Blood Eosinophils Counts and Comorbidities in Stable COPD Patients: an Exploratory Study

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2. Samenvatting

2.1. Context

Er ontwikkelt zich een groeiende interesse voor de waarde van het absolute aantal bloedeosinofielen (blood eosinophil counts of BEC) als biomerker bij Chronische Obstructieve Longziekte (COPD). Er heerst echter nog steeds een onenigheid over de beïnvloedende factoren, waaronder de aanwezigheid van comorbiditeiten, op deze biomerker. Bovendien werd de relatie tussen de BEC en de comorbiditeiten, beschreven in de Global Initiative for Obstructive Lung Disease (GOLD) richtlijn van 2022 enerzijds, en de relatie tussen de BEC en de multidimensionele predictieve instrumenten (de Charlson Comorbiditeiten Index (CCI) en de COPD specifieke comorbiditeiten test (COTE) index), die tevens comorbiditeiten bevatten, anderzijds nog zelden of niet bestudeerd.

2.2. Onderzoeksvraag

Bestaat er een correlatie tussen het absolute aantal bloedeosinofielen (BEC) van COPD patiënten en hun aantal GOLD-comorbiditeiten, hun CCI-score en hun COTE-indexscore?

2.3. Methode

Een retrospectieve exploratieve studie met gegevens uit het elektronisch medisch dossier (EMD) van twee Vlaamse huisartsenpraktijken werd opgezet om deze lineaire correlatie a.d.h.v. een Pearson correlatietest te onderzoeken.

2.4. Resultaten

Tachtig COPD patiënten, waaronder 48 mannen en 32 vrouwen, met een gemiddelde leeftijd (SD) van 73 (9,6) jaar en een gemiddelde éénsecondewaarde (SD) van 65,4 (17,5) % voorspelde waarde, werden geïncludeerd in deze studie. De BEC waarden waren niet normaal verdeeld en varieerden van nul tot 667 cellen/µL met een mediaan (IQR) van 181,5 (211) cellen/µL. In totaal werden 194 GOLD-comorbiditeiten beschreven, waarvan arteriële hypertensie het frequentst voorkwam (in 56.25% van alle patiënten). Elke patiënt had er gemiddeld (SD) 2,5 (1,7). Er kon echter geen significante lineaire correlatie gevonden worden tussen de patiëntspecifieke BEC waarden en hun aantal GOLD-comorbiditeiten (r = 0,089; p = 0,431; N = 80), noch hun CCI-uitkomstscores (r = 0,196; p = 0,081; N = 80), noch hun COTE-index scores (r = 0,102; p = 0,369; N = 80).

2.5. Conclusie

Deze studie toonde geen significante lineaire correlaties tussen het absolute aantal bloedeosinofielen (BEC) en het aantal GOLD-comorbiditeiten, en de resultaten van de predictieve instrumenten, de CCI en COTE-index. Grootschaligere studies zijn nodig om deze relaties verder te onderzoeken.

2.6. Sleutelwoorden

COPD – absoluut aantal bloedeosinofielen – comorbiditeiten

3. Abstract

3.1. Background

There is increasing interest in the absolute blood eosinophil counts (BEC) as a biomarker in Chronic Obstructive Pulmonary Disease (COPD). However, there is still controversy about the influencing factors of the BEC, including comorbidities. Furthermore, the relations between BEC and the comorbidities described in the 2022 Global Initiative for Obstructive Lung Disease (GOLD) guideline on the one hand, and BEC and the frequently used multidimensional predictive tools (Charlson Comorbidity Index (CCI) and COPD specific comorbidity test (COTE) index), also containing comorbidities, on the other hand, are still rarely or not studied.

3.2. Hypothesis

Is there a correlation in COPD patients between the BEC on the one hand and the number of GOLD comorbidities, the CCI score, and the COTE index score on the other?

3.3. Methods

A retrospective exploratory study on data from Electronic Health Records (EHR) of two Flemish primary care practices was designed to investigate the linear correlation (using a Pearson correlation test) between the BEC values of COPD patients and their total number of comorbidities described in the 2022 GOLD guideline, and their outcome scores of the CCI and the COTE index.

3.4. Results

Eighty eligible COPD patients (48 male; mean (SD) age is 73 (9.6) years; mean (SD) FEV_1 is 65.4 (17.5) % predicted) were included in this study. The BEC was right-skewed and ranged from zero to 667 cells/µL with a median (IQR) of 181.5 (211) cells/µL. A total of 194 GOLD comorbidities were identified with arterial hypertension being the most prevalent (in 56.25% of all patients). Each patient had a mean (SD) of 2.425 (1.71) comorbidities. However, no significant linear correlation could be found between the patient-specific BEC and their number of GOLD comorbidities ($r = 0.089$; $p = 0.431$; $N = 80$), neither their CCI outcome scores ($r = 0.196$; $p = 0.081$; $N = 80$), nor their COTE index scores ($r = 0.102$; $p = 0.369$; $N = 80$).

3.5. Conclusion

There were no linear correlations between the absolute number of BEC and the results of the predictive tools, CCI and COTE index, and the number of GOLD comorbidities. Future, larger-scale studies are needed to investigate and validate these objectives.

3.6. Keywords

COPD – blood eosinophil counts – comorbidities

4. Introduction

4.1. Chronic Obstructive Lung Disease

4.1.1. Definition and symptoms

Chronic Obstructive Pulmonary Disease (COPD) is a heterogenous disorder that often occurs in primary care practice. The 2023 Global Initiative for Chronic Obstructive Lung Disease (GOLD) defines this disease as follows: "*Chronic Obstructive Pulmonary Disease (COPD) is a heterogeneous lung condition characterized by chronic respiratory symptoms (dyspnea, cough, sputum production and/or exacerbations) due to abnormalities of the airways (bronchitis, bronchiolitis) and/or alveoli (emphysema) that cause persistent, often progressive, airflow obstruction."* [1] A parallel definition was established in 2004 by The European Respiratory Society (ERS) and the American Thoracic Society (ATS) and is still in use today. They state that "*COPD is a preventable and treatable disease state characterised by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases, primarily caused by cigarette smoking. Although COPD affects the lungs, it also produces significant systemic consequences.*" [2] The two definitions are largely similar. However, the ATS/ERS guideline emphasises the additional systemic consequences of the condition. [2]

COPD patients typically present symptoms such as dyspnoea, coughing, and sputum production (as mentioned above), but also wheezing, fatigue, weight and muscle loss are frequent. [1, 3] The symptoms can vary over time, are individually different and often accompanied by other complaints caused by systemic inflammatory consequences, more specifically aggravations of comorbidities. A sudden increase in intensity of symptomatology and a concomitant deterioration of the general health status of the COPD patient is defined as a 'COPD lung attack' or exacerbation. Both the symptoms of COPD and the episodes of exacerbation and their frequency or intensity are patient-specific and regularly under-recognized and under-reported. [4, 5]

4.1.2. Epidemiology

Chronic respiratory diseases (CRD), including COPD, are a major cause of morbidity and mortality worldwide. [3, 6] According to the Global Burden of Disease Study (GBD) of 2017, COPD is the seventh most frequent cause of years of life lost (YLLs) worldwide. The same study described approximately three million deaths each year due to COPD. [7] Moreover, the World Health Organization (WHO) predicts that COPD will be the third leading cause of death by 2030. [8] COPD currently has a prevalence of just over 10% [1] and it is continuing to rise, as the global number of patients with COPD increased by almost 50% in the last three decades [6]. In contrast, the incidence decreased slightly by

around 0.7% and, the age-standardized prevalence rate is falling. These phenomena can possibly be explained by the growing world population. [6] COPD usually occurs in the second half of life and 10% of people over the age of 45 are thought to suffer from COPD. [9, 10] In their guideline for COPD, Het Nederlands Huisartsen Genootschap (NHG) describes a prevalence of 35.6 COPD diagnoses per 1000 patients and an incidence of 0.23% and 0.21% per year for women and men, respectively, in the Netherlands primary care practices. [11] In Belgium, it is estimated that approximately 800,000 people suffer from this disease, but merely half of them have been diagnosed. [12] Previously, COPD used to be considered as a disease that predominantly affected smoking men, but due to the increasing number of women smoking the gender distribution of COPD is now thought to be roughly equal. [8, 10]

