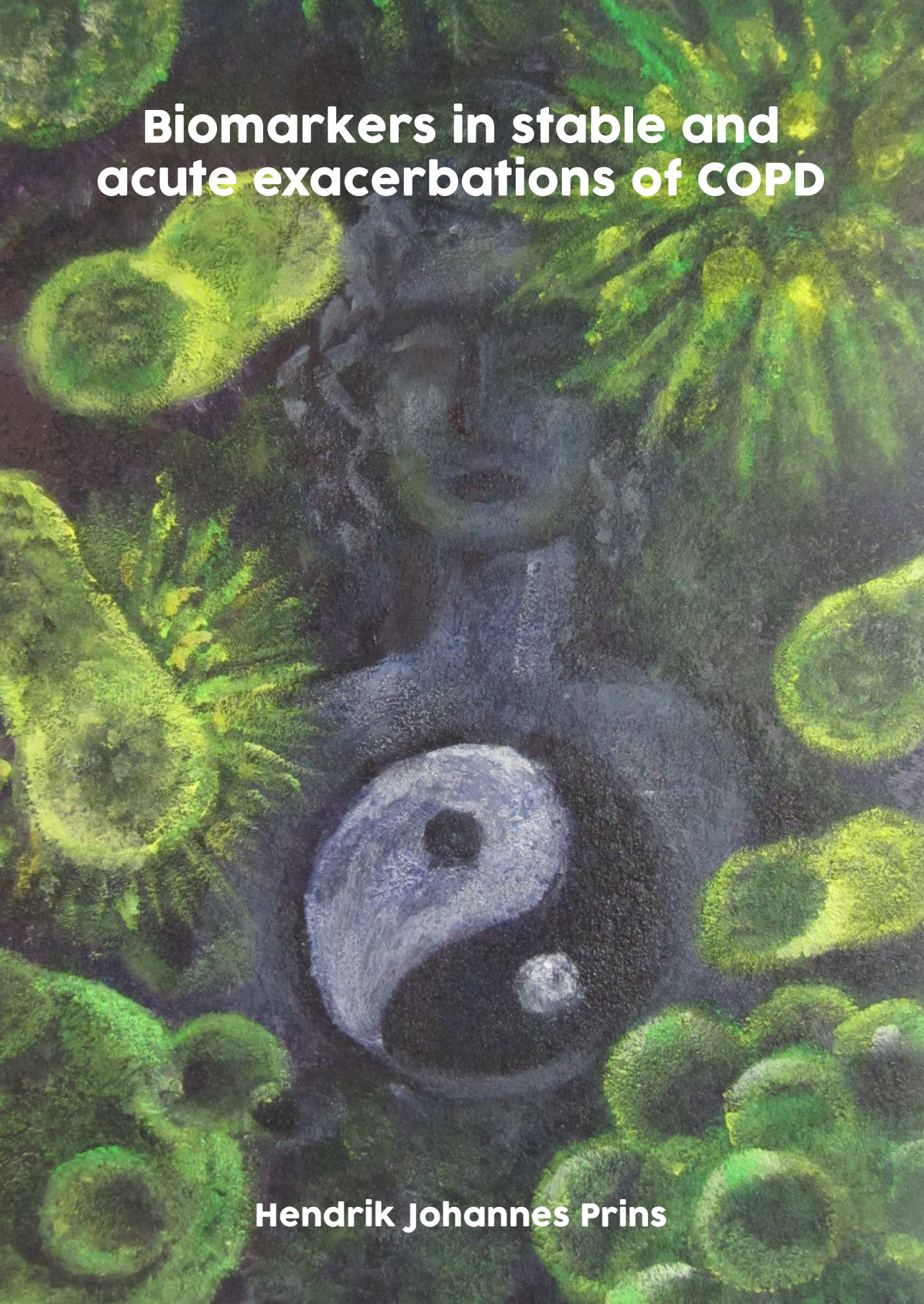
Biomarkers in stable and acute exacerbations of COPD



Hendrik Johannes Prins

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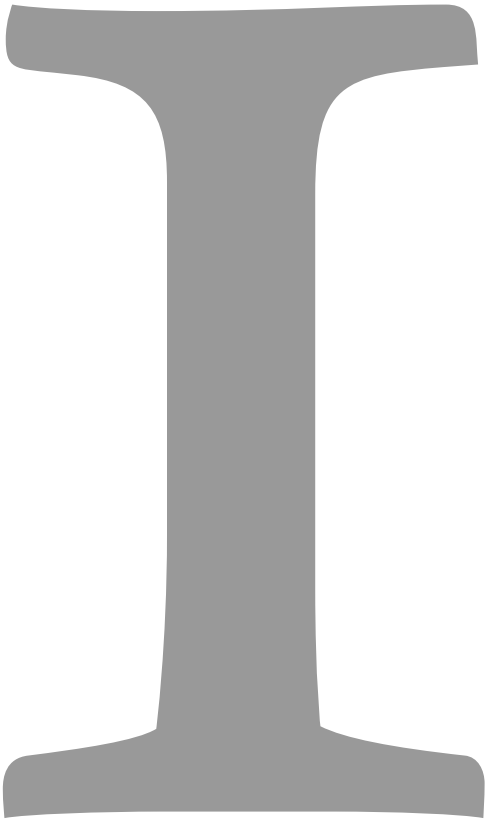
CHAPTER 1



General introduction

Background

Chronic obstructive pulmonary disease (COPD) is defined as 'a common, preventable and treatable disease that is characterised by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities, usually caused by significant exposure to noxious particles or gases'.[1] COPD is an umbrella term for small airways disease and parenchymal destruction, the relative contribution of which varies from person to person, resulting in chronic airflow obstruction.[1] The primary cause of COPD is persistent tobacco smoke exposure (including second hand smoking or passive smoking). Other agents associated with COPD are air pollution, occupational exposure to organic or anorganic dust, chemicals, and/or fumes.[1] COPD affects 380 million people worldwide, representing 12% of all adults over 30 years of age.[2] In the Netherlands, almost 600,000 patients have COPD; each year, about 27,000 new cases are identified, and 6,800 COPD patients died in 2017.[3] The burden of disease is explained by three important factors. First, patients suffer from the common symptoms of COPD such as shortness of breath, cough and sputum expectoration. These symptoms vary over time, and are associated with problems in performing basic daily activities.[4-6] Second, patients suffer from exacerbations of COPD (AECOPD); and third, include the non-pulmonary symptoms including weight loss, muscle wasting, osteoporosis and depression, but also comorbidities such as cardiovascular disease.[7;8] These symptoms are at least in part explained by low-grade systemic inflammation probably resulting from spill-over of multiple pro-inflammatory markers into the circulation.[9;10] Chronic airway and low-grade systemic inflammation are key to the progression of COPD and COPD-associated non-pulmonary co-morbidities.[8;11]

Symptoms of COPD

Patients with COPD experience symptoms to a greater or lesser extent, and these symptoms impose a significant burden on the individual patient.[1] As explained above, the most frequent respiratory symptoms are chronic cough with or without sputum production and shortness of breath.[12] Symptoms may vary during the day and during the week[12]. Perception of symptoms has a key impact on health-related quality of life (HRQL) and affects the ability to perform the activities of daily living.[13] In addition, dyspnoea is a better predictor of mortality than FEV1.[14] Another common symptom of COPD is fatigue, which has a profound negative impact on the physical, emotional, cognitive and social functioning of patients with COPD.[15] During AECOPD there is an increase in symptom severity.[16] Most common symptoms during AECOPD are dyspnoea, cough, increase of sputum production or purulence; and fatigue. Anthonisen et al classified exacerbations according to 3 cardinal symptoms - dyspnoea, sputum

production, and sputum purulence - in order to test the benefit of antimicrobial therapy in AECOPD.[17] This system has widely been implemented since, and has been used in a significant number of trials using all sorts of diary cards.[18-21] Most cards include the here for mentioned criteria but there is a large variability in the text of these cards; some do not use all three cardinal symptoms and none of these cards or questionnaires has been proper validated, making comparison across studies virtually impossible. Eventually, an extensive questionnaire designed for measurement of symptoms was validated for this purpose.[22] Although a major disadvantage is that written questionnaires can be challenging for poorly educated or illiterate patients whereas visual analogue scales do not have this problem.[23] We recently validated a VAS scale in bronchiectasis and a slightly modified version of this questionnaire was also earlier used in a COPD population.[24;25] On both occasions the questionnaire was generally well accepted by patients.

AECOPD

Exacerbations are a major contributor to the burden of disease as well as to disease progression.[7] Exacerbations are defined as an acute worsening of respiratory symptoms that results in additional therapy.[1] Additionally exacerbations are responsible for a substantial economic and social burden.[1;26;27] As much of COPD burden of disease for the patients as well as for society is associated with exacerbations, attention should be given to optimal treatment and prevention of these events.[28;29] Although considerable progress has been made in recent years in our understanding of the nature and causes of AECOPD, many questions have remained unanswered. AECOPD is heterogeneous in nature and severity. Often the difference between pneumonia, acute congestive heart failure and AECOPD is hard to make as these disease entities show a large overlap in symptoms. They affect similar patient populations; and they often co-exist.[30] It is therefore that scientists and clinicians are looking for ways to differentiate accurately between these conditions.[31] The common denominator in all three diagnoses is dyspnoea. In AECOPD this is caused by an increase of airflow limitation especially due to an increased airway inflammation.[7;31] In clinical practice it may be difficult to establish the exact cause of the exacerbation. Attempts have been made with increasing success to reliably cluster patients with AECOPD based upon the aetiology. The aim of creating these groups is to improve prognostic prediction, establish more effective treatment strategies and move toward precision medicine in the treatment of AECOPD.[32] Known subtypes of exacerbations include those with bacterial and/or viral origin; those associated with high eosinophilic inflammation; and those associated with increase of chemical air pollution.[33;34] Respiratory tract infections are generally believed to be the most common cause of AECOPD. Infections can be subdivided according to aetiology - viral infection, bacterial infection or a combination of both.[7] The most common cause

of infection-generated AECOPD are the ones triggered by upper respiratory tract viral infections, like the common cold; virally triggered AECOPD are more prevalent in the winter months.[35] In other patients eosinophilic inflammation seems to contribute to the AECOPD.[34] Although eosinophilic inflammation in chronic lung disease is usually associated with asthma, studies have indicated that approximately one third of the patients with COPD have sputum eosinophilia.[36] Regardless of the aetiology of the AECOPD, the final common pathway is an increase of airway inflammation which leads to increased air flow limitation.[7] Despite the fact that every subtype of AECOPD displays these symptoms, the treatment for AECOPD should target the underlying mechanism responsible for the exacerbation as this approach may help tailor treatment thereby reducing side effects, improving response to treatment and the overall prognosis.[37]

Current treatment for AECOPD

Main goals for the treatment of AECOPD are to minimize the negative impact of the current AECOPD and prevent the development of subsequent events[1]. Frequent exacerbations have deteriorating impact on lung function, quality of life and long term prognosis.[1] Depending on the severity and aetiology of an exacerbation, three classes of medication are most commonly used: bronchodilators, corticosteroids; and antimicrobial agents.[1]

Bronchodilators

There is no high quality evidence from RCT's that support the use of bronchodilators in the form of short acting inhaled $beta_2$-agonists with or without short-acting anticholinergics for the treatment of AECOPD, yet there is a widespread belief that bronchodilators have a beneficial effect on AECOPD and it has become a common therapeutic practice.[1;38]

Corticosteroids

In COPD, inhaled or systemic (intravenous or oral) corticosteroids can be used during an AECOPD.[1;39;40] A five day course of oral corticosteroids is likely to be sufficient for the treatment of AECOPD.[1;41] Current guidelines conclude that systemic corticosteroids can improve lung function (FEV1), oxygenation, and shorten recovery time and hospitalization.[1;39] However the prescription of systemic corticosteroids does not influence outcomes such as mortality, long-term decline in lung function or re-exacerbations while they are associated with significant side effects.[39]

Antimicrobial agents

Current guidelines recommend antimicrobials to patients with AECOPD who have two or more cardinal symptoms: increase in dyspnoea, increased sputum volume and increased sputum purulence.[1] However, it has been shown that sputum purulence is not a good marker of bacterial infection.[42-44] Moreover evidence shows that up to 64% of the exacerbations are induced by viruses.[45] As a consequence, the use of patient-reported sputum purulence might result in overuse of antibiotics in AECOPD.[46] It is evident that unnecessary prescription of antibiotics for respiratory illness leads to side effects, emerging antimicrobial resistance and higher medical costs.[47] Yet on the other hand a recent Cochrane review showed that antibiotics in AECOPD have been proven effective in reduction of treatment failure, although it should be noted that the studies included in this review did have some limitations regarding concomitant corticosteroid use and the populations studied consisted of heterogeneous groups of in and out patients. In addition improvements shown are marginal, and have no influence on length of hospital stay or mortality.[48]

Biomarkers

Despite the evidence that AECOPD has heterogeneous aetiology, and treatment options are potentially diverse, current treatment is universally standard, and not individually tailored.[49] In the past, exacerbation treatment was guided upon clinical presentation as well as on disease severity. Disease severity according to the GOLD classification although recently modified, still hinges to a large extent on the FEV1 relative to predicted values to classify disease severity (GOLD classes 1-4). Both have significant disadvantages. The clinical impression is subjective and variable within and across physicians, and unfortunately GOLD staging poorly reflects symptom severity.[50] In an effort to improve the latter system, the GOLD initiative has added symptoms, limitations and exacerbation frequency to their staging system, yet this still does not provide us with a solid roadmap for exacerbation treatment.[1] For this we need additional tools, such as biomarkers. Biomarkers can be defined as biological molecules that may reflect disease activity and fluctuate in accordance with disease state, while representing biologically plausible pathways.[51] Today, many biomarkers in a wide variety of sample types are available such as exhaled breath condensate, sputum, nasal wash, blood, broncho-alveolar lavage, and lung biopsies.[51] Many of these markers show statistically significant associations with AECOPD. Yet it leaves us with the question whether they represent a marker of disease activity or whether they are clinically useful biomarkers able to discriminate between the different exacerbation subtypes. In this thesis we have focused our attention to blood-based biomarkers to provide tailored care for each patient and each exacerbation in order to prevent unnecessary exposure to - or adverse

withholding of drugs thereby diminishing population wide antibiotic prescription and possible reducing antimicrobial resistance.

Currently there are four known types of exacerbations that can be identified using biomarkers; bacterial; virus-induced; eosinophilic inflammation-associated exacerbations; and the fourth type, termed "pauci-inflammatory exacerbation.[34] The latter is classified as an exacerbation without a clear cause. Unfortunately, to date there are only two biomarkers that have prospectively been validated to differentiate between bacterial, viral, or eosinophil-associated exacerbations. Blood eosinophilia has been used to characterise an exacerbation that is associated with increase in eosinophilic inflammation[52]. In this trial, patients were enrolled into a randomized biomarker-directed double-blind placebo controlled trial. In the intervention group patients received corticosteroids or placebo according to blood eosinophil count whereas patients in the control group where all treated with corticosteroids. This resulted in a reduction of 49% of corticosteroid use without an increase in treatment failure or worsening of symptoms. Even more in eosinophil biomarker negative patients corticosteroids treatment resulted in worse outcomes compared with placebo. Another study a cut-off of $0{\cdot}3 \times 10^9$ eosinophils was used to administer or withhold systemic corticosteroids. In this multi-centre RCT the investigators showed that eosinophil-guided therapy led to reduction of systemic corticosteroid exposure without an increase of treatment failure.[53] Procalcitonin (PCT) has been prospectively validated to guide therapy in bacteria-associated AECOPD. PCT is a protein that is secreted by a large array of host cells during systemic inflammation under the influence of inflammatory cytokines and microbial toxins[54]. A recent meta-analysis showed that the use of PCT as biomarker to guide antibiotic therapy is associated with a 35.5% reduction in antimicrobial consumption compared to standard therapy without an increase in length of hospital stay or adverse events. In most studies a Procalcitonin cut-off value of >0.25 μg/L was used. Although this reduction was significant, as many as 80.1% of the patients in the standard therapy group were treated with antimicrobial agents.[55] PCT guided antimicrobial treatment has not been widely adopted, probably because PCT is more expensive than for instance CRP and is not rapidly and widely available at the time of an AECOPD.[56] Another biomarker that could be useful for the detection of bacterial-associated AECOPD is C-reactive protein (CRP). CRP is an acute phase protein that is synthesized by hepatocytes under the influence of IL-6, IL-1β and tumour necrosis factor α (TNF α) in response to infections, tissue injury and inflammation.[57] CPR has been validated as a biomarker for the reduction of antimicrobial prescription in respiratory tract infections without compromising patient's recovery.[58;59] In a placebo controlled clinical trial investigating the use of doxycycline in severe AECOPD it was shown that doxycycline was superior to placebo on day 10 in terms of clinical cure yet this difference disappeared after 30 days.[25] However in a subgroup analysis patients with

CRP ≥50mg/L retained the beneficial treatment effect after 30 days. In patients with CRP <50mg/L there was no significant beneficial treatment effect of doxycycline after 10 days or after 30 days. In another randomized controlled placebo controlled clinical trial investigating amoxicillin/clavulanate in AECOPD.[60] It was shown that patients treated with amoxicillin/clavulanate had significant higher cure rates compared to placebo. However in patients with a CRP lower than 40 mg/L clinical success with placebo was significantly higher than in patients with a CRP ≥ 40 mg/L. These studies show that CRP might be a promising biomarker for the initiation or withholding of antibiotics in AECOPD. However until our study, this biomarker has not been prospectively validated for this purpose in AECOPD.

Other potential biomarkers that could be used for the differentiation between viral and bacterial exacerbations are Interferon-γ-inducible protein 10 (IP-10and Serum Amyloid A(SAA) IP-10 is a chemokine that is released in response to interferon-γ and tumour necrosis factor-α by bronchial epithelial cells, monocytes, lymphocytes, and neutrophils.[61] Human rhinovirus (HRV) replicating in bronchial epithelial cells triggers the production the production of IP-10.[61] HRV is known to be the most frequent isolated respiratory virus in AECOPD.[62] IP-10 has shown to be a marker of viral associated AECOPD yet so far has never been prospectively validated.[34;63] SAA is an acute phase protein whose expression is induced by Il-1 and Il-6.[64] SAA can be used for the detection of bacterial associated AECOPD and might be of extra use in discriminating for bacterial inflammation when combined with CRP yet so far this has not yet been prospectively validated in AECOPD.[34;65] Despite these encouraging results IP-10 and SAA where not prospectively validated for their use in AECOPD in this thesis although SAA was used to differentiate between AECOPD and subclinical pneumonia.

Inflammation in COPD

As was mentioned before airway inflammation is central to the pathophysiology of COPD and contributes to tissue damage and destruction in the airways. This is underlined by the fact that an abundance of inflammatory cell such as macrophages, neutrophils and T-cells are present in the lungs of COPD patients.[66;67] Due to cigarette smoke and other inhaled irritants, surface macrophages and the epithelial lining of the lung release chemotactic mediators which attract circulating white blood cells from the innate as well as from the adaptive immune system in the lung.[66] If the exposure to noxious particles or gasses persists long enough it becomes self-perpetuating leading although the pathogenesis of this phenomenon is not yet fully understood.[11] Although it is clear that bacterial colonization play a role in this process as airway bacterial load is associated with increased airway inflammation leading to a more rapid decline in lung function.[68;69] One of the key inflammatory cells in COPD is the neutrophil. All

patients with COPD have airway neutrophilia, regardless of clinical phenotype (chronic bronchitis, emphysema, and even eosinophilic COPD).[70] Neutrophils are recruited to lung tissue under the influence of cytokines such as IL-8, IL-6 and TNFα which is produced by alveolar macrophages and epithelial cells under the influence of infections or airpolution.[71;72] The neutrophils in the lung produce a variety of granule proteins such as myeloperoxidase, neutrophil elastase, proteinases, as well as MMP-8 and MMP-9, which leads to degradation of extracellular matrix (ECM) which degradation is linked to all clinical facets of COPD.[11;73;74] Another cell that may play a role in a part of the patients with COPD is the eosinophil. Although traditionnaly eosinophilic inflammation is regarded as a feature of asthma there is evidence that shows that het eosinophil may play a role in COPD pathogenisis.[75] Unfortunately inflammation is not confined to the lung. Possibly due to spill over of inflammatory markers form the lung systemic low-grade inflammation arises.[76] This may play a vital role in COPD associated co-morbidity.[66] As COPD is a heterogeneous disease so is the systemic inflammation, it is highly complex and many cytokines and mediators are involved.[76] Another hypothesis for systemic inflammation in COPD is that ultra-fine particles that are inhaled to the lung due to cigarette smoke or air pollution are able to translocate into the systemic circulation directly activate a systemic response of inflammation.[77] Known markers of low-grade inflammation in COPD are TNF-α, IL-1β, IL-6, IL-8 and CRP.[78;79]

Conclusion

COPD is a complex disease with pulmonary and extra pulmonary manifestations. It is heterogeneous in nature and characterized by persistent respiratory symptoms, airflow limitation and frequent exacerbations. Inflammation is one of the key driving pathophysiological features driving the progress of COPD. During AECOPD there is an increase of inflammation that can be divided in to four different subgroups: eosinophil associated, viral associate and bacterial associated and pauci-inflammatory AECOPD. By using specific biomarkers some these subgroups can be identified which may have significant impact on treatment options. There are currently two interventions that can be given or withheld based upon biomarkers. Antibiotics and corticosteroids. Both are controversial in the treatment of AECOPD as one has significant side effects and the other might cause antimicrobial resistance whereas improvements due to these therapies is marginal. Extensive research is therefore required to evaluate the role of these treatment modalities in ACOPD and to what extend biomarkers may play a role in this process.

Outline of the thesis

The principal objective of this thesis was to reduce the use of antibiotics in the management of AECOPD without compromising patient safety. A randomized clinical trial was carried out to determine whether CRP-guided antibiotic treatment could safely reduce the amount of antibiotics prescribed for the treatment of AECOPD compared to sputum purulence guided strategy (as formulated in the GOLD strategy) and what the influence of the CRP-guided strategy would be on 30-day treatment failure rate, exacerbation recovery and adverse events. This study is presented in **chapter 2**. The design of this study makes it applicable to a great number of patients in clinical practice and has several features that mimic the real-life situation: first, patients are often pre-treated with systemic corticosteroids and/or antibiotics, this was not an exclusion in our study. Second the additive treatment including systemic corticosteroids was standardized. Finally patients needing assisted ventilation were not excluded from participation.

The next two chapters discuss how different biomarkers can be used in the diagnosis and management of AECOPD and the differentiation between subclinical CAP and AECOPD. In **chapter 3** we used data from the CATCH study to compare the influence of peripheral blood eosinophilia (cut-off ≥2% of white blood cell count) on length of hospital stay and clinical outcome of patient with AECOPD. In **chapter 4** we investigated the serum levels of C-reactive protein, Procalcitonin and Serum Amyloid A in patients with AECOPD without radiological evidence of CAP on their chest X-ray and whether these biomarkers may predict the presence of radiological abnormalities (i.e. infiltrates) on a low-dose CT-thorax (LDCT).

As mentioned earlier, symptoms may vary over time in COPD. Therefore a simple tool to quantify symptom changes over time would be an asset, both in research in AECOPD as well as in clinical practice. Although many questionnaires are available to measure the impact of symptoms on daily life and well-being, a short and easy to complete questionnaire that solely focusses on symptoms was lacking. We therefore developed the COPD Lower Respiratory Tract visual analogue score (c-LRTI-VAS) as described in **chapter 5.** We describe the validation of this questionnaire in both stable and exacerbated patients. Patients with AECOPD were derived from the study described in chapter 2, whereas patients with stable COPD were recruited during routine check-up visits in the outpatient department.

Airway inflammation underlies tissue remodelling and subsequent airflow limitation and may also cause low-grade systemic inflammation due to spill over of multiple pro-inflammatory markers into the circulation. Therefore limiting airway inflammation might be an important way to reduce disease progression in COPD. Although tetracyclines

are primarily considered antimicrobial agents, these compounds have important immunomodulatory effects through an effects on host Matrix Metalloproteases. In **chapter 6** we describe a placebo controlled randomized clinical trial in which we investigate the anti-inflammatory effect of the tetracycline compound doxycycline on induced sputum and serum inflammatory markers in patients with stable COPD without airway bacterial colonization. We recruited patients without bacterial airway colonization to evaluate the anti-inflammatory properties of doxycycline without addressing the antimicrobial effects doxycycline might have.

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CHAPTER 2

CRP-guided Antibiotic Treatment in acute exacerbations of COPD admitted to Hospital

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Abstract

Introduction: the role of antibiotics in acute exacerbations of COPD (AECOPD) is controversial, a biomarker identifying patients who benefit from antibiotics is mandatory. We performed a RCT in patients with AECOPD comparing CRP-guided antibiotic treatment to patient reported symptoms according to GOLD strategy in order to show a reduction of antibiotic prescription

Methods: patients hospitalized with AECOPD were randomized to receive antibiotics based according the GOLD strategy or according to the CRP (≥50 mg/L) strategy.

Results: 101 patients were randomized to the CRP-group and 119 to GOLD-group. Fewer patients in the CRP-group were treated with antibiotics 31.7% versus 46.2% in the GOLD-group (p=0.028) (adjusted OR, 0.178 95%CI 0.077-0.411, p= 0.029). Thirty-day treatment failure rate was equal (CRP-group 44.5% vs GOLD-group 45.5%; (p=0.881) (adjusted OR 1.146 95%CI 0.649-1.187 p=0.630) as was time to next exacerbation (CRP-group 32 days, versus GOLD-group 28 days (p=0.713) (adjusted HR0.878 (95%CI 0.649-1.187 p=0.398). Length of stay was similar in both groups (CRP-group 7 days versus GOLD-group 6 days (p=0.167). On day 30 no difference in symptoms score, quality of life or serious adverse events was detected.

Conclusion: CRP as a biomarker to guide antibiotic treatment in severe AECOPD leads to a significant reduction of antibiotic treatment. In the present study no differences between both groups in adverse events were found. Further research is needed for the generalizability of these finding

Introduction

Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) are associated with substantial morbidity and mortality.[1] On average a patient with COPD suffers from 1.5 exacerbations a year.[2] Viruses and bacterial infections are the most important triggers in AECOPD.[3] Coinfection of viruses and bacteria has been detected in 25% of exacerbations.[4] Molecular techniques including polymerase chain reaction can detect viral infections more accurately as trigger of AECOPD.[2] Yet, still in about a third of severe exacerbations a specific infectious agent cannot be identified.[5]

Treatment of AECOPD usually consists of corticosteroids and bronchodilators. The current GOLD strategy advises to add or withhold antibiotic treatment based upon patient reported sputum purulence.[6] This strategy assumes that both sputum purulence is a good marker of bacterial infection and that the patients' assessment of sputum colour is reliable. However, both assumptions are controversial. We have shown before that sputum colour reported by patients is not a reliable marker of bacterial presence or bacterial load in AECOPD.[7;8] As a consequence, the widespread implementation of the GOLD strategy, that dictates use of patient reported sputum purulence, may result in overuse of antibiotics in AECOPD. It is evident that unnecessary prescription of antibiotics for respiratory illness leads to higher medical costs, side effects and emerging resistance to antibiotics.[9] This is underlined by the fact that the frequency of antibiotic resistance in bacteria among different countries is proportional to their relative rate of antibiotic use.[9] Reduction of resistance (up to 30%) can be achieved by implementing recommendations that discourage antibiotic treatment.[10] Therefore, a better identification of patients with AECOPD who actually benefit from antibiotics is mandatory. A biomarker like serum C-Reactive Protein (CRP) may help in selecting these patients. CRP is an acute phase protein and a sensitive biomarker for systemic inflammation and tissue damage.[11] Although CRP is not disease specific, it can aid to clinical decision making to guide antimicrobial use. This was reported by a study in patients with lower respiratory tract infections.[12] CRP levels are significantly higher during AECOPD compared to baseline levels, especially if a bacterial origin is likely.[13] A previous study has shown that patients with an AECOPD admitted to hospital with a CRP (≥50 mg/L) showed a trend to benefit more from antibiotics than patients with low CRP values.[14] We therefore hypothesized that CRP-guided antibiotic therapy may lead to a reduction of antibiotic therapy within 24 hours after admission compared to patient reported sputum purulence strategy (as formulated in the GOLD strategy) in patients with AECOPD admitted to hospital without increasing the rate of treatment failures or adverse events within 30 days.

Material and Methods

Study design and oversight

The CRP-guided Antibiotic Treatment in COPD exacerbations admitted to the Hospital study (CATCH) was an investigator initiated multicentre randomized controlled open intervention clinical trial performed in two hospitals in the Netherlands from July 2011 - February 2015 (clinicaltrials.gov NCT01232140). Consecutive patients with AECOPD who needed hospitalization according to GOLD strategy were screened and enrolled at the emergency department or medical wards within 24-hours after presentation.[6] Inclusion and exclusion criteria can be found in the supplementary data. All patients provided written informed consent.

Randomization and Intervention

Eligible patients were randomly assigned to receive either biomarker-directed (CRP-group) antibiotic therapy or GOLD strategy directed (GOLD-group) antibiotic therapy.[6] Randomization was performed with block sizes of fifty. Treatment allocation was concealed with a pre-specified computer-generated randomisation list by an independent statistician. Patients were randomly assigned to one of two groups by sealed, opaque envelops. Envelopes were numbered with consecutive unique study numbers. After obtaining informed consent the physician in charge opened the envelope and acted according to the randomization result. Subjects assigned to CRP-group were treated days if CRP on admission was ≥50 mg/L. In patients with CRP <50 mg/L, no antibiotic was prescribed. Within 24 hours CRP levels were re-evaluated. If CRP level rose ≥50 mg/L patients were also treated with amoxicillin/clavulanic acid. Subjects in the GOLD-group were also treated with amoxicillin/clavulanic acid for 7 days if they reported increased sputum purulence in combination with increased dyspnoea and/or increased sputum volume, or if this was observed by the attending physician in the first 24 hours after admission in order to minimize protocol violations. If patients were not able to expectorate sputum and remained to do so for the first 24 hour after admission, they were considered to be non-purulent. Medical staff treating subjects allocated to GOLD guided treatment were blinded for the CRP results in the first 24 hours. If allergy to penicillin was reported another antibiotic was prescribed. In with amoxicillin/clavulanic acid 625 mg 3 times a day for 7 addition, all patients were treated with corticosteroids (oral prednisolone 60 mg for three days, followed by 30 mg for 7 days) and bronchodilators. Supplemental oxygen and physiotherapy were added at the discretion of the attending physician.

Procedures

After informed consent was obtained, baseline blood samples were drawn and baseline variables were collected. Patients were treated according to their randomization results and discharged from the hospital as deemed appropriate by the attending physician.

