the influenza virus can use this aspect of the original antigenic sin as a potential way of escaping from the host's immune system.(46)

A remarkable finding in our study is that we observed a large difference in the humoral response between H1N1 and H3N2, with higher post-vaccination titers of H3N2. This could possibly be explained by the finding of Nath et al. who observed lower responses in those who had received the same influenza vaccine in the past. (17) In our cohort the H1N1 virus, that was used for the development of the vaccine and that was provided at the start of our cohort, when the majority of the patients were included, was similar to the virus included in the vaccines in the previous years. This was not the case for the H3N2 virus. However, we would then also expect a higher pre-vaccination titer to H1N1 compared to the pre-vaccination response to H3N2 and this was not the case.

Limitations of the current study include that the patients in our cohort were more severe, due to including a large number of patients during a severe COPD exacerbation, which limits the generalization to the more mild COPD patients. Another factor that could influence the results is the use of inhaled corticosteroids (ICS). Nath et al. showed that absolute post-vaccination titers were significantly lower in persons using ICS. While this might be explained by the systemic absorption of ICS, it is also feasible that use of ICS may be simply a marker of COPD severity.(17) In the current cohort we could not study this finding for ICS use, since almost all patients in our cohort were on ICS (>80%). Therapy adherence to ICS in our overall COMIC cohort was dependent on the type of medication prescribed, inhalation device and several disease-specific and quality of life factors.(22,23,25) The overall adherence to the different ICS prescribed in our study was optimal (≥ 75-≤125%) in 59% of patients, sub-optimal ≥ 50-<75% in 17%, poor (<50%) in 10% and we observed overuse (>125%) to ICS medications in 14%. Due to the small number of patient with a low exposure to ICS, we were not able to perform any subgroup analyses on ICS dosage. Based on the sample size calculation we needed 600 patients for this study, and although we included 795 patients within the cohort, for the current analyses we could only include 578 patients. This small difference in sample size does by no means lower the power far enough to explain the non-significant p-values.

We should also keep in mind that we have measured the response to vaccination when they first entered the cohort. The follow up extends to over 8 years. It can be assumed that they were vaccinated every year. We do not know whether they remained in their initial response group. Finally, our study lacks information about influenza associated morbidity and mortality. Therefore, we cannot exclude that achieving seroprotective titers could lead to lower influenza associated AECOPD, CAP and mortality, which then did not lead to lower overall morbidity/mortality. This was, however, not the main purpose of our study since we were interested in immune competence as a marker for overall morbidity and mortality.

In conclusion, in the COMIC study, a large well-characterized COPD cohort study, about 40% of the patients achieved seroprotective titers to H1N1 and H3N1 after the yearly influenza vaccination. Achieving seroprotection, as a surrogate marker of being immune-competent, was not associated with lower morbidity and mortality. Whether this means that the immune status is not a relevant pheno/endotype in COPD patients for the course of their COPD or that seroprotection to the influenza strains tested is just not a good (surrogate) marker to define the immune status in COPD patients needs to be further studied.

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General discussion

The main objective of this thesis was the search for markers associated with morbidity (defined as severe Acute Exacerbation of COPD (AECOPD), Community Acquired Pneumonia (CAP)) and mortality in patients with COPD. We investigated various biomarkers in stable state COPD and during severe AECOPD. We studied the stability in eosinophil categorisation in subsequent severe AECOPD for different cut-offs, and the association of statin use with subsequent morbidity and mortality was investigated. Finally, the relationship between an impaired immune response to the seasonal influenza vaccination on the one hand and morbidity and mortality on the other hand was studied. In this chapter the findings of this thesis will be reviewed and put in perspective, and suggestions for future research will be provided.

Underlying chronic and systemic inflammation play an important role in the pathophysiology of COPD and its progression.(1) Impaired lung function resulting in lower FEV_1 in COPD is independently associated with cardiovascular disease (CVD).(2) Furthermore CVD is a common cause for morbidity and mortality in COPD. The systemic spill-over effect of the pulmonary inflammation is suggested to be partially responsible for the increased CVD in COPD. There is a rationale for a treatment that has a positive effect on this ongoing (systemic) inflammation as well as on the, sometimes not yet recognised, cardiovascular conditions in patients with COPD. Besides proven effect on reducing cardiovascular disease by treating hypercholesterolemia, statins have other pleiotropic anti-inflammatory effects as well. In **chapter 2** we studied the association of statin use in COPD patients in the COMIC study with subsequent COPD morbidity and all-cause mortality within a minimum of three years of follow up in comparison to patients who did not use statins. Our study initially showed that statin use seemed to be associated with better survival in multivariate analyses which was also seen in some other mainly retrospective and observational studies. However, observational studies are at risk for different forms of biases. A potential important bias is the immortal time bias. The immortal time consists of the time between inclusion into the cohort and the moment of first starting the statin. The study subject has to survive this time before exposure to the statin can occur. In this period the patient is classified as a statin user while in fact he or she is not (yet). With the immortal time bias a non-existing survival benefit, associated with statin use is introduced.(3) Therefore we performed a prespecified sensitivity analysis in which we excluded the 62 patients who started using a statin for at least 90 consecutive days ≥ 3 months after inclusion, to prevent the risk of immortal time bias. Now statin use was not associated with all-cause mortality anymore. Furthermore, our study showed that statin use also was not associated with time till first hospitalisation for an