4.1.3. Etiology and Pathogenesis

COPD is a progressive, heterogenous lung disease characterised by chronic inflammation caused by the frequent inhalation of harmful gases. Chronic tobacco use is the main, but not the only, causative risk factor. [1, 3] Hence, at present COPD could be taxonomically subdivided into a few etiotypes. [1] Other outdoor or indoor air pollution, whether work-related or not (including chemical or (in)organic dusts, gases released during the combustion of various substances or liquids, and pesticides), can promote the development of this disease in non-smokers. A history of chronic inhalation of noxious particles (exogenous or environmental risk factor) in interaction with the presence of patient-specific, endogenous or genetic risk factors (e.g. genetic predisposition, age, gender, (pre- and postnatal) lung development, concomitant lung diseases such as asthma, paediatric lung infections etc.) can predispose current smokers as well as non-smokers, and ex-smokers to the development of COPD in life. [3, 4] In addition, less than half of smokers develop COPD at some point in their lives. [3]

Little is known about the underlying and complex mechanisms in the pathogenesis of COPD, but chronic inflammation seems to be a key feature. [10] The inflammation in the lung parenchyma, vasculature and the small peripheral airways is coordinated by several (still insufficiently understood) immunological processes, lymphocyte-, neutrophil- and eosinophil-associated. [9, 13] Surface receptors on epithelial lining of the small airways and on pulmonary macrophages recognise ligands, previously released after tissue damage by particles of chronically inhaled toxic agents (cigarette smoke or oxidative stress) or harmful pathogens (microbes). [13] This triggers the increase of macrophages, eosinophils, activated neutrophils and lymphocytes and the release of inflammatory mediators, and growth factors leading to three major pathophysiological mechanism: airway remodelling, mucus hypersecretion and emphysema. [5, 14, 15] The airway remodelling (fibrosis) causes narrowing of the small airways and airflow limitation. [5, 14, 15] The mucus hypersecretion is mostly T-cell guided, where emphysema is provoked by inflammatory cells released proteases. [5, 14,

15] Finely lung parenchyma, more specifically the alveolar attachments, are damaged in such a way that airspace enlargement and subsequently air trapping or hyperinflation occur. [5, 14, 15] This phenomenon can be seen on imaging and results in a deficient gas exchange. [5, 14, 15] An illustrative representation of neutrophil- and eosinophil-mediated cellular processes is nicely presented by Brightling et al. [13]

4.1.4. Diagnostics and staging

In patients with typical symptoms, recurrent lower respiratory tract infections and/or exposure to causally harmful substances, the diagnosis of COPD can be suspected but needs confirmation by spirometry. An irreversible airflow limitation is established when the Tiffeneau Index (TI) or FEV_1/FVC (forced expiratory volume in 1 second over forced vital capacity) after bronchodilation is less than 70%. [1, 3] The use of a fixed cut-off however is coming under increasing pressure because it could lead to misdiagnosis. Patients of advanced age are often overdiagnosed and overtreated, whereas COPD in younger patients (<45 years) is often underdiagnosed when strictly applying the fixed cut-off. An age-dependent approach using the lower limits of normal (LLNs) was proposed by the ERS (European Respiratory Society) GLI (Global Lung Function Initiative) Spirometry Task Force as an alternative. [3, 16, 17] They recommend considering cut-off levels below the lower fifth percentile of the post-bronchodilator TI (FEV_1/FVC) as deviating. As reference values, the normal distribution of LLNs within the general healthy population is used. [3, 16]

4.1.5. Treatment

As stated earlier, COPD is a heterogeneous disease characterized by a variety of clinical presentations and underlying pathophysiological mechanisms. As a result of this heterogeneity, every patient needs a personalized therapeutical approach. [18, 19] In the recent 2023 GOLD guidelines, the previous ABCD subdivision was replaced by an ABE classification tool. Based on the FEV_1 (% of predicted), patients are classified into three GOLD grades, representative of the severity of their airflow limitation. The intensity of the symptoms reported by the patients and their disease experience are taken into account and classified, using two types of tools: the modified Medical Research Council (mMRC) dyspnea scale or the COPD Assessment Test (CAT). Depending on the provided answers and their exacerbation history, patients are categorized into three groups (A, B or E). This classification is the foundation for the pharmacological decision-making process of newly diagnosed patients. In the follow-up phase, the degree of dyspnea and/or the occurrence of exacerbations are used to adjust therapy. Bronchodilatation, using long-acting (LA) or short-acting, (SA) bronchodilators, is the main pharmacological approach. These medications improve symptoms by dilation of the airways and reduce the risk of future exacerbations. Both anticholinergics (muscarinic antagonists, MA) and bèta$_2$-

mimetics (bèta$_2$-agonists, BA), whether or not combined, are used. [1] COPD patients belonging to group E, in whom exacerbations occur regularly (two or more times a year) or in whom hospitalisation has been necessary in the last year because of such an exacerbation, inhaled corticosteroids (ICS) can be added to the LABA-LAMA treatment in certain circumstances, if the blood eosinophil counts is ≥300 cells/µL. [1] However, the non-pharmacological treatment of COPD is as if not more important. Smoking cessation is the foremost step in the therapeutical approach to COPD, since, with or without the help of pharmacotherapy, it is the only intervention that favourably influences the course and prognosis of COPD. In addition, education, self-management, vaccination, pulmonary rehabilitation, oxygen therapy, and interventional therapies could improve the psychological and physical condition of the patient. [3]

4.2. Blood eosinophils in COPD

4.2.1. Eosinophilic airway inflammation and eosinophilic COPD

Eosinophils are granulocytes, innate bilobed immune cells produced during haematopoiesis by the bone marrow. They account for about 1% to 4% of the total white blood cell count. [21] Besides their role in the innate immune system, they also fulfil other functions during illness and health. The so-called LIAR (Local Immunity And/or Remodelling/Repair) hypothesis stated by Lee, et al. describes their role in remodelling, fibrosis, metabolic homeostasis, and the adaptive immune system. [19, 28] Eosinophils, like their counterparts, neutrophils, and macrophages, are recruited across the bronchial vascular endothelium to the airways by local pro-inflammatory mediators and growth factors, such as interleukin-5 (IL-5) and GM-CSF and induce a type II (Th2-mediated or eosinophilic) immune response (figure 1). [21] Other chemokines, such as CCL5, IL-3, IL-4, IL-13 and IL-33 are also involved in the type 2 eosinophilic inflammatory process. [20] As stated earlier, inflammation plays an important pathobiological role in the development of COPD. In the past, neutrophils were identified as the main driver of inflammation in COPD but more recently, another inflammatory entity has emerged: 'the eosinophilic COPD phenotype'. [18–20] This phenotype is characterised by the presence of eosinophilic airway inflammation, but the exact definition of this entity remains to be determined as studies used different criteria when researching this subgroup of COPD patients. [20] Defining eosinophilic COPD is further complicated by the fact that the proposed thresholds for establishing the presence of eosinophilic airway inflammation are within the normal range limits. Currently, BEC ≥300 cells/µL is the most widely used cut-off. [3]

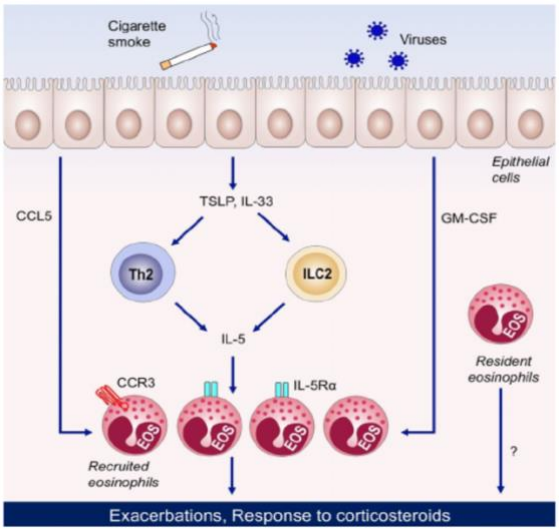


Figure 1: Developmental cascade for type 2 eosinophilic inflammation in COPD. Eosinophils are attracted to lung tissue by inflammatory mediators including chemokine CCL5, growth factor GM-CFS and IL-5, post-exposure to inhaled toxicants and pathogens. CCL5: C-C motif ligand 5; CCR3: C-C chemokine receptor type 3; GM-CSF: granulocyte-macrophage colony stimulating factor; ILC2: type 2 innate lymphoid cell; IL-5: interleukin-5; IL-33: interleukin-33; IL-5R α: interleukin-5 receptor alpha Th2: T helper-2 cell; TSLP: thymic stromal lymphopoietin.; Barnes PJ et al. 2019 [10] Reproduced with permission from John Wiley and Sons (Allergy) on 12 April 2023 (License number: 5526620151548).

