Biomarkers in stable and acute exacerbations of 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.

Reference List

1. Vollenweider DJ, Frei A, Steurer-Stey CA et al. Antibiotics for exacerbations of chronic obstructive pulmonary disease. Cochrane Database Syst Rev 2018; 10:CD010257.
2. Bafadhel M, McKenna S, Terry S et al. Acute exacerbations of chronic obstructive pulmonary disease: identification of biologic clusters and their biomarkers. Am J Respir Crit Care Med 2011; 184(6):662-671.
3. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. 2019. 2019.
4. Brusse-Keizer MG, Grotenhuis AJ, Kerstjens HA et al. Relation of sputum colour to bacterial load in acute exacerbations of COPD. Respir Med 2009; 103(4):601-606.
5. Daniels JM, de Graaff CS, Vlaspolder F et al. Sputum colour reported by patients is not a reliable marker of the presence of bacteria in acute exacerbations of chronic obstructive pulmonary disease. Clin Microbiol Infect 2010; 16(6):583-588.
6. Goossens H, Ferech M, Vander SR et al. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet 2005; 365(9459):579-587.
7. Mathioudakis AG, Chatzimavridou-Grigoriadou V, Corlateanu A et al. Procalcitonin to guide antibiotic administration in COPD exacerbations: a meta-analysis. Eur Respir Rev 2017; 26(143).
8. Ulrich RJ, McClung D, Wang BR et al. Introduction of Procalcitonin Testing and Antibiotic Utilization for Acute Exacerbations of Chronic Obstructive Pulmonary Disease. Infect Dis (Auckl) 2019; 12:1178633719852626.
9. Daniels JM, Snijders D, de Graaff CS et al. Antibiotics in addition to systemic corticosteroids for acute exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2010; 181(2):150-157.
10. Llor C, Moragas A, Hernandez S et al. Efficacy of antibiotic therapy for acute exacerbations of mild to moderate chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012; 186(8):716-723.
11. Hewitt R, Farne H, Ritchie A et al. The role of viral infections in exacerbations of chronic obstructive pulmonary disease and asthma. Ther Adv Respir Dis 2016; 10(2):158-174.
12. Soler N, Esperatti M, Ewig S et al. Sputum purulence-guided antibiotic use in hospitalised patients with exacerbations of COPD. Eur Respir J 2012; 40(6):1344-1353.
13. Adamson SL, Burns J, Camp PG et al. Impact of individualized care on readmissions after a hospitalization for acute exacerbation of COPD. Int J Chron Obstruct Pulmon Dis 2016; 11:61-71.
14. Brusselle G, Pavord ID, Landis S et al. Blood eosinophil levels as a biomarker in COPD. Respir Med 2018; 138:21-31.
15. Wedzicha JA. Role of viruses in exacerbations of chronic obstructive pulmonary disease. Proc Am Thorac Soc 2004; 1(2):115-120.
16. Butler CC, Gillespie D, White P et al. C-Reactive Protein Testing to Guide Antibiotic Prescribing for COPD Exacerbations. N Engl J Med 2019; 381(2):111-120.
17. Miravitlles M, Moragas A, Hernandez S et al. Is it possible to identify exacerbations of mild to moderate COPD that do not require antibiotic treatment? Chest 2013; 144(5):1571-1577.

18. Woolnough K, Wardlaw AJ. Eosinophilia in Pulmonary Disorders. Immunol Allergy Clin North Am 2015; 35(3):477-492.
19. Saha S, Brightling CE. Eosinophilic airway inflammation in COPD. Int J Chron Obstruct Pulmon Dis 2006; 1(1):39-47.
20. Narendra DK, Hanania NA. Targeting IL-5 in COPD. Int J Chron Obstruct Pulmon Dis 2019; 14:1045-1051.
21. Barnes PJ. Inflammatory endotypes in COPD. Allergy 2019; 74(7):1249-1256.
22. Varricchi G, Bagnasco D, Borriello F et al. Interleukin-5 pathway inhibition in the treatment of eosinophilic respiratory disorders: evidence and unmet needs. Curr Opin Allergy Clin Immunol 2016; 16(2):186-200.