AECOPD and time till first CAP. Our study results are in accordance with the first large randomized controlled trial by Criner et al. in which 885 patients with COPD randomly were assigned to either daily simvastatin 40mg or placebo and where no differences between exacerbation rate or time till first exacerbation were observed during follow up between the two groups and as part of the secondary outcome no difference in mortality was observed as well.(4) However, in that study patients with diabetes and CVD were excluded and exactly these patients are the ones that may have a high prevalence of systemic inflammation with underlying subclinical cardiovascular disease and therefore benefit from the immune-modulatory effects of statins.(5) Therefore, the search did not stop and meanwhile various new studies have been performed. In a systematic review and meta-analysis and in a methodological review of observational studies, both including studies with abovementioned limitations, the risk of death associated with statin use was reduced.(6,7) Also in a recently published small single-centre RCT, simvastatin at a dose of 40mg daily significantly prolonged time to first COPD exacerbation and reduced exacerbation rate.(8) Nonetheless, standardized statin use is still not recommended in the different COPD guidelines. The anti-inflammatory effects of statins are also being investigated in other diseases. For instance in a secondary analysis of a multi-centre randomized clinical trial in ARDS patients on the ICU, in the hyperinflammatory subphenotype, the administration of 80mg simvastatin vs. placebo resulted in a significantly improved survival attributed to the anti-inflammatory effects of statins,(9) while in their prior analysis in the overall group no difference was found.(10) Translating this to the COPD patients, statins will probably not be beneficial for all the COPD patients. However, when selecting COPD patients with, or at risk for CVD and having a high inflammatory profile, statin use could be a beneficial treatment and future, preferably prospectively randomized research, should focus on this group. Which biomarkers should be used to define this high inflammatory state is open for debate. The biomarkers MR-proADM and Fibrinogen could be part of them.

This brings us to **Chapter 3** and **Chapter 4** in which we studied the biomarkers MR-proADM and Fibrinogen with subsequent morbidity and mortality in patients with COPD. MR-proADM is the biologically-inactive midregional fragment of the adrenomedullin (ADM) prohormone.(11) The pluripotent regulatory peptide ADM is widely expressed throughout the body and acts as both a hormone and a cytokine.(12) ADM potentially has comprehensive effects such as vasodilatory, diuretic, anti-inflammatory and antimicrobial. In preclinical and animal models ADM has been shown to reduce hypoxic pulmonary vascular remodelling, inhibit

bronchoconstriction, and downregulate pro-inflammatory factors.(13) With these compensatory and regulatory capacities ADM tries to achieve homeostasis. Despite these beneficial and protective effects the upregulation of ADM should be interpreted as the apparent need for these compensatory/regulatory effects due to the underlying severity of the illness at that moment.(14) Therefore, elevated levels of the easy measurable MR-proADM in blood has the potential to be associated with and predict negative outcomes in COPD. In **chapter 3** we pooled the data of 1285 patients from two large European COPD cohorts, the COMIC and PROMISE-COPD cohort. We performed a pooled analysis to study the relationship of stable state MR-proADM with subsequent severe AECOPD and CAP. We concluded that COPD patients with a MR-proADM level above the cut-off level (>0.87nmol/l) had a significantly higher risk for a severe AECOPD compared with those with low level MR-proADM. For CAP the results were less clear since in univariate analysis there was an association with a 93% higher risk, however after correction for confounders the association was no longer significant and the HR was much lower although we cannot exclude that the smaller power was partially the cause of this. So, in apparently stable state COPD, 25% of the patients have an elevated level of MR-proADM. These patients have a 30% higher risk for a subsequent severe AECOPD in an overall group in which one-third of the patients had at least one severe AECOPD during 3 years of follow up. Thus, independent of other known factors associated with subsequent AECOPD, such as exacerbation history, lung function and comorbid status, the biomarker MR-proADM can be used to select patients at a higher risk for this morbidity of severe AECOPD. For mortality prediction we previously demonstrated stable state MR-proADM to be an independent predictor of mortality in COPD.(15) Another important biomarker associated with mortality in COPD is Fibrinogen. The correlation between these two biomarkers has not been studied yet, and neither the combined use of these two biomarkers for mortality prediction. In **chapter 4** we compared MR-proADM to Fibrinogen in COPD for the first time. Fibrinogen is a key component of the coagulation cascade via thrombin-mediated conversion of Fibrinogen to fibrin. Furthermore, Fibrinogen is also an acute-phase reactant with its synthesis being up-regulated in response to inflammatory mediators such as IL-6.(16) First, our study showed that the correlation between MR-proADM and Fibrinogen was, although significant, strikingly low. This strongly suggests that both biomarkers reflect different biological processes and as such they encompass a different part of the underlying (systemic) inflammation in COPD. Second, stable state Fibrinogen proved to be a predictor for mortality in our large COPD cohort as well. In our multivariate analysis, a doubling of Fibrinogen concentration shows

a 2.4 fold increased risk of dying. This confirms again the predictive capacity of this FDA-approved biomarker in COPD.(17) Third, doubling of MR-proADM concentration shows a 2.2 fold increased risk of dying. Interestingly, when adding stable state MR-proADM to the one-year mortality prediction model already including Fibrinogen this resulted in a significant increase of the predictive capacity of the model (AUC from 0.78 to 0.83). As stated both biomarkers probably reflect different biologic processes so there is a rationale for combining both biomarkers to increase the predictive capacity of the model. However, when adding Fibrinogen to the one-year mortality prediction model already including MR-proADM, this did not result in a significant increase of the predictive capacity of the model (AUC from 0.82 to 0.83) suggesting that MR-proADM alone might be a better biomarker than Fibrinogen for predicting mortality.

Overall, elevated ADM in stable state COPD probably represents a higher demand on the regulatory mechanisms ascribed to ADM. This higher demand is provoked by a possible higher continuous, or an at that moment active underlying (systemic) inflammation, hypoxia, bacterial exposition or colonization and cardiovascular instability/comorbidity in contrast to patients with non-elevated ADM levels. The higher ADM, measured in stable state could implicate that the patient is either at risk for impending disease instability or is already somewhat unstable, which in the end could lead to a negative outcome. With our findings we conclude that elevated stable state MR-proADM in COPD patients is associated with higher risk for subsequent AECOPD and higher level of stable state MR-proADM is a better biomarker than stable state Fibrinogen to be used for mortality prediction. Are we there to introduce stable state MR-proADM as a biomarker in COPD? We believe not yet. First, stable state MR-proADM has never been evaluated with morbidity in COPD until now. The independent predictive capability of MR-proADM as an independent predictor for AECOPD in COPD patients still has to be established in a prediction model, in which other known important predictors are taken into account. In this way also the additive prognostic value of MR-proADM to other known predictors can be studied. For stable state MR-proADM as a predictor for mortality this already has been done. In a study also pooling the data of the COMIC and the PROMISE-COPD studies, adding MR-proADM to the BOD, ADO and the updated ADO index significantly increased the predictive power of all the three indices.(18) Second, before a biomarker in COPD can be successfully translated to clinical translation and implementation, Hollander et al. suggested the 5 SAVED questions to be addressed: Superior, Actionable, Valuable, Economical, Clinical deployable.(19) The evolving studies of MR-proADM in COPD have now resulted in evaluating prediction for morbidity and mortality, in combination with multi-

dimensional indices, in defining cut-off levels and even in validation studies and with this it addresses some of the SAVED questions but not all. To do so we believe the focus of future research of MR-proADM in COPD has to include the following;