The prevalence of this phenotype (eosinophilic COPD) is substantial, yet an accurate percentage is not known as the results of prevalence studies vary. [18] A meta-analysis and systematic review performed by Hong-Xia Wu et al. with nineteen trials and more than 40,000 COPD patients included, showed that the prevalence of eosinophilic inflammation (defined as eosinophil levels of >2% in blood) ranged from 19% to 67%, with an average of 55%. [18] Other sources mention values between 20% and 55%. [20–22] The gold standard for assessing eosinophilic airway inflammation is sputum analyses, however it is an expensive and a difficult test to perform. As sputum eosinophils are correlated with the eosinophil numbers measured in the blood, even during exacerbations (when eosinophil levels are often already elevated) BEC has emerged as a surrogate, more accessible biomarker for eosinophilic airway inflammation. [18–20, 23–29] BEC is a better more accurate alternative than tissue eosinophil infiltrates. [20] Finally, there is such a thing as fractional exhaled nitric oxide (FeNO) which is also a biomarker of eosinophilic inflammation, used in asthmatic patients. [20]

4.2.2. Eosinophils and inhaled corticosteroids in stable COPD

According to the landmark RCTs (randomized controlled trials), TRILOGY, TRINITY, TRIBUTE, IMPACT, and ETHOS, a triple therapy (combination of LABA, LAMA and ICS) in COPD patients with high disease burden, reduces the risk of exacerbations, improves quality of life (QOL), and lowers all-cause mortality

compared to a bronchodilator monotherapy, dual bronchodilator therapy or ICS-bronchodilator combination therapy. [30–34] ICS therapy in these frequently exacerbating patients with a high number of eosinophils found in the peripheral blood reduces the likelihood of a COPD exacerbation [21, 30] Blood eosinophil count as biomarker has a predictive value regarding the effect size of ICS for reducing the risk of exacerbation. [20, 23, 35, 36] Therefore, the GOLD guideline advises the use of the absolute number of blood eosinophils per microliter as a therapeutic marker in stable COPD patients to guide clinical decisions. [1] In patients with a BEC exceeding ≥300 cells/µL, or frequent exacerbations (≥2 moderate exacerbation per year or a need for hospitalisation) or a concomitant asthma, treatment with ICS is strongly recommended. Other considerations for starting an ICS (such as BEC between 100 and 300cells/µL or 1 moderate exacerbation in the last year) and avoidances for their use are listed in the 2023 GOLD report. [1] The cut-offs (<100, and ≥300 eosinophils/µL) were determined using data models showing a continuous relation between BEC and the effectiveness of ICS inhalation therapy in preventing exacerbations. [3] However, these thresholds are not pure fixed cut-offs, but rather indicative and in addition below the usual upper limit of labs (around 500 cells/µL). [3, 37] Inhaling corticosteroids also has its side-effects and risks. This manifests itself in a higher prevalence of oral candida infections, the development of pneumonia, hoarseness, cataract, bruising of the skin, osteoporosis, and fractures. [3, 19, 21, 38] Therefore ICS should only be used by those patients with expected benefit and should be stopped in those with no indication (or with serious side-effects).[39] BEC can also be used to guide the withdrawal of ICS as in stable COPD patients without eosinophilic inflammation, ICS can be stopped with no additional exacerbation risk. [39, 40]

4.2.3. BEC as biomarker in stable COPD

The WHO defines a biomarker as "*any substance, structure or process that can be measured in the body or its products and that can influence or predict the incidence of an outcome or disease*". [41] Furthermore, a quality biomarker must be readily available, safe, inexpensive, stable, and reproducible. [27, 42] The clinical use of BEC as biomarker in COPD is very versatile. As stated earlier, they can be used for inflammatory phenotyping, but they can also be used as predictor of exacerbation risk. Studies show that the presence of blood eosinophilia tends to make the patient more prone to COPD exacerbations by causing airway remodelling. [21, 42] Although some evidence including post hoc analyses does not support this. [23, 43] Determining the number of eosinophils may be clinically important since: in 28% of COPD exacerbations, eosinophils are thought to underlie the acute clinical deterioration [44]; higher eosinophil levels in the blood (≥300 cells/µL) in COPD patients seem to predict a lower mortality risk [23], but make them more susceptible to readmission and are linked with a lower QOL [42]; eosinophilic exacerbation appears to have better outcomes in terms of survival, infection and duration of hospital stay [45, 46]; and treatment with corticosteroids would have a

positive effect on these eosinophilic COPD patients' recovery and clinical outcome [47]. All these insights led to eosinophilia being considered as one of the three treatable components of COPD, namely pulmonary abnormalities besides extrapulmonary comorbidities and environmental factors. [21]

Based on disease inflammatory phenotyping (eosinophilic COPD versus neutrophilic COPD), medical researchers try to personalise the treatment. [27] It seems that BEC would be a predictor of patient's response to corticosteroid treatment, during exacerbation and clinical stability. Higher values would predict therapeutic success. [47–50] This combined with the fact that BEC are easily accessible, inexpensive, and safe to use, makes them a very promising biomarker. However, there is still insufficient knowledge about the stability and influencing factors, such as patient characteristics or comorbidities, on BEC. [50] The absolute number of eosinophils (or BEC) can be inter-individually variable over time. [23, 38, 51, 52] Therefore a single eosinophilic measurement may not be fully reliable to determine whether a patient should receive ICS. Nevertheless, when patients are stratified into three groups (<100, 100 to 300 and >300 eosinophils/µL) according to their baseline eosinophil count, after one year almost 70% of the patients remained in their original group. [53] Furthermore, the use of ICS (in contrast to oral corticosteroids) would not affect the BEC. [54]

In addition, BEC can be influenced by various other factors, including different types of medication, the presence of parasitic or helminthic infections, autoimmune diseases or allergic predisposition, and haematological disorders... [20] Moreover, there are several eosinophilic pulmonary diseases typified by a pulmonary eosinophilia, including asthma, hypereosinophilic syndrome (HES), eosinophilic chronic rhinosinusitis (CRS), acute eosinophilic pneumonia (AEP), chronic eosinophilic pneumonia (CEP), allergic bronchopulmonary aspergillosis (ABPA), eosinophilic granulomatosis with polyangiitis (EGPA), and hypereosinophilic obliterative bronchiolitis (HOB). However, these do not always have an increased BEC. [20, 55]

4.3. COPD comorbidities

4.3.1. BEC and COPD comorbidities

People with COPD often suffer from more than just the pulmonary disorder, as a wide range of co-occurring comorbidities exists. According to Vanfleteren et al., almost all COPD patients (nearly 98%) suffer from at least one comorbidity. [56] The prevalence of the individual comorbidities or their combinations fluctuates between different studies. [56] Vanfleteren et al investigated the prevalence of 13 comorbidities (anaemia, mycardial infarction, underweight and muscle wasting, depression and anxiety, renal insufficiency, obesity, dyslipidemia, hypertension, atherosclerosis and hyperglycaemia) in COPD patients. The latter three occurred most frequently (each in about 50% of patients). An

illustrative histogram with the exact frequencies of occurrence can be found in the article available online by Vanfleteren et al. [56] Only 2.3% of patients had none of the 13 comorbidities. [56]

Another summarised list of COPD-comorbidities can be found in the 2022 GOLD report: cardiovascular diseases like heart failure, arrhythmias, ischaemic heart disease (IHD), hypertension and peripheral artery disease (PAD), gastroesophageal reflux disease (GERD), osteoporosis, metabolic syndrome (MS), diabetes mellitus (DM), bronchiectasis, obstructive sleep apnea (OSA), lung cancer, anxiety, depression, and cognitive impairment. [3] Divo et al. integrated the COPD comorbidities in a graphical expression based on their prevalence and risk of death, called the “Comorbidome". [57] The figure can be found in the article of Divo et al. [57] Comorbidities that are situated closer to the centre of the circle have a greater association with the risk of death. [57] Conditions with a significant increase in mortality were placed within the dotter circle. [57] The larger the circle of a comorbidity, the greater its prevalence in COPD patients from the studied cohort. [57] These comorbidities and the general health status of the patient can influence the course of the pulmonary disease, the effectiveness of the treatment, and their prognosis. [57] Depending on the GOLD grade, the causes of death (respiratory or due to another comorbidity) are distributed differently. [58] Lung cancer accounts for most cancer deaths worldwide, whereas cardiovascular diseases (CVD) including hypertension are the most common comorbidity in COPD patients. Some conditions may be partly due to COPD, others have a similar mechanism or clinical presentation. Concomitant, sometimes temporary, respiratory diseases, in addition to lung cancer and obstructive sleep apnoea syndrome, are also frequently highlighted: pneumonia, asthma, pulmonary embolism, pulmonary hypertension, pulmonary fibrosis... [3, 5] In a multicentre study following more than 1,600 COPD patients, as many as twelve comorbidities were found to have a negative impact on survival and their presence implies a higher risk of death. [57] Other host-specific characteristics are not considered comorbidities but nevertheless play a major role in the outcome of COPD patients. Examples of these include smoking status and history, age, gender, medication use, lifestyle, etc. [57] The simultaneous presence of various comorbidities (clusters of comorbidities) often renders the formulation of an individual patient-specific therapeutic strategy very challenging. [56]

