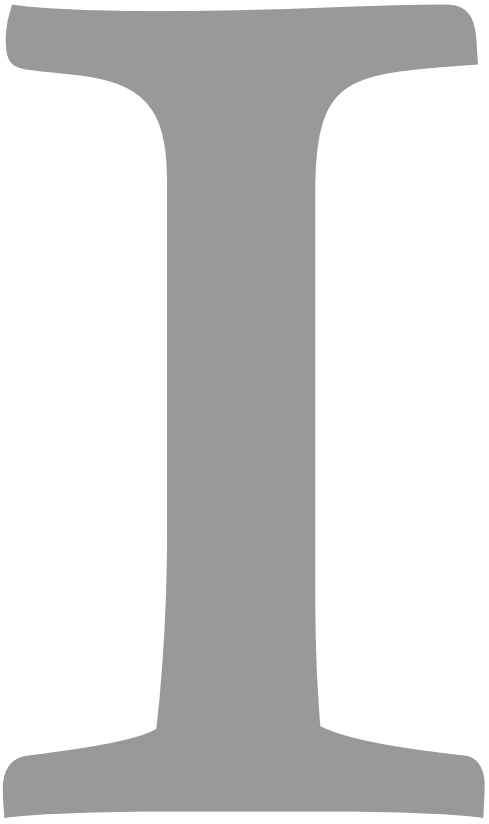
Biomarkers in stable and acute exacerbations of COPD

Prins, Hendrik J

DOI:
10.33612/diss.136484081

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.

Reference List

1. Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. 2019. 2019.
2. Adeloye D, Chua S, Lee C et al. Global and regional estimates of COPD prevalence: Systematic review and meta-analysis. J Glob Health 2015; 5(2):020415.
3. Nationaalkompas.nl, available from URl: https://www.volksgezondheidenzorg.info/onderwerp/copd/cijfers-context/sterfte#node-sterfte-copd-naar-leeftijd-en-geslacht. 2019.
4. Corlateanu A, Covantev S, Mathioudakis AG et al. Prevalence and burden of comorbidities in Chronic Obstructive Pulmonary Disease. Respir Investig 2016; 54(6):387-396.
5. Roche N, Chavannes NH, Miravitlles M. COPD symptoms in the morning: impact, evaluation and management. Respir Res 2013; 14:112.
6. Tsiligianni I, Kocks J, Tzanakis N et al. Factors that influence disease-specific quality of life or health status in patients with COPD: a review and meta-analysis of Pearson correlations. Prim Care Respir J 2011; 20(3):257-268.
7. Aaron SD. Management and prevention of exacerbations of COPD. BMJ 2014; 349:g5237.
8. Agusti A, Faner R. Systemic inflammation and comorbidities in chronic obstructive pulmonary disease. Proc Am Thorac Soc 2012; 9(2):43-46.
9. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. Eur Respir J 2009; 33(5):1165-1185.
10. Chen W, Thomas J, Sadatsafavi M et al. Risk of cardiovascular comorbidity in patients with chronic obstructive pulmonary disease: a systematic review and meta-analysis. Lancet Respir Med 2015; 3(8):631-639.
11. Barnes PJ. Inflammatory mechanisms in patients with chronic obstructive pulmonary disease. J Allergy Clin Immunol 2016; 138(1):16-27.
12. Kessler R, Partridge MR, Miravitlles M et al. Symptom variability in patients with severe COPD: a pan-European cross-sectional study. Eur Respir J 2011; 37(2):264-272.
13. Lopez-Campos JL, Calero C, Quintana-Gallego E. Symptom variability in COPD: a narrative review. Int J Chron Obstruct Pulmon Dis 2013; 8:231-238.
14. Nishimura K, Izumi T, Tsukino M et al. Dyspnea is a better predictor of 5-year survival than airway obstruction in patients with COPD. Chest 2002; 121(5):1434-1440.
15. Kouijzer M, Brusse-Keizer M, Bode C. COPD-related fatigue: Impact on daily life and treatment opportunities from the patient's perspective. Respir Med 2018; 141:47-51.
16. Miravitlles M, Anzueto A, Legnani D et al. Patient's perception of exacerbations of COPD--the PERCEIVE study. Respir Med 2007; 101(3):453-460.
17. 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.
18. Seemungal TA, Harper-Owen R, Bhowmik A et al. Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. Eur Respir J 2000; 16(4):677-683.