Evaluating the COPD patient evolved from the determination of FEV1, leading to the 2011 GOLD guideline offering a new system of assessment and treatment of patients with COPD according to current control and future risk.(20) In the 2019 guideline Blood eosinophil count was introduced as a biomarker for estimating the efficacy of inhaled corticosteroids (ICS) for the prevention of exacerbations. (21) Both major changes were finetuned in the most recent GOLD guideline.(22) This indeed led to changes in the treatment algorithms. However, overall in daily practice the majority of patients largely receive the same therapy. Furthermore, very little is known about the overall follow up strategies of the COPD patient. Maybe patients with an elevated (systemic) inflammatory profile and patients with a (biomarker assessed) prognostic profile at risk for subsequent morbidity and mortality, both using MR-proADM, should have different treatment and surveillance and advanced care planning strategies. It seems plausible then that patients at risk for subsequent morbidity and mortality should be treated and followed more intensively in second line while patients not at risk can be followed less intensively and in primary care. Furthermore, it would be interesting to evaluate whether this personalized strategy will lead to changes in the inflammatory and prognostic profile of the patient. When a patient still remains at higher risk for morbidity and mortality a thorough search needs to be done for the cause of it and maybe early deployment of preventive rehabilitation (or prehabilitation) programs can be introduced for prevention of this negative outcome, whereas this also could be the moment for starting advanced care planning. These questions and strategies have of course to be studied prospectively first. Regarding MR-proADM guided treatment and follow-up strategies in areas other than COPD, indeed these kinds of studies have proven to be feasible. An example: as stated, elevated MR-proADM is associated with negative outcome in other medical conditions as well, such as COVID-19 and sepsis.(23) A randomized controlled multicenter interventional clinical trial proved the utility of MR-proADM to guide decisions on hospitalization or out-patient treatment in the Emergency Department (ED) for patients with suspected infections.(24) In the MR-proADM guided arm the hospital admission rate was 17% lower than in the control arm (40.6% vs. 57.6%, p=0.024) without significant differences in the various safety endpoints. In a patient-level simulation model this MR-proADM decision algorithm in the ED has the potential for significant cost savings.(25) Regarding serial measurements of biomarkers in COPD, indeed less is known and studies addressing this topic with

serial longitudinal measurements within one patient during the course of the disease are needed and will be a next step in our future research. So, only when these type of studies are done in COPD, MR-proADM (or other biomarkers) can be evaluated for optimal clinical translation and implementation. Regarding serial measurements, in this thesis we have a first impression of serial blood eosinophil count measurements in subsequent AECOPD in patients in the COMIC study.

This brings me to **chapter 5** and **chapter 6** of this thesis. The blood eosinophil count, which is correlated to sputum eosinophil levels, can serve as a surrogate marker for eosinophilic inflammation in the lung.(26) In AECOPD, the blood eosinophil count predicts oral or intravenous steroid responsiveness and various clinical trials evaluated successfully eosinophil guided strategies for the use of steroid therapy during AECOPD.(27–30) This paves the way for a more frequent testing of the blood eosinophil count during AECOPD. Less is known about its prognostic accuracy to predict future morbidity and mortality. Furthermore it will be important to know if one can rely on one (historically) established blood eosinophil count at AECOPD or that it has to be established at every new AECOPD. Therefore in **chapter 5** first we studied whether this eosinophil count at AECOPD could also be a marker associated with subsequent morbidity and mortality. Different cut-off levels for the blood eosinophil count and eosinophil count as a continuous measure were used. In our cohort univariate analyses showed an association of some cut-off levels with survival, not with morbidity. However, after correcting for confounders (mainly age and lung function) this disappeared. Steroid use can influence the eosinophil count. Therefore we performed a prespecified sensitivity analyses in which we excluded patients with recent prior steroid use and again no association with morbidity and mortality was seen. Furthermore, this study showed that having a severe AECOPD is associated with a poor prognosis with almost half of the patients having a subsequent severe AECOPD within 3 years of follow up and 43% of the patients dying. In the search for biomarkers to assist in identifying these patients our study shows that the blood eosinophil count measured at AECOPD is not suited for this purpose.

In **chapter 6** we determined the stability in eosinophil categorisation between two subsequent severe AECOPDs employing frequently used cut-off levels for the blood eosinophil count. Depending upon the used cut-off and expression, the overall stability in eosinophil categorisation varied between 70%-85% during two subsequent AECOPDs. From patients who were eosinophilic at the first AECOPD only 34%-45% remained eosinophilic at the subsequent AECOPD while 9%-21% of patients being non-eosinophilic at the first AECOPD became eosinophilic