As previously mentioned, the influencing determinants of BEC, such as patient characteristics or comorbidities, have not yet been sufficiently described. [50] For example in the LEAD (Lung, hEart, sociAl, boDy) study, Hartl et al. described in a general population that the following potential confounders were significantly associated with a high BEC (>75th percentile): age ≤18 years, asthma or COPD, metabolic syndrome, male gender, positive skin prick test, obesity, and active smokers. [59] However, having an older age is also seen as an influencing factor in other studies. [60] When COPD

patients, asthma patients and COPD- or asthma-free controls were compared, Caspard et al. observed that BEC were higher in men and in patients with higher BMI, and lower in certain ethnicities. [61] An increased BEC with increasing age and in active smokers or former smokers was only seen in the control group and the control group plus asthma patients, respectively. [61] Moreover, contradicting the information from Hartl et al., Caspard et al. observed no significant difference between the BEC value in COPD patients and controls suffering neither from COPD, nor asthma. [61] Later, in a comprehensive review and meta-analysis of 91 studies of patients with and without respiratory disease by Benson and Hartl, the factors of the LEAD study mentioned above were supplemented by allergic rhinitis and an increased IgE count. [62] The ProAR (Program for Control of Asthma in Bahia) study showed similar results in non-asthmatic people. [62, 63] In a study of Amaral et al., metabolic syndrome (MS), heart disease and stroke were all associated with higher BEC (and not with FeNO or C-reactive protein (CRP)) in general population (with or without respiratory diseases). [50] Moreover, eosinophilic COPD seems to be more common in men, ex-smokers and patients with a higher BMI, and a history of ischaemic heart diseases, whereas certain comorbidities such as heart failure, arterial hypertension and diabetes mellitus are less common in these eosinophilic COPD patients. [18] In contrast, Pongdee et al. described an association between BEC and the prevalence of the following (sometimes already mentioned) comorbidities in non-COPD patients: hyperlipidaemia, hypertension, chronic kidney disease (CKD), diabetes mellitus, cancer, cardiovascular diseases (transient ischaemic attack (TIA) or stroke, heart failure (HF), coronary heart disease (CHD), arrhythmias). [60] In addition, CVD deaths were also observed at and here, too, an association with BEC was found. [60] In addition, in a COPD cohort an association was found between higher eosinophil values (eosinophils >2%) and increased age, male gender and severe concomitant asthma. In these patients, a lower prevalence of some comorbidities was observed (anemia and heart attack). [64] Other studies agree on the influence of age, active smoking status and gender of the included COPD patients on their eosinophil counts, and add the relation between high eosinophil counts (≥2% or ≥150 cells/µL) and better symptom control (lower BODE (body mass index, airflow obstruction, dyspnoea, exercise capacity) index, better mMRC and SGRQ (St George's Respiratory Questionnaire)), as well as better spirometry values (FEV1 % predicted) and fat free mass index to the list. [65] Finally, cumulations of influencing factors were also associated with higher measured BEC by Benson and Hartl. [62]

4.3.2. Multidimensional predictive tools including comorbidities

Age and FEV_1 are the most important prognostic factors in COPD, but unfortunately, they are progressive and irreversible. [66] However, in addition to the spirometry data, multidimensional tools also appear to be a good way of estimating disease severity and predicting treatment response and patient survival in COPD. [66] In 1987, Dr. Mary Charlson and her colleagues developed a prognostic tool to estimate (not only COPD) patients' survival based on comorbidities. The Charlson Comorbidity Index (CCI) calculates the patient's ten-year survival based on the presence or absence of sixteen comorbidities, and current age (scored from zero to six points) (table 1). Charlson et al., used the one-year adjusted relative risks from their longitudinal study to give each comorbidity a weighted score with a maximum score of thirty-seven. [67]

Charlson Comorbidity Index	
Comorbidity	**Score**
Myocardial infarction	1
Congestive heart failure	1
Peripheral vascular disease	1
Cerebrovascular accident or transient ischemic attack	1
Dementia	1
COPD	1
Connective tissue disease or rheumatic disease	1
Peptic ulcer disease	1
Diabetes mellitus	1 uncomplicated 2 end-organ damage
Liver disease	1 mild 3 moderate or severe
Hemiplegia or paraplegia	2
Chronic kidney disease	2 moderate or severe
Leukemia	2
Lymphoma	2
Solid tumor	2 localized 6 metastatic
AIDS or HIV	6
Age (years)	
50-59	1
60-69	2
70-79	3
≥80	4

Table 1: Charlson Comorbidity Index (CCI) weighted scores of patients' comorbidities. If the comorbidity is not present or the patient is less than 50 years old, then the score is zero for that item. The maximum score is 37 points. *"Liver disease: severe = cirrhosis and portal hypertension with a history of variceal bleeding; moderate = cirrhosis and portal hypertension without variceal bleeding; mild = chronic hepatitis (or cirrhosis without portal hypertension). Chronic kidney disease: severe = kidney transplant history, uremia, and dialysis treatment; moderate = creatinine >3 mg/dL."* For further information on the subclassifications within the comorbidities, please refer to the supplementary table with the included ICD-10 codes (table 11).; Charlson ME et al. 1987 [67]

However, in COPD, the most widely studied tool for predicting survival outcomes is the BODE (body mass index, airflow obstruction, dyspnea, and exercise capacity) index. This BODE index is a scoring system designed in 2004 by Celli et al. using four clinical parameters (BMI, FEV_1, the mMRC dyspnea scale, and the six minute walk distance). [68] This scoring system was the first to combine several determinants to predict mortality, whereas previously only the FEV_1 was used, to estimate the risk of mortality specifically in COPD patients. [69] A simplified and easier to apply in the clinic derivative of this multidimensional calculator, is the BODEx (body mass index, airflow obstruction, dyspnea, and exacerbations) index, in which the six minute walk test is replaced by the number of COPD exacerbations requiring a hospitalization in the last year (table 2). The prognostic value of the BODEx index is practically equal to the one of the BODE index. [70]

		BODEx index score			
Variable		**0**	**1**	**2**	**3**
B	BMI (kg/m^2)	>21	≤21	-	-
O	FEV_1 (%pred)*	≥65	50-64	36-49	≤35
D	mMRC dyspnea scale	0-1	2	3	4
Ex	Number of exacerbations**	0	1-2	≥3	-

Table 2: Variables of the BODEx index. The BODEx (body mass index, airflow obstruction, dyspnea, exacerbations) index is a simplified version of the BODE (body mass index, airflow obstruction, dyspnea, exercise capacity) index score and is used to predict the four-years survival rate in COPD patients. *FEV_1 (%predicted) is the post bronchodilator value. **The number of severe COPD exacerbations requiring a hospitalization in the last year. BMI: body mass index; FEV_1: forced expiratory volume in one second; mMRC dyspnea scale: modified Medical Research Council.; Soler-Cataluña JJ et al. 2009 [70]

The combination of the BODEx index and the CCI, the so-called CODEX (age adjusted Charlson Comorbidity Index score, airflow obstruction, dyspnea, and exacerbations) index, has also been developed and studied (table 3). This index calculates the mortality risk (after three and twelve months) and the risk of hospital readmission after an exacerbation. [71] Finally, the COTE index (COPD specific comorbidity test) calculates the mortality risk in COPD patients in the presence of twelve specific, disease-modifying comorbidities (table 4). [72]