Patients were monitored during 1 year with scheduled visits at 1 and 6 months. Additional telephone interviews were performed at 3 and 12 months. When patients were unable to attend a scheduled visit, they were contacted by telephone. Patients were instructed to contact the investigator(s) responsible for the study immediately if there was any change in their health status. During each visit, patients were asked to report any respiratory event that required antibiotic therapy, systemic corticosteroids and/or hospitalization elsewhere, in order to capture all exacerbations and other adverse events. Serum CRP was measured by nephelometry on a Beckman Synchron DxC 800 analyser (CRP latex Reagent, Beckman Coulter Inc; Fullerton, CA) on the day of admission. Because serum CRP levels peak at about 36 h after onset of infection, this test was repeated on the second day of the admission. The highest value was used to determine whether or not a patient should be treated with antibiotics.

The Lower Respiratory Tract Infection Visual Analogue Score (LRTI-VAS) is a condition-specific questionnaire that has been used to assess the severity of symptoms in COPD.[14] The score has been shown to be a reliable tool for symptom measurement in bronchiectasis and is currently being validated for COPD.[15] A detailed description can be found in the supplementary data. Subjective improvement in quality of life was recorded using the Clinical COPD Questionnaire (CCQ).[16;17] A detailed description can be found in the supplementary data.

Endpoints

The primary endpoint was antibiotic treatment started during the first 24 hours after admission. Secondary endpoints were 30-day treatment failure rate, length of hospital stay, time to next exacerbation, difference in symptoms score, quality of life after 30 days and safety profile. Treatment failure was defined as absence of resolution of symptoms and signs, worsening of symptoms and signs, occurrence of new symptoms and signs associated with the primary or with a new infection, or death of any cause after randomization in the study.[18] Time to next exacerbation was defined as the interval between hospital discharge and the end of the study or time to additional course of antibiotics and/or corticosteroids for worsening of symptoms and signs. Subjective improvement in symptoms and quality of life was recorded using the Lower Respiratory Tract Infection Visual Analogue Scale (LRTI-VAS) and Clinical COPD Questionnaire (CCQ) at admission and after 1 month.

We also assessed 1-year treatment failure rate, the number of exacerbations after 1 year and 1 year mortality rate.

Safety

A planned safety analysis performed by the Data Safety Monitoring Board after enrolment of 100 patients showed no significant differences with regard to benefit or to adverse effects and therefore the study was continued.

Statistical analysis

The primary outcome measure was a reduction in antimicrobial prescriptions in the experimental arm compared to the control group. Based on previous data it was estimated that 20% reduction in antibiotic use (60% in the GOLD-group versus 40% in the CRP-group) would be clinically relevant.[14;19] In order to detect this difference with a power of 0.8 and α of 0.05, and a 15% drop-out a total of 220 patients was needed. Continuity correction was used as the primary outcome was based upon a percentage. The primary analysis was performed based on intention-to-treatment (ITT) principle. The secondary analysis of per-protocol treatment (PPT) included participants who met the criteria of the ITT population, received the allocated treatment, and had no other protocol violation. IBM SPSS Statistics for Windows, Version 22.0 (Armonk, NY: IBM Corp.) and R version 3.4.1 for Windows was used for data management and statistical analysis. Data are presented as median (IQR) unless stated otherwise. Continuous data were analysed with the Student's t test or Mann–Whitney U test, when appropriate. Categorical characteristics were compared by using the χ^2 test. Multivariable logistic regression was used to calculate odds ratio (OR) and adjust for confounders. Multivariable Cox regression was used to calculate Hazard ratio (HR). Kaplan-Meier's log-rank test was used to compare differences in 30-day treatment failure. In case of skewed distributions, continuous variables were logarithmically transformed for further analyses. For the construction of the confidence intervals we used the bootstrap method based on 1000 bootstraps. Overall statistical significance was set at a 2-tailed P value <0.05.

Results

A total of 1650 patients with COPD were screened for inclusion; 220 (13.3%) were eligible and were randomized (figure 1). One hundred and one patients were assigned to the CRP-group and 119 to the GOLD-group; the data of these patients were used for ITT analysis. For the per-protocol treatment (PPT) 12 patients were excluded (figure 1).

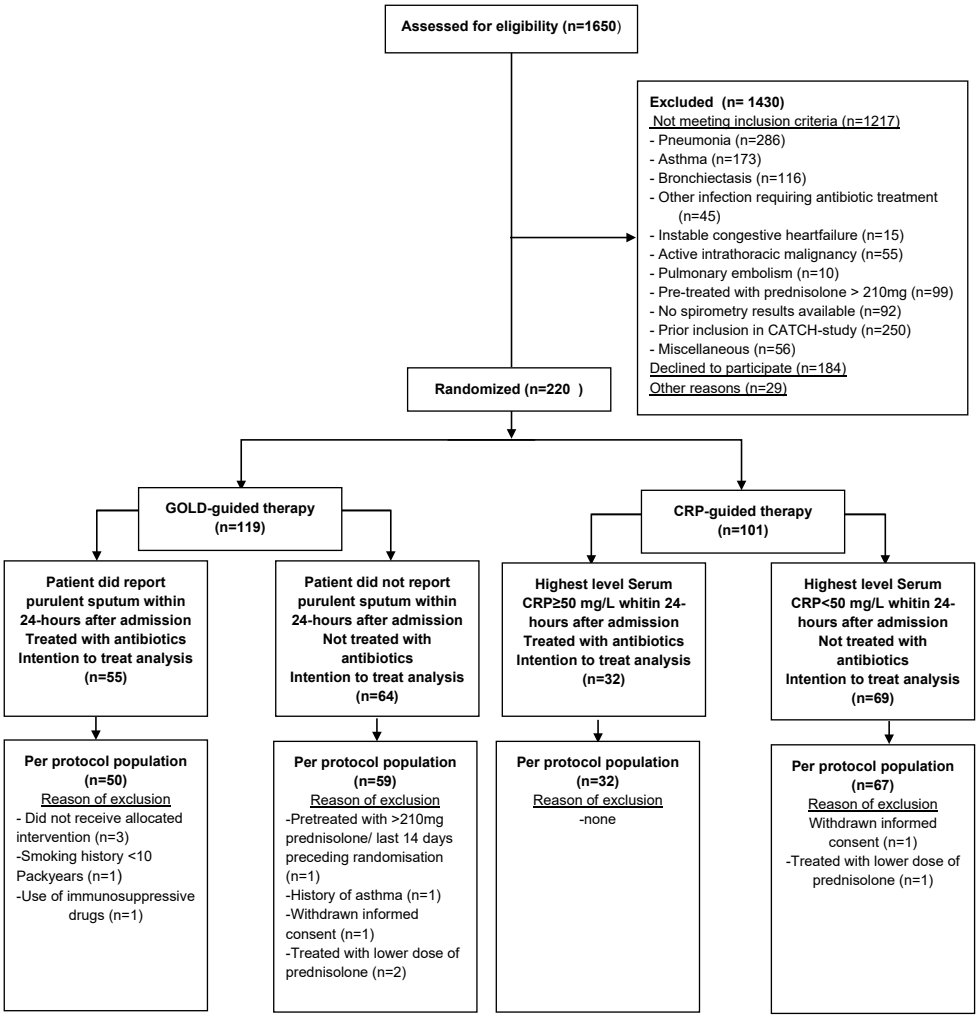


Figure 1: CONSORT flow diagram describing the screening and randomisation of participants. GOLD: Global Initiative for Chronic Obstructive Lung Disease; CRP: C-reactive protein.

The baseline characteristics of both groups in the ITT analysis are summarized in table 1 and were well balanced except for gender and sputum purulence. Also, the patient characteristics of the PPT groups were comparable (table1 supplementary data).

Table 1 Baseline characteristics

	GOLD-group (n=119)	CRP-group (n=101)
Age (SD) years	70.8 (11.8)	68.4 (12.0)
Gender male No. (%)[a]	67(56.3)	41(40.6)
Current Smoking No. (%)	35(29.4)	38 (37.6)
Pack years (SD) years	45.1(34.2)	40.5 (23.0)
BMI (SD) kg/m²	25.1(5.7)	25.0 (5.3)
FEV1 (SD) L[b]	1.21(0.54)	1.14 (0.44)
FEV1 (SD) % predicted	46(17)	45 (16)
FVC (SD) L[b]	2.9(1.04)	2.6 (0.9)
FVC (SD) % predicted[b]	85 (22)	84 (21)
FEV1/FVC ratio (IQR) %[b]	40(31-49)	37(31-52)
Number of exacerbations in the last year No. (IQR)	1 (1-2)	2 (1-2)
Type of exacerbation[c]		
Type 1 No. (%)	48 (40.3)	50 (49.5)
Type 2a purulence present No. (%)	7 (5.9)	12 (11.9)
Type 2b purulence not present No. (%)	25 (21.0)	14 (13.9)
Type 3 No. (%)	39 (32.8)	25 (24.8)
Sputum purulence present No. (%)[a]	55 (46.2)	62 (61.4)
Positive sputum culture at admission No. (%)	43 (36.1)	38 (37.6)
Co-morbidities		
Ischaemic heart disease No. (%)	19 (16.0)	15 (14.9)
Heart failure No. (%)	18 (15.1)	16 (15.8)
Cerebrovascular disease No. (%)	12 (10.1)	10 (9.9)
Diabetes mellitus No. (%)	11 (9.2)	10 (9.9)
Pre-treatment[d]		
Inhaled corticosteroids No. (%)	100 (84.0)	80 (79.2)
Pre-treatment with systemic corticosteroids No. (%)	58 (48.7)	52 (51.5)
Pre-treatment with antibiotics No. (%)	38 (31.9)	41 (40.6)
Short-acting beta adrenoceptor agonist No. (%)	68(57.1)	57(56.4)
Short-acting muscarinic antagonist No. (%)	29(24.4)	23(24.2)
Long-acting beta adrenoceptor agonist No. (%)	93(78.2)	71(70.3)
Long-acting muscarinic antagonist No. (%)	68(57.1)	61(60.4)
Vital parameters at admission		
Respiratory rate (IQR) per minute	20(16-24)	20 (18-24)
Temperature (IQR) °C	37.1(36.6-37.7)	37.1 (36.7-37.5)
Laboratory results at admission		
WBC (SD) 109/L	11.0(3.9)	10.7 (4.3)
Blood eosinophil count (IQR) 109/L	0.0 (0.0-0.2)	0.0 (0.0-0.1)
CRP (IQR) mg/L[e]	27(6.7-98)	19(5.6-75)
CRP≥50mg/L No. (%)[e]	49(41.2)	32(31.7)
Assisted ventilation		
None No. (%)	110(92.4)	93(92.1)
Non-invasive ventilation No. (%)	8(6.7)	7(6.7)
Invasive ventilation No. (%)	1(0.8)	1(1.08)

All data are represented as mean (SD) unless specified otherwise.
Definition of abbreviations: BMI: body mass index (kg/m2), FEV1: forced expiratory volume 1 second, FVC: Forced Vital Capacity, WBC: white blood cell count, CRP: C-reactive protein SD: standard deviation, IQR: inter quartile range.
[a]: p-value <0.05, [b]: Last recorded post bronchodilator value in a stable state before admission, [c]: according to Anthonissen [19], [d]: in the two weeks prior to randomisation [e]: Highest level recorded in the first 24-hours

Primary endpoint

In the ITT population antibiotics were prescribed in 32 patients (31.7%) of the CRP-group and 55 patients (46.2%) of the GOLD-group (p=0.028) (table 2). By using the CRP strategy, an absolute antibiotic reduction of 14.5 % was achieved which was a 31.4% reduction compared to the GOLD guided antibiotic strategy. This remained significant after correction for statistically significant confounders including sputum purulence, gender and FVC (L) (adjusted OR, 0.178 95%CI 0.077-0.411, p= 0.029). In the per protocol treatment a comparable result was found (table 2 supplementary data). Forty patients who were initially not treated with antibiotics were prescribed antibiotics due to treatment failure during admission. Twenty-one patients (30.4%) in the CRP group compared to 19 patients (29.7%) in the GOLD group (p=0.925).

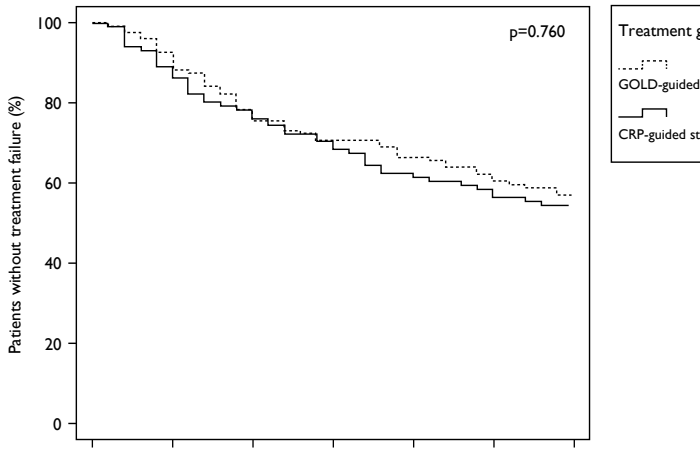
Table 2 Primary and secondary endpoints

	GOLD-group (n=119)	CRP-group (n=101)	Difference	95% bootstrap CI	p-value
Primary endpoint					
Patients treated with antibiotics No. (%)	55 (46.2)	32 (31.7)	-14.5	-1.9;-26.9	0.028
Secondary endpoints					
30-day treatment failure rate No. (%)	53 (44.5)	46 (45.5)	1.0	-14.7;11.7	0.881
Time to next exacerbation days (IQR)	28 (3-209)	32 (0-327)	4	-57.9;19.1	0.713
Length of stay days (IQR)	6 (4-8)	7 (4-9)	1.0	-0.1;2.7	0.167
CCQ score change on day 30 (IQR)	-1.00 (-1.95;-0.20)	-0.90 (-1.40;-0.1)	-0.1	-0.54; 0.16	0.336
LRTI-VAS score change on day 30 (IQR)	-8.5 (-14.0;-3.0)	-7.5 (-15.0;-2.0)	1.0	-2.3;2.9	0.723

All data are represented as median (IQR) unless specified otherwise. Differences are represented as percentages or differences in medians.
Definition of abbreviations: CCQ: Clinical COPD Questionnaire, LRTI-VAS: Lower Respiratory Tract Infection Visual Analogue Scale

Treatment failure rate

Treatment failure on day 30 was similar between the groups; 46 patients (45.5%) in the CRP-group versus 53 patients (44.5%) in the GOLD-group (p=0.881) (figure 2).



No. at risk							
GOLD-guided strategy group	119	105	90	84	79	72	66
CRP-guided strategy group	101	87	77	69	62	57	55
No of exacerbations							
GOLD-guided strategy group	0	14	29	35	40	47	53
CRP-guided strategy group	0	14	24	32	39	44	46

Figure 2: Kaplan-Meier Curve for treatment failure over 30 days. GOLD: Global Initiative for Chronic Obstructive Lung Disease; CRP: C-reactive protein.

Similar results were found after adjusting for confounders (OR 1.146 95%CI 0.649-1.187 p=0.630). Day 10 treatment failure rates showed no difference between the two groups; CRP-group 24 patients (23.8%) had treatment failure versus 29 patients (24.4%) in the GOLD-group (p=0.916). During 1 year of follow up 78 patients (77.2%) had treatment failure compared to 102 patients (85.7%) in the GOLD-group (p=0.104 e-figure 1).

Time to next exacerbation

Time to next exacerbation in the CRP-group was 32 days (IQR 0-327) versus 28 days (IQR 3-209) in the GOLD group (p=0.713) (figure 3A). No difference was found in HR after adjusting for confounders. The adjusted HR of time to next exacerbation in the CRP guided group was calculated to be 0.878 (95%CI 0.649-1.187 p=0.398) compared to the GOLD-group. The number of exacerbations in the year after randomization was similar in both groups; CRP-group 1 exacerbation (IQR 0-3) versus 2 exacerbations (IQR 1-4) in the GOLD-group (p=0.109) (figure 3B).

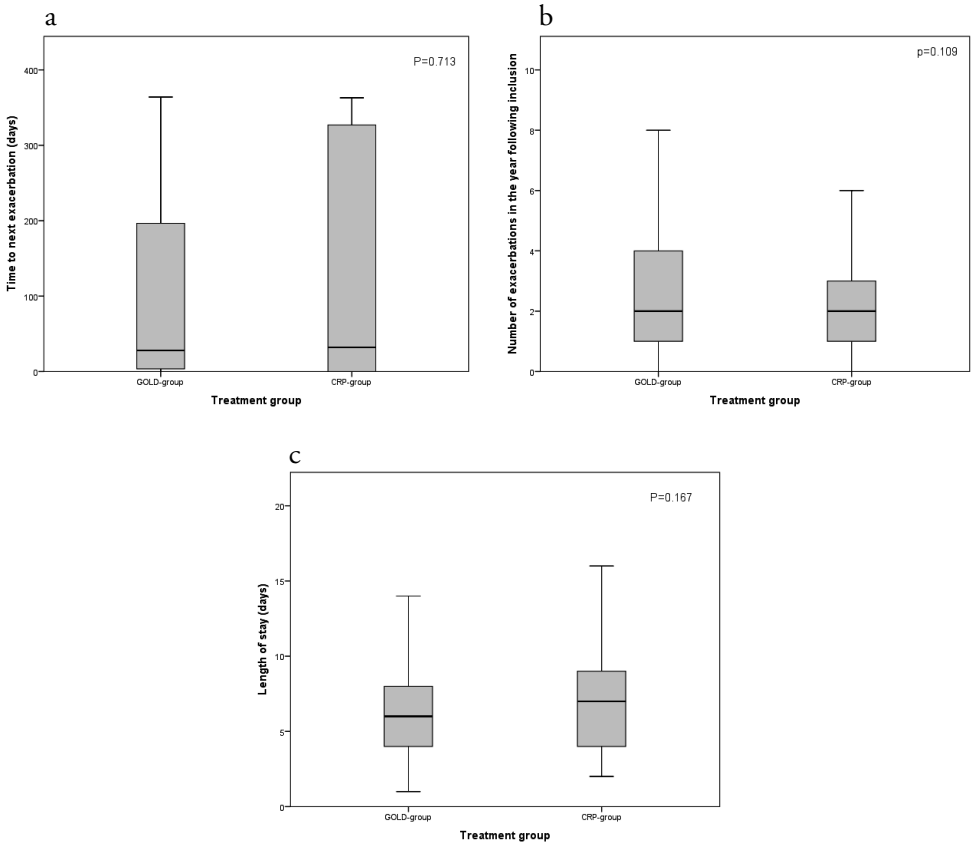


Figure 3: Comparison of treatment groups for a) time to next exacerbation, b) number of exacerbations in the year following inclusion and c) hospital length of stay. GOLD: Global Initiative for Chronic obstructive Lung Disease; CRP: C-reactive protein.

Length of hospital stay

Median length of hospital stay (LOS) in the CRP-group was 7 days (IQR 4-9) versus 6 days (IQR 4-8) in the GOLD-group (p=0.167) (figure 3C). The variable length of hospital stay was logarithmically transformed to accomplish a normally distributed variable. Linear regression analysis showed mean difference between the treatment groups of 0.14. The resultant ratio of geometric means after back transformation was 1.15 (95%CI: 0.98-1.34). After correction for gender, purulence and FVC in multivariable analyses this ratio changed to 1.12 (95%CI: 0.95-1.31)

Symptoms and Quality of Life scores

Baseline CCQ was equal in both groups, median 3.80(IQR 3.10-4.20) in the CRP-group compared to 3.55(IQR 3.00-4.05) in the GOLD-group. LRTI-VAS score was median 23 (IQR 21-28) in the CRP-group compared to 24 (IQR 18-27) in the GOLD-group (p=0.186).

The median change in total CCQ score on day 30 was -1.00(IQR -1.95--0.20) in the GOLD-group versus -0.90(IQR -1.40--0.10) in the CRP-group (p=0.289). The median change in LRTI-VAS was -8.5(IQR -14.0--3.0) in the GOLD-group versus -7.5(IQR -15.0--2.0) in the CRP-group (p=0.723). Additional scores CCQ and LRTI-VAS are noted in table 3 and 4 in the supplementary data.

Safety

In one year of follow-up serious adverse events occurred in 48 patients (40.3%) in the GOLD-group (64 events) compared to 42 patients (41.6%) in the CRP-group (53 events) one year after inclusion (p=0.851). During the same period 41 patients had 49 adverse events, events were evenly distributed among both groups. Adverse reactions related to the study medication occurred in 5 patients (4.2%) in the GOLD-group compared to 1 patient (2%) in the CRP-group (p=0.145).
All-cause mortality after one year was equal in both groups. Twenty patients (16.8%) died in the GOLD-group compared to 9 patients (8.9%) in the CRP-group (p=0.082). Thirty-day mortality in the GOLD-group was 5 patients (4.2%) compared to 1 patient (1.0%) in the CRP-group (p=0.145). Five patients died of AECOPD and one patient died of inoperable colon carcinoma.

Discussion

CRP-guided antibiotic therapy for patients hospitalized with AECOPD was associated with a 14.5% decrease of antibiotic use at admission compared with GOLD-guided antibiotic therapy. The CRP-guided strategy was not associated with an increase of adverse events or 30-day treatment failure rates. Finally, similar outcomes between groups were observed with regards to exacerbation recovery (difference in QoL and respiratory symptoms) and time to next exacerbation.

The current GOLD strategy advocates the use of antibiotics during exacerbations in patients with increased sputum purulence.[6] Reported or witnessed sputum purulence as a criterion to guide antimicrobial prescription, has several shortcomings. Regardless of sputum discoloration or purulence, studies have shown that antibiotics can improve short term outcomes.[20] A recent guideline by the ERS/ATS conditionally recommends the use of antibiotics in patients with AECOPD.[21] Yet this guideline only provides a treatment advice for ambulant patients and did not take into account a recent randomized controlled clinical trial not showing effect of antibiotics in ambulant patients.[22] The improvements ascribed to antibiotics are therefore marginal and antibiotics may be associated with increased morbidity.[20] Adverse effects of antibiotic treatment are gastrointestinal complications as diarrhea, allergic reactions and an increase in bacterial

resistance.[23;24] The results of the current study are in line with the findings of a previous study from our group which showed a trend towards more benefit in patients with a CRP ≥50 mg/L.[14] Similar results were found in another study using a cutoff point of 40 mg/L in which patients with a moderate AECOPD who were treated with antibiotics or placebo.[25]

Another biomarker used in AECOPD is Procalcitonin (PCT). A recent meta-analysis showed that PCT guided antibiotic treatment is associated with a 35.5% reduction of antibiotic use without an increase in LOS or adverse events.[26] This larger reduction may be explained by the observation that 80.1% of the patients in the control groups of the included studies were treated with antibiotics. In the current study only 46.2% of the patients in the control group were treated with antibiotics and only 31.7% of the patients in the intervention group were treated with antibiotics which is lower compared to PCT-guided strategy. There are some advantages of using CRP as a biomarker. First, serum CRP may better reflect bacterial infection in the lower airways. We have shown earlier that CRP is related to the presence of potential bacterial pathogens in sputum, whereas PCT is not.[27] Secondly, CRP is cheap and available in hospitals all over the world, whereas PCT is more costly and mainly used in research settings. As a consequence, implementation of CRP-guided antibiotic treatment might very well be cost-effective as it requires no changes in laboratory infrastructure.

Our data suggest that CRP guided antibiotic therapy is able to reduce antimicrobial pressure, while maintaining the patient safety. Although the effect size of reduced antibiotic consumption was less than the 20% we anticipated, a significant reduction of 14.5% was nonetheless found in the CRP-guided treatment strategy compared to the GOLD-guided treatment strategy. Perhaps the effect size was less than expected, because the proportion of participants with sputum purulence in the GOLD-group (46.2%) was less than in the CRP-group (61.4%; p=0.025). This was further emphasized by two other studies showing a sputum purulence of 53% and 59% respectively.[25;28]Thirty-day treatment failure rates were comparable in both groups and comparable to earlier research.[14] Time to next exacerbation was equal in both groups. In our study time to next exacerbation was considerably shorter compared to other studies. This reduction may be explained by a different definition of time to next exacerbation as well as the fact that both these studies were performed in GP practices.[22;25] In the present study LOS was the same in both groups, but shorter than was found in another study. This can be explained by a different study design as patients in our study were treated with oral corticosteroids instead of standardized intravenous corticosteroids for 6 days.[14] Of course these secondary endpoints should be interpreted with caution as the study was not powered on these outcomes. However, in the present study no important differences between the two groups were detected.

The strengths of our study are the real-life design of the study including patients with all GOLD classes, patients who were pre-treated with antibiotics and/or systemic corticosteroids as well as treatment naïve patients and finally patients needing assisted ventilation were not excluded from participation. The second strength of this study is the fact that all patients were treated uniformly with corticosteroids and bronchodilators. The third strength of the study was the fact that medical staff treating subjects allocated to GOLD guided treatment were blinded for the CRP results in the first 24 hours, thereby reducing the risk of starting antibiotics based upon CRP levels instead of patient reported sputum colour. One potential limitation to this study is the fact that patients, hospital staff and investigator were not blinded for the results of randomization allowing for a risk of performance bias. A second possible limitation is the low bacterial resistance in the country in which the study was performed. This might limit the generalisability. A third limitation is the imbalance at baseline regarding sputum purulence and gender, this might have influenced our results although after correction for confounders results remained statistically significant. The fourth limitation is that the results regarding patients requiring assisted ventilation must be interpreted with caution, as the groups were too small to draw a firm conclusion.

The primary objective of the present study was to find a reliable method to reduce overtreatment with antibiotics in AECOPD. Clearly antimicrobial pressure promotes antibiotic resistance.[9] Antibiotic resistance in bacteria among different countries is proportional to their relative rate of antibiotic use.[9] Reduction of resistance up to 30% can be achieved by implementing specific recommendations that discourage antibiotic treatment.[10] In a recent study the appropriate use of antibiotics in the management of hospitalized patients with AECOPD in 13 European countries was studied.[29] Overall in 86% of admissions antibiotics were prescribed but only 61.4% cases met the GOLD criteria justifying antibiotic prescription. This misuse of antibiotics also depends on the assumption that we fully rely on the reported "purulence" of sputum by the patient which is probably a highly unreliable parameter.[8] Beliefs, expectations and incentives are important drivers of antibiotic overuse among physicians. A fundamental change in behaviour among physicians is urgently needed to curb the daunting emergence and spread of antimicrobial resistance. For this purpose, biomarkers may be helpful to guide antibiotic treatment in AECOPD.

In conclusion, the present study shows that using serum CRP (cut-off value 50mg/L) to direct antibiotic treatment can lead to a significant reduction of antibiotic use in patients with severe AECOPD. Implementation of this strategy could contribute to the battle against emerging bacterial resistance. However, a prerequisite for implementation of this strategy is safety. Although we observed no negative effects of the CRP-guided strategy on treatment failure, length of stay and adverse events, this study was underpowered for the assessment of these endpoints. Future studies are required to resolve this issue.

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Chapter 2 supplementary data

Inclusion and Exclusion criteria.

The inclusion and exclusion criteria that were used for the CRP-guided Antibiotic Treatment in COPD exacerbations admitted to the Hospital study (CATCH) study were:

Inclusion criteria

- Age 40 years and older. No upper age limit will be employed.
- Written informed consent obtained.
- AECOPD according to the GOLD guideline. An exacerbation of COPD is defined as an event in the natural course of the disease characterized by a change in the patient's baseline dyspnoea, cough, and/or sputum that is beyond normal day-to-day variations, is acute in onset, and may warrant a change in regular medication in a patient with underlying COPD.
- Criteria for hospital admission according to the GOLD.
  - marked increase in symptoms (i.e. resting dyspnoea)
  - severe underlying COPD
  - onset of new physical signs (cyanosis, oedema)
  - failure to respond to initial medical management
  - significant co morbidities
  - frequent exacerbations
  - newly occurring arrhythmias
  - diagnostic uncertainty
- Former of current smoker with a minimum smoking history of 10 pack years.
- Patients have to be capable of ingesting oral medication.
- Patients have to be mentally capable of participating in the study (able to complete questionnaires and perform lung function tests).
- Life expectancy ≥ 30 days.

Exclusion criteria

- Pregnant or lactating women, or women of childbearing age not using an acceptable method of contraception.
- Pre-treatment with corticosteroids (cumulative dose >210 mg) for the present exacerbation.
- Strong clinical suspicion of pneumonia
- Progression or new radiographic abnormalities on the chest X-ray.
- Cystic fibrosis.
- Tuberculosis.
- Immunodeficiency disorders such as AIDS, humoral immune defect, ciliary dysfunction etc., and the use of immunosuppressive drugs (>30 mg prednisolone/day maintenance dose or equivalent for more than 4 weeks).

- Recent or unresolved lung malignancy.
- Other disease likely to require antibiotic therapy, such as recurrent sinusitis or urinary tract infection.
- Significant gastrointestinal or other conditions that may affect study drug absorption.
- Instable congestive heart failure or recent stroke.
- Newly diagnosed pulmonary embolism

Questionnaire description

LRTI-VAS description:

The Lower Respiratory Tract Infection Visual Analogue Scale(LRTI-VAS) consists of a set of 4 horizontal lines with 2 anchor points, one at each extreme, each line representing a different symptom - dyspnoea, fatigue, cough and sputum colour. Each symptom is scored from 1 to 10, the patients being unaware of the numbers. Higher scores indicate more severe symptoms. Separate scores were calculated for each symptom, with a total score consisting of all symptom scores added.