23. Eltboli O, Bafadhel M, Hollins F et al. COPD exacerbation severity and frequency is associated with impaired macrophage efferocytosis of eosinophils. BMC Pulm Med 2014; 14:112.
24. Moqbel R. Eosinophils, cytokines, and allergic inflammation. Ann N Y Acad Sci 1994; 725:223-233.
25. Rutgers SR, Timens W, Kaufmann HF et al. Comparison of induced sputum with bronchial wash, bronchoalveolar lavage and bronchial biopsies in COPD. Eur Respir J 2000; 15(1):109-115.
26. Beeh KM, Beier J, Kornmann O et al. Long-term repeatability of induced sputum cells and inflammatory markers in stable, moderately severe COPD. Chest 2003; 123(3):778-783.
27. Sutherland ER, Pak J, Langmack EL et al. Safety of sputum induction in moderate-to-severe chronic obstructive pulmonary disease. Respir Med 2002; 96(7):482-486.
28. Bafadhel M, McKenna S, Terry S et al. Blood eosinophils to direct corticosteroid treatment of exacerbations of chronic obstructive pulmonary disease: a randomized placebo-controlled trial. Am J Respir Crit Care Med 2012; 186(1):48-55.
29. Sivapalan P, Lapperre TS, Janner J et al. Eosinophil-guided corticosteroid therapy in patients admitted to hospital with COPD exacerbation (CORTICO-COP): a multicentre, randomised, controlled, open-label, non-inferiority trial. Lancet Respir Med 2019; 7(8):699-709.
30. Bafadhel M, Pavord ID, Russell REK. Eosinophils in COPD: just another biomarker? Lancet Respir Med 2017; 5(9):747-759.
31. Yousuf A, Ibrahim W, Greening NJ et al. T2 Biologics for Chronic Obstructive Pulmonary Disease. J Allergy Clin Immunol Pract 2019; 7(5):1405-1416.
32. Gibson PG. Variability of blood eosinophils as a biomarker in asthma and COPD. Respirology 2018; 23(1):12-13.
33. Negewo NA, McDonald VM, Baines KJ et al. Peripheral blood eosinophils: a surrogate marker for airway eosinophilia in stable COPD. Int J Chron Obstruct Pulmon Dis 2016; 11:1495-1504.
34. Bafadhel M, Greening NJ, Harvey-Dunstan TC et al. Blood Eosinophils and Outcomes in Severe Hospitalized Exacerbations of COPD. Chest 2016; 150(2):320-328.
35. Ko FWS, Chan KP, Ngai J et al. Blood eosinophil count as a predictor of hospital length of stay in COPD exacerbations. Respirology 2019.
36. Russell R, Beer S, Pavord ID et al. The acute wheezy adult with airways disease in the emergency department: a retrospective case-note review of exacerbations of COPD. Int J Chron Obstruct Pulmon Dis 2019; 14:971-977.

37. Hasegawa K, Camargo CA, Jr. Prevalence of blood eosinophilia in hospitalized patients with acute exacerbation of COPD. Respirology 2016; 21(4):761-764.
38. Tine M, Biondini D, Semenzato U et al. Reassessing the Role of Eosinophils as a Biomarker in Chronic Obstructive Pulmonary Disease. J Clin Med 2019; 8(7).
39. Shoenfeld Y, Gurewich Y, Gallant LA et al. Prednisone-induced leukocytosis. Influence of dosage, method and duration of administration on the degree of leukocytosis. Am J Med 1981; 71(5):773-778.
40. Abidi K, Khoudri I, Belayachi J et al. Eosinopenia is a reliable marker of sepsis on admission to medical intensive care units. Crit Care 2008; 12(2):R59.
41. Abidi K, Belayachi J, Derras Y et al. Eosinopenia, an early marker of increased mortality in critically ill medical patients. Intensive Care Med 2011; 37(7):1136-1142.