Variable		CODEX index score 0	1	2	3
C	CCI	0-4	5-7	≥8	-
O	FEV_1 (%pred)*	≥65	64-50	49-36	≤35
D	mMRC dyspnea scale	0-1	2	3	4
EX	Number of exacerbations**	0	1-2	≥3	-

Table 3: Variables of the CODEX index. The CODEX (age adjusted Charlson Comorbidity Index score, airflow obstruction, dyspnea, exacerbations) estimates the risk of readmission and also the mortality risk for recently hospitalized COPD patients. *FEV_1 (%predicted) is the post bronchodilator value. **The number of severe COPD exacerbations requiring a hospitalization in the last year. CCI: Charlson Comorbidity Index; FEV_1: forced expiratory volume in one second; mMRC dyspnea scale: modified Medical Research Council.; Almagro P et al. 2014 [71]

COTE index Comorbidity	Score
Lung, oesophageal, pancreatic, and breast cancer*	6
Anxiety*	6
All other cancers	2
Liver cirrhosis	2
Atrial fibrillation/flutter	2
Diabetes with neuropathy	2
Pulmonary fibrosis	2
Congestive heart failure	1
Gastric/duodenal ulcers	1
Coronary artery disease	1

Table 4: Comorbidities and corresponding values used for calculating the COTE index. The COTE (COPD specific comorbidity test) index computes the risk of death in COPD patients based on the presence of twelve specific concomitant comorbidities. *Only valid on female patients.; Divo M et al. 2012 [72]

5. Rationale and aims

Increasing attention is being paid to the influence of comorbidities as well as to the impact of eosinophils on the disease course, prognosis, and therapy possibilities in COPD patients. The BEC is a biomarker already used in clinical practice for guiding therapeutical decision making in stable COPD patients, despite the insufficient knowledge about the relation of this marker with co-existing comorbidities in COPD patient specifically. Nor is there sufficient clarity yet on their association with widely used indices such as the CCI and the COTE index. In addition, COPD is regularly diagnosed and treated in the primary care setting. The BEC are easy to determine by general practitioners (GP) as well. Furthermore, they have a global view of patients' comorbidities. This ensures that primary care has an important function in providing optimal patient-specific care in COPD.

In this retrospective, exploratory study, the comorbidities, and absolute eosinophil values (BEC) of COPD patients from two Flemish general practices were incorporated. Based on these data we looked at the association between the number of comorbidities (as described in the GOLD guideline of 2022), and also predictive tool outcome scores (CCI and COTE index) and the blood eosinophil values (BEC).

6. Research question

Is there a correlation in COPD patients between the BEC on the one hand and the number of GOLD comorbidities, the CCI score, and the COTE index score on the other?

7. Methods

7.1. Study design

A retrospective, non-interventional study was set up to determine the relation of BEC and the number of comorbidities. This study design allows us to first test the research question in a practically feasible way based on already available medical data from the elective medical records of COPD patients. Further prospective research will be subsequently required to validate the value of the results obtained.

7.2. Study activities

The completion of the entire study (literature review, protocol description, ethics committee application, data collection and analysis) took place from January 2021 to April 2023.

7.3. Study population

Eligible patients were recruited from two primary care practices in Flanders, Belgium:

- Group practice ‘De nieuwe Baan’, Cypriaan Verhavertstraat 111, 1500 Halle. One of the three general practitioners (Dr. Inès Van Rossem) is associated with the Department of Family Medicine and Chronic Care VUB.
- Group practice ‘Groepspraktijk Merchtem’, Oudstrijdersstraat 64-66, 1785 Merchtem. One of the five general practitioners (Dr. Julie Yap) is associated with the department of Family Medicine and Chronic Care VUB.

7.4. Inclusion and exclusion criteria

The following inclusion criteria were applied to the two patient groups:

- COPD patients.
- The availability of an accurate global medical record (GMD: globaal medisch dossier).
- The availability of a blood analysis with a measurement of the absolute number of eosinophils.

The exclusion criteria for this study were:

- Patients younger than 18 years.
- Patients with concomitant asthma.
- Blood analyses with the absolute number of eosinophils measured four weeks before to four weeks after following situations: hospitalisation for respiratory problems, respiratory tract infection, COPD exacerbation, systemic corticosteroid (oral or intra-venous) or antibiotic therapy.

7.5. Ethics committee

This retrospective study was approved by the ethics committee of UZ Brussels (EC-number: EC-2021-354; BUN: 1432021000643) on 22nd December 2021. A GDPR (General Data Protection Regulation) contract and a data protection clause were established and added to the application. For more information concerning this subject reference is made to the corresponding study protocol and ethics committee file. Since this is a retrospective study based on already existing data, informed consent was not required.

7.6. Data acquisition

From January 2022, eligible COPD patients of the two group practices were selected for this retrospective study and their data were integrated in a global Microsoft Excel database. The database was locked on 10th January 2023. Clinical patient data regarding patient characteristics, medical history, comorbidities, diagnosis, and other necessary patient information were obtained from the COPD patients’ electronic health records (EHR) of the two primary care practices. The most recent

available values have been used. The collected patient information implemented in the study database contained:

- **Patient characteristics**
  - Age (year).
  - Gender (M/F).
  - Height (m), weight (kg) and BMI (kg/m^2).
  - Ethnicity (Caucasian, North African, other African, Asian, South American).
- **Smoking status**
  - Never smoked/ current tobacco user/ ex-smoker.
  - Number of packyears (PY).
- **COPD data**
  - The modified Medical Research Council (mMRC) score (0-4) and exacerbation history.
  - Spirometry data of most recently available spirometry:
    - Pre- and post-bronchodilator Tiffeneau index (TI = FEV_1/FVC).
    - Pre- and post-bronchodilator FEV_1 (% predicted) and corresponding GOLD grade (1-4).
- **Blood analysis**
  - Percentage (%) and absolute number (cells/µL) of white blood cells and eosinophils.
- ***Current treatment***
  - Bronchodilator therapy: long/short-acting, anticholinergics/beta2-mimetics, combinations.
  - Inhaled corticoids (ICS).
  - Other COPD medication taken on daily basis.
- **Comorbidities**
  - The presence of the comorbidities implemented in the Charlson comorbidity index (CCI) (table 1) [67], the COTE index (table 4) [72] and the comorbidities mentioned in the 2022 GOLD guideline (table 7) [3].

7.7. Data analysis

The descriptive statistics and all statistical analyses were performed with IBM SPSS statistics version 28 (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0. Armonk, NY: IBM Corp.). To check the linear correlation between BEC values and CCI scores, COTE index scores and GOLD comorbidities (all continuous variables), a Pearson correlation test was chosen. An independent t-test was selected to check the difference between the mean values of the CCI scores, the COTE index scores, and GOLD comorbidities at BEC <300cells/µL on the one hand and ≥300cells/µL on the other. A *p*-value of <0.05 was considered significant.

8. Results

8.1. Patient characteristics

A total of 80 eligible cases (of which 40% were women and 60% men) were included in this retrospective study. The mean age was seven years higher in men. The vast majority (nearly 94%) of patients were over 59 years. More than half of the patients used to smoke, where nearly 44% of all cases still smoke. A small minority were passively exposed to toxic gases and would never have smoked. Only three patients had no Caucasian ethnicity. These were also all men. In table 5, a complete overview of the patient characteristics can be found. The mean FEV_1 (SD) was 65.4 (17.5)% predicted.

	Male	Female	Total
Gender, N (%)	48 (60)	32 (40)	**80**
Age			
Mean, years (SD)	76 (8.9)	69 (9)	**73 (9.6)**
Ranges, N (%)			
<50	0	1	1 (1.25)
50-59	0	4	4 (5.00)
60-69	11	10	21 (26.25)
70-79	23	14	37 (46.25)
≥80	14	3	17 (21.25)
Smoking status, N (%)			
Current smoker	14	21	**35 (43.75)**
Former smoker	32	11	**43 (53.75)**
Never smoked	2	0	**2 (2.50)**
BMI in kg/m² mean (SD)	27.8 (6.0)	24.5 (5.0)	**26.5 (5.9)**
Etnicity, N (%)			
Caucasian	45	32	**77 (96.25)**
Nord-African	3	0	**3 (3.75)**

Table 5: Patient characteristics. Total figures are displayed in orange. SD: Standard deviation; MBI: Body Mass Index.