CCQ description:

The Clinical COPD Questionnaire is a health-related quality of life questionnaire that has been widely used in both COPD and asthma research and includes 10 items across three domains: symptoms, activity and impact. Higher scores indicate more severe symptoms. It has been validated extensively and can accurately monitor the course of recovery of outpatients as well as that of inpatients with AECOPD

Supplementary data table 1 Baseline characteristics per protocol population

	GOLD (n=109)	CRP (n=99)
age (SD) years	71.1(11.8)	68.4(12.1)
Gender male No (%)[a]	65(59.6)	41(41.1)
Current Smoking No (%)	33(30.3)	36(36.4)
Packyears (SD) years	46.9(34.4)	40.5(23.2)
BMI (SD) kg/m²	24.6(5.4)	24.9(5.2)
FEV1 (SD) L [b]	1.23(0.56)	1.14(0.44)
FEV1 (SD) % pred [b]	46(18)	45(16)
FVC (SD) L [ab]	2.91(1.05)	2.64(0.86)
FVC (SD) % pred[b]	85(22)	83(21)
FEV1/FVC ratio (IQR) % [b]	39(31-49)	38(31-52)
Number of exacerbations in the last year(IQR) No	1(1-2)	1(1-2)
Anthonissen type exacerbation.		
Type 1 No (%)	44(40.4)	48(48.5)
Type 2a purulence present No (%)	6(5.5)	12(12,1)
Type 2b purulence not present No (%)	23(21.1)	14(14.1)
Type 3 No (%)	36(33.0)	25(25.3)
Sputum purulence present No (%) [a]	50(45.9)	60(60.6)
Positive sputum culture at admittance No (%)	40(36.7)	37(37.4)
Co-morbidities		
Ischaemic heart disease No (%)	17(15.6)	15(15.2)
Heart failure No (%)	17(15.6)	16(16.2)
Cerebrovasculair disease No (%)	10(9.2)	10(10.1)
Diabetes mellitus No (%)	8(7.3)	10(10.1)
Pre-treatment		
Inhaled corticosteroids usage No (%)	90(82.6)	78(78.8)
Pretreatment with systemic corticosteroids No (%)	54(49.5)	51(51.5)
Pretreatment with antibiotics No (%)	30(27.5)	40(40.4)
Vital parameters		
Respiratory rate (IQR) per minute	20(16-24)	20(18-24)
Temperature (IQR) °C	37.0(36.6-37.6)	37.1(36.7-37.5)
Laboratory results day 1		
WBC (SD) 10x9/L	11.0(4.03)	10.7(4.3)
Blood eosinophil count (IQR) 10x9/L	0.0(0.0-0.2)	0.0(0.0-0.01)
CRP (IQR) mg/L[C]	27(6.7-104)	19(5.6-83)
CRP≥50mg/L No (%)[c]	47(43.1)	32(32.3)
Assisted ventilation		
none No (%)	100(91.7)	91(91.9)
Non-invasisve ventilation No (%)	8(7.3)	7(7.1)
Invasive ventilation No (%)	1(0.9)	1(1.0)

All data are represented as mean (SD) unless specified otherwise.
Definition of abbreviations: BMI: body mass index (kg/m2),
FEV1: forced expiratory volume 1 second, FVC: Forced Vital Capacity,
WBC: white blood cell count, CRP: C-reactive protein SD: standard deviation, IQR: inter quartile range
[a]: <0.05, [b]: Last recorded postbronchodilator value in a stable state before admission, [c]: Highest level recorded in the first 24 hours

Supplementary data table 2 Primary & secondary outcomes per protocol population

Primary outcome	GOLD (n=109)	CRP (n=99)	Difference	95% CI bootstrap interval	p-value
Patients treated with antibiotics No (%)	50(45.9)	32(32.2)	-13.7	-1.4;-26.9	0.046
Secondary outcome					
30-day treatment failure rate No (%)	46(42.2)	45(45.5)	3.3	-10.8;16.2	0.637
Time to next exacerbation (IQR) days	28(5-210)	34(0-328)	6	-54.6;22.6	0.916
Length of stay(IQR) days	6(4-8)	7(4-9)	1	-0.1;2.7	0.157
CCQ score change on day 30	-1.0(-2.0;-0.2)	-0.9(-1.40;-0.1)	0.1	-0.53;0.16	0.655
LRTI-VAS score change on day 30, (IQR)	-9(-14;-5)	-7(-15;-2)	2	-3.3;2.2	0.540

All data are represented as mean (SD) unless specified otherwise. Differences are represented as percentages or differences in medians.
Definition of abbreviations: CCQ: Clinical COPD Questionnaire, LRTI-VAS: Lower Respiratory Tract Infection Visual Analogue Scale

Supplementary data table 3 Symptom score LRTI-VAS and CCQ at admission

	GOLD (n=100)	CRP (n=90)
LRTI-VAS score t=0(IQR)	24(18-27)	23(21-28)
Dyspnea t=0(IQR)	7(6-9)	8(7-9)
Fatigue t=0(IQR)	8(5-9)	8(6-9)
Cough t=0(IQR)	5(4-7)	6(5-8)
Sputum purulence t=0(IQR)	3(1-5)	3(1-5)
Clinical COPD Questionnair total t=0(IQR)	3.55(3.00-4.05)	3.80(3.10-4.20)
CCQ symptoms t=0(IQR)	4.00(3.00-4.50)	3.75(3.25-4.5)
CCQ mental t=0(IQR)[a]	2.00(1.00-3.00)	2.50(1.50-3.50)
CCQ functional t=0(IQR)	4.00(3.25-4.75)	4.25(3.00-4.75)

Definition of abbreviations: CCQ: Clinical COPD Questionnaire, LRTI-VAS: Lower Respiratory Tract Infection Visual Analogue Scale [a]: p=<0.05

Supplementary data table 4 Symptom score change on day 30 LRTI-VAS and CCQ

	GOLD (n=80)	CRP (n=78)	p-value
LRTI-VAS score change on day 30(IQR)	-8.5(-14.0--3.0)	-7.5(-15.0--2.0)	0.831
dyspnoea change on day 30(IQR)	-2.0(-5.0-0.0)	-2.0(-5.0-0.0)	0.856
fatigue change on day 30(IQR)	-2.0(-4.0-0.0)	-1.0(-3.5-0.0)	0.104
cough change on day 30(IQR)	-3.0(-4.5-0.0)	-2.0(-5.0-0.0)	0.611
sputum purulence change on day 30(IQR)	-1.0(-3.0-0.0)	-1.0(-3.0-0.0)	0.696
Clinical COPD Questionnaire total change on day 30(IQR)	-1.00(-1.95--0.20)	-0.90(-1.40;-0.10)	0.289
CCQ symptoms change on day 30(IQR)	-1.25(-2.13-0.00)	-0.75(-1.75;-0.25)	0.289
CCQ mental change on day 30(IQR)	-0.50(-1.50-0.00)	-0.50(-1.50;-0.00)	0.728
CCQ functional change on day 30(IQR)	-1.00(-2.13--0.13)	-0.75(-1.25;-0.00)	0.192

CHAPTER 2.1

2.1

Safety of CRP-guided antimicrobial treatment in hospitalized AECOPD

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To the editor,

We thank Dr Miravitlles and colleagues for their interest in our work.[1] They express concern about our failure rate - 24% at 10 days, and 45% at day 30; they feel that 31% and 46% of patients treated with antimicrobials is low in this high-risk population of hospitalized patients. Although treatment failure is high in our study, it reflects the severity of our population. Indeed the proportion of patients on antimicrobials is lower than the out-patient study population in a recently published trial from the UK however our COPD population is more severe and consists of hospitalised patients.[2] Their concern is safety - have we caused harm in our patients by withholding antimicrobial treatment? First, in our study population, there was no significant difference in failure rates at days 10 and 30 between the CRP and GOLD group - which strongly argues against their point that antimicrobial treatment might have prevented harmful events; see table 1.

Table 1 Antimicrobial prescription and outcome stratified according to GOLD and CRP guidance

	Antimicrobial treatment (n=87)			No antimicrobials (n=133)		
	GOLD group (n=55)	CRP group (n=32)	p-value	GOLD group (n=64)	CRP group (n=69)	p-value
10-day treatment failure rate, No. (%)	6 (10.9)	4 (12.5)	0.822	23 (35.9)	20 (29.0)	0.392
30-day treatment failure rate, No. (%)	10 (31.3)	17 (30.9)	0.974	36 (52.2)	36 (56.3)	0.637
Time to next exacerbation, days (IQR)	34 (22;72)	55 (15;121)	0.761	19 (7;68)	17(6;53)	0.792
Length of stay, days (IQR)	6 (5-8)	6 (5-9)	0.933	7 (4-11)	6 (4-9)	0.077

Neither failure during admission, nor relapse was significantly different between both study arms. Indeed, relapses among patients with AECOPD admitted to hospital are common especially among individuals with a low FEV-1, but antimicrobial treatment especially among those that had low inflammatory markers may not necessarily prevent this.[3] Slow recovery and early relapse have also been associated with increased inflammation, e.g. reflected by persistently increased CRP, and in patients characterised by chronic bronchitis, but whether these individuals might benefit from antimicrobial treatment if their CRP is below a given threshold, has not been addressed in clinical studies.[4,5] An earlier study suggested that patients with CRP >50 mg/L benefit more from antibiotic treatment compared to patients with CRP below this threshold.[6] Second, they argue that perhaps the active study arm treated with co-amoxiclav as the primary antimicrobial agent might have been inadequate. Although antimicrobial susceptibility data have not been listed in our paper, *Pseudomonas* spp or other high-risk pathogens were covered if retrieved from sputum, and patients known with colonisation with high-

risk pathogens were provided with tailored antimicrobial regimens.
Third, we agree with Dr Miravitlles and colleagues that our study was not powered to demonstrate safety beyond all reasonable doubt for patients in whom antimicrobial treatment was withheld based on the CRP decision rule alone.[7] Besides the initial reduction of antibiotics of more than 30% (from 46.2% to 31.7%) associated with the CRP algorithm, around 30% of the patients were additionally treated with antibiotics due to treatment failure (equally distributed between the two groups). Importantly, this did not result in an increase of adverse events or length of hospital stay.
Our study provides preliminary data suggesting safety, and therefore argues in favour of a larger international multicentre trial to address this question more definitively for patients with exacerbated COPD that are hospitalized.
Antimicrobial treatment may cause serious harm – first of all, for individuals themselves. [8] Differences across geographic regions suggest that out-patient antimicrobial prescription is at least in part culturally, not scientifically triggered [9]. Indeed Spain, Cyprus and Mongolia do worse than some other locales, e.g. the Netherlands. If we fail to reduce our antimicrobial footprint, sooner or later we will lose the war on antimicrobial resistance.[10]

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CHAPTER 3

3

Blood eosinophilia as a marker of early and late treatment failure in severe acute exacerbations of COPD

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Abstract.

Introduction: Blood eosinophilia is frequently encountered in patients with AECOPD. However the impact of blood eosinophilia at admission in patients with AECOPD on outcome on the short and long term has not been extensively studied which was the objective of the present study.

Methods: We used data of 207 exacerbations from a randomized clinical trial on antibiotic prescription based upon CRP-levels versus GOLD guided strategy and analysed the impact of blood eosinophils (≥2% of total white cell count and absolute eosinophil count ≥300 cell/microliter) at admission on clinical outcome.

Results: 207 patients were included of whom 39 (18·8%) had eosinophilia ≥2% and 23 patients (11.1%) had a peripheral blood eosinophil counts ≥300cell/microliter. Eosinophilia was associated with shorter median length of stay in the eosinophilic groups (≥2% and ≥300 cell/microliter) compared to the non-eosinophilic groups. Early treatment failure (within 10 days) was reduced in both the eosinophilic groups (≥2% and ≥300 cell/microliter). Late treatment failure (day 11-30) was equal in the eosinophilic groups as well as in the non-eosinophilic groups. Relapse (day 31-180), was more frequent in both eosinophilic groups (≥2% and ≥300 cell/microliter), however in the latter group this did not reach statistical significance. Eosinophilia ≥2% was a risk factor for having relapse (eosinophilia ≥2%: HR= 2·351; 95%CI 1·335-4·139), whereas eosinophilia ≥2% was associated with a lower risk factor for having early treatment failure (HR=0·339 95%CI 0·122-0·943).

Conclusion: Blood eosinophilia at hospital admission in patients with an AECOPD is associated with higher short-term treatment success. However, blood eosinophilia ≥ 2% predicts a less favourable outcome on the long term.

Introduction

Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) are associated with significant morbidity and mortality.[1] Current guidelines advocate the use of systemic corticosteroids in all exacerbations of COPD to shorten recovery time, improve arterial hypoxemia, improve lung function, decrease length of stay and reduce treatment failure.[1-4] Yet, these benefits are limited and systemic steroids have no effect on mortality, while they are associated with significant side effects.[4] Exacerbations are heterogeneous with respect to aetiology and so is airway inflammation, which accompanies exacerbations.[5] Most exacerbations are associated with neutrophilic airway inflammation, but a significant proportion of exacerbations shows eosinophilic airway inflammation.[5] It has been demonstrated that patients with stable COPD and eosinophilic airway inflammation respond well to systemic glucocorticoid therapy.[6] A recent trial showed that peripheral blood eosinophil count exceeding ≥2% of total white blood cell count (WBC) can be used to direct systemic corticosteroid treatment during an AECOPD.[7] In a subgroup analysis of another study it was also shown that patients with blood eosinophilia benefit most from treatment with corticosteroids compared to non-eosinophilic patients.[8] As blood eosinophil count seems a valid biomarker of eosinophilic airway inflammation, this raises the question whether blood eosinophilia can also be used to predict outcome in patients who are hospitalized with severe AECOPD.[5] We hypothesized that blood eosinophilia ≥2% of WBC as well as ≥300 eosinophils cell/microliter is associated with an improved response to systemic corticosteroids in patients with severe AECOPD resulting in a shortened length of stay (LOS), compared to otherwise well-matched patients with AECOPD without blood eosinophilia. In addition, we investigated whether blood eosinophilia is related to the occurrence of early and late treatment failure as well as relapse after 30 days. Some of the results of these studies have been previously reported in the form of an abstract.[9]

Methods

Two hundred and nine participants were enrolled, 183 at the Northwest Clinics in Alkmaar, and 26 in the Medisch Spectrum Twente, Enschede, the Netherlands between July 2011 and September 2014, as part of the CRP-guided Antibiotic Treatment for acute exacerbations of COPD admitted to Hospital (CATCH) study. The methods and design of this trial have been described in detail and can be found at clinicaltrials.gov (NCT01232140). The local ethics boards approved the study protocol, and all patients provided written informed consent. Patients in this study were randomised to receive antibiotics or not, based on either C-Reactive Protein (CRP) levels or on GOLD criteria.[1] In the CRP group a cut-off level of ≥ 50 mg/L was used. In the GOLD group, patients

with increased sputum purulence were prescribed antibiotics.[1] The main outcome in this study was the reduction of antibiotic consumption. Apart from antibiotics patients with an AECOPD were treated with oral corticosteroids (OCS) for 10 days (first 3 days 60 mg and last 7 days 30 mg of prednisolone). The study population consisted of patients diagnosed with COPD stages I–IV as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD), with an acute exacerbation as defined by GOLD[1]. Inclusion criteria were: age above 40 years; AECOPD requiring hospital admission according to GOLD guidelines, and former or current smokers with a minimum smoking history of 10 pack years.[1] Exclusion criteria were: pre-treatment with oral corticosteroids exceeding a total dose of 210 mg prednisolone during the last 14 days preceding the presentation with AECOPD for hospitalization, this was done to exclude patients with a chronic exacerbation of COPD. Other exclusion criteria consisted of pneumonia visualized on a chest X-ray, Immunocompromised patients, patients with active lung cancer and patients with pulmonary embolism were excluded.

Blood eosinophilia

Blood was collected at admittance, in K2EDTA tubes (Vacutainer, Becton Dickinson, and Plymouth, UK). Peripheral blood smear were measured using a Sysmex XE-2100 Hematology Analyzer (Sysmex Corporation, Kobe, Japan) for cell differentiation.
Patients were grouped according to blood eosinophil count: ≥2% or <2%. For the purpose of a subgroup analysis a new group was created within the group of patients with blood eosinophilia ≥2% consisting of patients with eosinophil count ≥4%. Based upon earlier studies we also performed an analysis of absolute eosinophil count ≥300 cell/microliter.[10-12]

Definition of Clinical Outcome

Treatment failure was defined as absence of resolution of symptoms and signs, worsening of symptoms and signs, occurrence of new symptoms and signs associated with the primary or a new infection, or death after randomization in the study.[13] Early treatment failure was defined as treatment failure within 10 days, late treatment failure was defined as treatment failure between day 11 and 30. Relapse was defined as a new exacerbation requiring antibiotics or systemic corticosteroids between day 31 and day 180.

Statistical analysis

SPSS, version 22.0 for Windows (IBM Corporation, Armonk NY) was used for data management and statistical analysis. Data are presented as median ± IQR unless stated otherwise. Differences between continuous variables were tested with students T-test or Mann-Whitney U test when appropriate; categorical variables were tested with the Pearson χ^2 test. Cox proportional hazard models were used to assess the association between eosinophil count group and treatment failure rates. Kaplan Meyer curves were

used to display the association between eosinophilia and treatment failure. All tests were 2-sided with a p-value for significance of <0.05.

Results

Patients characteristics

We included 209 patients in the study (figure 1). All patients were hospitalized with an AECOPD. The mean follow-up was 174 days (SD 30 days). Two of these patients were excluded because no blood was tested for eosinophils at admittance. Fourteen (6·7%) patients died during follow-up of 180 days. Thirty-nine (18·8%) patients had peripheral blood eosinophil counts ≥ 2%. Of this group 16 patients (7.7%) had a peripheral blood eosinophil counts ≥ 2% of WBC but without an absolute eosinophilic blood count ≥300 eosinophils/microliter. Twenty-three patients (11.1%) had a peripheral blood eosinophil counts ≥ 2% as well as an absolute eosinophilic blood count ≥300 eosinophils/microliter.

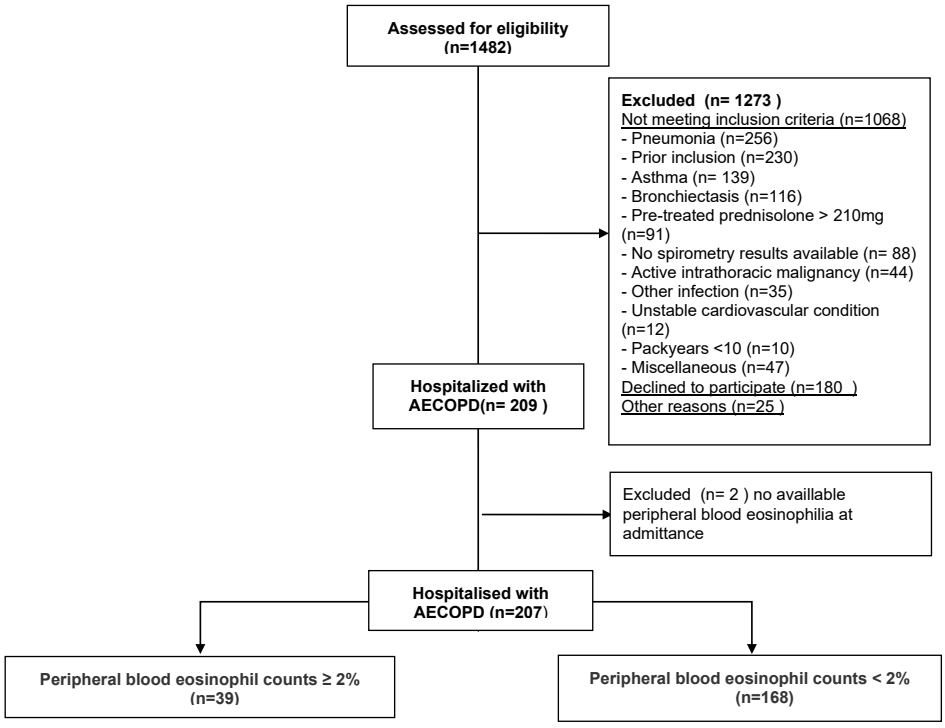

Figure 1: Trial profile

In the eosinophilic group>2%, the median eosinophil count was 3·1% (IQR 2·6-4·9%), whereas in the non-eosinophilic group (<2% eosinophils) this was 0·1 % (IQR 0·0-0·5%). Absolute median eosinophil count in the eosinophilic group was 0·37x10^9/L (IQR 0·21-0·53x10^9/L) and in the non-eosinophilic group 0·01x10^9/L (IQR 0·00-0·05 x10^9/L). Baseline characteristics are outlined in table 1.

Table 1. Baseline characteristics

	Blood eosinophils		
	≥ 2% (n=39)	<2 % (n=168)	p-value
Age, years (mean, sd)	70.4 (8.7)	69.7 (11.5)	0.721
Gender, % male	59.0	46.4	0.158
Absolute eosinophil count (x10^9/L, IQR)	0.37(0.21-0.53)	0.01(0.00-0.05)	0.000
Peripheral blood Eosinophilia (%, IQR)	3.1% (2.6-4.9)	0.1(0.0-0.5)	0.000
BMI *, kg/m^2 (mean, SD)	25,3 (5.0)	24,9 (5.3)	0.693
Pack years, (median, IQR)	40 (29-60)	40 (25-50)	0.255
Current smoker, n (%)	9 (23.1)	60 (35.9)	0.126
Number of exacerbations in the past two years, n (median, IQR)	3 (1-6)	3 (1-4)	0.502
Pre-treatment oral corticosteroids, n (%)	16 (41.0)	87 (51.8)	0.226
Cumulative oral corticosteroids dose last 14 days, mg (median, IQR)	105(70-165)	90(60-180)	0.966
Inhaled corticosteroids, n (%)	28 (71.8)	144 (85.7)	0.037
Cumulative inhaled corticosteroid dose ug (median, IQR)	500(400-1000)	675(400-1000)	0.520
Antibiotics at admission, n (%)	11 (28.2)	65 (38.7)	0.140
FEV1 †, liters (mean, SD)	1.31 (0.48)	1.15 (0.50)	0.069
FEV1 % pred, (mean, SD)	50.6 (16.0)	44.6 (16.6)	0.064
FVC ‡, liters (mean, SD)	3.0 (1.0)	2.7 (1.0)	0.143
FVC % pred, (mean, SD)	89.0 (23.9)	83.4 (21.0)	0.152
FEV1/FVC %, (mean, SD)	42.5 (13.6)	39.9 (12.4)	0.248

* BMI: body mass index (kg/m^2), † FEV1: forced expiratory volume 1 second, ‡ FVC: Forced Vital Capacity, SD: standard deviation, IQR: inter quartile range.

Absolute number of eosinophils as well as percentage eosinophils of WBC did not differ between patients with and without pre-treatment with systemic corticosteroids: in the pre-treated group the median absolute eosinophil count was 0·01 x10^9/L (IQR 0·00-0·15 x10^9/L) and in the non-pre-treated group median 0·04 x10^9/L (IQR 0·00-0·16 x10^9/L; p=0·157). Similarly, the percentages eosinophils were 0·1% (IQR 0·0-1·4%) and 0·04% (IQR 0·0-1·7%; p=0·09), respectively. Baseline characteristics did not differ in the absolute eosinophil count ≥300 cell/microliter group compared to <300 cell/microliter group (data not shown).

Length of stay and mortality

The median length of stay was 5 (IQR 4-6) days in the eosinophilic group as compared to 7 (IQR 5-10) days (p=0.001) in the non-eosinophilic group. In-hospital mortality was numerically higher in the non-eosinophilic group (5 (3%) patients died) as compared to the eosinophilic group (none died (0%)) (p=0.275). During the follow-up of 180 days mortality rate was similar in both groups: in the non-eosinophilic group 12 (7.1%) patients died, in the eosinophilic group 2 (5.1%, p=0.652). Results regarding the patients with an eosinophilic blood count ≥300 and <300 eosinophils/microliter can be found in table 2.

Table 2: Results absolute eosinophil count

	Eosinophils ≥ 300/ microliter (n=23)	**Eosinophils < 300 / microliter (n=184)**	p value
Length of stay	4(4-6)	7(5-10)	**0.012**
Early treatment failure n,(%)	1(4.3)	49(26.6)	**0.019**
Late treatment failure n,(%)	7(31.8)	35(25.9)	0.563
Relapse n,(%)	10(66.7)	46(46)	0.135
Number of exacerbations	1(0-2)	0(0-1)	0.174
Mortality n,(%)	1(4.3)	13(7.1)	0.652
In hospital mortality n,(%)	0(0)	5(2.7)	0.424

All data are represented as median (IQR) unless specified otherwise

Treatment failure

Treatment failure rates for eosinophilia ≥ 2% and <2% as well as total blood eosinophilia ≥ 300 and <300 /microliter are depicted in figure 2 and were markedly different over time.

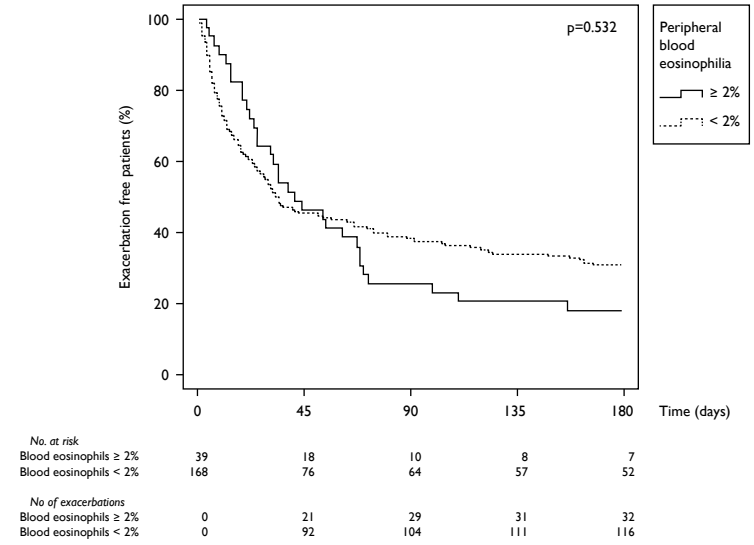

Figure 2a: Kaplan Meyer Curve day 0-180 eosinophilia≥ 2% and <2%

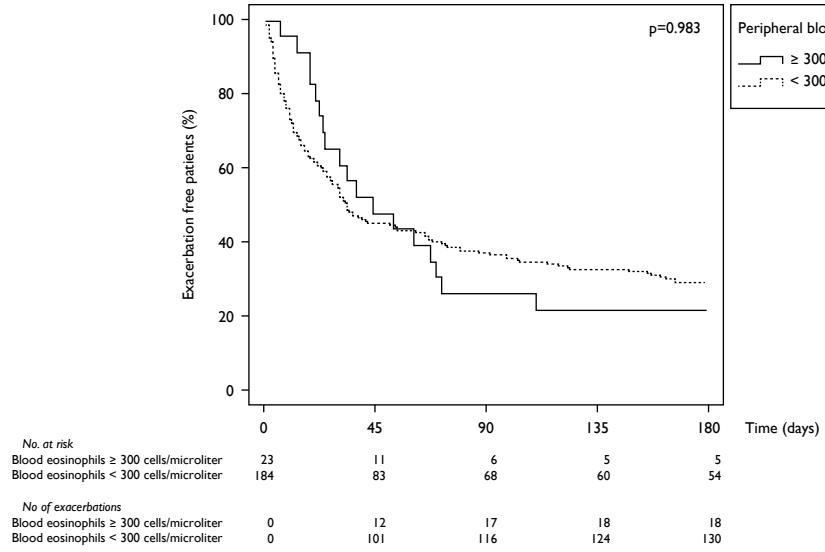

Figure 2b: Kaplan Meyer Curve day 0–180 eosinophilia≥ 300 and <300 eosinophils/microliter

Early treatment failure rates were higher in the non-eosinophilic group 46 (27·4%) compared to 4 (10·3%) patients in the eosinophilic group (p=0·024; supplementary data figure 1). Late treatment failure rates were equal: 10 (28·6%) patients had treatment failure in the eosinophilic group and 32 (26·2%) patients in the non-eosinophilic group (p=0·783;supplementary data figure 2). Relapse rates were higher in the eosinophilic group with 18 (72·0%) patients compared to 38 (42·2%) patients in the non-eosinophilic group (p=0·008; supplementary data figure 3). The median total number of treatment failure events measured after 180 days post-inclusion was also higher in the eosinophilic group with 2 (IQR 1-2) events compared to 1 (IQR 0-2) event in the non-eosinophilic group (p=0·042). Results regarding treatment failure and relapse in the patients with an eosinophilic blood count ≥300 and <300 eosinophils/microliter can be found in table 2 Cox proportional hazard analysis revealed that eosinophilia (≥2%) at admittance was associated with a lower risk factor for having treatment failure in the first 10 days (hazard ratio HR= 0·339 95%CI 0·122-0·943). Eosinophilia at admittance was not a risk factor for developing late treatment failure (hazard ratio for eosinophilia ≥2% =1·094 95%CI 0·538-2.225; p= 0·804). Blood eosinophils ≥2% at admittance was a risk factor for relapse (hazard ratio for eosinophilia ≥2%= 2·351; 95%CI 1·335-4·139).

Increased eosinophilia

In the group of patients with eosinophilia ≥2%, 26 (66·7%) patients had eosinophil percentages between 2-4% and 13 (33·3%) patients had ≥4%. Early treatment failure rate was not different between ≥4% eosinophil group (1 patient 7·7%) compared to the eosinophilia 2-4% group (3 patients 11·5%) (p=0·709). However, late treatment failure

was higher in the ≥ 4% eosinophil group, which was observed in 6 patients (50%) as compared to 4 patients (17·4%), p=0·043) in the 2-4% eosinophil group. Relapse rates were lower in the ≥4% group compared to the 2-4% group, respectively 2 (33·3%) patients and 16 (84·2%) patients (p=0·016). Overall treatment failure was not different in patients with ≥4% eosinophils, 9 (69·2%) patients compared to patients with an eosinophilia 2-4%, 23 (88·5%) patients (p=0·140).

Discussion

This study demonstrates two important new findings related to blood eosinophilia, which was present in 19% of patients with an AECOPD at presentation to the hospital regardless whether they were pre-treated with systemic corticosteroids or antibiotics: better short-term treatment response, but more exacerbations in the 31–180 days thereafter although the latter was not observed in the eosinophilic group ≥300 eosinophils/microliter. Eosinophilia was also associated with a shorter length of hospital stay, as was also observed in another study.[14]

The decrease in early treatment failure in the eosinophilic group might be explained by the observation that oral corticosteroids had a more prominent effect in patients with eosinophilic inflammation.[7] Similarly, discontinuation of oral corticosteroids may lead to a surge in circulating eosinophils and accumulation of eosinophils in the bronchial mucosa and underlies a new exacerbation and thus an increased relapse rate.[15,16] This is further supported by the finding that patients with a relatively high number of circulating eosinophils (≥4%) were more likely to experience late treatment failure. In another study no increased relapse rates were observed in eosinophilic patients, which may have been due to exclusion of patients with more than 4 hospitalizations for any reason.[14] This might have led to an under-representation of patients with high eosinophilia and frequent exacerbations. The increased number of relapse exacerbations in the eosinophilic group are in line with an earlier study and might partly be explained by the fact that in our study in the eosinophilic group less patients were treated with ICS.[17] An earlier study showed that ICS might lower the exacerbation frequency in patients with eosinophilia.[18] The observation that an increase in relapse was not seen in the absolute eosinophil count ≥300 cell/microliter group might be explained by the small sample size.

Nineteen percent of our patients had blood eosinophilia at baseline despite the use of systemic steroids in the last two weeks in 41%. This signifies that systemic steroids do not fully abrogate blood eosinophilia.