19. Lorenz J, Busch W, Thate-Waschke IM. Moxifloxacin in acute exacerbations of chronic bronchitis: clinical evaluation and assessment by patients. J Int Med Res 2001; 29(2):61-73.
20. Vijayasaratha K, Stockley RA. Reported and unreported exacerbations of COPD: analysis by diary cards. Chest 2008; 133(1):34-41.
21. Calverley PM, Rennard S, Nelson HS et al. One-year treatment with mometasone furoate in chronic obstructive pulmonary disease. Respir Res 2008; 9:73.
22. 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.
23. Mudgalkar N, Bele SD, Valsangkar S et al. Utility of numerical and visual analog scales for evaluating the post-operative pain in rural patients. Indian J Anaesth 2012; 56(6):553-557.
24. 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.
25. 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.
26. Criner RN, Han MK. COPD Care in the 21st Century: A Public Health Priority. Respir Care 2018; 63(5):591-600.
27. Wouters EF. The burden of COPD in The Netherlands: results from the Confronting COPD survey. Respir Med 2003; 97 Suppl C:S51-S59.
28. Llanos JP, Ortega H, Germain G et al. Health characteristics of patients with asthma, COPD and asthma-COPD overlap in the NHANES database. Int J Chron Obstruct Pulmon Dis 2018; 13:2859-2868.
29. Seemungal TA, Donaldson GC, Paul EA et al. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1998; 157(5 Pt 1):1418-1422.
30. Celutkiene J, Balciunas M, Kablucko D et al. Challenges of Treating Acute Heart Failure in Patients with Chronic Obstructive Pulmonary Disease. Card Fail Rev 2017; 3(1):56-61.
31. Chen YW, Leung JM, Sin DD. A Systematic Review of Diagnostic Biomarkers of COPD Exacerbation. PLoS One 2016; 11(7):e0158843.
32. Stockley RA, Halpin DMG, Celli BR et al. Chronic Obstructive Pulmonary Disease Biomarkers and Their Interpretation. Am J Respir Crit Care Med 2019; 199(10):1195-1204.
33. Annesi-Maesano I. Air Pollution and Chronic Obstructive Pulmonary Disease Exacerbations: When Prevention Becomes Feasible. Am J Respir Crit Care Med 2019; 199(5):547-548.
34. 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.
35. Donaldson GC, Goldring JJ, Wedzicha JA. Influence of season on exacerbation characteristics in patients with COPD. Chest 2012; 141(1):94-100.
36. Tashkin DP, Wechsler ME. Role of eosinophils in airway inflammation of chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2018; 13:335-349.

37. Sidhaye VK, Nishida K, Martinez FJ. Precision medicine in COPD: where are we and where do we need to go? Eur Respir Rev 2018; 27(149).
38. Crisafulli E, Barbeta E, Ielpo A et al. Management of severe acute exacerbations of COPD: an updated narrative review. Multidiscip Respir Med 2018; 13:36.
39. Walters JA, Tan DJ, White CJ et al. Systemic corticosteroids for acute exacerbations of chronic obstructive pulmonary disease. Cochrane Database Syst Rev 2014;(9):CD001288.
40. Gunen H, Hacievliyagil SS, Yetkin O et al. The role of nebulised budesonide in the treatment of exacerbations of COPD. Eur Respir J 2007; 29(4):660-667.
41. Walters JA, Tan DJ, White CJ et al. Different durations of corticosteroid therapy for exacerbations of chronic obstructive pulmonary disease. Cochrane Database Syst Rev 2018; 3:CD006897.
42. 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.
43. 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.
44. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med 2008; 359(22):2355-2365.
45. 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.
46. Lopez-Campos JL, Hartl S, Pozo-Rodriguez F et al. Antibiotic Prescription for COPD Exacerbations Admitted to Hospital: European COPD Audit. PLoS One 2015; 10(4):e0124374.
47. 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.
48. Vollenweider DJ, Frei A, Steurer-Stey CA et al. Antibiotics for exacerbations of chronic obstructive pulmonary disease. Cochrane Database Syst Rev 2018; 10:CD010257.
49. Lopez-Campos JL, Agusti A. Heterogeneity of chronic obstructive pulmonary disease exacerbations: a two-axes classification proposal. Lancet Respir Med 2015; 3(9):729-734.
50. Westwood M, Bourbeau J, Jones PW et al. Relationship between FEV1 change and patient-reported outcomes in randomised trials of inhaled bronchodilators for stable COPD: a systematic review. Respir Res 2011; 12:40.
51. Yoon HI, Sin DD. Biomarkers of therapeutic response in patients with chronic obstructive pulmonary disease: a critical review of the literature. Drugs 2011; 71(14):1821-1837.
52. 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.
53. 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.
54. Aloisio E, Dolci A, Panteghini M. Procalcitonin: Between evidence and critical issues. Clin Chim Acta 2019; 496:7-12.