8.2. Blood eosinophil count

As shown in table 6 all patients' BEC ranged from zero to 667 cells/µL. The mean in women was 200 cells/µL (SD 148.2), whereas in men it was 209.5 cells/µL (SD 146.2). An independent t-test showed that the difference in mean BEC value between women (M = 209.63; SD = 146.247) and men (M = 199.90; SD = 148.195) was not significant (t (78) = 0.289; p = 0.773). In table 6 the BEC values were divided into three ranges (cf. the ranges used by the GOLD guideline [1, 3]), with half of these are classified between ≥100 and <300 cells/µL.

Blood eosinophils counts	
Range, cells/µL	0-667
Mean, cells/µL (SD)	203.8 (146.6)
Median, cells/µL (IQR)	181.5 (211)
GOLD ranges in cells/µL, N (%)	
<100	23 (28.75)
≥100 - <300	39 (48.75)
≥300	18 (22.50)

Table 6: Blood eosinophil counts. SD: standard deviation; IQR: interquartile range.

The histogram in figure 2 demonstrates the BEC data is not normally distributed with skewness of 0.920 with a standard error of 0.269 and an outliner to the right. The normality test (Kolmogorov-Smirnov) is found to be significant (p = 0.015), rejecting the null hypothesis and therefore the BEC values are not normally distributed.

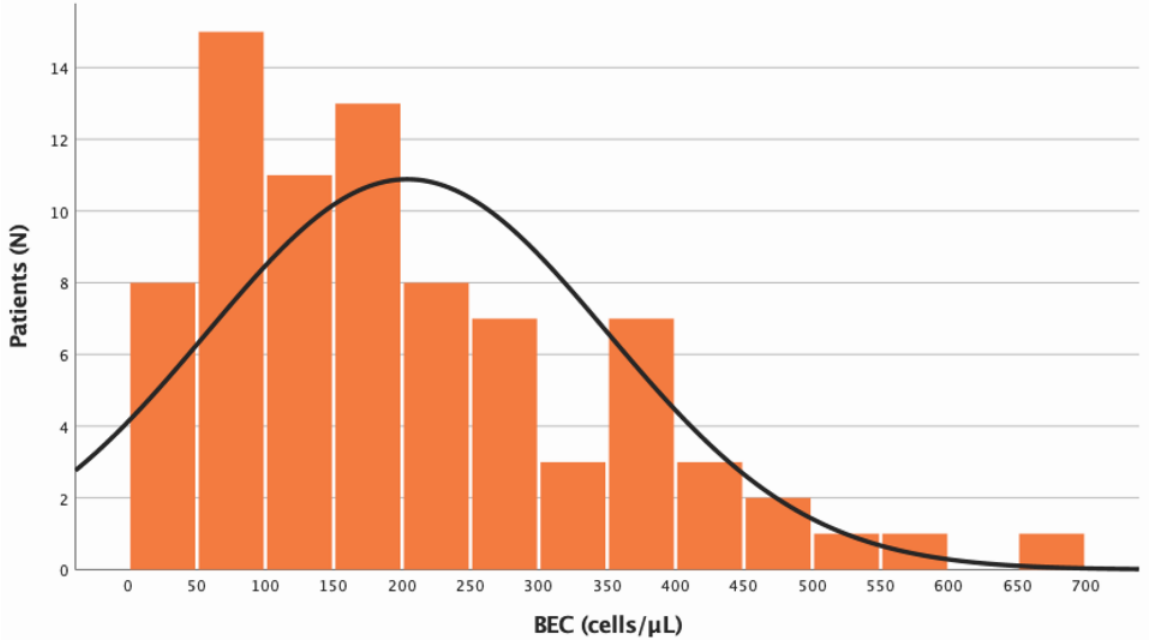


Figure 2: BEC histogram conducted in SPSS: x-axis shows the BEC (cells/µL), and y-axis shows the number of patients (total of 80 patients). The BEC data is not normally distributed with a skewness of 0.920 and a standard error of 0.269. Tests of normality were significant and the null-hypothesis could be rejected. BEC: Blood eosinophil counts.

8.3. Multidimensional predictive tools including comorbidities

Charlson Comorbidity Index	
Comorbidity, N (%)	**Total**
Myocardial infarction	28 (35.00)
Congestive heart failure	15 (18.75)
Peripheral vascular disease	10 (12.50)
CVA or TIA	9 (11.25)
Dementia	5 (6.25)
COPD	80 (100)
CTD or rheumatic disease	4 (5.00)
Peptic ulcer disease	10 (12.50)
Diabetes mellitus	11 (13.75)
Uncomplicated	9 (11.25)
End-organ damage	2 (2.50)
Liver disease	4 (5.00)
Mild	3 (3.75)
Moderate or sever	1 (1.25)
Hemiplegia or paraplegia	1 (1.25)
CKD (moderate or severe)	0 (0)
Leukemia	1 (1.25)
Lymphoma	0 (0)
Solid tumor	20 (25.00)
Localized	18 (22.50)
Metastatic	2 (2.50)
AIDS or HIV	1 (1.25)
Age (years)	
50-59	4 (5.00)
60-69	21 (26.25)
70-79	37 (46.25)
≥80	17 (21.25)
CCI results	**Total**
Range	**1-15**
Mean (SD)	**5.79 (2.39)**
Median (IQR)	**5 (3)**
10-years survival rate in %, N (%)	
96	1 (1.25)
90	3 (3.75)
77	7 (8.75)
53	14 (17.50)
21	18 (22.50)
2	12 (15.00)
0	25 (31.25)

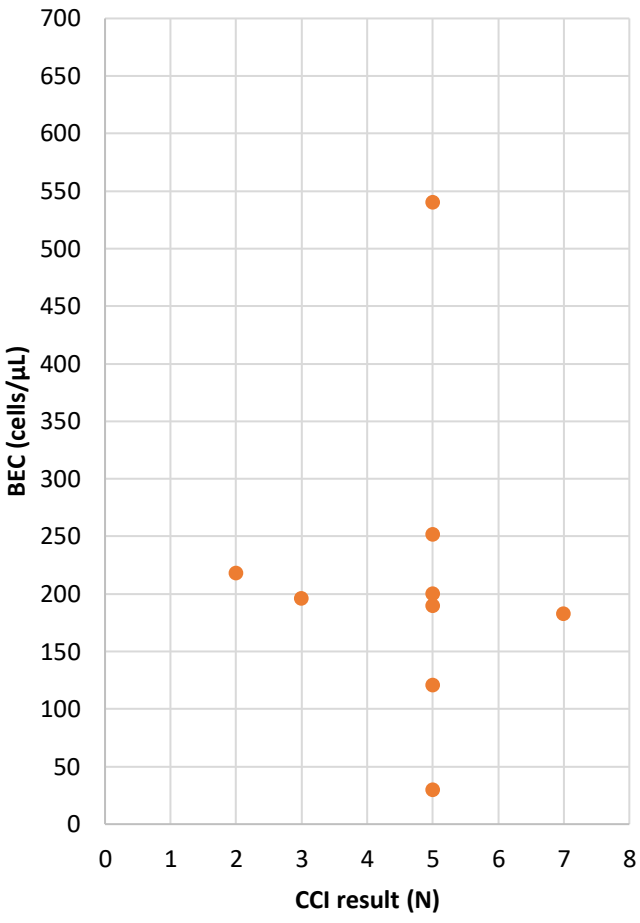


Table 7: Charlson comorbidity index (CCI) results: the prevalences of all comorbidities used in the CCI, age ranges and the calculated 10-years survival rates. CVA: cerebral vascular accident; TIA: transient ischemic attack; CTD: connective tissue disease; CKD: chronic kidney disease; SD: standard deviation; IQR: interquartile range.
Figure 3: Charlson comorbidity index (CCI) scatter plot: x-axis shows the patient specific CCI result, and y-axis their BEC (cells/µL). BEC: blood eosinophil counts.

Table 7 lists how many each comorbidity, included in the CCI, occurs in this group of 80 patients. 35% of the patients have had a myocardial infarction in the past, making this pathology (besides COPD), the most frequent among the listed comorbidities, followed by the history of having solid tumours and congestive heart failure. The CCI scores of the 80 patients ranged from one to fifteen and have a mean of nearly six. The CCI scores, like the BEC above, were not normally distributed. The bottom of the table shows the 10-years survival rates calculated based on the CCI score. Slightly more than 86% of patients had a 10-years survival rate of 53% or less. This corresponds to three quarters of women and almost 94% of men. As suggested by the scatter plot in figure 3, no clear correlation can be noticed visually between the CCI score and the patients' BEC.