at the subsequent AECOPD. Our results demonstrate unequivocally that the eosinophil variability in subsequent AECOPDs within individuals leads them to belong to different classifications in consecutive AECOPDs. This is in accordance with our other findings in that there is only a very weak correlation between the blood eosinophil counts measured at 2 subsequent severe AECOPDs. In a recent non-inferiority, multicentre, double-blind, placebo- controlled, randomised controlled trial a blood eosinophil-guided strategy for oral prednisolone use for COPD exacerbations in primary care in the UK leading to reduced systemic glucocorticoid use demonstrated non-inferiority to standard of care.(27) This will accelerate the implementation of systematic use of the blood eosinophil count at AECOPD. Our results suggest that, independent of which cut-off will be used, when making a choice regarding systemic steroid treatment dependent on eosinophil numbers during a severe AECOPD, one cannot rely on the eosinophil count measured during an earlier severe AECOPD. The eosinophil count has to be determined at every new severe AECOPD. For eosinophil guidance in the treatment of AECOPD to become common practice, this would have major implications, among others, for COPD self-management interventions, as this should then lead to measuring eosinophils at the moment of each AECOPD. The implications and the potential of this strategy is described in **chapter** 7 in which we introduce the potential for personalised and biomarker-guided COPD self-treatment approaches. We believe that the integration of a self-treatment approach guided by blood eosinophil count should be considered to safely reduce the systemic exposure and toxicity of universal prednisolone therapy. As a consequence, health care providers of patients with COPD and patients with self-management action plans should have easy access to a (point-of-care) tool also in the outpatient clinic to determine their eosinophil count at each AECOPD onset that should not delay treatment. This could also be a blue print for future implementation of biomarker guided antibiotic treatment in AECOPD. With these upcoming biomarkers we are at the beginning of a more personalised and biomarker-guided COPD self-treatment approach embedded in COPD self-management.

Considering eosinophil guided therapy in AECOPD, our study also showed that systemic steroid use immediately prior to the severe AECOPD lowered the eosinophil numbers but did not normalize them (10-22% were still labelled as eosinophilic depending on the criterion used) and did not relevantly influence the stability of the eosinophil categorization. Whether these patients will benefit from another course of systemic steroids is not known. Frequent use of systemic steroids however can have side effects such as pneumonia, hyperglycaemia, osteoporosis

and venous thromboembolism(31,32) and may contribute to an impaired immune status. Specific immune deviations in COPD have been suggested previously.(33) Since infectious disease are highly prevalent in COPD and in their exacerbations we hypothesized that an impaired immune status could be associated with a higher risk for CAP, AECOPD and mortality.

This brings me to **chapter 8** in which we studied the hypothesis that COPD patients who do not achieve seroprotective levels after influenza vaccination, are a less immune-competent group with a higher risk of morbidity and mortality. The immune system, both the humoral and cellular response, is vitally important to protect against pathogens, without overshoot or immune deviation. We used achieving seroprotection following the yearly influenza vaccination as a surrogate marker to determine whether patients had an impaired immune status. Our study showed that only a minority of patients (around 40%) achieved seroprotective titers to H1N1 and H3N1 after the yearly influenza vaccination. This however was not associated with lower morbidity and mortality. Seroprotection can therefore not be used as a marker for morbidity and mortality. The Committee for Proprietary Medicinal Product (CPMP) criteria state that post-vaccination serum is considered seroprotective if the hemagglutination inhibition (HAI) antibody titer is ≥ 40.(34) The antibody titer measured by the HAI assay is an established correlate of protection for inactivated influenza vaccines because this vaccination leads to increased HAI titers and higher HAI titers are correlated with protection against influenza virus infection. However, one has to keep in mind that the HAI titer only measures part of the humoral immune response stimulated by inactivated influenza vaccines; other immune mechanisms may also play a role in the protection conferred by inactivated influenza vaccines. In particular, the HAI assay does not capture other immune mechanisms that may be protective, including antibodies that target the hemagglutinin stalk, antibody-dependent cell-mediated cytotoxicity antibodies, and anti-neuraminidase antibodies.(35) So seroprotection measures only a small part of the total immune response achieved. If this is enough to define someone's overall immune status is not that clear. Maybe other parts of the immune system and immune response, such as the (specific) immunoglobulins with their subclasses, have to be involved. This will be a topic of our further research. Over 300 distinct primary immune deficiency diseases have been described in which more than half result in antibody deficiency. The vast majority of patients with antibody deficiency lack defined genetic defects and carry the diagnosis of common variable immune deficiency (CVID).(36) For these, criteria are defined for diagnosis. The major problem is however, that

there is no clear definition or test to determine and define a persons, i.e. COPD patient's, overall immune status. If the seroprotection is indeed a marker for the immune status then in COPD this is not associated with morbidity and mortality. If it is not a (good) marker for the immune status then we should search for other ones. Immune dysfunction in patients with COPD is modulated by various critical parameters in the lung microenvironment that result in chronic inflammation and facilitates recurrent respiratory infections. This complexity is explained in a review by Bhat et al.(37) Maybe the COPD patients with elevated (bio-) markers indicating chronic and systemic inflammation are the ones at risk for this immune dysfunction. Further studies are needed to determine whether there is indeed an existent COPD phenotype with a reduced immunocompetence, how to define it, whether it is associated with worse outcomes, and whether these patients need another approach for prevention and treatment and follow-up.

COPD is accompanied with high morbidity and mortality. This thesis mainly was about search for (bio-) markers associated with severe AECOPD, CAP and mortality. Eventually this is about prognostication. Future research should be on developing a prognostic tool, possibly embedded in an artificial intelligence model encompassing multiple known and unknown variables, for a better prognostication for morbidity and mortality for our individual COPD patients at diagnosis and with serial measurements also over time. This is only useful when we attach consequences to this prognostication, such as in allocation of treatment and/or guidance strategies etc. and if these strategies subsequently influence this prognostication. This sort of interventional studies have to be performed. Further, the role of the immune system in COPD is more and more a topic of interest in recent research. Immune impairment on the one hand and an overactive immune system on the other hand. Autoimmunity as a pathophysiological basis for COPD and it's progression is intriguing. Understanding the immune system with it's (imperfect) immune responses in COPD may open doors for new treatment options in COPD.

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Appendices

Summary

In this thesis, we searched for markers associated with morbidity and mortality in a large prospective single-centre chronic obstructive pulmonary disease (COPD) cohort study from Enschede, the Netherlands: the COMIC study. A total of 795 patients were included with a follow-up period of at least three years. Morbidity was defined as hospitalized (severe) Acute Exacerbations of COPD (AECOPD) and Community Acquired Pneumonia (CAP). Mortality was defined as all-cause mortality.