55. 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).
56. 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.
57. Boncler M, Wu Y, Watala C. The Multiple Faces of C-Reactive Protein-Physiological and Pathophysiological Implications in Cardiovascular Disease. Molecules 2019; 24(11).
58. Cooke J, Butler C, Hopstaken R et al. Narrative review of primary care point-of-care testing (POCT) and antibacterial use in respiratory tract infection (RTI). BMJ Open Respir Res 2015; 2(1):e000086.
59. Andreeva E, Melbye H. Usefulness of C-reactive protein testing in acute cough/respiratory tract infection: an open cluster-randomized clinical trial with C-reactive protein testing in the intervention group. BMC Fam Pract 2014; 15:80.
60. 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.
61. Spurrell JC, Wiehler S, Zaheer RS et al. Human airway epithelial cells produce IP-10 (CXCL10) in vitro and in vivo upon rhinovirus infection. Am J Physiol Lung Cell Mol Physiol 2005; 289(1):L85-L95.
62. Wilkinson TM, Hurst JR, Perera WR et al. Effect of interactions between lower airway bacterial and rhinoviral infection in exacerbations of COPD. Chest 2006; 129(2):317-324.
63. Quint JK, Donaldson GC, Goldring JJ et al. Serum IP-10 as a biomarker of human rhinovirus infection at exacerbation of COPD. Chest 2010; 137(4):812-822.
64. Sack GH, Jr. Serum amyloid A - a review. Mol Med 2018; 24(1):46.
65. Bu XL, Cao GQ, Shen LL et al. Serum Amyloid-Beta Levels are Increased in Patients with Chronic Obstructive Pulmonary Disease. Neurotox Res 2015; 28(4):346-351.
66. Barnes PJ. Cellular and molecular mechanisms of chronic obstructive pulmonary disease. Clin Chest Med 2014; 35(1):71-86.
67. Austin V, Crack PJ, Bozinovski S et al. COPD and stroke: are systemic inflammation and oxidative stress the missing links? Clin Sci (Lond) 2016; 130(13):1039-1050.
68. Donaldson GC, Seemungal TA, Patel IS et al. Airway and systemic inflammation and decline in lung function in patients with COPD. Chest 2005; 128(4):1995-2004.
69. Wilkinson TM, Patel IS, Wilks M et al. Airway bacterial load and FEV1 decline in patients with chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2003; 167(8):1090-1095.
70. Jasper AE, McIver WJ, Sapey E et al. Understanding the role of neutrophils in chronic inflammatory airway disease. F1000Res 2019; 8.
71. Khair OA, Davies RJ, Devalia JL. Bacterial-induced release of inflammatory mediators by bronchial epithelial cells. Eur Respir J 1996; 9(9):1913-1922.
72. Chung KF. Cytokines in chronic obstructive pulmonary disease. Eur Respir J Suppl 2001; 34:50s-59s.
73. Kawabata K, Hagio T, Matsuoka S. The role of neutrophil elastase in acute lung injury. Eur J Pharmacol 2002; 451(1):1-10.

74. 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.
75. Bateman ED, Reddel HK, van Zyl-Smit RN et al. The asthma-COPD overlap syndrome: towards a revised taxonomy of chronic airways diseases? Lancet Respir Med 2015; 3(9):719-728.
76. Sinden NJ, Stockley RA. Systemic inflammation and comorbidity in COPD: a result of 'overspill' of inflammatory mediators from the lungs? Review of the evidence. Thorax 2010; 65(10):930-936.
77. Nemmar A, Hoylaerts MF, Hoet PH et al. Possible mechanisms of the cardiovascular effects of inhaled particles: systemic translocation and prothrombotic effects. Toxicol Lett 2004; 149(1-3):243-253.
78. Garcia-Rio F, Miravitlles M, Soriano JB et al. Systemic inflammation in chronic obstructive pulmonary disease: a population-based study. Respir Res 2010; 11:63.
79. Celli BR, Locantore N, Yates J et al. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012; 185(10):1065-1072.