It is still unclear how eosinophils contribute to the pathogenesis of AECOPD. Eosinophils can release granular contents that contain cytotoxic and inflammatory mediators. Activated eosinophils are also an important source of reactive oxygen species,

which together with the granular contents induce local tissue damage and direct immune response.[19] It is currently unknown what causes the recruitment and possibly activation of eosinophils during AECOPD, but there are several possible candidate mediators that are associated with exacerbations. IL-33 is an alarmin, which is released during virus-induced exacerbations, and has been shown to recruit and activate eosinophils.[20] Likely, IgA directed against microorganisms will increase and may lead to enhanced secretory IgA and secretory component. Both IgA and secretory component are potent activators of eosinophils.[21-23] Eosinophils might also contribute to the pathogenesis of COPD by a defective efferocytosis of apoptotic eosinophils, leading to an increased number of sputum eosinophils. Subsequently, with failure of the apoptotic pathway, these eosinophils become necrotic and release toxic intracellular pro-inflammatory mediators leading to more influx of eosinophils. An increase of defective efferocytosis has been related to severity and frequency of COPD exacerbations.[24] Which of these processes or whether other mechanisms are involved awaits further studies.

Eosinophils are derived from the bone marrow under the influence of granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-3 and IL-5, with IL-5 as most specific to the eosinophil lineage.[25, 26] IL-5 is also known for its pivotal role in homing, activation and prevention of apoptosis of eosinophils.[26-28] Therefore, IL-5 cytokine and IL-5-receptor blocking agents are of interest to reduce eosinophilic inflammation. Recently, Benraluzimab, an anti-interleukin-5 receptor monoclonal antibody has been investigated in patients with stable COPD and sputum eosinophilia.[10] Benralizumab was able to reduce the number of eosinophils in blood and sputum effectively, but failed to reduce the exacerbation rate. In a post-hoc subgroup analysis, patients with peripheral blood eosinophil count exceeding 200 cells per μL had fewer exacerbations, improved health status, symptoms and lung function.[10] This, however, does not exclude a potential role for these biologics after treatment for an exacerbation, preventing relapses upon withholding oral corticosteroids.

The strength of the present study is that this is the first study in which all the patients received a standardized corticosteroid treatment, which provides a unique insight into the role of eosinophils in AECOPD. Moreover, the fact that only 2 patients were excluded from the present study due to missing data, makes the risk of a selection bias small. Another strong point of this study is the fact that all patients had a known history of COPD confirmed by spirometry and a smoking history of at least 10 pack-years. Patients with a prior history of asthma or other respiratory disease were excluded leaving a homogeneous population of patients with COPD. Yet, we cannot entirely rule out the possibility that some patients who were included in this study did have a form of atopy or asthma in their childhood.

The present study also has several limitations. First, the study was not primarily designed to investigate the effect of peripheral blood eosinophilia as a biomarker for outcome of AECOPD. The results should therefore be interpreted with caution and this study

should be regarded as exploratory. Secondly, the percentage of patients treated with inhaled corticosteroids before admittance in the non-eosinophilic group was slightly but significantly higher, which may have influenced the number of blood eosinophils at baseline in the non-eosinophilic group.[29] Another potential pitfall is the high number of patients pre-treated with systemic corticosteroids. Systemic corticosteroids can lower the number of circulating eosinophils leading to a lower percentage of eosinophils and can increase the number of circulating neutrophils.[7, 30] Therefore, due to the pre-treatment with corticosteroids a shift of patients from the eosinophilic group into the non-eosinophilic group might have occurred leading to an underestimation of the observed effects. This is further emphasized by the fact that on day 30 there was a significant increase in the absolute numbers of eosinophils as well as an increase of the percentage eosinophils of WBC compared to day 0 in non-eosinophilic patients (data not shown).

Conclusions

In this study we have observed that blood eosinophilia at admittance is associated with a shorter length of stay and lower 10-days failure rate. However, compared to the group with eosinophils <2%, the group with eosinophilia had higher long-term relapse rates after stopping systemic corticosteroids although this did not reach significance in the >300 eosinophils/microliter. We suggest that peripheral blood eosinophilia indicates a distinct phenotype in COPD and it would be of interest to investigate in a randomized study whether initiation and continuation of corticosteroid therapy in patients hospitalized with AECOPD can based upon peripheral blood eosinophilia.

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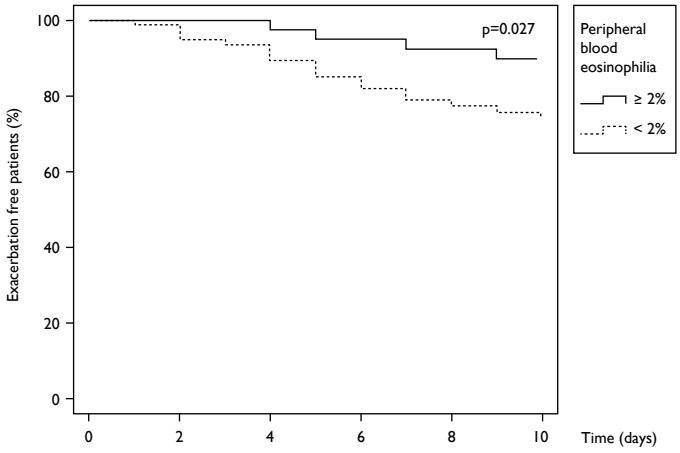
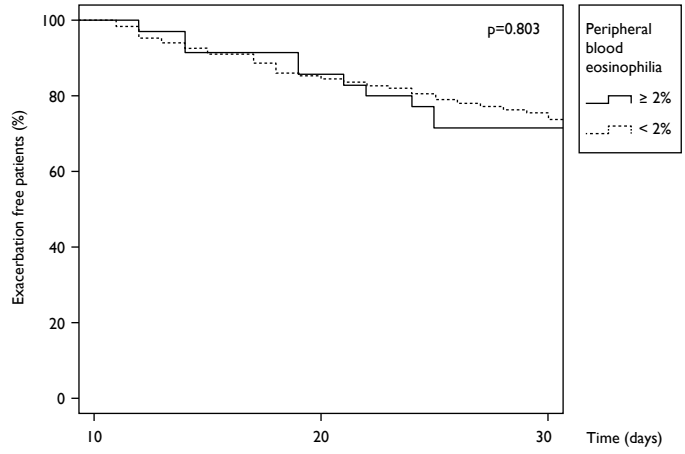
Chapter 3 supplementary data



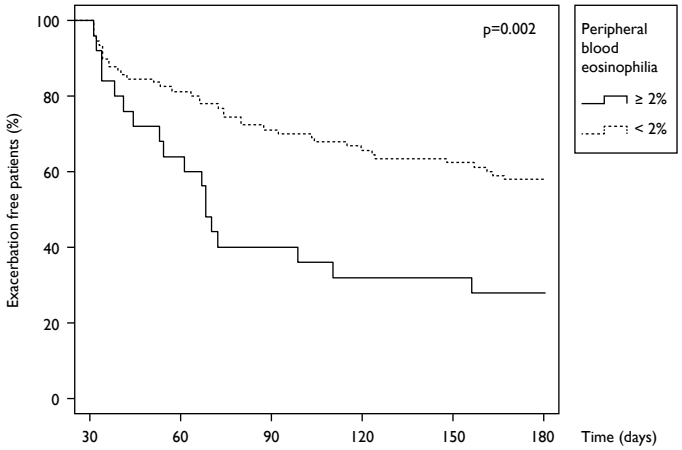
No. at risk						
Blood eosinophils ≥ 2%	39	39	38	37	36	35
Blood eosinophils < 2%	168	158	150	138	138	122
No of exacerbations						
Blood eosinophils ≥ 2%	0	0	1	2	3	4
Blood eosinophils < 2%	0	10	18	30	30	46

Figure 1: supplemental data day 0-10. Kaplan Meyer Curve day 0-10 eosinophilia≥ 2% and <2%.



No. at risk			
Blood eosinophils ≥ 2%	35	30	25
Blood eosinophils < 2%	122	103	90
No of exacerbations			
Blood eosinophils ≥ 2%	0	5	10
Blood eosinophils < 2%	0	19	32

Figure 2: supplemantary data day 10-30. Kaplan Meyer Curve day 10-30 eosinophilia≥ 2% and <2%.



No. at risk						
Blood eosinophils ≥ 2%	25	16	10	8	8	7
Blood eosinophils < 2%	90	73	64	59	56	38
No of exacerbations						
Blood eosinophils ≥ 2%	0	9	15	17	17	18
Blood eosinophils < 2%	0	17	26	31	34	52

Figure 3: supplementary data day 30-180. Kaplan Meyer Curve day 30-180 eosinophilia≥ 2% and <2%

CHAPTER 4

4

Role of low dose computed tomography scans and biomarkers in patients hospitalized with acute exacerbations of COPD

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Submitted

Abstract

Introduction: Acute exacerbations of COPD (AECOPD) and community-acquired pneumonia (CAP) often coexist. Although Chest X-rays may differentiate between both diagnoses, chest X-rays are known to underestimate the incidence of CAP in AECOPD. Therefore, the true incidence of CAP in patients with AECOPD is not known. In this exploratory study, we prospectively investigated the occurrence of infiltrative changes using low-dose CT-scan and correlate these with biomarkers.

Methods: Patients with AECOPD requiring hospitalisation in whom pneumonia was excluded using chest X-ray underwent additional LDCT-thorax and C-reactive protein (CRP), Procalcitonin (PCT), and Serum Amyloid A (SAA) on admission were assessed.

Results: Of the 100 patients that were included, 24 patients had one or more radiographic abnormalities suggestive for pneumonia. The inter-observer agreement between two readers (Cohen's Kappa) was 0.562 (95%CI 0.371-0.752 p<0.001). Biomarkers were significantly higher in the group with CT abnormalities compared to group without: CRP was 20.5 (IQR 8.8-81.5) mg/L compared to 76 (IQR 21.5-148.0) mg/L (p=0.018), PCT was 0.06 (IQR 0.04-0.08) µg/L compared to 0.09 (IQR 0.06-0.15) µg/L (p=0.007), SAA was 16 (IQR 3-89) µg/ml compared to 95 (7-160) µg/ml (p=0.019). Sensitivity and specificity for all three biomarkers where poor for detecting pneumonia by LDCT in this population. The area under the ROC curve was 0.659 (95% CI: 0.521-0.796) for CRP, 0.664 (95%CI: 0.526-0.801) for PCT, and 0.687 (95%CI: 0.566-0.808) for SAA.

Conclusion: in a quarter of patients with AECOPD without infiltrate(s) on the chest X-ray, additional infiltrative changes compatible with acute-phase lung involvement were detected by LDCT. Although the three investigated biomarkers were significantly higher in the group with abnormalities present on LDCT, they were not able to reliably detect or exclude CAP in this specific population.

Introduction

Acute exacerbations of chronic obstructive pulmonary disease (AECOPD) are associated with short term and long-term reductions in quality of life and lung function, as well as increased risk of death.[1-4] On average a patient with COPD suffers from 1.5 exacerbations a year.[5] Moreover, AECOPD requiring hospital admission represents a significant prognostic factor for reduced survival across all stages of COPD severity.[6]

Patients with COPD have an increased risk of community-acquired pneumonia (CAP); indeed, CAP is the most frequent infectious complication in COPD.[7] Moreover, AECOPD and CAP have a large overlap in clinical symptoms making it hard to diagnose CAP in COPD based on clinical signs and symptoms alone.[8] Establishing a correct diagnosis is important for the guidance of antibiotic and other therapies. Misdiagnosing pneumonia could have great impact for patients, whereas over diagnosing pneumonia leads to unnecessary prescription of antibiotics, and with increasing antimicrobial pressure, antimicrobial resistance may ensue.[9;10] Hence, clinicians have sought for new biomarkers that together with clinical assessments can improve the diagnostic accuracy of CAP in patients with COPD presenting with an acute exacerbation. Potential biomarkers that are used in AECOPD to detect bacterial inflammation are C-reactive protein (CRP), Procalcitonin (PCT) and Serum Amyloid A (SAA).[11;12] These biomarkers may help in the detection of clinically relevant bacterial infections at an early stage of the disease. Yet it is not known whether these biomarkers can differentiate between AECOPD and CAP. Therefore current practice for detecting pneumonia in patients with AECOPD is the chest X-ray. One study has shown that 20% of patients admitted to hospital with AECOPD had abnormalities on the chest X-ray consistent with pneumonia.[13] Although this is probably an underestimation of the true incidence of pneumonia in patients with AECOPD, as chest X-rays have limited sensitivity and specificity. Moreover the interpretation of chest X-ray is often difficult with pre-existing cardiopulmonary disease.[14;15] Chest CT scanning outclasses chest radiography and is now considered the gold standard for diagnosing CAP.[16;17] However, standard CT delivers higher radiation doses than do conventional diagnostic radiography.[18] An alternative for conventional chest CT scan is the low-dose CT-scan (LDCT), with acceptable image quality and more acceptable radiation exposure. LDCT-scans have been shown to detect CAP if the chest radiograph does not reveal findings that explain the patient's clinical presentation.[19]

In this exploratory study we prospectively aimed to investigate whether biomarkers correlate with radiological abnormalities compatible with acute-phase lung involvement in patients with AECOPD admitted to hospital using LDCT in whom CAP was excluded using chest X-ray. We also investigated the inter-observer variation in LDCT of the infiltrative changes,

Material and methods

Hundred patients were included between November 2011 and March 2014 as part of the CRP guided Antibiotic treatment of acute exacerbations of COPD admitted to Hospital study (CATCH study) (Clinical trial.gov NCT01232140).[20] The local ethics boards approved the study protocol, and all patients provided written informed consent. The study population consisted of patients diagnosed with COPD stages I–IV as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD).[21] Inclusion criteria dictate that patients had acute exacerbation as defined by GOLD requiring hospital admission according to GOLD guidelines, age above 40 years; all study participants were former or current smokers with a minimum smoking history of 10 pack years.[21] Exclusion criteria were: pre-treatment with oral corticosteroids exceeding a total dose of 210 mg prednisolone during the last 14 days preceding the presentation with AECOPD for hospitalization, chest X-ray findings compatible with CAP, immuno-compromised patients, patients with active lung cancer and patients with pulmonary embolism.

Procedures

Before informed consent was obtained a chest X-ray was performed to rule out CAP. Immediate after informed consent was obtained, baseline blood samples were drawn and baseline variables were collected. LDCT was performed within 12 h after informed consent was obtained using a standardized protocol. LDCT were performed on a CT PhilipMX 8000 (16 slice) with the following settings: 90 kV en 25 mAs. CTDI 0.8 mGy and CT Somatom Definition Flash (128 slice Dual source CT) with the following settings: 120 kV 20 mAs, CTDI 1.35 mGy.

Image analysis

The images were independently read by two radiologists (EB and FJR). If there was a dispute between both radiologists considering the presence of an infiltrate, a third radiologist was consulted as an adjunctor (PdJ). The readers had no knowledge of clinical or laboratory data, other than the age and sex of the patient. One or a combination of abnormalities (segmental, peribronchovascular or scattered ground-glass, reticular opacity or consolidation) was used for the diagnosis of CAP as assessed by LDCT scanning.[22]

Biomarker measurements

Blood samples for CRP, PCT and SAA were collected into Vacutainer® plain tubes (Becton Dickinson, Plymouth, UK). Blood samples were centrifuged at 2000g for 10 minutes in order to separate the serum form the cellular fraction. Serum was analysed immediately (CRP, PCT) or stored at -70°C until analysis (serum amyloid). Serum CRP

was measured by nephelometry on a Beckman Synchron DxC 800 analyser (CRP latex Reagent, Beckman Coulter Inc; Fullerton, CA); PCT was measured with Time-Resolved Amplified Cryptate Emission (TRACE) technology on a Kryptor Compact analyser (Thermofisher – B.R.A.H.M.S. GmbH Heningsdorf, Germany) using PCT sensitive KRYPTOR reagent (Thermofisher – B.R.A.H.M.S. GmbH Hennigsdorf, Germany). Serum amyloid A (SAA) was measured with an in house sandwich ELISA. The ELISA test was calibrated against WHO standard 92/680; reference values were established as < 4.2 mg/L.[23] The test was performed at the Laboratory of Medicine of the University Medical Centre Groningen (UMCG, The Netherlands).

Statistical analysis

In this pilot study our primary objective was to establish whether levels of CRP, PCT and SSA correlate with infiltrative changes on LDCT. As we were unaware of how many patients would have infiltrative changes on their LDCT and what this would do to the biomarker levels we were not able to make a sample size calculation. Initially, 100 patients underwent a LDCT scan. Statistical analysis was performed with the SPSS Package Program (SPSS version 22.0). Data was given as median (25^{th} 75^{th} quartile). Chi-square test and Mann-Whitney U test were used to compare groups. The inter-observer agreement was measured using Cohen's Kappa: κ<0.20 indicates poor agreement, κ of 0.21–0.40 fair, κ of 0.41–0.60 moderate, κ of 0.61 0.80 good, and κ of 0.81–1.00 indicates very good agreement between two observers. Receiver operating characteristics analysis was used to analyse the diagnostic accuracy of biomarkers. Overall statistical significance was set at a 2- tailed p value <0.05.

Results

A total of 592 patients presented at your emergency department with AECOPD were screened for inclusion. One hundred patients (16.8%) were eligible and gave informed consent (figure 1).

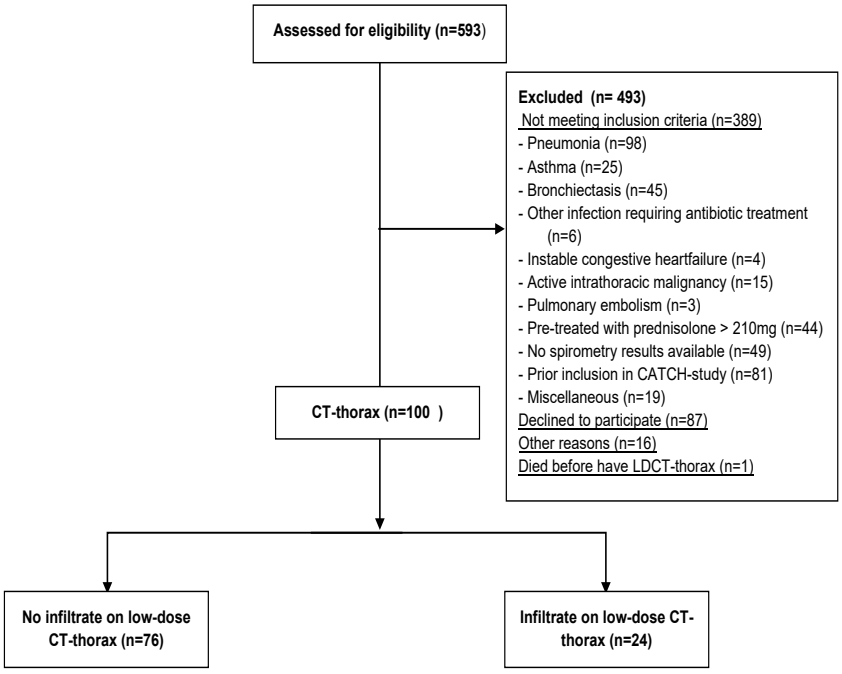

Figure 1: Trial profile.

Twenty-four patients (24%) had radiological abnormalities compatible with acute-phase lung involvement detected by LDCT without abnormalities visible on chest X-ray (figure 2).

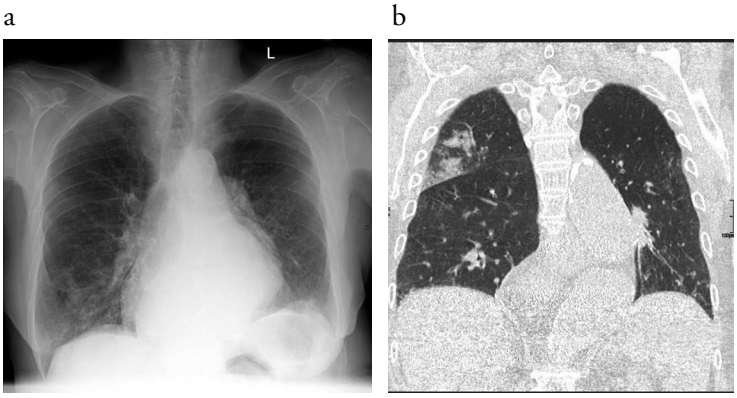

Figure 2: 79 year old female was presented to our emergency room with symptoms of AE-COPD. a) Conventional chest X-ray without evidence of radiological abnormalities. b) Low-dose CT coronal plane of the same patients with alveolar consolidation of the right upper lobe.

Baseline characteristics of both groups are summarized in table 1; no significant differences were observed between both groups. Results regarding sputum cultures are presented in table 2.

Table 1 baseline characteristics

	No radiological abnormalities present(n=76)	Radiological abnormalities present(n=24)	p value
Gender, male (%)	38(50)	12(50)	1.000
Age	71(62-77)	68(64-77)	0.707
FEV1, liters(IQR)[a]	1.13(0.82-1.46)	1.17(0.87-1.43)	0.710
FEV1, % pred(IQR)[a]	45(35-59)	42(34-61)	0.965
FVC, liters(IQR)[a]	2.76(2.05-3.63)	2.73(2.16-3.44)	0.803
FVC, %pred(IQR)[a]	82(74-99)	81(71-103)	0.954
FEV1/FVC, % (IQR)[a]	39.3(31.4-48.9)	38.5(30.6-47.0)	0.834
BMI, kg/m2 (IQR)	24.2(21.2-27.8)	23.6(21.8-27.8)	0.916
Current smoking, n (%)	22(28.9)	9(37.5)	0.430
Pack years, n(IQR)	43(24-53)	30(21-50)	0.207
Number of exacerbations last year, n(IQR)	1(1-2)	1(1-2)	0.311
Prior pneumonia, n (%)	12(15.8)	3(12.5)	0.694
History of heart failure, n (%)	4(5.3)	2(8.3)	0.581
Diabetes mellitus, n (%)	8(10.5)	1(4.2)	0.343
Pre-treatment with antibiotics, n (%)	31(40.8)	10(41.7)	0.939
Pre-treatment with systemic corticosteroids, n (%)	38(50.0	11 (48.8)	0.722
ICS usage, n (%)	18(23.7)	5(20.8)	0.722
Respiratory rate min (IQR)	20(16-24)	24(18-24)	0.151
Heart rate/ min (IQR)	89(78-102)	95(79-104)	0.412
Systolic blood pressure, mmHg (IQR)	148(131-162)	137(120-157)	0.108
Diastolic Blood pressure, mmHg (IQR)	86(71-93)	78(67-88)	0.252
Temperature C°(IQR)	37.2(36.7-37.7)	37.5(36.8-37.8)	0.440
Oxygen saturation, % (IQR)	94(92-96)	93(91-94)	0.041
CCQ at admittance, (IQR)	3.8(3.2-4.1)	3.8(3.1-4.3)	0.360
c-LRTI-VAS at admittance, (IQR)	23(19-27)	25(23-27)	0.816
Positive sputum culture at admittance, n(IQR)	25(32.9)	10(41.7)	0.432
CURB-65 score at admittance			
CURB65 0-1, n (%)	53(69.7)	17(70.8)	0.630
CURB65 2, n (%)	20(26.3)	5(20.8)	
CURB65 3-5, n (%)	3(3.9)	2(8.3)	

All data are represented as median (IQR) unless specified otherwise.
Definition of abbreviations: BMI: body mass index (kg/m2),
FEV1: forced expiratory volume 1 second, FVC: Forced Vital Capacity,
ICS: Inhaled corticosteroids, CCQ: clinical COPD Questionnaire, c-LRTI-VAS: COPD lower respiratory questionnaire visual analogue score
[a]: Last recorded post bronchodilator value in a stable state before admission

Table 2 Sputum culture

	No radiological abnormalities present(n=76)	Radiological abnormalities present(n=24)	p value
Representative sputum culture, n(%)[a]	25(32.9)	10(41.7)	0.432
Isolated sputum			
	No infiltrate (n=25)	Infiltrate (n=10)	p value
H. influenzae, n (%)	10(40.0)	3(30.0)	0.580
S. pneumoniae, n (%)	6(24.0)	3(30.0)	0.714
H. parainfluenzae, n (%)	6(24.0)	2(20.0)	0.799
S. aureus, n (%)	3(12.0)	4(40.0)	0.061
Pseudomonas species, n (%)	1(4.0)	2(20.0)	0.127
M. catharrhalis, n (%)	5(20)	0(0.0)	0.127
E.coli, n (%)	1(4.0)	2(20.0)	0.127
S. malthophilia, n (%)	1(4.0)	0(0.0)	0.521

[a] Sputum was representative according to the Bartlett criteria: sputum sample with >25 polymorph nuclear leukocytes and <10 squamous epithelial cells per low-power field was defined as a sputum sample representative of the lower airways

Inter-observer variation

The observed proportional agreement (P_0) in low-dose CT-scan judgment of observed abnormalities by radiologists A and B was (84.0%). The proportional agreement by chance (P_e) was 63.5%, resulting in a moderate κ of 0.562 with a 95% confidence interval of 0.371 to 0.752 (p<0.001). The proportional agreement in positive cases (P_{pos}= 69.6%) was lower than the proportional agreement in negative cases (P_{neg}= 88.3%).

Biomarkers

All biomarkers that we measured were significantly higher in the group with radiological abnormalities on the LDCT compared to those without. CRP was 20.5 (IQR 8.8-81.5) mg/L in the group without radiological abnormalities compared to 76(21.5-148.0) mg/L (p=0.018) in the group with abnormalities (figure 3a), PCT was 0.06 (IQR 0.04-0.08) µg/L in the group without radiological abnormalities compared to 0.09 (IQR0.06-0.15) µg/L (p=0.007) in the group with radiological abnormalities (Figure 3b). SAA was 16 (IQR 3-89) µg/ml in the group without radiological abnormalities compared to 95 (7-160) µg/L (p=0.019) in the group with abnormalities (figure 3c).

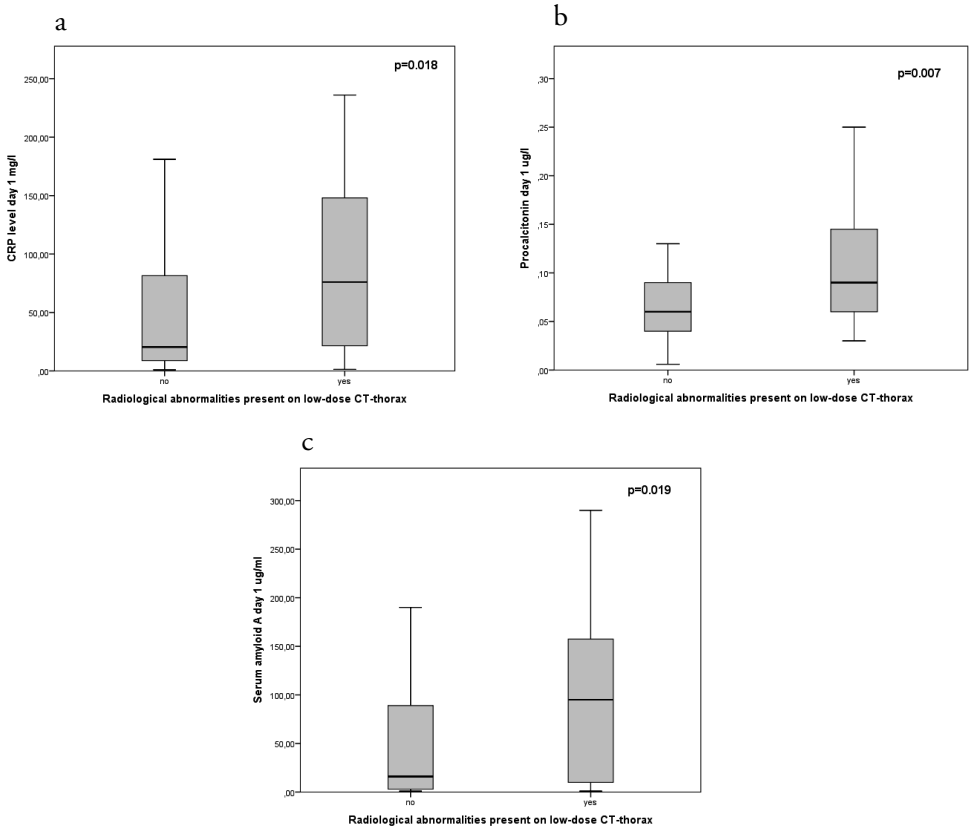

Figure 3: Biomarker level at admittance in patients with and without radioloical abnormalities present on LDCT. a) CRP, b) PCT and C) SAA

The area under the ROC curve was 0.659 (95% CI: 0.521-0.796) for CRP, 0.664 (95%CI: 0.526-0.801) for PCT and 0.687 (95%CI: 0.566-0.808) for SAA (figure 4). The optimal cut-off value to identify radiological abnormalities was 43mg/L for CRP, with a sensitivity of 0.70 and a specificity of 0.68. The optimal cut-off point for PCT to identify radiological abnormalities was 0.05ug/L with a sensitivity of 0.78 and a specificity of 0.47. The optimal cut-off point for SAA to identify radiological abnormalities was 27 µg/L with a sensitivity of 0.70 and a specificity of 0.65.

Discussion

The present exploratory study shows that in 24% of the patients with AECOPD admitted to hospital without evidence of CAP on chest X-ray had evidence of infiltrative changes compatible with acute-phase lung involvement on LDCT. In patients with radiological abnormalities on LDCT the levels of CRP, PCT, and SAA were higher although they

were unable to reliably exclude or confirm the diagnosis of CAP.
The incidence of infiltrative changes in AECOPD not detected by chest X-ray was considerably higher in an earlier study.[22] The higher number of infiltrates found in this study can be explained by a difference in baseline population. Our study population consisted of patients with AECOPD; the aforementioned study enrolled individuals from the general population with fever and cough.
The κ value of agreement between both radiologists was moderate (κ 0,562) and lower compared to previous research.[22] The difference in Cohen's kappa can be explained by a difference in technique used, in our study Low-dose CT was used whereas in the study of Syrjala et al. high resolution (HR) CT was used.[22] HR images are of higher quality making agreement between radiologists more likely. The κ value of agreement of LDCT was similar to that of chest radiography in patients presenting to a primary care facility with acute cough (κ 0.45 (95%CI 0.36-0.54)).[24]
The biomarkers reported were elevated in the group with consolidations, although they were considerably lower compared to other studies in patients with radiographically confirmed pneumonia.[25;26] The biomarkers reported in the group without radiological abnormalities were comparable with an earlier study in patients with AECOPD.[11] CRP, SAA, and PCT showed poor sensitivity and specificity for the prediction of radiological abnormalities on low dose CT. In part this might be due to the fact that all biomarkers in this study are used in the detection of bacterial infection, whereas these radiological abnormalities can also be caused by viral infections.[11;27] This might subsequently lead to a lower level of chosen biomarkers compared to bacterial infection.[28;29] Obviously imaging technology is not a reliable way to identify or predict causative pathogens causing CAP, though a diffuse bilateral pattern may suggest a viral origin of CAP, and might conceivable help to reduce unnecessary use of antibiotics.[30]
A potential strength of this study is that all data were collected prospectively thereby not resulting in selection bias. Another strength is the fact that radiologists were blinded for clinical data except for age and gender so this could not have influenced their judgement.
A potential limitation in design of our study was that a 12 hour time gap between the initial chest X-ray and the LDCT was allowed. Infiltrative changes may progress over time, which may have led to progression or emergence of new radiological abnormalities.
The second limitation is the prescription of antibiotics or systemic corticosteroids prior to inclusion. We cannot exclude that these pre-treated patients may have altered biomarker performance or that remnants of infiltrates invisible to chest X-ray still can be detected on CT. The third potential limitation is the fact that CT cannot discriminate between viral and bacterial CAP as only invasive local microbiological samples would have provided this diagnosis.[30] As we did not gather these data, results regarding the biomarker levels and their correlation with LDCT findings should be interpreted with caution. A fourth weakness is that this is an explorative study not powered to detect differences in biomarkers and therefore, the results should be interpreted with caution.