Both chronic inflammation and cardiovascular comorbidity play an important role in the morbidity and mortality of patients with COPD. Besides their proven effect on reducing cardiovascular disease, statins have other pleiotropic anti-inflammatory effects as well. Therefore, statins could be a potential adjunct therapy in COPD patients. In **chapter 2**, the use of statins, defined as having a statin for at least 90 consecutive days after inclusion, was not associated with time till first severe AECOPD and CAP in COPD patients in the COMIC study. Statin use resulted in a better overall survival (corrected hazard ratio 0.70 (95% CI, 0.51 - 0.96) in multivariate analysis), but in the sensitivity analysis, excluding immortal time bias, this association disappeared. This means that there is no role for standardized statin prescription for all our COPD patients, other than the recommendations from national and international guidelines for cardiovascular risk management.

Chapter 3-6 are about the blood biomarkers MR-proADM, Fibrinogen and the eosinophil count. Underlying chronic and systemic inflammation play an important role in the pathophysiology of COPD, its progression and its associated comorbidity. In **chapter 3** we evaluated for the first time the association of stable state MR-proADM with subsequent severe AECOPD and CAP in a pooled analysis in 1285 COPD patients of two large COPD cohort studies, the COMIC study and the PROMISE-COPD study. Our study showed that 25% of the patients had a stable state MR-proADM above the frequently used cut-off level of 0.87nmol/l and that this was associated with a 30% higher risk for subsequent severe AECOPD in a population in which 34% of the patients had at least one severe AECOPD during 3 years of follow up. This was independent of other known factors associated with AECOPD, such as exacerbation history, lung function and comorbid status. For time till first CAP, only COMIC data (n=795) was available. Patients with high level stable state MR-proADM had a significantly higher risk for a CAP compared with COPD patients with low level MR-proADM in univariate analysis (HR 1.93; 95% CI,

1.24 - 3.01) but, after correction for age, lung function and previous AECOPD, the association was no longer significant (cHR 1.10; 95% CI, 0.68 - 1.80).

In **chapter 4**, we focused on mortality prediction in COPD and found that both stable-state MR-proADM and stable-state Fibrinogen, two validated and sensitive biomarkers, were significantly associated with mortality. When both fibrinogen and MR-proADM were included together in the survival model, a doubling in fibrinogen and MR-proADM levels gave a 2.2 (95% CI 1.3–3.7) and 2.1 (95% CI 1.5–3.0) fold increased risk of dying, respectively. Although the two biomarkers seemed correlated to each other, the actual correlation was only very weak (r=0.2; p=<0,001). This strongly suggests that both biomarkers reflect different biological processes and as such they encompass a different part of the underlying (systemic) inflammation in COPD. Despite their weak correlation, combining both biomarkers did not substantially improve predictive performance when assessed using AUC or c-statistic. When adding MR-proADM to a one-year prediction model already including Fibrinogen, this resulted in a significant increase of the predictive capacity of the model (AUC increased from 0.78 to 0.83). In contrast, adding Fibrinogen to the one-year mortality prediction model already including MR-proADM, this did not result in a significant increase of the predictive capacity of the model (AUC increased from 0.82 to 0.83). These findings suggest that MR-proADM is a better predictor for mortality than Fibrinogen.

A relevant subgroup (20-40%) of the COPD population has an eosinophilic phenotype, probably linked to underlying type 2 inflammation, similar to that observed in patients with asthma. It is suggested that this eosinophil inflammation may be associated with future AECOPD and CAP risk.(43) However less is known about its prognostic accuracy to predict future morbidity and mortality when measured at AECOPD. In **chapter 5** we studied the association of the blood eosinophil count measured at severe AECOPD with subsequent morbidity and mortality. We analysed this for different frequently used cut-off levels and for eosinophil count as a continuous variable. Our multivariate analyses and sensitivity analyses (excluding patients with recent systemic steroid use), revealed that none of the four eosinophil classifications nor eosinophil count as continuous variable were associated with time till all-cause mortality, next severe AECOPD or next CAP. Despite the high rates of morbidity and mortality observed in COPD patients following a severe AECOPD—where nearly 25% developed at least one subsequent episode of CAP, almost 50% experienced another severe AECOPD, and 43% died within three years—the blood eosinophil count measured during

the initial exacerbation did not prove to be a reliable prognostic marker for these outcomes. This highlights that, although eosinophilic inflammation may play a role in the underlying disease mechanisms and can inform treatment decisions, eosinophil levels measured during severe AECOPD lack predictive value for future disease burden or mortality.

Despite this, elevated blood eosinophil count during an AECOPD remain clinically relevant as they are associated with steroid responsiveness. Due to this association it has been shown to be a promising biomarker for establishing personalised treatment strategies to reduce corticosteroid use, either inhaled or systemic, in COPD. Eosinophil levels seem relatively stable over time in stable state, but little is known whether this is also true in subsequent severe AECOPD. In **chapter 6** we studied the stability of eosinophil categorisation across 2 subsequent severe AECOPDs for different frequently used cut-off levels. Depending upon the used cut-off and expression of the eosinophil, the overall stability in eosinophil categorisation varied between 70%-85% during two subsequent AECOPDs. From patients who were eosinophilic at the first AECOPD only 34%-45% remained eosinophilic at the subsequent AECOPD while 9%-21% of patients being non-eosinophilic at the first AECOPD became eosinophilic at the subsequent AECOPD. There was only a weak correlation between the blood eosinophil counts measured at 2 subsequent severe AECOPDs ($r=0.19$, $p=0.003$ for the absolute counts, $r=0.22$, $p=0.001$ for the relative counts). Recent steroid use did not influence the eosinophil categorisation stability. Although it did lower the eosinophil numbers, it did not normalize them (10-22% were still labelled as eosinophilic depending on cut-off used). Our study concludes that the eosinophil variability in subsequent AECOPDs leads to category changes. Therefore, if eosinophil guided strategies for steroid use for AECOPD will be implemented, one cannot rely on the eosinophil count measured during an earlier severe AECOPD. The eosinophil count has to be determined at every new severe AECOPD. If our results can be extrapolated also to moderate AECOPD then we recognize that this potentially will have major implications for COPD self-management interventions, as this should then lead to measuring eosinophils at the moment of each AECOPD. Therefore, in **chapter 7** we proposed the potential for the introduction of personalised and biomarker-guided COPD self-treatment approaches, while addressing the hurdles to be overcome. We emphasized that the integration of necessary refinements and tailoring of self-treatment approaches will be an ongoing and evolving process, with practical and logistical challenges to overcome. Recent advances in biomarker-guided antibiotic therapy for AECOPD