42. Steer J, Gibson J, Bourke SC. The DECAF Score: predicting hospital mortality in exacerbations of chronic obstructive pulmonary disease. Thorax 2012; 67(11):970-976.
43. Holland M, Alkhalil M, Chandromouli S et al. Eosinopenia as a marker of mortality and length of stay in patients admitted with exacerbations of chronic obstructive pulmonary disease. Respirology 2010; 15(1):165-167.
44. Rahimi-Rad MH, Asgari B, Hosseinzadeh N et al. Eosinopenia as a Marker of Outcome in Acute Exacerbations of Chronic Obstructive Pulmonary Disease. Maedica (Buchar) 2015; 10(1):10-13.
45. Hogan SP, Waddell A, Fulkerson PC. Eosinophils in infection and intestinal immunity. Curr Opin Gastroenterol 2013; 29(1):7-14.
46. Kolsum U, Donaldson GC, Singh R et al. Blood and sputum eosinophils in COPD; relationship with bacterial load. Respir Res 2017; 18(1):88.
47. Torres A, Peetermans WE, Viegi G et al. Risk factors for community-acquired pneumonia in adults in Europe: a literature review. Thorax 2013; 68(11):1057-1065.
48. Titova E, Christensen A, Henriksen AH et al. Comparison of procalcitonin, C-reactive protein, white blood cell count and clinical status in diagnosing pneumonia in patients hospitalized with acute exacerbations of COPD: A prospective observational study. Chron Respir Dis 2019; 16:1479972318769762.
49. Brendish NJ, Malachira AK, Beard KR et al. Hospitalised adults with pneumonia are frequently misclassified as another diagnosis. Respir Med 2019; 150:81-84.
50. Capelastegui A, Espana PP, Bilbao A et al. Etiology of community-acquired pneumonia in a population-based study: link between etiology and patients characteristics, process-of-care, clinical evolution and outcomes. BMC Infect Dis 2012; 12:134.
51. Daniels JM, Schoorl M, Snijders D et al. Procalcitonin vs C-reactive protein as predictive markers of response to antibiotic therapy in acute exacerbations of COPD. Chest 2010; 138(5):1108-1115.
52. Karakioulaki M, Stolz D. Biomarkers in Pneumonia-Beyond Procalcitonin. Int J Mol Sci 2019; 20(8).
53. Zapata-Arriaza E, Mancha F, Bustamante A et al. Biomarkers predictive value for early diagnosis of Stroke-Associated Pneumonia. Ann Clin Transl Neurol 2019; 6(9):1882-1887.
54. Self WH, Courtney DM, McNaughton CD et al. High discordance of chest x-ray and computed tomography for detection of pulmonary opacities in ED patients: implications for diagnosing pneumonia. Am J Emerg Med 2013; 31(2):401-405.

55. Waterer GW. The Diagnosis of Community-acquired Pneumonia. Do We Need to Take a Big Step Backward? Am J Respir Crit Care Med 2015; 192(8):912-913.
56. Claessens YE, Debray MP, Tubach F et al. Early Chest Computed Tomography Scan to Assist Diagnosis and Guide Treatment Decision for Suspected Community-acquired Pneumonia. Am J Respir Crit Care Med 2015; 192(8):974-982.
57. Smith-Bindman R, Lipson J, Marcus R et al. Radiation dose associated with common computed tomography examinations and the associated lifetime attributable risk of cancer. Arch Intern Med 2009; 169(22):2078-2086.
58. Garcia-Vazquez E, Marcos MA, Mensa J et al. Assessment of the usefulness of sputum culture for diagnosis of community-acquired pneumonia using the PORT predictive scoring system. Arch Intern Med 2004; 164(16):1807-1811.
59. Garcha DS, Thurston SJ, Patel AR et al. Changes in prevalence and load of airway bacteria using quantitative PCR in stable and exacerbated COPD. Thorax 2012; 67(12):1075-1080.