AECOPD and CAP share many of the symptoms, and ruling out CAP using a chest X-ray is often difficult. We have shown that LDCT is able to detect additional radiological abnormalities; in this study we were unable to assess the potential clinical impact of finding these additional abnormalities suggesting CAP. Biomarkers are increased in patients with infiltrative changes on their LDCT. Yet CRP, PCT, nor SAA carried sufficient weight to confirm or exclude the diagnosis of CAP in this specific population. Therefore further research is necessary to determine whether the presence of infiltrative changes on LDCT not visible on chest X-ray are relevant to the management or prognosis of patients with AECOPD.

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CHAPTER 5

5

COPD-Lower Respiratory Tract Infection Visual Analogue Score (c-LRTI-VAS) validation in stable and exacerbated COPD patients

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Submitted

Abstract

Introduction: We developed the COPD-lower respiratory tract infections – visual analogue score (c-LRTI-VAS) in order to easily quantify symptoms during exacerbations in patients with COPD. This study aimed to validate this score.

Methods: In our study patients with stable COPD as well as those with an acute exacerbation of COPD (AECOPD) were included. The results of c-LRTI-VAS were compared with other markers of disease activity (lung function parameters, oxygen saturation and two health related quality of life questionnaires (St Georges Respiratory Questionnaire (SGRQ) and Clinical COPD Questionnaire (CCQ)) and validity, reliability and responsiveness were assessed.

Results: Eighty-eight patients with clinically stable COPD and 102 patients who had an AECOPD completed the c-LRTI-VAS questionnaire. When testing on two separate occasions for repeatability, no statistically significant difference between total scores was found 0.143 (SD 5.42) (p=0.826). Internal consistency was high across items (Cronbach's Alpha 0.755). Correlation with SGRQ and CCQ total scores was moderate to high. After treatment for hospitalized AECOPD, the mean c-LRTI-VAS total score improved 8.14 points (SD 9.13; p=<0.001).

Conclusions: c-LRTI-VAS showed proper validity, responsiveness to change and moderate to high correlation with other questionnaires. It therefore appears a reliable tool for symptom measurement in COPD

Introduction

Chronic obstructive pulmonary disease (COPD) is the fourth leading cause of mortality worldwide and an important cause of morbidity.[1] COPD is a common preventable and treatable disease that is characterized by persistent respiratory symptoms and airflow limitation and chronic low-grade local and systemic inflammation that is due to airway and/or alveolar abnormalities usually caused by significant exposure to noxious particles or gases.[1;2] Clinical measures such as FEV_1 or oxygen saturation correlate only moderately with functional capacity of patients with COPD.[3;4] The main determinants of a patient's health-related quality of life (HRQL) appear to be the degree of dyspnoea, fatigue, muscle wasting, sleep- and mood disturbances.[5;6] Measurement of these symptoms and signs is very useful in monitoring COPD patients. It is a strong predictor of future disease outcome and potentially modifies treatment management.[7] To this end many questionnaires have been developed to measure the impact of symptoms on quality of life.[8;9] Although several questionnaires exist that measure symptoms in a subdomain until now no specific questionnaire that solely focusses on symptoms are scarce.[10] The incentive for the development of a practical health status instrument, the COPD-Lower Respiratory Tract Infection- Visual analogue score (c-LRTI-VAS) arose from routine clinical management of COPD in daily practice where it was recognized that clinicians require a simple tool that will help them objectively to identify a worsening or relief of symptoms during an exacerbation instead of measuring the impact of symptoms on daily life and well-being. It was thought that such an instrument would encourage clinicians to focus on disease activity more than focusing on the impact of symptoms. The LRTI-VAS was used before to quantify symptoms in an acute exacerbation of COPD (AECOPD) and was recently validated in non-CF bronchiectasis.[11-13] On both occasions the LRTI-VAS was generally well accepted by patients and showed a high response rate. Furthermore, the LRTI-VAS proved to be easily processed.
The aim of this study was to validate the c-LRTI-VAS for assessment of symptoms in COPD patients in stable condition and during an AECOPD.

Material and Methods

Study population

Data from a clinically stable population were obtained between November 2011 and November 2014. Data from patients with an AECOPD were available from a randomized clinical trial we preformed between July 2011 and February 2015 (the CATCH study Clinical trial.gov NCT01232140). The study population consisted of patients diagnosed with COPD stages I–IV as defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD), and a minimum smoking history of 10

pack years.[1] Patients with a recent change in medication, immunocompromised patients or patients with respiratory disease other than COPD were excluded. Reliability was measured during a clinically stable situation: measurement of responsiveness required the presence of an exacerbation according to GOLD.[1] To guarantee clinical stability each participant was instructed to report to the researchers without delay any changes in their clinical condition pointing towards an exacerbation. In order to test for responsiveness, an additional criterion was added: the presence of an acute exacerbation of COPD according to GOLD criteria requiring hospitalization.[1] All patients provided written informed consent in both patients groups.

Development of the c-LRTI-VAS

The initial specifications for the c-LRTI-VAS identified that the questionnaire should only contain the symptoms that physicians consider to be the most important for estimating the clinical status of the airways. Therefore item generation was performed based upon Anthonisen criteria with the addition of the symptom fatigue.[14] Fatigue was added as being one of the most prominent symptoms in COPD.[15] A VAS scale was chosen to meet the specification of simplicity. The c-LRTI-VAS is short (4 items) and easy to complete (figure 1). It takes patients approximately 1 minute to complete the questionnaire, and assistance is generally not required. Patients were instructed to recall their experiences during the last day. They respond to each question using a VAS scale. The scale ranges from 1 to 10, the subjects being unaware of the numbers. Higher scores indicate more severe symptoms. Four symptom domains are scored: shortness of breath, tiredness, cough and sputum colour. Separate scores are calculated for each symptom and a total score is provided, consisting of all symptom scores added up. Similar weight is assigned to all symptom domains. For the present study, a Dutch version was used.

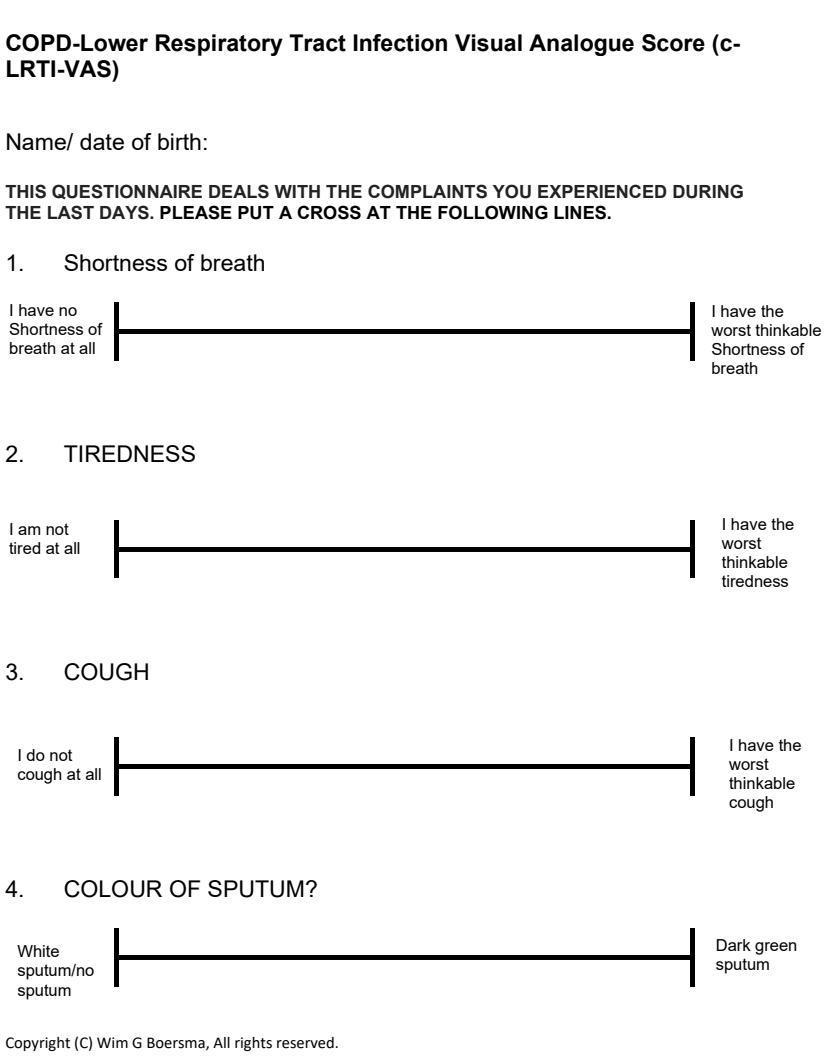


Figure 1 COPD-Lower Respiratory Tract Infection Visual Analogue Scale (c-LRTI-VAS)

Other Questionnaires

Clinical COPD Questionnaire (CCQ): a disease specific questionnaire that consists of 10 items. The items are divided into 3 different domains (functional state, symptoms and mental state) which can be scored separately, added together they provide a total score. Representing the impairment of quality of life. The CCQ requires about 4 minutes to complete.[16;17]

St George Respiratory Questionnaire (SGRQ): a condition specific HRQL-measure, that consists of 76 items. These items are partitioned into three sections (symptoms, activity, impact), which are scored separately and can be added up to provide a total score, ranging from 0 to 100%, zero indicating no impairment of quality of life. The SGRQ requires about 10 minutes to complete.[9]

Study visits

All participants with stable COPD visited our outpatient clinic on two separate occasions 30 days apart. On both occasions participants were asked to complete the LRTI-VAS, the CCQ and SGRQ. In addition spirometry was measured. In case of participants with AECOPD the first study visit was scheduled within 24 hour after the admission for AECOPD and after 30 days patients visited our outpatient clinic. On both occasions patients were asked to complete the c-LRTI-VAS, CCQ and SGRQ. In addition arterial oxygen saturation was measured using a fingertip pulse oximeter (Beurer GmbH Y23/003700, Ulm, Germany). The study was approved by the local Medical Ethics Committee (METC-Noord Holland registration number M010-071) and conducted in the Northwest Hospital, Alkmaar. All patients provided their written informed consent.

Sputum colour analysis

Sputum samples were collected on the first day after admission and one month after admission. At the laboratory for microbiology, sputum colour was assessed with a previously validated five-point sputum colour chart (BronkoTest; Heredilab Inc., Salt Lake City, UT, USA) by specifically instructed analysts.[18] These data were used to assess the correlation between reported sputum colour compared to objectified sputum colour

Cross sectional validity

Patients with clinical stable COPD and patients with an AECOPD completed the c-LRTI-VAS, the CCQ and the SGRQ on two separate occasions. In addition, in patients with stable COPD spirometry was performed as well as pulse oxygen saturation measurement on both occasions. Correlation of c-LRTI-VAS, CCQ and SGRQ, FEV_1, FVC and oxygen saturation was calculated in order to test validity. Internal consistency was calculated in order to test the degree of association between the questionnaire items.

Longitudinal validity

In the group of patients with stable COPD the c-LRTI-VAS, CCQ and SGRQ administered and re-administered after 1 month. At both occasions patients conducted spirometry and pulse oxygen saturation measurement. These data were used to assess re-test reliability. Patients were excluded if they had an exacerbation, an increase of respiratory symptoms due to heart failure or upper respiratory infection or change in smoking status. An exacerbation was defined as an acute event characterized by worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and lead to a change in medication.[1]

Responsiveness

Patients with an AECOPD completed the c-LRTI-VAS, the CCQ and the SGRQ on the first day of their exacerbation. The Questionnaires were re-administered after 30

days. The responsiveness of the c-LRTI-VAS was assessed by comparing changes in score in the CCQ and c-LRTI-VAS.

Statistical analysis

Our sample size regarding patients with an AECOPD was based upon the data of Daniels et al.[12] An effect size of 5 was expected with a SD of 12. With alpha being 0.05 and beta being 0.20 a sample size of 92 patients on each measuring moment was needed. Our sample size regarding patients with stable COPD we assumed a moderate correlation (0.3) between the scores on $t = 1$ and $t = 2$. With alpha being 0.05 and beta being 0.20 a sample size of 85 patients. In the group of patients with AECOPD a higher number of drop-out was anticipated therefore we decided to include an additional 10 patients. In the group of patients with stable COPD an additional 3 patients were added.

Data analysis was performed using SPSS 20.0 (SPSS inc, USA). Data are expressed as means (SD) unless stated otherwise. Paired T-test was used to compare LRTI-VAS domain and total scores on two occasions during clinical stability and at the start and end of an exacerbation. In case of skewed distribution, Wilcoxon's signed ranks test was used. Pearson's correlation and the intra-class correlation coefficient (ICC) was used to assess validity. Internal consistency of the LRTI-VAS was measured by applying Cronbach's alpha to each of the component scores at entry; accepting >0.7 as sufficient. Nominal and ordinal variables were expressed using frequency tables, modus and median. Interval/ratio variables were expressed in terms of mean, SD and confidence intervals. Bland and Altman graphs were made to assess the agreement between day 1 and day 30. When comparing two variables, a p value of < 0.05 was considered as statistically significant.

Results

Two hundred and six patients were included; 88 of whom were clinically stable and 102 had an exacerbation (figure 2). Patients characteristics are shown in table 1.

Table 1 baseline characteristics

	AECOPD (n=102)	Clinically stable (n=88)
Gender male (%)	44 (43.1)	56(63.6)
Age, years (SD)	68.8(10.4)	69(12.5)
Current smoking n (%)	32(31.4)	20(23.5)
Pack years	38.3(18.4)	37.8(15.1)
FEV1 % pred	46.8(16.9)	54.2(16.6)
FVC % pred	84.1(21.7)	91.5(16.5)
FEV1/FVC %	41.1(12.4)	44.3(11.6)
GOLD classification		
Stage I n (%)	7(6.9)	7(8.0)
Stage II n (%)	35(34.3)	42(47.7)
Stage III n (%)	45(44.1)	37(42.0)
Stage IVn (%)	15(14.7)	2(2.3)
number of exacerbations last year median (IQR)	1(1-2)	0(0-1)

All data are represented as mean (SD) unless specified otherwise
Definition of abbreviations FEV1: forced expiratory volume 1 second, FVC: Forced Vital Capacity

Eighty-six patients in the exacerbation group and 77 in the stable group completed all 3 questionnaires on two occasions. Median c-LRTI-VAS score during stable state was 11(IQR 7-16) and during AECOPD mean 23.2(SD 6.2) Median CCQ score during stable state was 2.25(IQR1.50-2.75) and during AECOPD 3.88(3.00-4.50). Mean SGRQ score during stable state was 44.1(SD 21.2) and during AECOPD 63.5(SD17.1) (please find results for the c-LRTI-VAS, CCQ and SGRQ domain scores in the supplementary data).

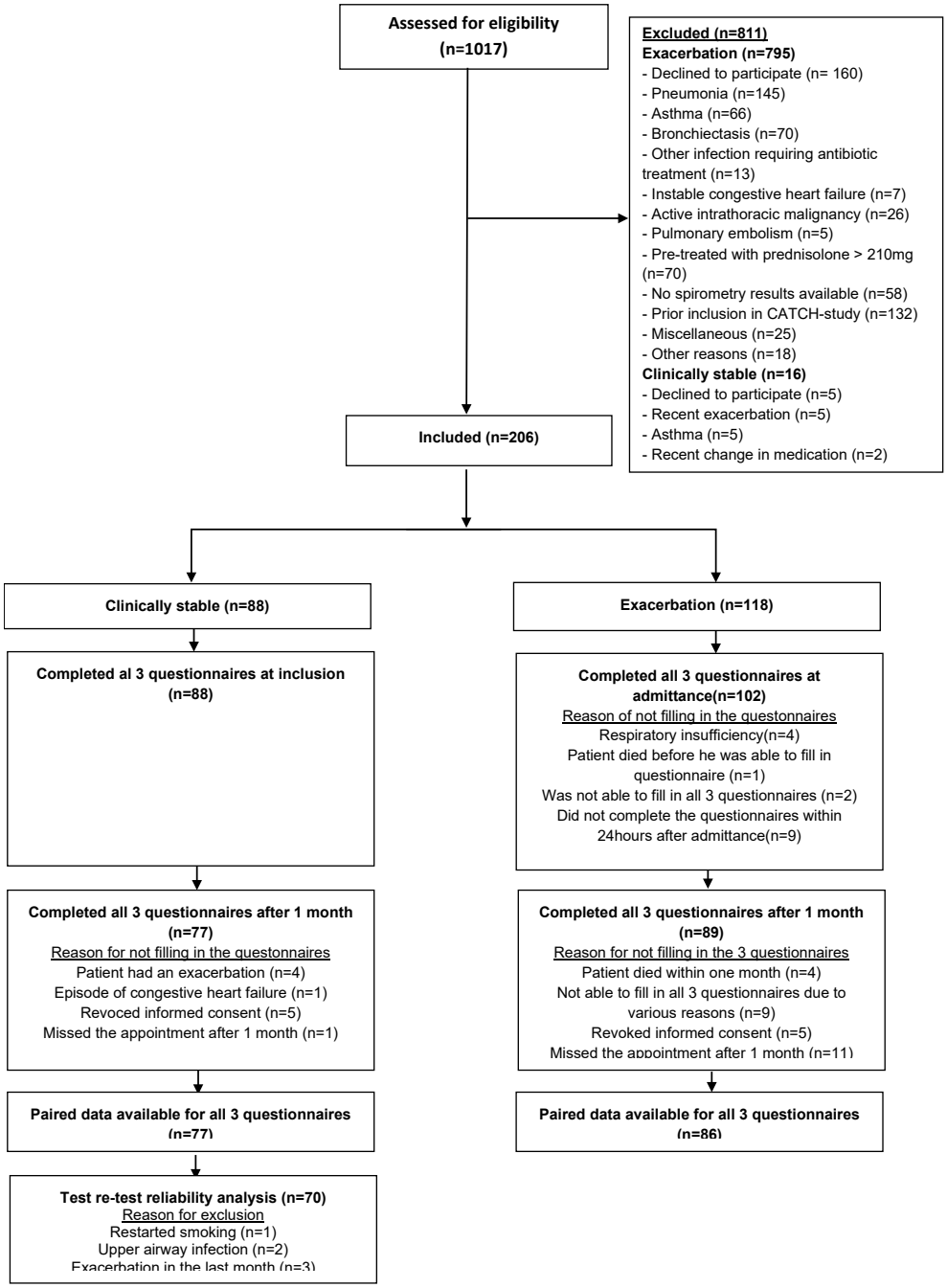


Figure 2 Trial profile

Test re-test reliability:
Seventy-seven patients with stable COPD completed the all three questionnaires on day 1 and day 30. Six patients were excluded due to various reasons (figure 2). Mean difference of the c-LRTI-VAS was 0.143 (SD 5.42) (p=0.826) (figure 3). The intra-class correlation coefficient was 0.667 (95%CI 0.733-0.892 p<0.001) for the total c-LRTI-VAS score. The intra-class correlation coefficient of the SGRQ was 0.953 (95%CI 0.924-0.970 p<0.001). The intra-class correlation coefficient of the CCQ was 0.871 (95%CI 0.793-0.919 p<0.001). The relation between c-LRTI-VAS score on T=0 and T=30 is shown in the Bland and Altman plots (figure 4). No systematic errors can be seen as the mean difference was 0.143 with an upper limit of agreement of 10.775 and a lower limit of agreement of -10.480.

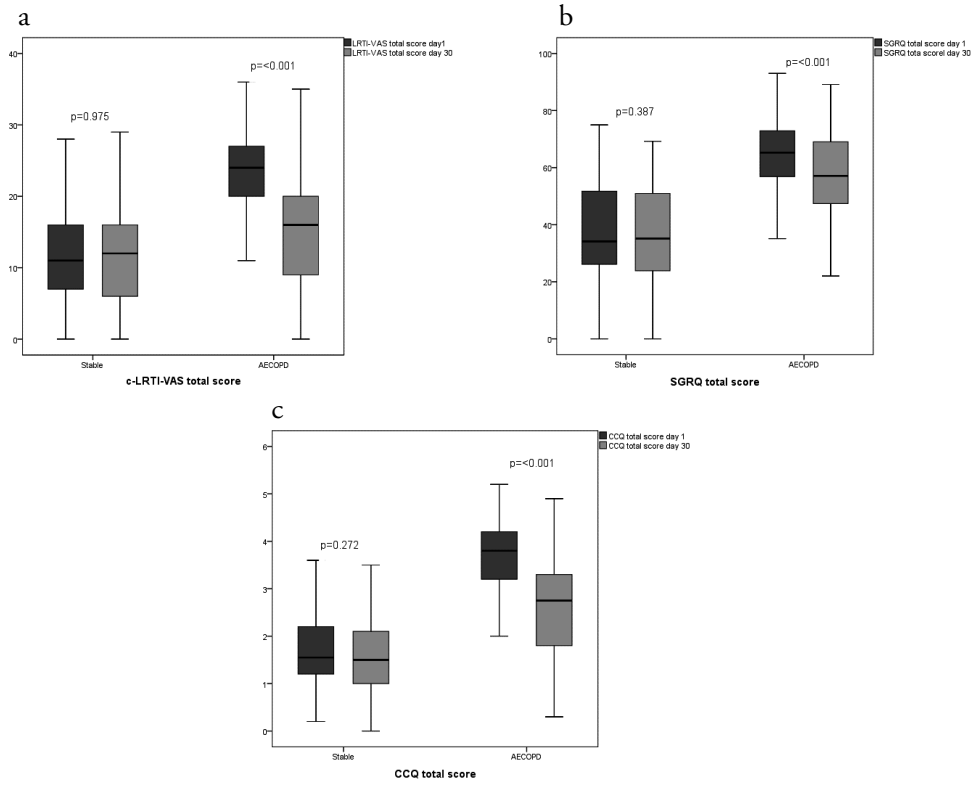


Figure 3: Scores of individual questionnaires during stable state and AECOPD at t=0 days and t=30 days. a) c-LRTI-VAS, b) SGRQ, c) CCQ.

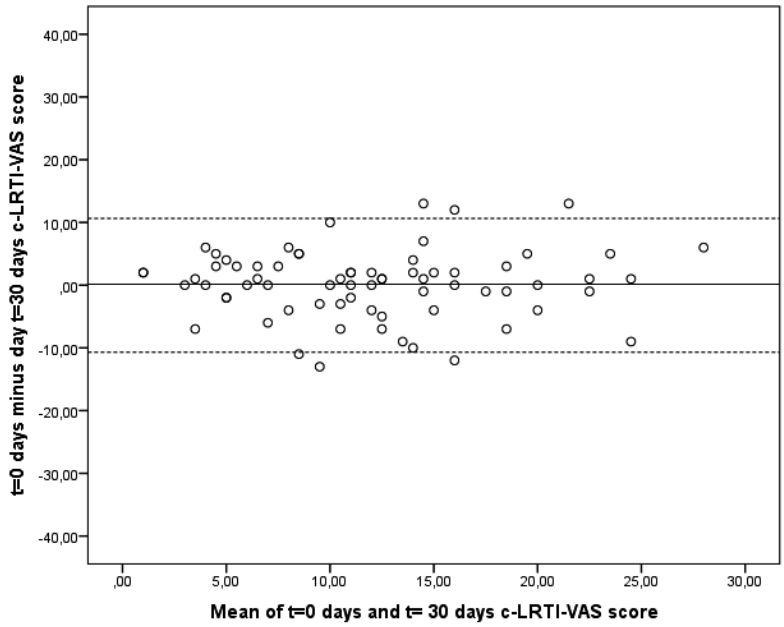
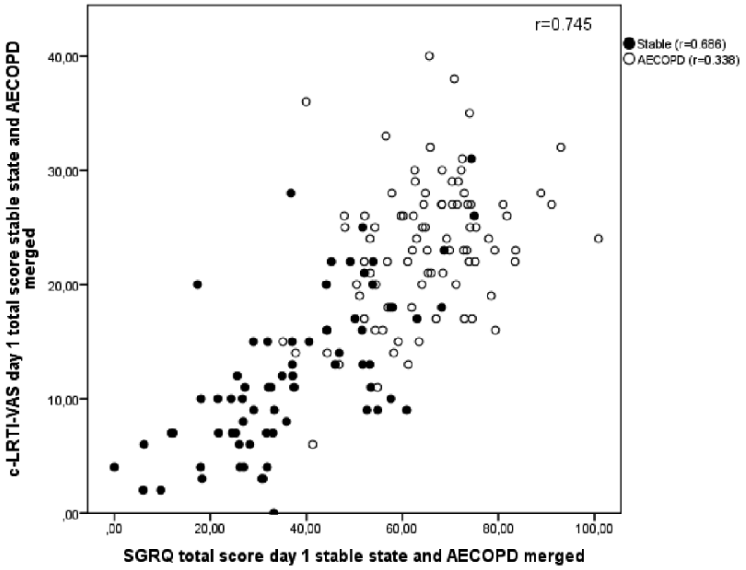


Figure 4: Bland-Altman plot c-LRTI-VAS stable state

Internal consistency:
For the validation of internal consistency of the c-LRTI-VAS, both datasets of the AECOPD as well as the stable situation were merged (n=190). Cronbach's α for the internal consistency for the 4 domains was 0.755, indicating a good consistency. Internal consistency increased when the item sputum purulence was deleted from the questionnaire to 0.803.
Cronbach's α for the internal consistency during AECOPD (n=102) for the 4 domains was 0.533. Internal consistency increased further when sputum purulence was deleted to 0.642. Cronbach's α for the internal consistency during stable state (n=89) for the 4 domains was 0.623 Internal consistency increased further when the item sputum purulence was deleted from the questionnaire to 0.676. Internal consistency of SGRQ was 0.818 and of the CCQ 0.783

Validity
The correlation coefficients between total scores on validated questionnaires (SGRQ and CCQ) are shown in figure 5. Correlation between FEV1, FVC, oxygen saturation, sputum colour and c-LRTI-VAS was low (r=0.071-0.377).

a



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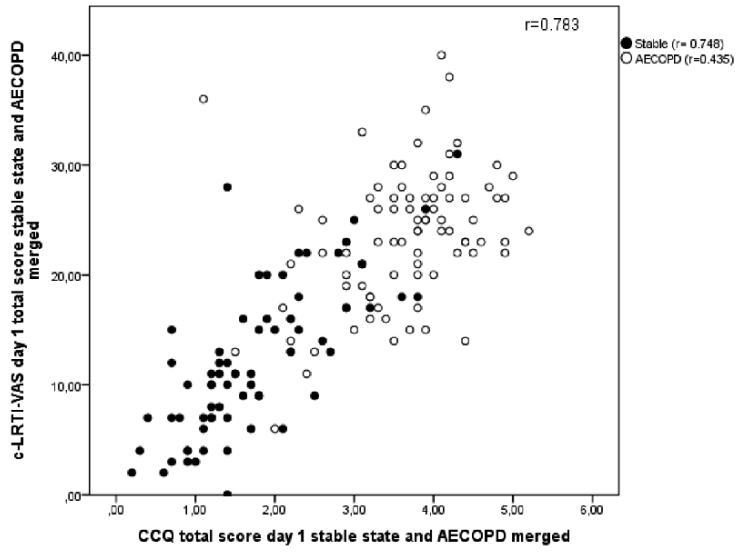

Figure 5 a) Correlation between c-LRTI-VAS and SGRQ during stable COPD and AECOPD. b) Correlation between c-LRTI-VAS and CCQ during stable COPD and AECOPD.

Responsiveness

Eighty-six patients completed the c-LRTI-VAS, CCQ and SGRQ at admission and 1 month later. Mean difference of the c-LRTI-VAS was 8.14 SD 9.13 (95%CI 6.16-10.12 p=<0.001). Responsiveness for individual GOLD stages was shown in table 2

Table 2 responsiveness of c-LRTI-VAS according to GOLD stages

	Median difference(IQR)	p-value
GOLD I (n=6)	-13.0(-19.5;-1.0)	0.075
GOLD II (n=32)	-7.5(-14.0;-2.0)	<0.001
GOLD III (n=35)	-6.5(-12.0;-1.0)	0.001
GOLD IV (n=13)	-8.0(-16;0.0)	0.013

Discussion

This study shows that the c-LRTI-VAS questionnaire is valid, reliable and promises to be responsive to changes in patients with COPD. The VAS instrument has been around for a long time and initially mainly used for the quantification of pain. It has been shown to be reliable and is widely used. The VAS in COPD has mainly been used for quantification of dyspnoea, but has also been validated for the quantification of quality of life in COPD.[19-21] We used the c-LRTI-VAS before to quantify symptoms in 223 patients with acute exacerbations of COPD and a slightly modified version of the LRTI-VAS was validated in a population of patients with bronchiectasis.[11;12] On all occasions, the c-LRTI-VAS was generally well accepted by patients, showed a high response rate and both patients and researchers appeared to quickly familiarise themselves with this questionnaire.

Currently many HRQL questionnaires are available such as the SGRQ, CCQ and COPD Assessment Test. All are comprehensive and do contain a domain of symptoms, but are not exclusively designed for measurements of symptoms.[8;9] Although such an instrument was developed in the form of the EXACT-pro, this questionnaire still has the shortcoming that it is less suitable for illiterate or poorly educated patients compared to a VAS instrument.[10;22] It was therefore thought that there is a need for a less extensive and time consuming questionnaire for patient-reported outcome in clinical settings that solely focusses on the most reported symptoms in COPD and that is suitable for poorly educated or illiterate patients. The items were generated based upon the Anthonisen criteria and fatigue as being one of the most prominent features in COPD.[14;15] Although other markers are able to monitor disease activity in AECOPD as pulmonary function tests, including peak flow or oxygen saturation, the c-LRTI-VAS is easy to administer and has a low burden on stable as well as on patients with an AECOPD. The VAS instrument has been used before in COPD, it has been used for the quantification of separate symptoms such as dyspnoea and cough as well as for the quantification of quality of life.[20;21;23] Yet is has never been used solely for the quantification of the most frequent symptoms in stable COPD as well as in AECOPD.