and blood eosinophil-guided prednisolone treatment highlight the need for COPD patients within a self-management program to have rapid, easy access to point-of-care tools establishing their biomarker(s) at AECOPD onset, without delaying treatment. Embedding these personalised and biomarker-guided COPD self-treatment approaches in self-management interventions, will contribute to lower the use of systemic steroids. Frequent use of systemic steroids can have side effects and can contribute to the comorbid status of the COPD patient and impair the immune status. Specific immune deviations in COPD have been suggested previously. We hypothesized that an impaired immune status could be associated with a higher risk for CAP, AECOPD and mortality. However, there is no clear definition or test to determine and define a COPD patient's overall immune status. In the study presented in **chapter 8** we studied the hypothesis that COPD patients who do not achieve seroprotective levels after influenza vaccination, are a less immune-competent group with a higher risk of morbidity and mortality. In total 578 patients included in the COMIC cohort had pre- and post-vaccination stable state blood samples drawn, in which influenza-vaccine specific antibodies were measured. Our results show that 42% of the patients achieved seroprotective levels to both H1N1 and H3N2 after vaccination. Seroprotective levels to H3N2 were markedly higher (96%) than to H1N1(43%). Having seroprotective levels to both H1N1 and H3N2 was not associated with less morbidity (severe AECOPD HR 0.91 (95% 0.66-1.25; p=0.56), CAP HR 1.23 (95% 0.75-2.00; p=0.41)) or lower mortality (HR 1.10 (95% 0.87-1.38; p=0.43)). While achieving seroprotection after vaccination can be considered a surrogate marker of being immunocompetent, this was not associated with lower morbidity and mortality. Whether this means that the immune status is not a relevant pheno/endotype in COPD patients for the course of their disease or that seroprotection is not an adequate (surrogate) marker to define the immune status in COPD needs to be further studied.

In **chapter 9** we discuss the results presented in the chapters 2-8 and place them in a broader context and suggestions for future research are provided.

Samenvatting

In dit proefschrift zochten we naar markers die verband hielden met morbiditeit en mortaliteit in een groot prospectief single-center chronisch obstructieve longziekte (COPD) cohortonderzoek uit Enschede, Nederland: de COMIC studie. In totaal werden 795 patiënten geïncludeerd met een follow-upperiode van ten minste drie jaar. Morbiditeit werd gedefinieerd als gehospitaliseerde (ernstige) acute exacerbaties van COPD (AECOPD) en community-acquired pneumonie (CAP). Mortaliteit was gedefinieerd als "all-cause" mortaliteit.

Zowel chronische ontsteking als cardiovasculaire comorbiditeit spelen een belangrijke rol in de morbiditeit en mortaliteit van patiënten met COPD. Naast het bewezen effect op het verminderen van cardiovasculaire aandoeningen hebben statines ook andere pleiotrope ontstekingsremmende effecten. Daarom zouden statines een potentiële aanvullende therapie kunnen zijn bij COPD-patiënten. In **hoofdstuk 2** was het gebruik van statines, gedefinieerd als het hebben van een statine gedurende ten minste 90 opeenvolgende dagen na inclusie, niet geassocieerd met de tijd tot de eerste ernstige AECOPD en CAP bij COPD-patiënten in de COMIC-studie. Statinegebruik resulteerde in een betere algehele overleving (gecorrigeerde hazard ratio 0,70 (95% BI, 0,51 - 0,96) in multivariate analyse), maar in de sensitiviteitsanalyse met uitsluiten van de immortal time bias verdween deze associatie. Dit betekent dat er geen rol is voor het standaard voorschrijven van statines voor al onze COPD-patiënten, anders dan de aanbevelingen van nationale en internationale richtlijnen voor cardiovasculair risicomanagement.

Hoofdstuk 3-6 gaan over de bloedbiomarkers MR-proADM, fibrinogeen en het eosinofielen getal. Onderliggende chronische en systemische ontsteking speelt een belangrijke rol in de pathofysiologie van COPD, de progressie ervan en de bijbehorende comorbiditeit. In **hoofdstuk 3** evalueerden we voor het eerst de associatie van in stabiele fase gemeten MR-proADM met daaropvolgende ernstige AECOPD en CAP in een gepoolde analyse bij 1285 COPD-patiënten van twee grote COPD-cohortstudies, de COMIC-studie en de PROMISE-COPD-studie. Onze studie toonde aan dat 25% van de patiënten een in stabiele fase gemeten MR-proADM had boven de frequent gebruikte cut-off waarde van 0,87 nmol/l en dat dit geassocieerd was met een 30% hoger risico op daaropvolgende ernstige AECOPD in een populatie waarin 34% van de patiënten ten minste één ernstige AECOPD had gedurende 3 jaar follow-up. Dit was onafhankelijk van andere bekende factoren die geassocieerd zijn met AECOPD, zoals exacerbatiegeschiedenis, longfunctie

en comorbiditeit. Voor de tijd tot de eerste CAP waren alleen COMIC-gegevens (n=795) beschikbaar. Patiënten in stabiele fase met een hoog niveau MR-proADM hadden een significant hoger risico op een CAP vergeleken met COPD-patiënten met een laag niveau MR-proADM in univariate analyse (HR 1,93; 95% BI, 1,24 - 3,01), maar na correctie voor leeftijd, longfunctie en eerdere AECOPD was de associatie niet langer significant (cHR 1,10; 95% BI, 0,68 - 1,80).