COTE index	
Comorbidity, N (%)	**Total**
Lung, oesophageal, pancreatic, and breast cancer*	3 (3.75)
Anxiety*	1 (1.25)
All other cancers	17 (21.25)
Liver cirrhosis	3 (3.75)
Atrial fibrillation/flutter	19 (23.75)
Diabetes with neuropathy	0 (0)
Pulmonary fibrosis	1 (1.25)
Congestive heart failure	15 (18.75)
Gastric/duodenal ulcers	10 (12.50)
Coronary artery disease	28 (35.00)
COTE results	**Total**
Range	**0-8**
Mean (SD)	**1.96 (2.14)**
Median (IQR)	**1 (3)**

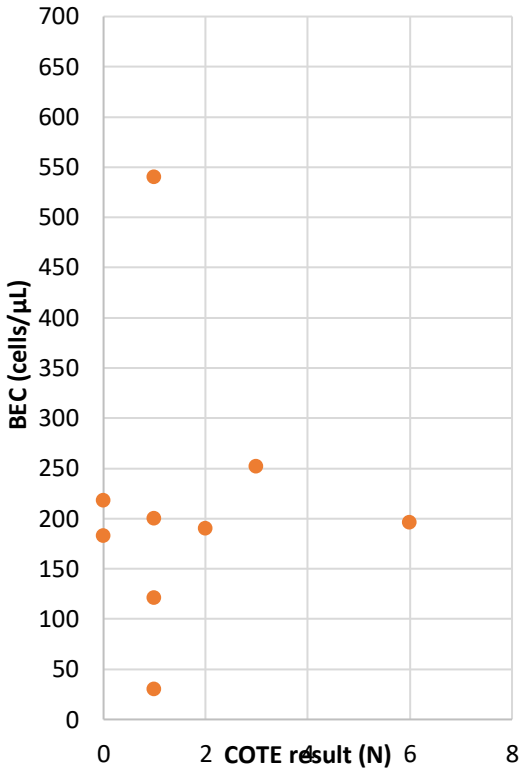


Table 8: COTE index results: the prevalences of all comorbidities used in the COTE index. COTE: COPD specific comorbidity test; SD: standard deviation; IQR: interquartile range. *Only valid for female patients.
Figure 4: COTE scatter plot: x-axis shows the patient specific COTE result, and y-axis their BEC (cells/µL). BEC: blood eosinophil counts.

A quarter of patients had a history of a (passive) malignancy. Breast cancer was described in only one female patient. Atrial fibrillation or flutter was the second most common pathology in the COTE index, after coronary artery diseases. The mean COTE index score was 1.96, with a range between zero and eight in men and zero and seven in women at a maximum possible score of 19 and 25, respectively. No normal distribution applied to the COTE index values either. The scatter plot for the COTE index results is shown in figure 4.

8.4. GOLD comorbidities

Table 9 shows all groups of COPD comorbidities listed by the 2022 GOLD guideline. [3] The 1999 WHO definition of metabolic syndrome was used to include this condition. [73] However, it was not counted as a separate entity as it includes several conditions including diabetes mellitus anyway. Only seven patients had none of the described GOLD comorbidities. Three quarters of the patients (including 40 of 42 men and 20 of 32 women) had one or more cardiovascular conditions (arrhythmia, ischemic heart disease, arterial hypertension, heart failure or peripheral vascular disease) described in the 2022 GOLD guideline. A total of 194 comorbidities were described, two-thirds of which were attributed to men. Each patient had a mean (SD) of 2.425 (1.71) comorbidities. Similarly here, tests of normality showed no normal distribution. The prevalence of all comorbidities in this group are presented in the histogram below (figure 6). Arterial hypertension was the most frequent comorbidity, occurring in 56.25% of patients. The box plot (figure 7) shows the distribution of the BEC per number of GOLD comorbidities per patient. The number of patients in each group is shown in in the bottom of the figure.

GOLD	
Comorbidity, N (%)	**Total**
Arrythmia	28 (35.00)
Ischemic heart disease	28 (35.00)
Arterial hypertension	45 (56.25)
Heart failure	15 (18.75)
Peripheral vascular disease	10 (12.50)
Osteoporosis	4 (5.00)
Anxiety*	2 (2.50)
Depression	14 (17.50)
Diabetes mellitus	11 (13.75)
Metabolic syndrome	8 (10.00)
GERD	19 (23.75)
Bronchiectasis	2 (2.50)
OSAS	9 (11.25)
Cognitive impairment	5 (6.25)
Lung cancer	2 (2.50)
GOLD results	**Total**
Comorbidity, N	**194**
Range	**0-7**
Mean (SD)	**2.425 (1.71)**
Median (IQR)	**2 (3)**

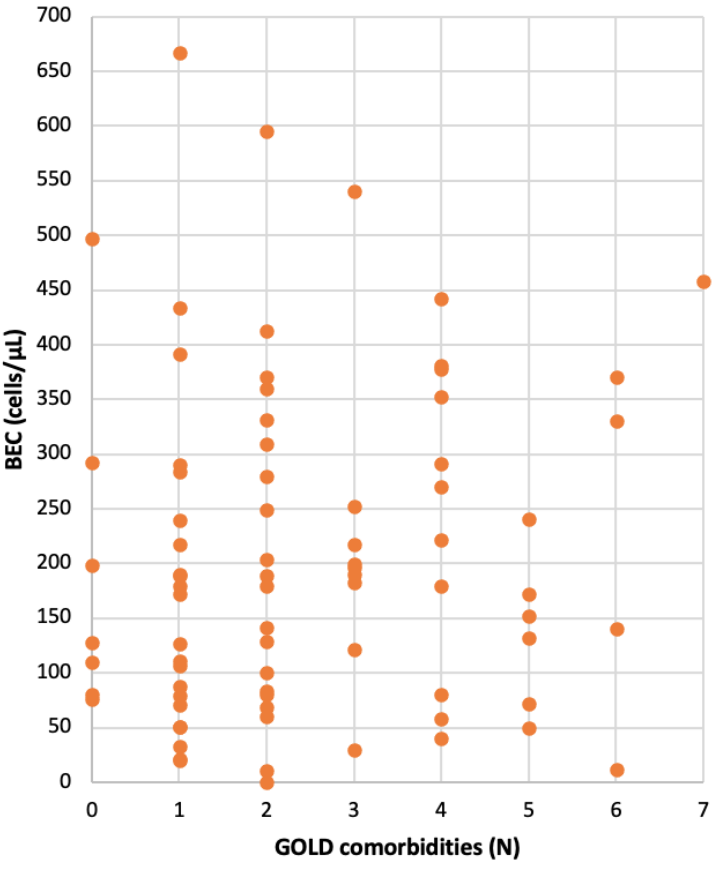


Table 9: Frequencies of identified 2022 GOLD comorbidities [3]**.** WHO 1999 definition of metabolic syndrome was used. [73] GERD: gastroesophageal reflux disease; OSAS: obstructive sleep apnea syndrome. SD: standard deviation; IQR: interquartile range. *Include both men and women.

Figure 5: GOLD scatter plot: x-axis shows the patient specific sum of GOLD comorbidities, and y-axis their BEC (cells/µL).

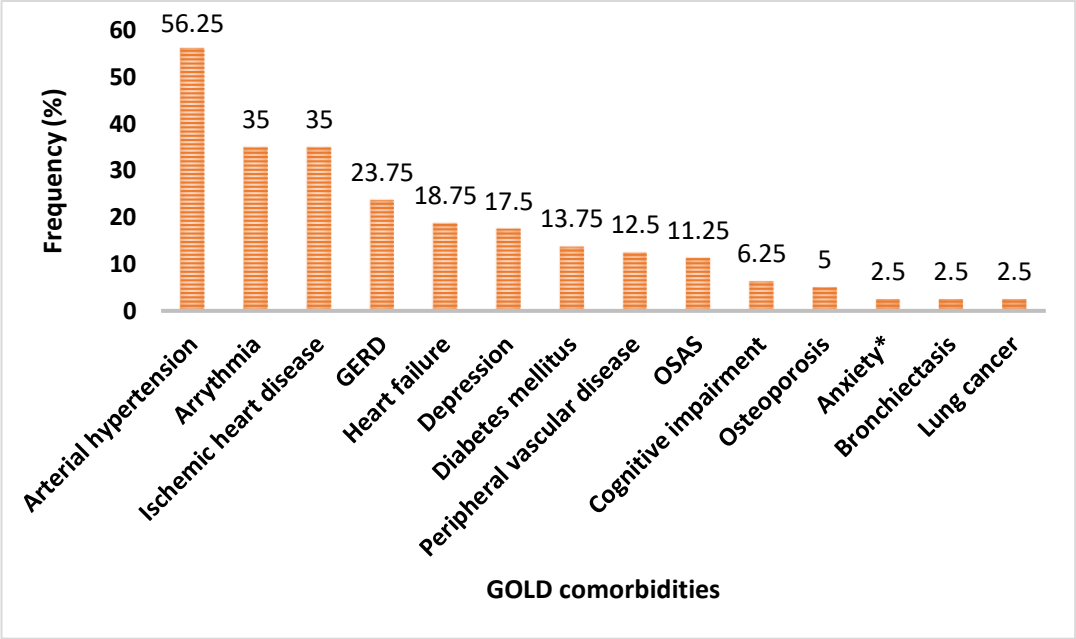


Figure 6: GOLD comorbidities histogram: x-axis shows the identified comorbidities described in the 2022 GOLD guideline [3], and y-axis their prevalences (%) in the study population (N=80). *Include both men and women.