In our population, subjects scored similar results on two separate occasions in clinically stable situation. Patients during an outpatient visit scored within 1.2-4.1 point on

the c-LRTI-VAS 10 point scale for shortness of breath, tiredness, cough, and sputum purulence. During an exacerbation scores for these symptoms increased to 5-8 points per item with a significant decrement 30 days after treatment for the exacerbation. Sputum purulence was only marginally increased during AECOPD compared to the recovered or stable state. This might be explained by the fact that patients' assessment of sputum colour is unreliable as was shown earlier.[18]

In COPD parameters such as lung function tests and oxygen saturation often do not correlate well with functional capacity and well-being.[3;4] The absence of this relation may explain the low correlations we found between these parameters. This does not disqualify these parameters as they have important predictive values in COPD.[24;25] Yet they do not play a significant role in quality of life as this is mainly defined by the presence and severity of symptoms as dyspnoea, cough and fatigue.

The strength of our study is that patients with all GOLD-classes were included, second strength is that the c-LRTI-VAS was validated for patients with stable as well as with AECOPD. Potential weaknesses were the high number of patients that were lost to follow-up. This potentially might have influenced our results. Another potential weakness is the generalisability of our results as this trial was performed in a hospital setting with patient admitted to hospital as well ambulant patients. It remains to be seen whether the LRTI-VAS is a useful tool in general practices.

The c-LRTI-VAS has shown to reliably measure shortness of breath, tiredness, cough and sputum colour, although sputum purulence proved not to contribute to the reliability and consistency of the questionnaire. This might be explained by the fact that not all patients routinely inspect their sputum, and so the answer given could be a "best guess", secondly sputum colour can change rapidly, especially during acute exacerbations and finally, sputum is not always homogeneous, which can be confusing. We therefore consider to adapt the c-LRTI-VAS by removing sputum purulence from the questionnaire. A potential replacement for sputum purulence might be anxiety as one of the most prominent features of patients with COPD.[5] Yet we do think that the LRTI-VAS in its current form is a potential valuable outcome measure when evaluating treatment effects in clinical trials as it is easy to complete and to implement as was shown in earlier trials.

Conclusion

The LRTI-VAS showed proper repeatability and responsiveness, moderate to high correlation with other validated questionnaires and a moderate internal consistency that was lowered by sputum purulence. The c-LRTI-VAS therefore meets all the criteria to be used in monitoring disease and can be used in clinical practice.

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Chapter 5 supplementary data

Table 1 c-LRTI-VAS, CCQ and SGRQ score at inclusion in stable state and during AECOPD

c-LRTI-VAS stable t=0		c-LRTI-VAS AECOPD t=0	
Shortness of breath (median, IQR)	4(2-6)	Shortness of breath (median, IQR)	7(6-9)
Tiredness (median, IQR)	3(1-6)	Tiredness (median, IQR)	8(5-9)
Cough (median, IQR)	2(1-4)	Cough (median, IQR)	6(5-8)
Sputum purulence (median, IQR)	1(0-2)	Sputum purulence (median, IQR)	2(0-5)
Total score (median, IQR)	11(7-16)	Total score (mean, SD)	23.2(6.2)
CCQ stable t=0		CCQ AECOPD t=0	
Symptoms (median, IQR)	2.25(1.50-2.75)	Symptoms (median, IQR)	3.88(3.00-4.50)
Mental (median, IQR)	0.50(0.00-1.50)	Mental (median, IQR)	2.50(1.38-3.50)
Functional (median, IQR)	1.50(0.75-2.50)	Functional (median, IQR)	4.00(3.19-4.75)
Total score (median, IQR)	1.70(1.20-2.30)	Total score (median, IQR)	3.70(3.18-4.1)
SGRQ stable t=0		SGRQ AECOPD t=0	
Symptoms (mean, SD)	44.1(21.2)	Symptoms (mean, SD)	63.5(17.1)
Activity (median, IQR)	66.1(47.5-73.8)	Activity (median, IQR)	86.3(73.1-92.5)
Impact (mean, SD)	26.6(17.7)	Impact (mean, SD)	55.4(18.0)
Total (mean, SD)	39.5(17.0)	Total (mean, SD)	64.9(13.8)

All data are represented as mean (SD) unless specified otherwise
Definition of abbreviations FEV1: forced expiratory volume 1 second, FVC: Forced Vital Capacity

CHAPTER 6

6

Effects of doxycycline on local and systemic inflammation in stable COPD patients, a randomized clinical trial

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Respiratory Medicine January 2016

Abstract

Introduction: Neutrophilic inflammation plays a causal role in Chronic Obstructive Pulmonary Disease (COPD). Neutrophil derived myeloperoxidase(MPO) matrix metalloproteinases(MMP's), and elastases are thought to contribute to the perpetuation of the disease. The tetracycline analogue doxycycline has been shown to inhibit neutrophil-mediated inflammation. It was thus reasoned that doxycycline may attenuate neutrophil-mediated inflammation in COPD

Methods: In this double blind randomized controlled trial the effect of a 3-week course of doxycycline on sputum and systemic inflammatory parameters was evaluated in stable COPD patients. In order to exclude inflammation by bacterial colonisation patients must have 2 negative sputum cultures in the previous year. The effect of doxycycline treatment on inflammatory markers (TNF-α, IL-1β and IL-6) and neutrophil specific markers in sputum (MPO, MMP's, and IL-8) and serum C-reactive protein was evaluated. Sputum was obtained by sputum induction with hypertonic saline.

Results: A total of 41 patients were included. Ten patients were excluded as they were not able to produce sputum at the first or second visit. Baseline characteristics were similar in the two groups. In the remaining patients doxycycline did not influence sputum MPO concentrations. Also MMP-8 and 9, IL-6 and IL-8 concentrations as well as lung function parameters were not affected by doxycycline. Systemic inflammation by means of CRP was also not influenced by doxycycline.

Conclusion: A three week course of doxycycline did not influence MPO sputum levels nor any of the other inflammatory sputum and systemic markers.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by a complex neutrophil-driven inflammatory airway disease which leads to chronic airflow limitation. COPD is a growing cause of morbidity and mortality worldwide, to this day no treatment is available that can stop-or even reverse the disease process. Neutrophils play an important role in the inflammatory process and are considered a critical factor in disease progression. This is supported by the correlation that has been found between airway neutrophilia and the rate of lung function decline and the finding that airway neutrophilia is related to small airway dysfunction.[1;2] Furthermore associations are found between COPD and markers of neutrophilic inflammation such as myeloperoxidase (MPO), matrix metalloproteinases (MMPs) and elastase.[1;3;4]

It has been shown that a sustained neutrophilic response contributes to the disease process indirectly through perpetuation of the inflammatory response, and directly through contributing to the airway remodelling.[5] This is exemplified by degradation of extracellular matrix (ECM).[6;7] This degradation results in the destruction of alveoli and airway remodelling which is a hallmark of moderate to severe COPD.[8] These observations characterize the neutrophilic inflammatory cascade as a candidate target for COPD.

Independent of the antibiotic properties, tetracyclines show comprehensive anti-inflammatory effects which include a strong effect on neutrophilic mediators, neutrophil and cytotoxic T cell recruitment.[9;10] A tetracycline analogue, doxycycline, markedly reduced airway inflammation in an animal experiment.[11;12] Moreover, a recent randomized clinical trial of doxycycline in patients with COPD showed clear reductions of C-reactive protein plasma levels (CRP) as well as improved lung function.[13] A critical question arises whether these positive effects observed were based upon a reduction of bacterial load or due to a direct anti-inflammatory effect. In the current proof of concept study we aimed to assess the anti-inflammatory effects of doxycycline on sputum inflammatory markers in COPD patients. To that end we performed a placebo controlled randomized clinical trial of doxycycline in patients without airway bacterial colonization during the last year.

Material and Methods

Study subjects

The study was conducted in the outpatient population of the department of pulmonary diseases of the Medical Centre Alkmaar, Alkmaar, and the Netherlands. The study protocol was approved by the local ethics committee and carried out in accordance with good clinical practice. The trial was registered at clinicaltrials.gov (NTC00857038).

Written informed consent was obtained from all the participants. The study was carried out between August 2009 and December 2010.

The present study is a randomized double blinded placebo-controlled study, designed to investigate the efficacy of doxycycline in addition to standard treatment in patients with stable COPD GOLD II-III.[14] Randomization was based on a one-on-one allocation by means of pre-numbered containers containing identical looking capsules with placebo or doxycycline. The allocation sequence was computer generated and was kept in a safe at the hospital pharmacy throughout the course of the study. Patients included were, age over 40 years and without airway bacterial colonization, defined as two negative sputum cultures or broncho-alveolar lavage cultures in the previous year. Exclusion criteria included respiratory diseases other than COPD, use of systemic corticosteroids or other immunosuppressive drugs within one month prior to inclusion in the study and allergy for tetracyclines or a history of substantial side effects. Subjects with an acute exacerbation as defined by Anthonisen et al, or an active respiratory or non-respiratory infection one month prior to the study were also excluded.[15] The primary end point was change in sputum myeloperoxidase (MPO) levels from baseline to end of 3 weeks. MPO was chosen because it is a well-known marker of neutrophil presence and activation.[16] Secondary endpoints were changes in sputum interleukin (IL)-6 and IL-8, as markers of inflammation and recruitment of neutrophils, sputum MMP-8 and MMP-9, as additional markers of neutrophil activation, sputum granzyme A as a marker of cytotoxic $CD8^+$/NK-cell activation, sputum TNF-α and IL-1β as markers of macrophage function, serum C-reactive protein (CRP) as a marker of systemic inflammation and lung function from baseline to end of 3 week treatment. Treatment dose and duration of 3 weeks was chosen based upon the trials of Lindeman et al, one week of treatment was added to ensure maximum effect because of the small sample size of the current study.[17;18]

At initial screening visit patients were asked to discontinue their inhalation corticosteroids if they used any. After this run-out period of 4 weeks, baseline testing and sputum induction were performed. Subsequently patients were randomized and treated with doxycycline 100mg/day or placebo for 21 days. Hundred milligram of doxycycline was chosen based on earlier research and availability of doxycycline 100mg as well as matching placebos. In the event of an acute exacerbation of COPD or other indication for use of steroids or antibiotics, patients were excluded from the study. Patients were also excluded if sputum induction was unsuccessful at the start or end of the study.

Sputum induction and analyses

Sputum induction was performed by inhalation of hypertonic saline 3% for 10 minutes. Saline concentrations were increased to 4% and 5% at intervals of 10 minutes, in

accordance with a protocol described before.[2] If patients were able to produce sputum spontaneously, sputum induction proceeded as planned, spontaneous produced sputum was not analysed to minimize bias. Induced sputum was processed by the whole-sample technique using dithiothreitol (DTT).[19] Total cell numbers were determined by counting manually in a Bürker counting chamber. Samples containing less than 50% viable cells, as assessed by Trypan blue dye exclusion, were excluded from analysis. The remaining samples were centrifuged for 5 minutes at 700*g*. Cells were then separated from the supernatant and cytocentrifuged at 500rpm for 2 minutes, dried and stained with Romanovski and Jenner-Giemsa. Squamous and non-squamous, macrophages, lymphocytes, neutrophils and eosinophils were identified. Differential counts are expressed as a percentage of non-squamous cells. For differential cell counts 200 non-squamous cells were enumerated in samples with more than 10% eosinophils. The number of non-squamous cells is increased to 500 or 1000 cells if the percentage of eosinophils is between 1% and 10% or less than 1% respectively. Sputum samples containing more than 75% squamous cells on differential cell counting were excluded from cell differential analysis. Supernatants were re-centrifuged and stored in aliquots at -80˚C until analysis.

MPO was measured by ELISA (DY3174; R&D Systems, Abingdon, UK)in sputum supernatant with lower limits of detection of 1 ng/ml being 2-fold the background absorbance.[20] Granzym A was determined with a PeliKine ELISA following the supplier's recommendations (Sanquin, Amsterdam, the Netherlands) lower limit of detection 41.25 pg/ml. IL-6, IL-8, IL-1β and TNF-α were determined by Luminex (BioRad reagents, Veenendaal, the Netherlands) lower limit of detection 0.12pg/ml, 0.093pg/ml, 0.5pg/ml and 0.28pg/ml respectively. Samples were measured in one assay run to limit variation. In all assays a bias by the sulphur-bridge reducing reagent DTT was ruled out based upon appropriate controls. To that end we showed for all parameters that a 1 in 50 dilution is required to dilute out effects of DTT. At dilutions ≥1 in 50 dilution the samples diluted out properly. Spike recoveries were between 80-100% for most.

MMP-8 and MMP-9 activity assays (Amersham Biosciences; Buckinghamshire, UK) were performed according to the recommendations of the supplier. These assays measure both active (mature) MMP as well as total MMP (already active plus activatable [*ie*, latent] pro-MMP) activity, but are insensitive to proteinase inhibitor complexes. In brief, MMP-8 or MMP-9, is captured by a specific antibody that has been immobilized on a microtiter plate. The amount of active MMP is measured directly by the incubation of the captured MMP with modified pro-urokinase (Ukcol), and subsequent activation of Ukcol is quantified by a chromogenic substrate (S-2444). Colour development is recorded at 405 nm at different time intervals. Total MMP activity (*i.e.*, the pro-MMP and active MMP forms) is assessed through the activation of pro-MMP by pre-incubation with 0.5 mmol/L p-aminophenylmercuric acetate for 2 h at 37°C before the addition of modified Ukcol and chromogenic substrate. Activity is expressed in

recombinant enzyme equivalents (recEEs) in nanograms per mL. The lower detection limits were 5.0 ng/mL recEE for MMP-8 and 2.7 ng/mL recEE for MMP-9.
DTT may interfere with MMP activity. In order to study the effect of DTT on MMP activity, standard curves of appropriate recombinant proteins were incubated with 0.1% DTT (Sputolysin) for 20 min at room temperature under the same conditions as sputum samples during processing. DTT concentration and incubation time was based on an earlier optimization procedure.[4] An incubation time longer than 20 minutes did not result in a higher recovery but may interfere with MMP activity. In order to study the effect of DTT on MMP activity, standard curves of appropriate recombinant proteins were incubated with 0.1% DTT (sputolysin) for 20 minutes at room temperature under the same conditions as sputum samples during processing. The presence of DTT did not affect MMP activities, indicating that DTT had no effect on the immunocapture activity assays used in this study (data not shown).

Statistical analysis

As being a proof of concept study, sample size was based upon a pilot trial design.[21] We decided to include 12 evaluable patients in each arm. Statistical evaluations were accomplished with Mann-Whitney test for difference in change amongst both groups in continuous variables and Wilcoxon test for difference in change within groups in continuous variables. Chi square test was used for dichotomous variables. Possible associations between sputum MPO, IL-6, IL-8 and cellular content of sputum were evaluated by Spearman's correlation. A p-value <0.05 was considered significant. SPSS 20.0 for PC (IBM) was used for the statistical analyses.

Results

One hundred and eleven patients with COPD were screened. Fifty-four patients refused to participate and 11 patients did not meet the inclusion criteria. One patient had an exacerbation during the run-in phase, and four patients had worsening of symptoms after

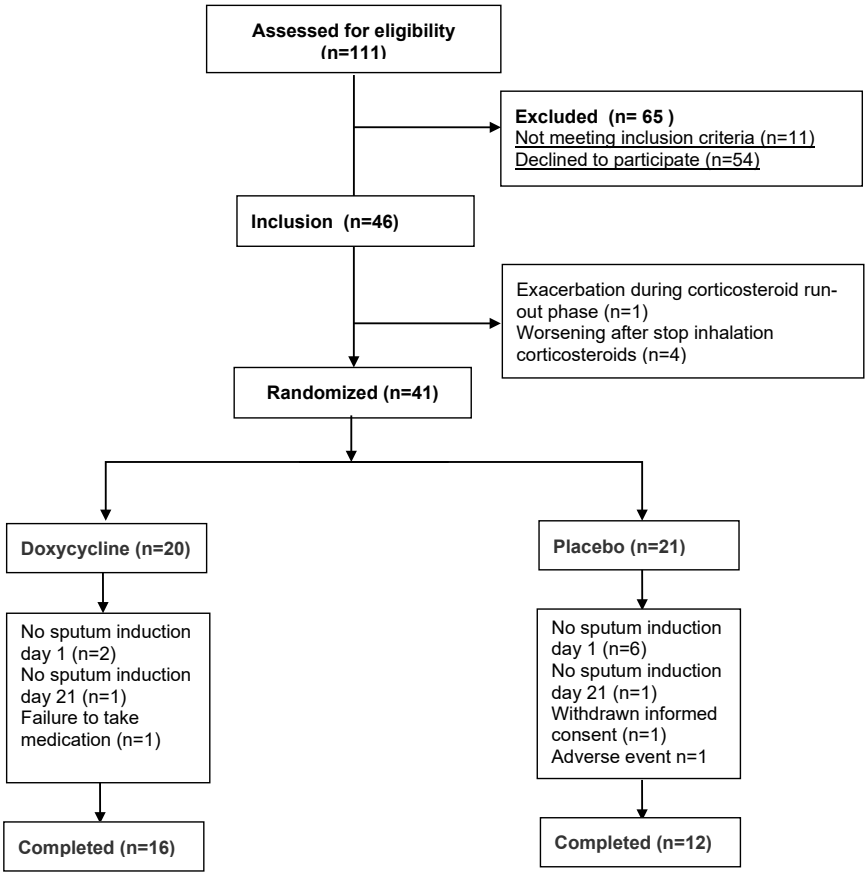

Figure 1 trial profile

Hence, 41 patients were randomized. In the doxycycline group two patients at day 1, and 1 patient at day 21 failed to produce sputum. In the placebo group six patients at day 1 and one patient at day 21 were not able to produce sputum after induction (p=0.108). One patient withdrew consent and one patient was excluded because of systemic corticosteroid because of non-bacterial arm inflammation in the placebo group, and one person failed to use his medication in the doxycycline group. One adverse event was reported in the placebo group. No adverse events were reported in the doxycycline group. The adverse event reported was non-bacterial inflammation of the arm and treated with systemic corticosteroids. Therefore study medication was discontinued.
The groups were similar with respect to age, gender, smoking history, use of inhaled corticosteroids, baseline sputum inflammatory markers, baseline cellular sputum components and lung function parameters although differences were found for baseline in FVC (Table 1).

Table 1: Baseline characteristics

	Placebo n=21	Doxycycline n=20	p-value
Age (years)	70 (63-75)	67 (63-72)	0.620
Male gender (n, %)	18 (85.7)	16 (84.2)	0.894
BMI (kg/m2)	26.5(24.7-29.7)	25.5(24.1-27.5)	0.303
FEV1 (L)	1.63(1.39-2.08)	1.88(1.64-2.17)	0.246
$FEV1\%_{pred}$	59(54-70)	60(52-66)	0.979
FVC (L)	2.94(2.70-3.35)	3.53(3.08-4.15)	0.023*
$FVC\%_{pred}$	75(69-87)	88(77-104)	0.055
FEV1/FVC ratio	55.6(43.4-63.0)	54.6(46.7-63.5)	0.732
Current smokers (N, %)	4 (19)	8 (40)	0.141
Pack years (N)	32(20-40)	37(24-46)	0.329
Number of exacerbations last year (n)	0(0-1)	0(0-1)	0.663
ICS usage (N, %)	11(52.4)	8(40)	0.473
SABA treatment (n, %)	6(28.6)	4(20.0)	0.595
LABA treatment (n, %)	11(52.4)	12(62.0)	0.823
SAMA treatment (n, %)	1(4.8)	0(0)	0.240
LAMA treatment (n, %)	12(57.1)	11(55.0)	0.890

All data are represented as median (IQR) unless specified otherwise
BMI: body mass index, FEV1: Forced expiratory volume in 1 second, FVC: Forced vital capacity. ICS: inhalation corticosteroids, SABA: Short-acting beta agonist, LABA: Long-acting beta agonist, SAMA: Short-acting muscarinic antagonist, LAMA: Long-acting muscarinic antagonist

Doxycycline as compared to placebo did not influence sputum MPO, IL-6 and IL-8 levels as well as MMP-8 and 9 activity at day 21 (Table 2 and Figure 2).

Table 2: Sputum biomarkers

	Placebo (n=12)			**Doxycycline (n=16)**		
	T= day 0	T= day 21	P-value	T= day 0	T= day 21	P-value
MPO (μg/ml)	9.2 (2.1-100.8)	6.9 (4.8-36.6)	0.768	6.1 (4.4-27.9)	6.4 (2.2-21.3)	0.910
IL-6 (pg/ml)	10.5 (0.1-226.7)	38.7 (0.9-232.9)	0.767	16.0 (4.8-102.8)	12.7 (1,0-62.9)	0.570
IL-8 (pg/ml)	2155 (521-4435)	1554 (543-10750)	0.718	797 (297-2945)	1268 (284-4033)	0.880
MMP-9 (mg/ml)	67.8 (26.9-198.7)	53.2 (10.8-108.8)	0.488	40.3 (8.4-146.7)	29.7 (7.7-96.7)	0.520
active MMP-9 (mg/ml)	7.0 (5.2-13.6)	6.8 (1.5-9.9)	0.326	2.8 (2.0-13.0)	3.1 (1.7-5.7)	0.631
MMP-8 (mg/ml)	247.6 (185.6-458.1)	265.7 (151.7-397.6)	0.488	275.9 (147.2-443.9)	264.2 (163.3-323.9)	0.468
active MMP-8 (mg/ml)	256.8 (147.9-518.9)	289.4 (151.2-422.6)	0.954	241.1 (123.2-395.9)	201.7 (102.7-348.9)	0.548

All data are represented as median (IQR).
Wilcoxon test for difference in change within groups in continuous variables was used.

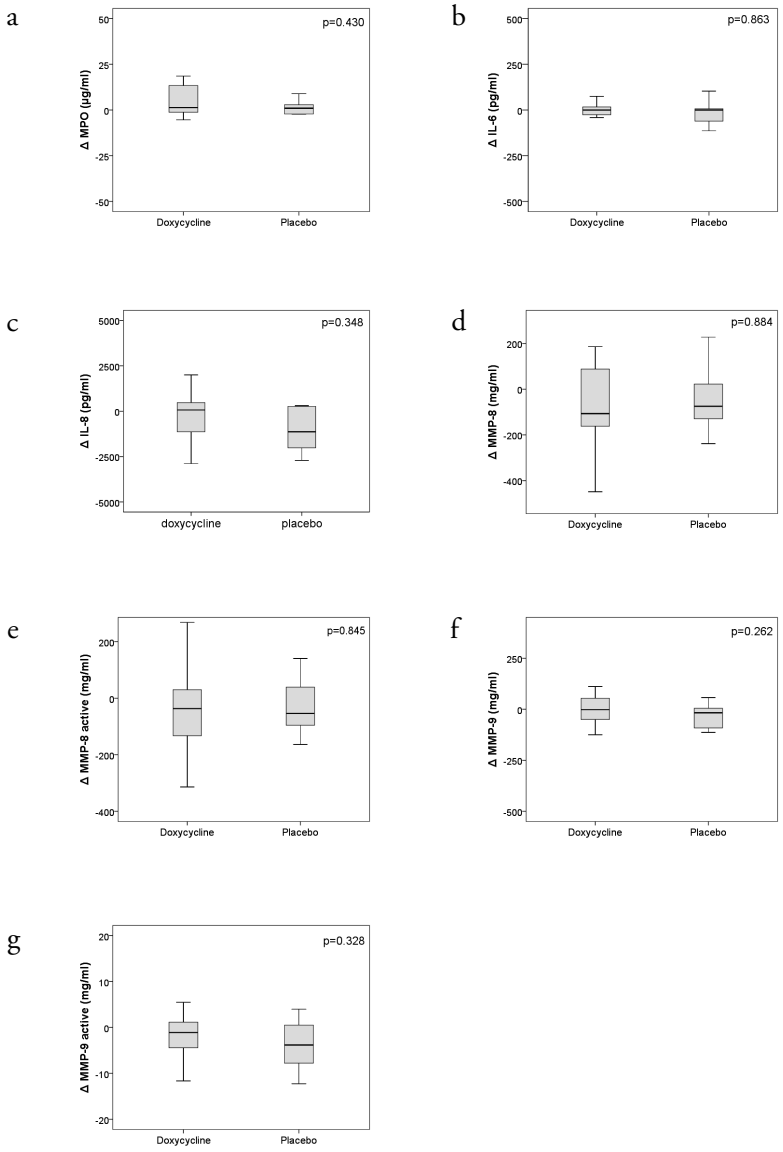

Figure 2: a) Delta sputum MPO concentration (day 0-21), b) delta sputum IL-6 concentration (day 0-21), c) delta sputum IL-8 concentration (day 0-21), d) delta sputum MMP-8 concentration (day 0-21), e) delta sputum MMP-8 active concentration (day 0-21), f) delta sputum MMP-9concentration (day 0-21), g) delta sputum MMP-9 active concentration (day 0-21)

Biomarker levels were similar in the doxycycline and placebo group at baseline as well as at day 21(Table 3). TNF-α, IL-1β and Granzym-A were all below the detection threshold of the assay for both time points. Doxycycline did not influence systemic inflammation represented by serum CRP, numbers of peripheral blood neutrophils or total leucocyte count (Table 3).

Table 3: Cellular components in sputum blood and serum CRP

	Placebo(n=12)			**Doxycycline(n=16)**		
	T=0	T=21 days	P-value	T=0 days	T=21 days	P-value
Total sputum cell count	15.5	12.1		17.8	13.3	
($x10^6$/ml)	(8.51-22.44)	(9.8-18.3)	0.705	(12.0-25.1)	(7.6-17.0)	0.108
Sputum neutrophil cell count	9.0	7.6		9.4	6.1	
($x10^6$/ml)	(4.5-14.2)	(3.4-11.4)	0.545	(5.1-18.4)	(3.1-10.0)	0.198
Relative sputum neutrophil	56.8	55.4		65.3	56.3	
count (%)	(37.0-69.3)	(46.0-61.9)	0.762	(37.7-74.5)	(35.9-62.0)	0.358
Total white blood cell count	7.3	7.1		7.2	6.6	
($x10^9$/ml)	(6.1-8.5)	(6.1-8.4)	0.945	(6.1-7.8)	(5.3-7.7)	0.241
Total blood neutrophil count	4.1	4.1		4.5	4.0	
($x10^9$/ml)	(3.5-5.0)	(3.3-4.6)	0.982	(3.7-4.8)	(3.4-4.6)	0.418
Relative blood neutrophil count	57.8	56.0		58.7	56.9	
(%)	(53.5-64.0)	(52.7-59.6)	0.890	(51.3-60.8)	(50.3-65.8)	0.705
Serum CRP (mg/L)	4.40	4.0		4.3	2.90	
	(2.80-5.70)	(2.40-6.60)	0.854	(1.70-6.30)	(1.80-4.40)	0.380

All data are represented as median (IQR), Wilcoxon test for difference in change within groups in continuous variables was used. CRP: C-reactive protein

Clear correlations were found between the number of neutrophils in sputum and sputum MPO at the start of the study (r=0.553 [p=0.005]) and at day 21 (r=0.544 [p=0.006]). Doxycycline therapy did not improve lung function parameters. Median change in FEV1 (L) in doxycycline group was -0.11 L (IQR 0.29-0.9L) whereas placebo had a median decrease of -0.3L (IQR -0.12-0.5L) [p=0.623]. Median change in FEV1 as percentage of predicted neither differed between doxycycline -4 %(IQR -10.0-3.0%) and placebo -1 %(IQR -4.0-2.0%) [p= 0.812]. FVC in litres as well as in percentage of predicted did not change within both groups, doxycycline -0.09L (IQR -0.32-0.1L) vs placebo -0.07L (IQR -0.19-0.14L) [p= 0.657], respectively, and doxycycline -2 %(IQR -8-3%) vs placebo -2%(-4.0-5.0%) [p= 0.623].

Discussion

COPD is a complex disease involving many types of immune responses that recruit many types of innate and adaptive immune cells, yet it is predominantly recognized as a neutrophilic inflammatory disorder.[3;22] MPO is a well-known marker of neutrophil presence as well as activation.[23;24] It is a heme-containing peroxidase expressed abundantly in neutrophils and to a lesser extent in monocytes. MPO is one of the principal enzymes released from secondary granules following neutrophil activation.[24] Although the generation of oxidants by MPO is beneficial in terms of the immune response to invading pathogens, there is considerable evidence that inappropriate stimulation of oxidant formation can result in host tissue damage.[25] Induced sputum represents the cellular pattern of the mucosa of the lower airways, and analysis of this sputum composition makes non-invasive measurement of airway inflammation possible[1]. Therefore, sputum MPO may be a potential non-invasive biomarker that reflects the severity or prognosis of COPD. A potential novel way to oppose this neutrophilic inflammation is doxycycline.[17;18;26] Doxycycline, besides its antibiotic properties has been shown to interfere with aspects of neutrophil-mediated inflammation as well as through inhibition of MMP's.[27] Although these anti-inflammatory effects of doxycycline never has been described in respiratory diseases, these effects where described in other chronic diseases such as abdominal aneurysm (AA) and periodontitis.[17;18,28] Several parallels exist between AA, periodontitis and COPD.[29;30] Both with regard to risk factors as well as to the underlying pathophysiology in which persistent inflammation and excess matrix turnover play a pivotal role.[29] It was therefore thought that doxycycline might reduce chronic inflammation in COPD. Therefore we designed this randomized controlled clinical trial that specifically investigates the anti-inflammatory effect of doxycycline on sputum inflammatory markers in stable patients with COPD without bacteria in their sputum. MPO, our primary outcome was not influenced by doxycycline treatment.

These findings are in line with an earlier trial which showed no effect of 100mg of doxycycline a day on MPO levels in patients with previous coronary artery surgery.[31] However, another study showed a decrease of MPO levels in obese patients with type 2 diabetes mellitus.[32] Although none of these trials studied the specific effect of doxycycline on MPO levels in sputum of stable patients with COPD, a possible explanation for our results might be that our population was specifically selected to be not colonized by PPM's. This is further emphasized by the relative low levels of MPO at baseline compared to other studies.[1] As is known levels of MPO as well as other pro-inflammatory cytokines are increased by higher bacterial load in sputum.[20] Another explanation could be the length of antibiotic treatment. Patients were only treated for 3 weeks, yet this seems not likely as another trial only treated patients with doxycycline for 10 days and saw a significant reduction in MPO levels in nasal secretions after 20 days of doxycycline using similar dosage as in our trial.[33]

Our data showed no effect of doxycycline on neutrophil content as well as on the amount of MMP8 and MMP9 in sputum. This is in contrast with earlier research in which doxycycline dose independently reduced MMP8 and MMP9 due to a reduced neutrophil content of the aneurysmatic wall of the aorta after two weeks of doxycycline (50, 100 or 300 mg once a day) in patients who underwent elective replacement surgery of the abdominal aneurysmatic aorta.[17;18]This difference in local inflammation by MMP-8 and MMP-9 can be explained by the low cell count as well as a low percentage of neutrophils in the induced sputum at the beginning of study compared to previous trials.[1;20] This might be due to the absence of bacterial colonization in our patient group. Another possibility is that doxycycline has a different mode of action in the lung compared to the aorta or periodontal disease.