In **hoofdstuk 4** richtten we ons op mortaliteitsvoorspelling bij COPD en vonden we dat zowel in stabiele fase gemeten MR-proADM als in stabiele fase gemeten fibrinogeen, twee gevalideerde en sensitieve biomarkers, significant geassocieerd waren met mortaliteit. Wanneer zowel fibrinogeen als MR-proADM gezamenlijk werden opgenomen in het overlevingsmodel, gaf een verdubbeling van de fibrinogeen en MR-proADM waardes respectievelijk een 2,2 (95% BI 1,3-3,7) en 2,1 (95% BI 1,5-3,0) keer verhoogd risico op overlijden. Alhoewel beide biomarkers aan elkaar leken gerelateerd, was er slechts een zeer zwakke correlatie ($r=0,2$; $p<0,001$). Dit suggereert sterk dat beide biomarkers verschillende biologische processen weerspiegelen en als zodanig een ander deel van de onderliggende (systemische) ontsteking bij COPD omvatten. Ondanks de zwakke correlatie resulteerde het combineren van beide biomarkers niet in een substantiële verbetering van de predictieve capaciteit vastgesteld met AUC of c-statistic. Wanneer MR-proADM werd toegevoegd aan een model voor mortaliteitsvoorspelling over één jaar dat al fibrinogeen bevatte, resulteerde dit in een significante toename van het voorspellende vermogen van het model (AUC steeg van 0,78 naar 0,83). Toen fibrinogeen werd toegevoegd aan het model voor mortaliteitsvoorspelling over één jaar dat al MR-proADM bevatte, resulteerde dit niet in een significante toename van het voorspellende vermogen van het model (AUC steeg van 0,82 naar 0,83). Deze bevindingen suggereren dat MR-proADM een betere voorspeller is voor mortaliteit dan fibrinogeen.

Een relevante subgroep (20-40%) van de COPD-populatie heeft een eosinofiel fenotype dat waarschijnlijk gelinkt is aan onderliggende type 2 ontsteking, gelijkend aan wat kan worden gezien bij patiënten met astma. Er wordt gesuggereerd dat deze eosinofiele ontsteking mogelijk is geassocieerd met risico op toekomstige AECOPD en CAP. Er is echter minder bekend over de prognostische nauwkeurigheid ervan om toekomstige morbiditeit en mortaliteit te voorspellen wanneer deze wordt gemeten bij AECOPD. In **hoofdstuk 5** bestudeerden we de associatie van het bloed eosinofielen aantal gemeten bij ernstige AECOPD met daaropvolgende morbiditeit en mortaliteit. We analyseerden dit voor verschillende

veelgebruikte afkappunten en voor het eosinofielen aantal als continue variabele. Onze multivariate analyses en sensitiviteitsanalyses (waarbij patiënten met recent systemisch steroïdengebruik werden uitgesloten), lieten zien dat geen van de vier eosinofielen classificaties of het eosinofielen aantal als continue variabele geassocieerd waren met tijd tot all-cause mortaliteit, volgende ernstige AECOPD of CAP. Ondanks de hoge waargenomen ziektelast in de vorm van morbiditeit en mortaliteit volgend op een ernstige AECOPD-waar in deze studie binnen 3 jaar follow-up ongeveer een kwart van de patiënten ten minste één daaropvolgende CAP ontwikkelde, bijna de helft ten minste één daaropvolgende ernstige AECOPD en drieënveertig procent van de patiënten stierf-bleek het bloed eosinofiel getal bij de initiële ernstige AECOPD niet een goede bewezen biomarker als prognosticum voor deze uitkomstparameters. Dit benadrukt dat, alhoewel eosinofiele inflammatie een rol speelt in het onderliggende ziektemechanisme en ons kan informeren over te nemen behandelbeslissingen, deze bloed eosinofielen aantal gemeten tijdens ernstige AECOPD niet gebruikt kan worden als predictieve waarde voor toekomstige ziektelast en mortaliteit.

Desondanks blijft verhoogde eosinofielen in het bloed ten tijde van een AECOPD klinisch relevant aangezien ze geassocieerd zijn met responsiviteit op steroïden. Vanwege deze associatie is aangetoond dat het een veelbelovende biomarker is voor het vaststellen van gepersonaliseerde behandelingsstrategieën om het gebruik van corticosteroïden, geïnhaleerd of systemisch, bij COPD te verminderen. Eosinofielen niveaus gemeten in stabiele fase bij COPD lijken relatief stabiel in de loop van de tijd, maar er is weinig bekend of dit ook geldt bij metingen bij opeenvolgende ernstige AECOPD. In **hoofdstuk 6** bestudeerden we de stabiliteit van de eosinofielen categorisatie over 2 opeenvolgende ernstige AECOPD's voor verschillende veelgebruikte afkappunten. Afhankelijk van de gebruikte afkappunten en expressie van de eosinofiel varieerde de algehele stabiliteit in eosinofielen categorisatie tussen 70%-85% tijdens twee opeenvolgende AECOPD's. Van patiënten die eosinofiel waren bij de eerste AECOPD bleef slechts 34%-45% eosinofiel bij de daaropvolgende AECOPD, terwijl 9%-21% van de patiënten die niet-eosinofiel waren bij de eerste AECOPD eosinofiel werden bij de daaropvolgende AECOPD. Er was slechts een zwakke correlatie tussen de bloed eosinofielen niveaus gemeten bij 2 opeenvolgende ernstige AECOPD's ($r=0,19$, $p=0,003$ voor de absolute tellingen, $r=0,22$, $p=0,001$ voor de relatieve tellingen). Recent steroïdengebruik had geen invloed op de eosinofielen categorisatie stabiliteit. Alhoewel het de eosinofielen aantallen wel verlaagde, normaliseerde het dit niet (10-22% werd nog steeds als eosinofiel gelabeld, afhankelijk van de