60. Bhat N, O'Brien KL, Karron RA et al. Use and evaluation of molecular diagnostics for pneumonia etiology studies. Clin Infect Dis 2012; 54 Suppl 2:S153-S158.
61. Burk M, El-Kersh K, Saad M et al. Viral infection in community-acquired pneumonia: a systematic review and meta-analysis. Eur Respir Rev 2016; 25(140):178-188.
62. Mohan A, Chandra S, Agarwal D et al. Prevalence of viral infection detected by PCR and RT-PCR in patients with acute exacerbation of COPD: a systematic review. Respirology 2010; 15(3):536-542.
63. Jartti T, Lehtinen P, Vuorinen T et al. Persistence of rhinovirus and enterovirus RNA after acute respiratory illness in children. J Med Virol 2004; 72(4):695-699.
64. Papi A, Bellettato CM, Braccioni F et al. Infections and airway inflammation in chronic obstructive pulmonary disease severe exacerbations. Am J Respir Crit Care Med 2006; 173(10):1114-1121.
65. Karhu J, Ala-Kokko TI, Vuorinen T et al. Lower respiratory tract virus findings in mechanically ventilated patients with severe community-acquired pneumonia. Clin Infect Dis 2014; 59(1):62-70.
66. Koo HJ, Lim S, Choe J et al. Radiographic and CT Features of Viral Pneumonia. Radiographics 2018; 38(3):719-739.
67. Miravitlles M, Ribera A. Understanding the impact of symptoms on the burden of COPD. Respir Res 2017; 18(1):67.
68. Miravitlles M, Garcia-Sidro P, Fernandez-Nistal A et al. The chronic obstructive pulmonary disease assessment test improves the predictive value of previous exacerbations for poor outcomes in COPD. Int J Chron Obstruct Pulmon Dis 2015; 10:2571-2579.
69. Figarska SM, Boezen HM, Vonk JM. Dyspnea severity, changes in dyspnea status and mortality in the general population: the Vlagtwedde/Vlaardingen study. Eur J Epidemiol 2012; 27(11):867-876.
70. Jones PW, Quirk FH, Baveystock CM. The St George's Respiratory Questionnaire. Respir Med 1991; 85 Suppl B:25-31.
71. Leidy NK, Wilcox TK, Jones PW et al. Standardizing measurement of chronic obstructive pulmonary disease exacerbations. Reliability and validity of a patient-reported diary. Am J Respir Crit Care Med 2011; 183(3):323-329.

72. Altenburg J, Wortel K, de Graaff CS et al. Validation of a visual analogue score (LRTI-VAS) in non-CF bronchiectasis. Clin Respir J 2016; 10(2):168-175.
73. van der Molen T, Willemse BW, Schokker S et al. Development, validity and responsiveness of the Clinical COPD Questionnaire. Health Qual Life Outcomes 2003; 1:13.
74. Anthonisen NR, Manfreda J, Warren CP et al. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. Ann Intern Med 1987; 106(2):196-204.
75. Szucs B, Szucs C, Petrekanits M et al. Molecular Characteristics and Treatment of Endothelial Dysfunction in Patients with COPD: A Review Article. Int J Mol Sci 2019; 20(18).
76. Barnes PJ. Inflammatory endotypes in COPD. Allergy 2019.
77. Jasper AE, McIver WJ, Sapey E et al. Understanding the role of neutrophils in chronic inflammatory airway disease. F1000Res 2019; 8.
78. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016; 138(1):16-27.
79. Khair OA, Davies RJ, Devalia JL. Bacterial-induced release of inflammatory mediators by bronchial epithelial cells. Eur Respir J 1996; 9(9):1913-1922.
80. Chung KF. Cytokines in chronic obstructive pulmonary disease. Eur Respir J Suppl 2001; 34:50s-59s.
81. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. Lancet 2004; 364(9435):709-721.
82. Saetta M, Turato G, Baraldo S et al. Goblet cell hyperplasia and epithelial inflammation in peripheral airways of smokers with both symptoms of chronic bronchitis and chronic airflow limitation. Am J Respir Crit Care Med 2000; 161(3 Pt 1):1016-1021.