IL-6 plays an important role in the progression of COPD. It is known that patients with stable COPD have higher plasma levels of IL-6 compared to healthy volunteers and levels of circulating IL-6 have also been shown to be associated with lung function impairment.[34] Tetracyclines are known inhibitors of IL-6.[35] In our trial IL-6 levels were not affected by doxycycline. This might be due to the relative low levels of IL-6 at the start of the study.[36] As is known IL-6 levels correlate to bacterial load in sputum.[37] Therefore our hypothesis is that doxycycline does not have an anti-inflammatory effect on IL-6 levels, yet the low levels observed before and after treatment with doxycycline are caused by the absence of bacteria. Yet this does not explain the effects of doxycycline observed in earlier research.[35]

In our trial we did not observe effect of doxycycline on lung function parameters nor on systemic inflammation. This is in contrast with earlier results shown by Dalvi et al.[13] These differences found may be explained by the shorter treatment period and the fact that they did not exclude patients with PPM in sputum, and that therefore the observed effect is antibacterial, this might also explain the differences found in lung function.

Modulation of inflammation in COPD might be one of the future keystones in the treatment of COPD. Although in our study we were not able to show effect of doxycycline on inflammation, other possible candidate to modulate inflammation in COPD are the group of macrolides, a group of broad spectrum antibiotics which has been shown to have significant immunomodulatory effects related to the macrolytic lactone ring.[38] Extensive research has been done with azithromycin showing a reduction in pro-inflammatory interleukins and TNF-α resulting in a decreased number of exacerbations and an increased time to next exacerbation.[39;40]

Shortcomings of this study are the small sample size and a high percentage of lost to follow-up due to the fact that subjects could not evacuate sputum during sputum induction. This is a strong argument that fits the assumption that in our trial we are looking at a distinct phenotype of COPD in which there is little bacterial inflammation and mucus hypersecretion. Although there is a certain amount of inflammation present, this is not influenced by doxycycline. Another shortcoming of this study is the absence

of a sputum culture at the start of the study. However earlier research showed that the amount of inflammation correlates well with the presence of bacteria.[4;41] This combined with the fact that at baseline low inflammation levels in sputum were present and the absence of PPM's in sputum cultures in the prior year, advocates the fact that our subjects were not colonized by PPM's.

This is the first placebo controlled randomized clinical trial that investigates anti-inflammatory effect of doxycycline in COPD patients. Strength of this study are the good correlation of the individual biomarkers as well as the methodology. Sputum was induced by the same study nurse at the same time of the day, so fluctuations of sputum quality throughout the day were excluded. A weakness of this study is the pilot design. Due to heterogeneity of the population regarding types of inflammation in COPD a higher number of patients might be needed to draw a more firm conclusion regarding doxycycline being an anti-inflammatory drug in COPD.

However in this study we were not able to show effect of a 3 week course of doxycycline on sputum MPO, or on any of the other sputum and serum inflammatory markers as well as lung function parameters in stable COPD patients without airway bacterial colonization. In spite of current findings, it might be possible that the tetracycline analogue doxycycline is able to attenuate inflammation in COPD patients with a latent infection or a more outspoken pro-inflammatory phenotype. On the basis of the current study we cannot recommend doxycycline as an anti-inflammatory agent for patients with stable COPD.

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CHAPTER 7

7

General conclusion and future perspectives

General discussion

The role of antibiotics in COPD and especially in acute exacerbations of COPD (AECOPD) is controversial. In AECOPD antimicrobials have been proven effective, but these improvements are marginal and have no effect on mortality.[1] This can be explained by the fact that exacerbations are often triggered by viral infection or are associated with eosinophilic inflammation.[2] Despite these findings current guidelines still advocate the use of antibiotic therapy in AECOPD if a patient suffers from sputum purulence in combination with increased dyspnoea and/or increase of sputum volume.[3] However, this antibiotic prescription strategy has several shortcomings. First, sputum purulence has not been shown to be a reliable marker for bacterial infection and assessment by patients for sputum purulence has been shown to yield inconsistent results.[4;5] Therefore, more reliable markers are necessary to identify those patients who will benefit most from antibiotic treatment in order to avoid complications and antimicrobial resistance associated with overuse of antibiotics.[6] Currently only Procalcitonin (PCT) has prospectively been evaluated as biomarker in AECOPD to determine if a patient needs antibiotic treatment.[7] However, PCT is expensive and health care providers have not introduced the test widely in routine clinical practice.[8] Another biomarker that could be helpful in predicting who will benefit from antibiotic therapy is C-reactive protein (CRP). CRP levels are significantly higher during AECOPD compared to baseline levels, especially if a bacterial infection is likely.[2] Previous studies have shown that patients with an AECOPD with an elevated CRP level showed a trend to benefit more from antibiotics than patients with low CRP values.[9;10] Therefore, in the CATCH study the primary objective was to evaluate whether CRP could act as a biomarker to initiate or to withhold antibiotic treatment in AECOPD without compromising safety.

CRP guided antibiotic treatment in severe AECOPD

In Chapter 2 we describe the trial that examines whether it is possible to reduce antibiotic treatment in patients with AECOPD admitted to hospital using the CRP-guided antibiotic treatment regime. Earlier studies have shown that the majority of exacerbations are provoked by viral infection, or are associated with eosinophilic inflammation.[2;11] Hence, based upon this evidence, antibiotic treatment of AECOPD might be redundant and inappropriate for most patients with AECOPD. However a recent Cochrane review argues that antibiotics in COPD can reduce treatment failure rates.[1] Nevertheless, it should be noted that studies included in this review have some limitations regarding the use of concomitant corticosteroid use and populations consisted of heterogeneous groups of in and out patients. Moreover, in the review it has been concluded that the inconsistent effects observed call for research into clinical

signs and biomarkers that can help identify patients who would benefit from antibiotics while withholding antibiotics for patients unlikely to benefit from antibiotics.[1] For this purpose, the CATCH study was designed and conducted. The real-life design of this trial is a unique feature; patients with all GOLD classes were included, as well as patients who were pre-treated with antibiotics and/or systemic corticosteroids, and finally, patients needing assisted (invasive and non-invasive) ventilation were not excluded from participation. This makes this study widely applicable to all patients with AECOPD admitted to hospital. Additionally, all patients were treated with systemic corticosteroids and bronchodilators. We followed patients for median one year, showing short as well as long-term safety of the CRP-guided treatment. This study thereby provides evidence that CRP guided antibiotic treatment for patients with AECOPD admitted to hospital is able to reduce antibiotic prescription at admission without compromising safety. Although our primary endpoint was not met, a significant reduction of antibiotic prescription at admittance was achieved. One of the reasons that this endpoint was not achieved could be the relative low number of patients with sputum purulence in the GOLD-guided group compared to the CRP-guided group as well as compared to other studies.[10;12] The observation that patients in our study had a high level of treatment failure might reflect the severity of disease in the cohort included. This is illustrated by the fact that relapses are common in patients with COPD admitted to hospital and antimicrobial treatment might not prevent this, especially not among those with low inflammatory markers.[9;13] However, there was a striking difference between patients treated with antibiotics and those that were not, in the CRP-group as well as in the GOLD-group. Patients who are treated with antibiotics had lower treatment failure rate. The most convenient way to explain this striking difference is attributing it to antibiotic treatment. However, this assumption might be too simple, as AECOPD has different aetiological perpetrators that cannot be treated with antibiotics whereas these causes, such as viral infection and eosinophilic inflammation are known to influence outcome in AECOPD.[14;15] Recently, a study investigating the potential role of CRP as an add on to clinical assessment, for the initiation or withholding of antibiotics in out-patients with AECOPD was published.[16] The authors showed a reduction of 20% (57.0 vs 77.4%) in antibiotic consumption in the first 4 weeks after randomization compared to usual care. Although this is considerably higher compared to our study, they looked at antibiotic consumption in the 4 weeks following randomization. Another explanation might be that in this study, patients in the intervention group were treated with antibiotics based upon other cut-off values and clinical judgement. This is a potential weakness of this study as the authors did not describe how many patients were treated based upon their CRP level and how many patients were treated based upon clinical signs and symptoms. The interpretation of clinical signs and symptoms is subjective and leaves room for interpretation, especially if this includes sputum purulence. Nevertheless, sputum purulence might be a predictor of clinical failure in patients not treated with antibiotics

although CRP (cut off value ≥40mg/L) might be a better predictor.[17] This last finding is in line with earlier work showing that doxycycline was superior compared to placebo in patients with an elevated level CRP (cut off value ≥50mg/L); doxycycline had a better and lasting treatment effect if given to patients with a high CRP level (cut off value ≥50mg/L) compared to placebo whereas it was equivalent in patients with a CRP value less than 50 mg/L. In the light of this evidence we think it would be unjustified to advocate antibiotic treatment for all patients with AECOPD. Patients should therefore be further stratified according to their individual inflammatory subtype and underlying etiologic cause of the exacerbation.

Eosinophilia in AECOPD

Airway eosinophilia is associated with a broad range of pulmonary diseases in small and large airways.[18] Traditionally, airway eosinophilia in obstructive pulmonary disease is associated with asthma; however, evidence suggests that up to 40% of the patients with COPD have some form eosinophilic inflammation.[19] Currently, the role of eosinophils in the pathogenesis has not been completely elucidated. Several different hypotheses have been proposed but none of these explain why some patients with COPD have eosinophilic inflammation whereas others do not. One of the hypotheses state that that under the influence of viral infection or tobacco smoke, epithelial cells produce thymic stromal lymphopoietin (TSLP), interleukin (IL)-33, granulocyte-macrophage colony stimulating factor (GM-CSF) and Chemokine (C-C motif) Ligand 5 (CCL5). TSLP and IL-33 recruits T helper-2 cells and type 2 innate lymphoid cells (ILC2) Th2 cells and ILC2 cells produce IL-5.[20;21] IL-5 is a cytokine that is involved in the maturation, chemotaxis, degranulation, and cytokine production of eosinophils.[22] CCL5 may attract eosinophils in the lungs while GM-CSF stimulates their survival.[21] Another possible source of eosinophilia in COPD is defective efferocytosis of apoptotic eosinophils, leading to an increased number of sputum eosinophils. Subsequently, with failure of the apoptotic pathway, these eosinophils become necrotic and release toxic intracellular pro-inflammatory mediators leading to more influx of eosinophils. An increase of defective efferocytosis has been related to severity and frequency of COPD exacerbation.[23] Regardless the source of eosinophil accumulation in the airway and lung parenchyma, these cells perform their harmful work by releasing proteins capable of causing tissue damage and bronchoconstriction.[24] Eosinophilic inflammation in COPD can be measured using induced sputum with bronchial wash, bronchoalveolar lavage and bronchial biopsies. Sputum induction is the least invasive of all these methods.[25] Although this method is safe, it has several disadvantages such as being time consuming and expensive; moreover, it has only moderate repeatability.[26;27] Peripheral blood eosinophilia has been shown to be a good surrogate marker for sputum eosinophilic

inflammation.[2] In addition it was shown that patients with predominantly eosinophilic inflammation could be identified using a peripheral blood eosinophilic count with a cut-off value ≥2% of total peripheral white blood cell count (WBC).[2] From these observations, we hypothesized that a high number of eosinophils may be a marker for initiating or withholding systemic corticosteroids in AECOPD. We found only two published trials showing that blood eosinophilia could be used as a biomarker to direct systemic corticosteroid therapy.[28;29] Although the design of both studies and population studied was quite different, both studies showed a significant reduction of corticosteroid use without an increase of adverse events. Despite these promising results, still some controversy surrounds eosinophilic inflammation in COPD as the role of eosinophils in COPD has not been fully elucidated.[30] Despite these uncertainties with the use of eosinophilic inflammation as a biomarker, it might be useful to identify patients that need treatment with corticosteroids or other eosinophilic inflammation modifying agents, such as small molecules that specifically target the cascade of eosinophilic inflammation. Examples of these agents are monoclonal antibodies against IL-5 or IL-5 receptor such as Mepoluzimab, Benraluzimab and Reslizumab.[31] In severe eosinophilic asthma these drugs have been proven to be effective in reducing exacerbations and some may have a glucocorticoid sparing effect.[31] However, compared to asthma the results of anti-IL5 therapy in COPD have been poor with only a small reduction of exacerbation frequency and some improvement in lung function. Benefits were associated with reduction in eosinophilic inflammation.[31] Additional research is needed to determine the role of monoclonal antibodies against IL-5 or its receptor in the field of COPD. Another critical point regarding the use of eosinophils as biomarker is that it can be influenced by many external factors such as nutrient intake, exercise, concomitant drugs, and the timing of testing.[32] These factors could falsely classify a person as non-eosinophilic thereby withholding a beneficial effect of corticosteroids. Nevertheless in stable COPD blood eosinophilia had good agreement between two measurements over a median of 28 days (Intra Class Correlation coefficient 0.8 95% CI =0.66-0.88).[33] In addition, the blood eosinophil biomarker status (using ≥2% WBC as a cut off) in stable state has an odds ratio of 5.5 (95%CI 2.7–11.0) for predicting blood eosinophil biomarker status at exacerbation. Moreover if a patient experienced a biomarker positive or negative exacerbation there is a good chance that a subsequent exacerbation occurs with the same biomarker status.[2] This shows that blood eosinophilia might be a useful tool in determining eosinophilic airway inflammation in AECOPD. This raised the question whether blood eosinophilia can also be used to predict outcome and prognosis in patients who are hospitalized with severe AECOPD. In chapter 3 we present the results of a post-hoc analysis. Here, we investigated the proposed surrogate marker for eosinophilic airway inflammation, blood eosinophilia ≥2% WBC at admittance, on the outcome of patients with AECOPD. We found that blood eosinophilia in patients with AECOPD was associated with a faster treatment success reflected by a shorter hospital

stay and lower early treatment failure rates. However, blood eosinophilia predicts a less favourable outcome on the long term in patients with blood eosinophilia ≥2% WBC - although the results were not significant in the ≥300 eosinophils/microliter group. Other research groups found similar results regarding short term prognosis.[34-36] Although we found a negative correlation between long term outcome and eosinophilia at admittance, a recent study showed no association between eosinophilia and hospital readmission in the next 12 months whereas others did.[34;35,37] The patients included in our post-hoc analysis represent a cross section of patients admitted with AECOPD, especially because patients, which were pre-treated with systemic corticosteroids and/or antibiotics, were not excluded from participation. Another strength of our study was that we tried to systematically exclude patients with asthma. Patients with a prior history of asthma were excluded, Inclusion criteria included having a smoking history of at least 10 pack years and a history of COPD confirmed by spirometry. Exclusion of asthma is especially important, as some claim that eosinophilia in COPD could be a manifestation of asthma with a fixed airway obstruction.[38] Another point that needs consideration is the high number of patients pre-treated with systemic or inhaled corticosteroids. This might have caused an underestimation of the number of patients observed with eosinophilia as corticosteroids rapidly reduce the number of circulating eosinophils.[39] Thereby leading to an additional underestimation of the current results. Another point of interest is eosinopenia. Eosinopenia defined as Eosinophils $<0.05\times10^9$/L in peripheral blood is known to be a diagnostic and prognostic factor in severely ill patients in the ICU.[40;41] Eosinopenia has also been used in AECOPD as part of the DECAF score.[42] The acronym DECAF stands for Dyspnoea, Eosinopenia, Consolidation, Academia and atrial Fibrillation. The DECAF scoring system has been developed to predict in-hospital mortality in AECOPD. Two other studies looked at the usefulness of eosinopenia for prediction of outcomes in AECOPD.[43;44] They found that patients with AECOPD admitted to hospital with eosinopenia had a worse prognosis compared to patients who had normal or high eosinophils regarding mortality and length of hospital stay and had a higher need for mechanical ventilation. Additionally, eosinopenia has been shown to be a sensitive and reliable marker for distinguishing between non-infectious and infection-associated sepsis in the intensive care unit setting.[45] This has not yet been shown in AECOPD. However it has recently been reported that patients with AECOPD who have high bacterial sputum load during AECOPD had a significant decrease in blood eosinophilic count compared to stable state, although there was no increase of blood eosinophilia in patients without bacterial infection.[46] This suggests that there might be a third group within the spectrum of eosinophilic inflammation; dichotomization of eosinophilic inflammation into normal and high in AECOPD according to Bafadhel may overlook the value of a very low eosinophil count in phenotyping of AECOPD.[2] Therefore, we argue that further research is needed to elucidate the role of eosinophils in AECOPD. It might be interesting to investigate

whether eosinopenia in combination with eosinophilia could be used for a new management algorithm for AECOPD regarding the prescription of systemic corticosteroid and/or antibiotics.

Pneumonia in AECOPD

Community acquired Pneumonia (CAP) is a frequent complication in patients with COPD.[47] However, diagnosing pneumonia in COPD can be challenging as exacerbations of COPD and CAP often co-exist and may symptomatically look alike.[48] Indeed, pneumonia is often misdiagnosed as AECOPD or vice versa.[49] Unfortunately using clinical signs and symptoms cannot differentiate between both. However it is important to make the correct diagnosis as misdiagnosing CAP could have major implications for an individual patient whereas over diagnosing CAP could lead to unnecessary use of antibiotics which in turn leads to extra costs, side effects and antimicrobial resistance.[6] As pneumonia and AECOPD often have bacterial aetiology, biomarkers can detect this type of inflammation.[2;50] C-reactive protein (CRP), Procalcitonin (PCT) and to a lesser extent, serum amyloid A(SAA) are biomarkers that are used in the detection of bacterial AECOPD and pneumonia.[2;51-53] Using these biomarkers as a diagnostic tool may increase the ability to detect clinically relevant bacterial infections at an early stage of the disease. However, the discriminative power of CPR, PCT and SAA to distinguish between AECOPD and CAP is questionable. In current clinical practice chest X-ray is the most frequently used radiological test to detect pneumonia despite of its shortcomings.[54] A CT-scan on the other hand is currently considered the gold standard for the detection of pneumonia in COPD, however it is not always immediately available, and it delivers a higher radiation dose than conventional diagnostic X-rays.[55-57] We were therefore interested in biomarkers that could improve diagnostic accuracy of CAP in patients with AECOPD in combination with clinical assessment and chest X-ray using low dose CT thorax as the reference standard. In Chapter 4 we describe the results of an exploratory study in which CRP, PCT and SAA were correlated with radiological abnormalities compatible with acute-phase lung involvement in patients with AECOPD admitted to hospital using low dose CT (LDCT) in whom pneumonia was excluded using chest X-ray; additionally, we also investigated the interobserver variation in LDCT of the infiltrative changes. The 100 patients included in this sub study were participants of the study described in chapter 2. We found that 24 patients had radiological abnormalities consistent with acute-phase lung involvement. These patients had significantly higher biomarker levels compared to patients without radiological abnormalities. Although these differences were statistically significant, they did not result into an area under the ROC curve that would reflect sufficiently high discriminatory power (0.659-0.687). Sensitivity between 0.70-0.78 and specificity between 0.47-0.68 were low. Inter

observer variation regarding the LDCT was moderate. From our study, we conclude that biomarker levels between patient with radiological abnormalities and those without have insufficient discriminatory power to rule out the diagnosis of pneumonia in this population, despite the fact that there was a statistically different level of inflammation between the groups. Our study has some potential limitations; first of all, it should be regarded as exploratory due to a limited number of participants. Secondly the diagnosis pneumonia should be based upon the detection of potential pathogenic microorganisms or viruses in lung parenchyma along with radiological abnormalities. Unfortunately we only performed a random sputum culture at admission, which can be indicative of the pathogen causing the pneumonia but certainly this approach has its limitations.[58] An alternative approach would be the use of polymerase chain reaction (PCR). This technique has the potential to revolutionise the treatment of infectious disease as clinicians are provided with almost "real time" information regarding the pathogen and microbial load present in sputum. It has demonstrated superior diagnostic accuracy compared to standard culture.[59;60] Moreover PCR is able to detect both bacterial and viral pathogens in CAP as well as in AECOPD.[61;62] In the case of a mixed infection with viral as well as bacterial pathogens it might have added value, although challenging for interpretation. A potential flaw would be to differentiate between past and present infections, especially as PCR may remain positive up to five weeks post infection by some respiratory viruses. In other words detection does not necessary mean active replication or infection.[63] The role of quantification by measuring the number of copies present in the specimen may only partly compensate this challenge. Indeed, pneumonia as well as AECOPD can be caused by a combination of viral and bacterial infection.[64;65] However in some cases of pneumonia, a positive PCR can help determine the nature of the pathogen although in other cases additional information is required. Another potential way to identify a possible pathogen is taking a closer look to the radiological abnormalities on the LDCT. Especially viral pneumonias may have distinct CT- patterns.[66] Regrettably definite diagnosis cannot be achieved by using imaging features alone, as not all patients present with typical patterns.[66] It might be interesting to see whether patients with specific infiltrative changes more compatible with viral infection may have lower bacterial associated biomarkers such as PCT, CRP and SAA. However, it is questionable if we be able to reliably confirm or reject the presence of viral infection solely based only upon CT-patterns without the use additional (PCR) test results. Unfortunately, we could not answer these questions with the results of this study; additionally, our study was probably underpowered to find any relation regarding this research question). Nevertheless, combining serum biomarkers with real-time PCR data might improve correlation of biomarkers with radiological abnormalities thereby improving diagnostic accuracy of CAP in this specific patient population. We conclude that the radiological abnormalities can be present in absence of an infiltrate on chest X-ray in patients with AECOPD, however we are unable to reliably confirm or rule out

CAP using CRP, PCT or SAA. In addition, it is still unknown whether the radiological abnormalities observed in our study could be of clinical significance. Should they be treated as CAP or should we treat these patients according to their CRP level as was shown in chapter 2 and discard the radiological findings considering these as irrelevant? It would be interesting to perform an additional analysis on our data to see whether or not the prognosis of patients with AECOPD without infiltrative changes on their chest X-ray is changed if they have radiological abnormalities present on their LDCT although our study might be underpowered to find such a relation.

Symptom measurement in AECOPD

The burden of symptoms in COPD is an important factor in the outcome of COPD.[67;68] Especially dyspnoea is correlated with mortality.[69] It is therefore important to measure symptoms in COPD, especially to monitor improvements or worsening during AECOPD. Traditionally symptoms have been measured as part of quality of life questionnaires.[70] Although some questionnaires solely measuring symptoms, most of these questionnaires have not been validated. Recently the questionnaire EXACT-Pro was proposed. This questionnaire is a properly validated instrument measuring the most common symptoms in COPD.[71] However, in our opinion this questionnaire has one shortcoming as it is elaborate, and therefore less suitable for illiterate persons or those with low educational level. For that reason we developed the COPD Lower Respiratory Tract Infection Visual Analogue Scale (c-LRTI-VAS). The c-LRTI-VAS was used earlier to quantify symptoms in 223 patients with AECOPD. A modified version of the c-LRTI-VAS was validated and turned out to be a reliable tool for symptoms measurement in patients with bronchiectasis.[9;72] In chapter 5 we describe a study in which the c-LRTI-VAS was validated for the measurement of symptoms in stable as well as in exacerbating patients. Patients with AECOPD were included from the trial described in chapter 2. Patients with stable COPD where included during routine check-up visits in the outpatient department. Test re-test during stable phase showed a minor non-significant difference. A moderate intra-class correlation coefficient was observed. Internal consistency using Cronbach's α was good. Internal consistency increased when the item sputum purulence was deleted. The c-LRTI-VAS showed a strong correlation with two reference questionnaires, the Saint George Respiratory Questionnaire (SGRQ) and the Clinical COPD Questionnaire (CCQ).[70;73] Potential strength of this study is that patients with all 4 GOLD classes were included. Not all patient categories showed responsiveness, although this is probably explained by a type 1 error, as there were considerably less patients included with GOLD class 1 and 4 compared to class 2 and 3. Another limitation is the genesis of the c-LRTI-VAS, as we decided to use the Anthonissen criteria as backbone for the c-LRTI-VAS in combination

with fatigiue.[74] In retrospect, it might have been better to convene a focus group of physicians and patients to inquire about symptoms they consider most important. This might have prevented the use of sputum purulence as part of the c-LRTI-VAS which showed to be a less suitable marker in this questionnaire. Nonetheless, the c-LRTI-VAS showed proper validity, responsiveness to change and moderate to high correlation with other questionnaires and can therefore be used for monitoring disease or treatment effect in clinical trials.

Airway and systemic inflammation in COPD

Chronic airway and low-grade systemic inflammation are key to the progression of COPD and COPD-associated non-pulmonary co-morbidities.[75] One of the key cells involved in this inflammatory process is the neutrophil.[76;77] All patients with COPD have airway neutrophilia, regardless of clinical phenotype (chronic bronchitis, emphysema, and even eosinophilic COPD).[78] Neutrophils are recruited to lung tissue directed by cytokines such as IL-8, IL-6 and tumour necrosis factor-α (TNFα). These cytokines are produced by alveolar macrophages and epithelial cells under the influence of smoking, infections or air pollution.[79;80] A sustained neutrophilic response contributes to the disease process indirectly through perpetuation of the inflammatory response, and directly by contributing to airway remodelling and degradation of extracellular matrix (ECM).[81] This break down of ECM is due to a variety of granule proteins secreted by neutrophils such as myeloperoxidase, neutrophil elastase, proteinases, as well as MMP-8 and MMP-9, which leads to degradation of ECM.[78;82;83] If the exposure to noxious particles or gasses persists long enough it becomes self-perpetuating although the pathogenesis of this phenomenon is not yet fully understood.[78] Apart from smoking cessation, no other interventions have been shown to slow down disease progression. Therefore much effort has been made to find other ways to modify neutrophilic inflammation in order to stop COPD disease progression.[84] A potential candidate for the modification of neutrophilic inflammation are tetracyclines. Besides their antibiotic properties, tetracyclines have anti-inflammatory effects on neutrophilic mediators, neutrophil recruitment, inhibition of matrix metalloproteinase (MMP's), and may also suppress cytokines such as TNFα, IL-1 *β,* IL-6 and IL-8 under certain conditions.[85;86] All of these mechanisms are key mediators in COPD related inflammation and progression.[86] Doxycycline, a tetracycline analogue, improved lung function parameters and reduced the systemic inflammatory marker CRP, in patients with stable COPD. However, the effects observed in this study might be due to resolution of mild occult infection which was not excluded prior to initiation of doxycycline in this specific study.[87] In chapter 6 we describe a double-blind placebo-controlled proof of concept study which aimed to assess the anti-inflammatory effects of doxycycline on sputum and serum inflammatory markers in clinically stable COPD

patients without airway bacterial colonization. We were not able to show any effect of doxycycline on sputum inflammatory markers (myeloperoxidase, IL-6, IL-8, MMP-8 and MMP-9), cellular components of induced sputum, or systemic inflammation (blood cellular components or CRP). There were no changes in lung function. These findings are in contrast with a recent trial in which patients with COPD were treated with doxycycline for 3 months.[88] In this study a significant improvement in lung function, improvement in quality of life, and a response in some systemic markers of inflammation were found. Although the systemic markers used in this study were similar to ours, they were measured in serum instead of in sputum. Based on these results and an improvement of pulmonary function tests the authors concluded that this reflected an improvement of pulmonary inflammation due to the anti-inflammatory effects of doxycycline. This interpretation might be overstretched. First of all, the observed effects may be due to the resolution of mild occult infection as colonization or infection was not excluded before entry of the study. This might also explain the improvement of pulmonary function tests.[87] Secondly, systemic inflammation is not exclusively caused by COPD induced spill-over from multiple pro-inflammatory markers into the circulation but systemic inflammation is also seen in diseases such as atherosclerosis, gingivitis, and aetes.[89] This makes a systemic biomarker less suitable to monitor low grade inflammation in the lung. Our study also has some limitations, first its limited sample size made it impossible to perform analysis of responders vs non responders. It might be possible that there are subgroups of patients with COPD who might benefit from doxycycline therapy. Another limitation is the high number drop outs due to inability to evacuate sputum during sputum induction. This resulted into a selection of patients with a distinctive phenotype of COPD with little bacterial inflammation and hypersecretion. Despite the results shown by other authors, based upon our study and the limitations of the other studies described above we cannot recommend doxycycline as an anti-inflammatory agent for patients with stable COPD. In general, even though tetracyclines might have beneficial effect in some COPD patients with chronic inflammation. To identify patients with a specific phenotype, further research is needed. The study design we used might be helpful thereby selecting patients with hypersecretion and bacterial inflammation, using doxycycline in a sub-antimicrobial dose, or using chemically modified tetracyclines that have lost their antimicrobial activity, but have retained their anticollagenase activity.[66;90] Another group of antimicrobial agents that reduces inflammation in COPD are the macrolides.[91;92] Macrolides are primarily known for their treatment of acute bacterial driven exacerbations of COPD, yet macrolides also possess anti-inflammatory and immunomodulatory activity.[93] Historically these properties have been used to reduce disease progression of diffuse pan-bronchiolitis yet has also been used successfully in the treatment of bronchiectasis, a disease that has some features in common with COPD[94]. In COPD, effectiveness of macrolides has recently been shown in reducing the frequency of AECOPD yet only in a specific population.[95;96]

Besides, macrolides are able to reduce treatment failure with 20% in patients with severe AECOPD.[97] Unfortunately, maintenance treatment with macrolides do not seem to inhibit decrease of lung function nor does it reverse the disease. Macrolides have significant side effects and could lead to macrolide resistance.[95;98-100] However, despite these limitations' macrolides are useful for some patients with COPD.

Future perspectives

COPD is traditionally regarded as one disease entity. It has increasingly become clear that COPD is an umbrella term rather than a narrow and specific clinical entity. Clearly, all phenotypes of COPD have airway obstruction as a common denominator. In the past this led to a standardization of treatment options based upon studies of the group as a whole leading to a standard therapy for every exacerbation. Today, we endorse the concept that COPD is a heterogeneous disease and therefore COPD treatment should no longer be based upon the common denominators of airway obstruction but instead treatment should also aim at underlying pathophysiological processes. This requires new diagnostic strategies and tests. However, we do not argue that old methods should discarded. We therefore advocate the use and combination of old methods such as, clinical characteristics by clinical measurements as well as reflected by questionnaire-based scoring systems, physiologic criteria, radiographic techniques, and microbiological diagnostic tests including cultures and PCR. Classical evaluations should be accompanied and combined with new methods such as comprehensive biomarker assay of blood, sputum exhaled breath condensate, nasal swabs, and broncho-alveolar lavage as well as microbiome analysis, genotype surveys, metabolomics and proteomics analysis. Using cluster analysis for this comprehensive set of data could lead to a further sub classification of COPD which in turn could lead to useful new therapeutic strategies for some patients. Another outcome of this strategy could be the more rational use of traditional COPD medication such as corticosteroids and antibiotics to achieve maximal benefit at the cost of minimal side effects. Using these new techniques could also be useful for the early identification of patients at risk for treatment failure in the period following exacerbation. Early relapse is a significant problem after severe exacerbations as was also seen in our study. Unfortunately a comprehensive system of COPD sub classification is still a long way to go, but working with the currently known sub classification of AECOPD as proposed by Bafadhel could be an interesting start [2]. Additionally, a subdivision of 3 types of eosinophilic inflammation (eosinopenia, normal eosinophil count, and an eosinophilic group) could be made for additional typing. This requires an international trial of patients with severe AECOPD treated with systemic corticosteroids according to their eosinophilic status and treated with antibiotics according to their CRP level compared to standard care in which patients

are treated with corticosteroids and antibiotics. Sample size should be calculated based on primary endpoints in this trial: safety profile should be high, a non-inferiority design with a small margin of inferiority. The difference in the cumulative dose of systemic corticosteroids and antibiotics consumed within 30 days after the exacerbation should be among the co-primary or secondary endpoints.