gebruikte cut-off). Onze studie concludeert dat de eosinofielen variabiliteit in daaropvolgende AECOPD's leidt tot categorieveranderingen. Daarom kan men, als eosinofielen gestuurde strategieën voor steroïdengebruik voor AECOPD zouden worden geïmplementeerd, niet vertrouwen op het eosinofielen aantal dat is gemeten tijdens een eerdere ernstige AECOPD. Het eosinofielen aantal moet bij elke nieuwe ernstige AECOPD worden bepaald. Als onze resultaten ook kunnen worden geëxtrapoleerd naar matige AECOPD, dan erkennen we dat dit mogelijk grote implicaties zal hebben voor COPD-zelfmanagement interventies, aangezien dit dan zou moeten leiden tot het meten van de eosinofielen op het moment van elke AECOPD. Daarom hebben we in **hoofdstuk 7** de potentie voorgesteld voor de introductie van gepersonaliseerde en biomarker-gestuurde COPD-zelfbehandelingsbenaderingen en benoemen we de obstakels die overwonnen moeten worden. We benadrukten dat de integratie van noodzakelijke verfijningen en het op maat maken van zelfbehandelingsbenaderingen een doorlopend en evoluerend proces zal zijn, met praktische en logistieke uitdagingen die overwonnen moeten worden. De recente ontwikkelingen in biomarker-gestuurde antibioticabehandeling in AECOPD en bloed eosinofielen gestuurde prednisolon therapie vereisen dat COPD-patiënten binnen een zelfmanagementprogramma gemakkelijk en snel toegang hebben tot point-of-care-tools die hun biomarker(s) vaststellen bij het begin van de AECOPD, zonder de behandeling uit te stellen. Het inbedden van deze gepersonaliseerde en biomarker-gestuurde COPD zelfbehandelingsbenaderingen in zelfmanagementinterventies zal bijdragen aan een lager gebruik van systemische steroïden. Frequent gebruik van systemische steroïden kan bijwerkingen hebben en kan bijdragen aan de comorbiditeit van de COPD-patiënt en de immuunstatus aantasten. Specifieke immuun afwijkingen bij COPD zijn eerder gesuggereerd. Wij stelden de hypothese op dat een verminderde immuunstatus geassocieerd zou kunnen zijn met een hoger risico op CAP, AECOPD en mortaliteit.

Er is echter geen duidelijke definitie of test om de algehele immuunstatus van een COPD-patiënt te bepalen en te definiëren. In de studie die in **hoofdstuk 8** wordt gepresenteerd, hebben we de hypothese bestudeerd dat COPD-patiënten die geen seroprotectieve niveaus bereiken na griepvaccinatie, een minder immuun competente groep zijn met een hoger risico op morbiditeit en mortaliteit. In totaal werden bij 578 patiënten die in het COMIC-cohort waren opgenomen, bloedmonsters van stabiele toestand vóór en na de vaccinatie afgenomen, waarin specifieke antilichamen tegen het griepvaccin werden gemeten. Onze resultaten laten zien dat 42% van de patiënten seroprotectieve niveaus bereikte

voor zowel H1N1 als H3N2 na vaccinatie. Seroprotectieve niveaus voor H3N2 waren aanzienlijk hoger (96%) dan voor H1N1 (43%). Seroprotectieve niveaus voor zowel H1N1 als H3N2 waren niet geassocieerd met minder morbiditeit (ernstige AECOPD HR 0,91 (95% 0,66-1,25; p=0,56) (CAP HR 1,23 (95% 0,75-2,00; p=0,41)) of lagere mortaliteit (HR 1,10 (95% 0,87-1,38; p=0,43)). Hoewel het bereiken van seroprotectie na vaccinatie kan worden beschouwd als een surrogaatmarker van immuun competent zijn, was dit niet geassocieerd met lagere morbiditeit en mortaliteit. Of dit betekent dat de immuunstatus geen relevant feno/endotype is bij COPD-patiënten voor het beloop van hun ziekte of dat seroprotectie geen adequate (surrogaat)marker is om de immuunstatus bij COPD te definiëren, moet verder worden onderzocht.

In **hoofdstuk 9** bespreken we de resultaten die in de hoofdstukken 2-8 worden gepresenteerd en plaatsen we deze in een bredere context en worden er suggesties gedaan voor toekomstig onderzoek.

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Wij is natuurlijk de vakgroep longziekten MST. Wat ben ik nog steeds blij en vereerd dat ik destijds in 2016 lid mocht worden van deze groep longartsen. We bestaan inmiddels uit 9 longartsen(aan nummer 10 wordt gewerkt) en een longfysioloog. We zijn een mooi team en hebben oog voor elkaar. We hebben nooit het belang van onderzoek uit het oog verloren en mogen trots zijn op ons onderzoeksbureau

en op al onze output. Het heeft mede de groep gebracht waar we nu staan en daar ben ik trots op.

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Zo, nu is het dan toch echt af. Tijd voor vakantie. Italy it is…….again!!!

Curriculum Vitae

Emanuel Citgez was born on February 23rd 1983 in Almelo and lived most of his live in beautiful Wierden. After graduating from secondary school in Almelo (Pius X College) in 2001 he moved to Groningen and started studying Medicine at the Rijksuniversiteit Groningen and obtained his medical degree in 2009. From 2009 till 2010 he worked as a resident in Ziekenhuis Groep Twente, Hengelo and there his enthusiasm for pulmonary medicine started. From 2010-2016 he started to work in Medisch Spectrum Twente Enschede as a resident to become a pulmonologist. Afterwards he started to work as a pulmonologist till 2017 in Antoni van Leeuwenhoek, Amsterdam. In 2016 he started his PhD project at the same department where he works now since April 2017 as a pulmonologist, the pulmonary department of Medisch Spectrum Twente, Enschede. He is married to Martina and they have three children, Loïs(2014), Isabel(2015) and Feline(2019).