83. Kawabata K, Hagio T, Matsuoka S. The role of neutrophil elastase in acute lung injury. Eur J Pharmacol 2002; 451(1):1-10.
84. Lakshmi SP, Reddy AT, Reddy RC. Emerging pharmaceutical therapies for COPD. Int J Chron Obstruct Pulmon Dis 2017; 12:2141-2156.
85. Henehan M, Montuno M, De BA. Doxycycline as an anti-inflammatory agent: updates in dermatology. J Eur Acad Dermatol Venereol 2017; 31(11):1800-1808.
86. Sapadin AN, Fleischmajer R. Tetracyclines: nonantibiotic properties and their clinical implications. J Am Acad Dermatol 2006; 54(2):258-265.
87. Dalvi PS, Singh A, Trivedi HR et al. Effect of doxycycline in patients of moderate to severe chronic obstructive pulmonary disease with stable symptoms. Ann Thorac Med 2011; 6(4):221-226.
88. Singh B, Ghosh N, Saha D et al. Effect of doxycyline in chronic obstructive pulmonary disease - An exploratory study. Pulm Pharmacol Ther 2019; 58:101831.
89. Bullon P, Newman HN, Battino M. Obesity, diabetes mellitus, atherosclerosis and chronic periodontitis: a shared pathology via oxidative stress and mitochondrial dysfunction? Periodontol 2000 2014; 64(1):139-153.
90. Golub LM, Suomalainen K, Sorsa T. Host modulation with tetracyclines and their chemically modified analogues. Curr Opin Dent 1992; 2:80-90.
91. Baines KJ, Wright TK, Gibson PG et al. Azithromycin treatment modifies airway and blood gene expression networks in neutrophilic COPD. ERJ Open Res 2018; 4(4).

92. van Eeden SF, Hogg JC. Immune-Modulation in Chronic Obstructive Pulmonary Disease: Current Concepts and Future Strategies. Respiration 2019;1-16.
93. Altenburg J, de Graaff CS, van der Werf TS et al. Immunomodulatory effects of macrolide antibiotics - part 1: biological mechanisms. Respiration 2011; 81(1):67-74.
94. Altenburg J, de Graaff CS, Stienstra Y et al. Effect of azithromycin maintenance treatment on infectious exacerbations among patients with non-cystic fibrosis bronchiectasis: the BAT randomized controlled trial. JAMA 2013; 309(12):1251-1259.
95. Cui Y, Luo L, Li C et al. Long-term macrolide treatment for the prevention of acute exacerbations in COPD: a systematic review and meta-analysis. Int J Chron Obstruct Pulmon Dis 2018; 13:3813-3829.
96. Altenburg J, van den Aardweg JG. [Maintenance treatment with macrolide antibiotics in COPD: not for everyone]. Ned Tijdschr Geneeskd 2019; 163.
97. Vermeersch K, Gabrovska M, Aumann J et al. Azithromycin during Acute Chronic Obstructive Pulmonary Disease Exacerbations Requiring Hospitalization (BACE). A Multicenter, Randomized, Double-Blind, Placebo-controlled Trial. Am J Respir Crit Care Med 2019; 200(7):857-868.
98. Seemungal TA, Wilkinson TM, Hurst JR et al. Long-term erythromycin therapy is associated with decreased chronic obstructive pulmonary disease exacerbations. Am J Respir Crit Care Med 2008; 178(11):1139-1147.
99. Berkhof FF, Doornewaard-ten Hertog NE, Uil SM et al. Azithromycin and cough-specific health status in patients with chronic obstructive pulmonary disease and chronic cough: a randomised controlled trial. Respir Res 2013; 14:125.
100. Simpson JL, Powell H, Baines KJ et al. The effect of azithromycin in adults with stable neutrophilic COPD: a double blind randomised, placebo controlled trial. PLoS One 2014; 9(8):e105609.