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CHAPTER 8

Summary

Summary

In **chapter 2** we presented the results of the CATCH trial (CRP-guided Antibiotic Treatment in acute exacerbations of COPD admitted to Hospital). In this trial patients hospitalized with an acute exacerbation COPD were randomized to receive antibiotics based according the GOLD strategy (patient reported sputum purulence) or according to the CRP (≥50 mg/L) strategy. Hundred and one patients were randomized to the CRP-group and 119 to GOLD-group. Fewer patients in the CRP-group were treated with antibiotics 31.7% compared to 46.2% in the GOLD-group (p=0.028) (adjusted OR 0.178; 95%CI 0.077-0.411). Thirty-day treatment failure rate was equal (CRP-group 44.5% vs GOLD-group 45.5%; (p=0.881) (adjusted OR 1.146; 95%CI 0.649-1.187) as was time to next exacerbation (CRP-group 32 days, versus GOLD-group 28 days (p=0.713) (adjusted HR 0.878; 95%CI 0.649-1.187). Length of stay was similar in both groups (CRP-group 7 days versus GOLD-group 6 days (p=0.167). On day 30 no difference in symptoms score, quality of life or serious adverse events was detected. In the present study no differences between both groups in adverse events were found. Based upon our results we concluded that the use of CRP as a biomarker to guide antibiotic treatment in severe AECOPD leads to a significant reduction of antibiotic treatment without compromising safety profile. Further research is needed for the generalizability of these findings.

In **chapter 3** we presented the results of a post-hoc analysis of 207 patients included in the CATCH trial. In this study we analysed the impact of blood eosinophils (≥2% of total white cell count and absolute eosinophil count ≥300 cell/microliter) at admission on clinical outcome in patients with severe AECOPD. Thirty-nine (18.8%) had eosinophilia ≥2% and 23 patients (11.1%) had a peripheral blood eosinophil counts ≥300cell/microliter. Eosinophilia was associated with shorter median length of stay in the eosinophilic groups (≥2% or ≥300 cell/microliter) compared to the non-eosinophilic groups. Early treatment failure (within 10 days) was reduced in both the eosinophilic groups (≥2% or ≥300 cell/microliter). Late treatment failure (day 11-30) was equal in the eosinophilic groups as well as in the non-eosinophilic groups. Relapse (day 31-180), was more frequent in both eosinophilic groups (≥2% or ≥300 cell/microliter), but in the latter group this did not reach statistical significance. Eosinophilia ≥2% was associated with a lower risk factor for having early treatment failure (HR 0.339; 95%CI 0.122-0.943) whereas eosinophilia ≥2% was a risk factor for having relapse (eosinophilia ≥2%: HR 2.351; 95%CI 1.335-4.139). We concluded that blood eosinophilia in patients with hospitalized AECOPD at admission is associated with higher short-term treatment success. However, blood eosinophilia ≥ 2% predicts a less favourable outcome on the long term.

In **chapter 4** we presented the result of a sub-study of the CATCH trial. In this exploratory study patients with severe AECOPD, in whom pneumonia was excluded using chest X-ray, underwent additional LDCT-thorax. C-reactive protein (CRP), Procalcitonin (PCT), and Serum Amyloid A (SAA) on admission were assessed and correlated with potential CT abnormalities. Of the 100 patients that were included, 24 patients had one or more radiographic abnormalities suggestive for pneumonia. The inter-observer agreement between two readers (Cohen's Kappa) was 0.562 (95%CI 0.371-0.752 p<0.001). Biomarkers were significantly higher in the group with CT abnormalities compared to group without: CRP was 20.5 (IQR 8.8-81.5) mg/L and 76 (IQR 21.5-148.0) mg/L (p=0.018), PCT was 0.06 (IQR 0.04-0.08) µg/L ando 0.09 (IQR 0.06-0.15) µg/L (p=0.007), SAA was 16 (IQR 3-89) µg/ml and 95 (7-160) µg/ml (p=0.019), respectively. The sensitivity and specificity of all three biomarkers where poor for detecting pneumonia by LDCT in this population. The area under the ROC curve was 0.659 (95% CI: 0.521-0.796) for CRP, 0.664 (95%CI: 0.526-0.801) for PCT, and 0.687 (95%CI: 0.566-0.808) for SAA. We concluded that in quarter of patients with severe AECOPD without infiltrate(s) on the chest X-ray, additional infiltrative changes compatible with acute-phase lung involvement were detected by LDCT. Although the three investigated biomarkers were significantly higher in the group with abnormalities present on LDCT, they were not able to reliably detect or exclude CAP in this specific population.

In **chapter 5** we presented the results of the validation study of the COPD-lower respiratory tract infections – visual analogue score (c-LRTI-VAS). The questionnaire was validated in patients with stable COPD as well as those with an acute exacerbation of COPD (AECOPD). The results of c-LRTI-VAS were compared with two health related quality of life questionnaires (St Georges Respiratory Questionnaire (SGRQ) and Clinical COPD Questionnaire (CCQ)). Validity, reliability and responsiveness were assessed. Eighty-eight patients with clinically stable COPD and 102 patients who had an AECOPD completed the c-LRTI-VAS questionnaire. When testing on two separate occasions for repeatability, no statistically significant difference between total scores was found 0.143 (SD 5.42) (p=0.826). Internal consistency was high across items Cronbach's Alpha 0.755. Correlation with SGRQ and CCQ total scores was moderate to high. After treatment for hospitalized AECOPD, the mean c-LRTI-VAS total score improved 8.14 points (SD 9.13; p=<0.001). We concluded that the c-LRTI-VAS showed proper validity, responsiveness to change and moderate to high correlation with other questionnaires. It therefore appears a reliable tool for symptom measurement in COPD.

In **chapter 6** we present the results of an exploratory double blind randomized controlled trial investigating the effect of a 3-week course of doxycycline on sputum and systemic inflammatory parameters in stable COPD patients presumably without

bacterial colonisation of the airways. The effect of doxycycline treatment on inflammatory markers (TNF-α, IL-1β and IL-6) and neutrophil specific markers in sputum (MPO, MMP's, and IL-8) and serum C-reactive protein was evaluated. Sputum was obtained by sputum induction with hypertonic saline. A total of 41 patients were included. Ten patients were excluded as they were not able to produce sputum at the first or second visit. Baseline characteristics were similar in the two groups. In the remaining patient's doxycycline did not influence sputum MPO concentrations. Also, MMP-8 and 9, IL-6 and IL-8 concentrations as well as lung function parameters were not affected by doxycycline. Systemic inflammation by means of CRP was also not influenced by doxycycline. Based upon our study we cannot recommend doxycycline for the reduction MPO sputum levels nor any of the other inflammatory sputum and systemic markers.

CHAPTER 9

9

Nederlandse samenvatting voor niet-ingewijden

Nederlandse samenvatting voor niet–ingewijden

Chronische obstructieve longziekten (COPD) is de medische benaming voor wat in de volksmond longemfyseem, chronische bronchitis of wel rokerslongen worden genoemd. COPD is een ziekte waarbij als gevolg van blootstelling aan tabaksrook of andere schadelijke stoffen (bijvoorbeeld luchtvervuiling) een ontstekingsreactie optreedt die de luchtwegen en longblaasjes onherstelbaar beschadigt. Deze ontstekingsreactie treedt niet bij iedereen op, sommige mensen zijn hiervoor vatbaarder voor dan andere, en ontwikkelen hierdoor COPD. Ten gevolge van de beschadiging aan de luchtwegen en longblaasjes gaat de rek uit de longen en vallen luchtwegen eerder dicht. Hierdoor heeft een persoon met COPD meer moeite met uitademen en blijft er veel lucht achter in de longen. Omdat een patiënt niet goed kan uitademen is er minder ruimte om opnieuw in te ademen. Dit veroorzaakt een gevoel van chronische benauwdheid. De mate van ernst van COPD wordt uitgedrukt in de Global initiative for chronic Obstructive Lung Disease (GOLD) classificatie. Dit is een systeem dat loopt van A tot en met D, waarbij A het minst ergst is en D het ernstigst. De mate van ernst wordt mede vastgesteld door longfunctieonderzoek, symptomen van kortademigheid bij inspanning en het aantal exacerbaties (longaanvallen) dat een patiënt heeft per jaar. Bij het longfunctieonderzoek wordt bekeken hoeveel lucht een patiënt in de eerste seconde maximaal kan uitblazen (Forced Expiratory Volume in 1 second, FEV-1). Naar mate de FEV-1 daalt is er sprake van een ernstiger COPD: stadium 1 is >80% van de voorspelde waarde, en stadium 4: <30%.

Bij de eerder genoemde ontstekingsreactie in de longen en luchtwegen die optreedt komen allerlei stoffen vrij. Deze stoffen gaan via het bloed dat door de longen loopt het hele lichaam in. Deze stoffen zijn betrokken bij het activeren van het immuunsysteem. Ze zetten het immuunsysteem als het ware “aan” en maken het hierdoor extra alert. Hierdoor ontstaat er ook in de rest van het lichaam ontsteking en hiermee ook schade. Dit heeft als gevolg dat er veel andere ziekten optreden bij mensen met COPD (bijvoorbeeld aderverkalking, botontkalking, suikerziekte en depressie).

COPD is met bijna 614.000 mensen die de ziekte hebben een veel voorkomend probleem in Nederland. Per jaar komen er ruim 37.000 nieuwe COPD-patiënten bij en overlijden er 6900 mensen per jaar aan COPD. De maatschappelijke kosten van COPD zijn enorm. Alleen al aan zorguitgaven kost COPD 912 miljoen euro per jaar. Hierin zijn niet meegenomen kosten voor ziekteverzuim of verlies van arbeidsproductiviteit. Deze kosten overtreffen veruit die van het zorggebruik voor COPD.

Kenmerkend voor het verloop van de ziekte COPD is het optreden van longaanvallen (exacerbatie COPD). Deze longaanvallen gaan gepaard met een plotselinge toename van klachten (benauwdheid, hoesten, verkleurd / pussig slijm opgeven en vermoeidheid). Longaanvallen kunnen veroorzaakt worden door een infectie (virus, bacterie of combinatie van beiden), dan wel samengaan met een ontstekingsreactie veroorzaakt door

een bepaalde witte bloedcel (de eosinofiele granulocyt). Tot slot is er ook nog een deel van de mensen die een longaanval krijgt door een onduidelijke oorzaak (bijvoorbeeld toename van luchtvervuiling). Een longaanval gaat vrijwel altijd gepaard met een toename van ontstekingsactiviteit in de longen. Gedurende de longaanval is er vrijwel altijd een verslechtering van de longfunctie (gemeten met de FEV-1). Daarnaast is er toename van ontstekingsactiviteit in de rest van het lichaam. Ontstekingsactiviteit kan zowel in de longen als in het bloed gemeten worden. In het bloed is dit het gemakkelijkst. Onder invloed van stoffen die vrijkomen bij infectie of ontsteking in de longen, wordt het lichaam klaargemaakt om de strijd aan te gaan met een mogelijke ziekteverwekker. Deze stoffen activeren de lever voor het aanmaken van zogenaamde acute fase eiwitten. Dit zijn eiwitten die betrokken zijn de afweer en die het immuunsysteem ondersteunen. Het meest bekende voorbeeld hiervan is C-Reactief proteïne (CRP). Dit eiwit wordt al jaren gebruikt voor het meten van ontstekingsactiviteit en is eenvoudig meetbaar in het bloed.

Een longaanval leidt vaak tot een bezoek aan de huisarts of zelfs ziekenhuis opname. Hierbij moet een patiënt vaak extra medicatie gebruiken. Vrijwel altijd zijn mensen met een longaanval minder actief of zelfs bedlegerig. Hierdoor verliezen ze kracht en conditie. In combinatie met het achteruitgaan van de longfunctie zorgt dit ervoor dat patiënten minder actief kunnen zijn. Dit zorgt voor toegenomen gevoel van benauwdheid en een verlies van kwaliteit van leven. Met name de benauwdheid zorgt ervoor dat patiënten nog minder gaan doen. Patiënten komen hierdoor vaak in een neerwaartse spiraal terecht. Het voorkomen en het beter behandelen van exacerbaties zou daardoor mogelijk voordeel kunnen opleveren voor patiënten met COPD.

De medicamenteuze behandeling van een exacerbatie COPD (longaanval) bestaat uit 3 onderdelen. Luchtwegverwijders, corticosteroïden en/of antibiotica. Luchtwegverwijders worden gebruikt in zowel de stabiele situatie als gedurende een longaanval en zorgen voor een maximale verwijding van de luchtwegen. Hierdoor is het ademhalen makkelijker. Het gebruik van corticosteroïden worden volgens de GOLD-richtlijn aangeraden voor alle patiënten met een exacerbatie. Op korte termijn geeft dit een verbetering van de longfunctie (FEV-1), stijgt de hoeveelheid zuurstof in het bloed en daalt de hersteltijd. Corticosteroïden blijken helaas geen invloed te hebben op de kans dat mensen overlijden na een longaanval. Daarnaast is het de vraag of dit middel voor alle patiënten met een longaanval geschikt is. Corticosteroïden hebben namelijk forse bijwerkingen zoals verslechtering van suikerziekte, botontkalking, spierafbraak, vocht vasthouden, dunne huid, en psychische veranderingen zoals slaapstoornis. Er zijn zelfs aanwijzingen dat sommige patiënten die behandeld worden met corticosteroïden bij een longaanval slechter af zijn dan patiënten die geen corticosteroïden krijgen.
Ten derde antibiotica, het gebruik hiervan bij een longaanval is omstreden. Desalniettemin is aangetoond dat antibiotica de kans op overlijden op de korte termijn vermindert, en

dat antibiotica de tijd tot falen van de behandeling – met een nieuwe longaanval - zou kunnen verlengen. Echter het lijkt zo te zijn dat niet alle patiënten met een longaanval behandeld moeten worden met antibiotica. De richtlijn van de GOLD zegt dat er 2 groepen met antibiotica behandeld moeten worden. Patiënten die beademing nodig hebben en patiënten die een toename van verkleuring (pussigheid) van het opgehoeste slijm hebben, in combinatie met toegenomen benauwdheid en/of hoesten. Helaas is echter gebleken dat verkleuring van het opgehoeste slijm vaak moeilijk door de patiënt kan worden vastgesteld. Indien het sputum verkleurd is, is dit helaas geen garantie voor de aanwezigheid van een bacteriële infectie. Daarnaast blijkt een groot deel van de longaanvallen niet veroorzaakt te worden door bacteriën, maar door bijvoorbeeld een virale infectie. Virussen reageren niet op antibiotica. Bovendien hebben antibiotica bijwerkingen zoals diarree, maagklachten en allergische reacties.
Tot slot is het grote gevaar van het overdadig voorschrijven van antibiotica dat bacteriën ongevoelig (resistent) worden voor antibiotica. Daarom is het van belang om te weten welke patiënten het meeste baat hebben bij antibiotica en welke patiënten hier niet mee behandeld moeten worden. Zoals eerder beschreven kan hoogte van de CRP concentratie in het bloed voorspellen of er sprake is van een bacteriële infectie. Uit eerder onderzoek is gebleken dat antibiotica mogelijk beter werkzaam zijn bij patiënten met een fors verhoogd CRP. Echter dit is tot op heden niet in de praktijk getest. Het primaire doel van dit proefschrift is dan ook onderzoeken of een verhoogd CRP een goed signaal is om al dan niet te starten met antibiotica bij patiënten met ernstige longaanval. Ten tweede hebben wij gepoogd meer inzicht te krijgen welke invloed een bepaalde witte bloedcel (de eosinofiele granulocyt), gemeten in het bloed, heeft op het verloop van de longaanval op korte en langer termijn. Ten derde hebben wij gekeken of mensen met een longaanval zonder aanwijzingen voor longontsteking op de longfoto toch een longontsteking zouden kunnen hebben op een CT-scan van de longen en of dit ook te voorspellen is door het meten van bepaalde ontstekingseiwitten in het bloed. Daarnaast hebben wij een vragenlijst met de meest voorkomende klachten van een longaanval getest op betrouwbaarheid en vergeleken met andere vragenlijsten bij patiënten met en zonder longaanval. Tot slot hebben wij bij patiënten met stabiel COPD zonder aanwijzingen voor aanwezigheid van bacteriën in de luchtwegen gekeken of we door gebruik van doxycycline gedurende 3 weken de typische ontstekingsreactie die hoort bij COPD in de longen kunnen remmen.

In **hoofdstuk 2** is onderzocht of CRP gemeten in het bloed een geschikt signaal is om patiënten met een longaanval al dan niet antibiotisch te behandelen met amoxicilline-clavulaanzuur. Bij een matig verhoogd CRP ≥50 mg/L werd gestart met dit antibioticum. Dit werd vergeleken met de behandeling volgens de richtlijn van de GOLD. Hierbij worden patiënten die verkleurd slijm ophoesten in combinatie met kortademigheid behandeld met dit antibioticum. Patiënten zonder verkleuring van het slijm dat zij

ophoesten worden niet antibiotisch behandeld. Door loting (randomisatie) werd bepaald of patiënten werden ingedeeld in de CRP-groep of in de GOLD-groep. Aanvullend werden alle patiënten behandeld met corticosteroïden en luchtwegverwijders. Daarnaast kregen de deelnemers dezelfde zorg als de niet-deelnemende patiënten. In de eerste plaats werd er gekeken of patiënten minder antibiotica gebruikten bij start van de behandeling. Ten tweede werd er gekeken of patiënten niet vaker faalden op de behandeling binnen de eerste 30 dagen. Ten derde werd er gekeken of de tijd tot de volgende exacerbatie veranderde. Ten vierde werd de opname duur vergeleken in beide groepen en tot slot werd er gekeken naar het verschil in klachten en kwaliteit van leven na 30 dagen.
In de CRP-groep bleek dat er minder mensen met antibiotica behandeld werden, 31.7% vergeleken met 46.2% in de GOLD-groep, zonder nadelige gevolgen voor de patiënt. Het percentage van patiënten die falen van behandeling binnen 30 dagen hadden was gelijk in beide groepen (CRP-groep: 44.5% en GOLD-groep 45.5%). De tijd tussen de opname en de volgende exacerbatie was gelijk in beide groepen (CRP-groep 32 dagen en GOLD-groep 28 dagen). De opnameduur in het ziekenhuis was iets langer in de CRP-groep dan in de GOLD-groep (CRP-groep: 7 dagen GOLD-groep: 6 dagen), echter dit verschil was zo klein dat dit waarschijnlijk in de dagelijkse praktijk geen verschil gaat opleveren. Kwaliteit van leven en de mate van klachtenvermindering in de eerste dertig dagen was gelijk in beide groepen. Samenvattend bleek dat een verhoogd CRP een goed signaal oplevert om te starten met antibiotica en dat dit leidt tot het minder voorschrijven van antibiotica zonder nadelen voor patiënten.

Hoofdstuk 3 behandelt de resultaten van een analyse die wij verrichtten naar de invloed van de eosinofiele granulocyt bij opname op de uitkomsten van patiënten met een ernstige longaanval. De eosinofiele granulocyt is een bepaalde witte bloedcel die bij patiënten met COPD verhoogd kan zijn. Wij gebruikten hiervoor als afkappunt ≥2% van het totale aantal witte bloedcellen of een absoluut aantal ≥300 eosinofielen/microliter. Alle patiënten werden behandeld met corticosteroïden. Dit is belangrijk omdat eosinofiele granulocyten hier erg goed op reageren: ze worden snel en effectief onderdrukt door deze medicijnen. Antibiotica gebruik was in beide groepen gelijk en had dus waarschijnlijk geen invloed op de uitkomsten. Wij keken naar opname duur, het percentage van patiënten met falen van de behandeling binnen 10 dagen (vroeg), binnen 11-30 dagen (laat) en binnen 31-180 dagen (terugval).
In 18.8% van de patiënten was er sprake van een toename van eosinofiele granulocyten (eosinofilie) in het bloed. Patiënten met eosinofilie waren gemiddeld korter opgenomen dan patiënten zonder eosinofilie (gemiddeld 5 dagen in de eosinofiele groep vergeleken met 7 dagen in de niet eosinofiele groep). Vroeg falen van de behandeling kwam vaker voor in de niet eosinofiele groep dan in de eosinofiele groep (26.6% vs 4.3%). Laat falen van de behandeling kwam even vaak voor in beide groepen, terwijl een terugval meer voorkwam in de groep met verhoogde eosinofiele granulocyten (72.0% vs 42.2%). Op

basis van deze analyse concludeerden wij dat eosinofilie bij opname in het ziekenhuis in verband met een longaanval gelinkt is aan een lager risico op falen van de behandeling binnen 10 dagen, terwijl ze een grotere kans hebben op het krijgen van een terugval na 30 dagen.

Hoofdstuk 4 betreft het onderzoek waarbij patiënten met een longaanval zonder aanwijzingen voor longontsteking op hun longfoto een aanvullende CT-scan kregen. Deze scan had als bijzonderheid dat er veel minder Röntgenstraling wordt gebruikt dan bij een gewone scan. De eventuele afwijkingen op deze scans werden door twee radiologen onafhankelijk van elkaar bekeken. Hierna werd er gekeken of ze dezelfde uitslag hadden bij één scan. Dit vertelde ons iets of de lage dosis CT goed beoordeeld kon worden. Uiteindelijk werd de uitslag vergeleken met drie ontstekingseiwitten die in het bloed gemeten kunnen worden, CRP, Procalcitonine (PCT) en Serum Amyloid A (SAA). Alle drie deze ontstekingsstoffen kunnen verhoogd raken, vaak ten gevolge van bacteriële infectie. De gedachte en hoop is dat deze stoffen als signaal kunnen werken en zo een nog niet zichtbare longontsteking op de longfoto kunnen voorspellen. Van elke signaalstof werd er onafhankelijk een sensitiviteit en een specificiteit berekend. Dit zijn belangrijke uitkomsten die wat vertellen over de waarde van de test. Sensitiviteit is een maat voor de gevoeligheid van een test, specificiteit bepaalt hoe specifiek een test is. Een hoge sensitiviteit betekent dat er een grote kans is dat een zieke patiënt een positieve test zal hebben. Een hoge specificiteit betekent dat er een grote kans is dat als een patiënt de ziekte niet heeft hij ook een negatieve testuitslag heeft. Beide waarden worden uitgedrukt in een percentage.

Het is belangrijk om een longontsteking vroeg vast te stellen, omdat een gemiste longontsteking voor een patiënt ernstige gevolgen kan hebben. Alle patiënten die werden opgenomen in verband met een longaanval kregen een lage dosis CT-scan van de longen en bij opname werden de drie signaalstoffen in het bloed bepaald.

Honderd patiënten van de in hoofdstuk 2 beschreven studie deden mee aan het onderzoek. Vierentwintig patiënten (24%) hadden op hun lage dosis CT-scan radiologische afwijkingen die kunnen passen bij longontsteking. De overeenstemming tussen de twee radiologen was gemiddeld (Cohen's Kappa 0.562). De signaalstoffen die gemeten werden waren hoger in de groep met patiënten die radiologische afwijkingen hadden. Het CRP was 20.5 mg/L in de groep zonder afwijkingen en in de groep met afwijkingen 76 mg/L. PCT was 0.06 µg/L in de groep zonder afwijkingen en in de groep met afwijkingen 0.09 µg/L. SAA was 16 µg/ml in de groep zonder afwijkingen en in de groep met afwijkingen 95 µg/ml. Sensitiviteit en specificiteit vielen tegen voor alle drie de testen. Daarom concludeerden wij dat hoewel de drie gemeten signaalstoffen gemiddeld duidelijk hoger waren in patiënten met radiologische afwijkingen op hun lage dosis CT-scan, zij onvoldoende sensitiviteit en specificiteit bezaten om betrouwbaar te voorspellen dat er sprake is van radiologische afwijkingen.

In **Hoofdstuk 5** toont de ontwikkeling en validatie van de COPD Lower Respiratory Tract Infection Visual Analogue score (c-LRTI-VAS). Dit is een vragenlijst die bestaat uit vier losse vragen over de mate van kortademigheid, vermoeidheid, hoesten en sputum kleur. Patiënten gaven de ernst van deze klachten weer op een rechte lijn. Na het invullen kan hier door de onderzoeker een cijfer van 0 tot 10 aan gegeven worden. Dit is een zogenaamde visueel analoge schaal (VAS). Uiteindelijk worden de scores opgeteld tot een totaalscore. De vragenlijst werd zowel getest bij patiënten met een longaanval als bij patiënten met stabiel COPD. De vragenlijst werd vergeleken met bekende uitkomsten van ziekteactiviteit zoals longfunctie, zuurstof in het bloed en een tweetal vragenlijsten die kwaliteit van leven meten. Ook werd de test op validiteit, herhaalbaarheid en op reactievermogen getest. De test liet zien dat bij stabiele patiënten met COPD de test na 30 dagen gelijk bleef.
De c-LRTI-VAS reageerde goed op veranderingen, dat wil zeggen, een duidelijk verschil tussen voor en 30 dagen na de behandeling voor de longaanval. De c-LRTI-VAS had een goede validiteit en in het vergelijk met de andere vragenlijsten liet hij redelijke resultaten zien. Het vergelijk van de c-LRTI-VAS met andere bekende uitkomsten van ziekteactiviteit was matig. Wij concludeerden op basis van ons onderzoek dat de c-LRTI-VAS een geschikte vragenlijst is voor het monitoren van klachten bij patiënten met COPD.

Hoofdstuk 6 behandelt het onderzoek naar de effecten van het antibioticum doxycycline op de ontstekingsactiviteit in de longen van patiënten met stabiel COPD. Doxycycline is een bekend antibioticum, maar heeft naast zijn antibiotische werking ook nog de mogelijkheid tot het remmen van ontstekingsactiviteit. Daarom is er in de opzet van deze studie voor gekozen om alleen patiënten voor deze studie te vragen die geen aanwijzingen hebben voor aanwezigheid van bacteriën in hun luchtwegen. Hierdoor kon er optimaal gekeken worden naar de ontstekingsremmende werking van doxycycline. De studie heeft een placebogecontroleerde dubbelblind gerandomiseerde opzet. Dit betekent dat de helft van de patiënten gerandomiseerd (loting) werd voor het krijgen van doxycycline en de andere helft kreeg placebo (neppil). Noch de patiënt noch de onderzoeker wisten gedurende het onderzoek of ze de werkzame stof of de neppil kregen. Patiënten werden drie weken behandeld. In dit onderzoek werd er alleen gekeken naar het effect van doxycycline op ontstekingssignaalstoffen in opgehoest slijm. In het bloed werd het ontstekingseiwit CRP bepaald als teken van ontsteking in het gehele lichaam. Indien patiënten niet spontaan slijm konden ophoesten werden zij verneveld met een sterke zout oplossing. Dit brengt een heftige hoestprikkel teweeg die vaak tot het ophoesten van sputum leidt - maar niet in alle gevallen.
Helaas vielen er gedurende de studie veel mensen uit, omdat ze niet in staat bleken om goed slijm op te hoesten. Bij de resterende patiënten die wel goed slijm op konden hoesten werden er verschillende ontstekingssignaalstoffen voor en na behandeling

gemeten. Geen van de ontstekingssignaalstoffen in het opgehoeste slijm werd verlaagd door doxycycline. Ook het CRP werd niet beïnvloed door doxycycline. Op basis van onze uitkomsten konden wij daarom doxycycline niet aanraden als ontstekingsremmer voor patiënten met stabiel COPD.

CHAPTER 10

Curriculum Vitae

Curriculum Vitae

Hendrik Johannes (Henk-Jan) Prins werd geboren in 1982 in Zwolle en groeide op in Hoorn. In 2000 ronde hij zijn HAVO af aan het Oscar Romero te Hoorn, in 2001 ronde hij zijn VWO af op het Luzac college te Alkmaar. In hetzelfde jaar startte hij met geneeskunde aan de universiteit van Utrecht.
In 2010 startte hij als ANIOS bij de longziekten in Alkmaar. In het jaar daarop startte hij, ook in Alkmaar met onderzoek doen onder leiding van dr. W.G. Boersma. Dit onderzoek resulteert uiteindelijk in dit proefschrift. Tot 2015 was hij werkzaam in Alkmaar. Hierna maakte hij de overstap naar de revalidatiegeneeskunde. In 2017 startte hij met zijn opleiding bij Adelante revalidatie onder verantwoordelijkheid van drs. W.G. Bakx en dr. E. de Klerk. In 2021 hoopt hij zijn opleiding af te ronden. Momenteel is hij werkzaam als AIOS revalidatiegeneeskunde in het MUMC+ in Maastricht. Henk-Jan woont samen met zijn verloofde Mariëlle en hun kinderen Lise-Lotte (2016) en Rose-Marijn (2018).

CHAPTER 11

Dankwoord

Dankwoord

Bij geen van de hoofdstukken uit dit proefschrift ben ik zo vaak opnieuw begonnen als bij het dankwoord. Dit met name omdat zoveel mensen hebben bijgedragen aan dit proefschrift. Daarom wil ik ook de mensen die niet in het bijzonder hieronder worden genoemd bedanken voor hun bijdrage. Graag had ik ook individueel alle proefpersonen aan mijn onderzoek willen bedanken, velen van u hebben op mij een diepe indruk gemaakt. Zonder jullie bijzondere bijdrage had dit onderzoek niet uitgevoerd kunnen worden. Ik ben u hiervoor zeer dankbaar!

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CHAPTER 12

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Publications

List of publications

Rommers GM, **Prins HJ**, Interdisciplinair opleiden: AIOS leren van elkaar. Nederlands tijdschrift voor revalidatiegeneeskunde June 2020

Prins HJ, Van der Werf TS, Boersma WG. Is CRP-guided antibiotic treatment a safe way to reduce antibiotic use in severe hospitalised patients with exacerbations of COPD? European Respiratory Journal October 2019

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