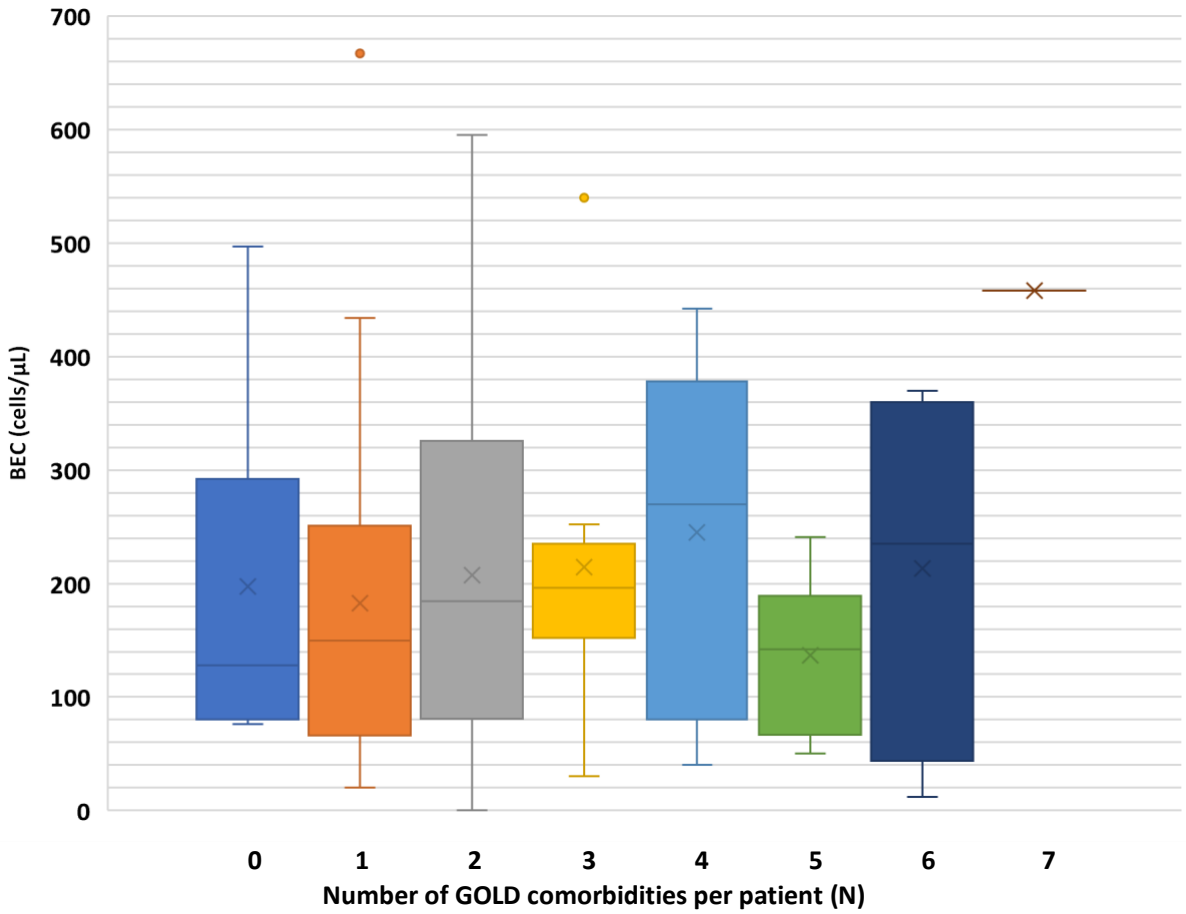


Figure 7: GOLD comorbidities box plot: x-axis shows the number of GOLD comorbidities per patient, and y-axis the blood eosinophil counts (BEC) in cells/µL. Groups 0 until 7 include 7, 22, 20, 9, 11, 6, 4, and 1 patient(s), respectively.

8.5. Independent t-test analysis and Pearson correlation

The patient group was divided into two classes based on their BEC (<300 and ≥300cells/µL). An independent t-test was used to check whether the two sample means differ significantly from each other. The variance of the two groups was equal (the Levene's test were not significant with p-values of 0.454, 0.447, and 0.297 for CCI, COTE index, and GOLD, respectively). The difference in mean CCI value between patients with a BEC <300 cells/µL (*M* = 5.52; *SD* = 2.366) and a BEC ≥300 cells/µL (*M* = 6.72; *SD* = 2.347) is not significant (*t* (78) = -1.907; *p* = 0.060). The mean scores between the two BEC groups were also not significantly different for the COTE index (*t* (78) = -0.961; *p* = 0.340) and the GOLD comorbidities (*t* (78) = -1.473; *p* = 0.145).

	T-test group statistics					Levene's test		Independent t-test	Pearson correlation	
	BEC*	**N**	**Mean**	**SD**	**Std. EM**	**Sig.**	**t (df = 78)**	**2-sided *p***	***r***	***p***
CCI	**<300**	62	5.52	2.366	0.300	0.454	-1.907	**0.060**	0.196	**0.081**
	≥300	18	6.72	2.347	0.553					
COTE	**<300**	62	1.84	2.082	0.264	0.447	-0.961	**0.340**	0.102	**0.369**
	≥300	18	2.39	2.330	0.549					
GOLD	**<300**	62	2.27	1.621	0.206	0.297	-1.473	**0.145**	0.089	**0.431**
	≥300	18	2.94	1.955	0.461					

Table 10: Independent t-test and Pearson correlation results. The t-tests, in which the BEC values were divided into two groups (<300 and ≥300cells/µL) were all not significant (*p*-value >0.05). The Pearson correlations between the absolute BEC values and the indices were also not significant. BEC: blood eosinophil counts *in cells/µL; N: number of cases; SD: standard deviation; Std. EM: standard error mean; Sig: significance; df: degrees of freedom; *r*: Pearson's r correlation coefficient; *p*: *p*-value.

There was a non-significant, very low to absent, positive correlation between BEC and CCI scores (*r* = 0.196; *p* = 0.081; *N* = 80), BEC and COTE index scores (*r* = 0.102; *p* = 0.369; *N* = 80), and BEC and GOLD comorbidities (*r* = 0.089; *p* = 0.431; *N* = 80). Consequently, there were no linear correlations between the absolute number of BEC on the one hand and CCI scores, COTE index scores and GOLD comorbidities on the other.

9. Discussion

There is still controversy about the relation between comorbidities in COPD patients (and additionally in the general population) and their BEC. Several studies are contradictory about the relation of different phenotypes or comorbidities and the BEC. In addition, the influence of BEC on outcomes in COPD patients remains inadequately studied. As described in the results above, this study detected no relation between BEC in (a select group of) COPD patients and the COTE index, the CCI and the number of GOLD comorbidities.

As described in other studies [62], the BEC distribution in this analysis is also right skewed. This study showed no difference between the BEC in men and women with stable COPD. However, this contradicts other studies both in the general population [59–62], as well as in asthma or COPD patients [61], and in a population where respiratory diseases were excluded [50], where a higher BEC is seen in men.

Furthermore, no correlation was found between BEC and the CCI scores. This was also described by Casanova et al. where, unlike this study, three BEC values over two years were used. [23]In addition, it has already been examined whether CCI scores differ between patients with an eosinophilic exacerbation and those with a non-eosinophilic exacerbation but, the Mann-Whitney U test showed no significant difference between these two groups. [74] Neither was there any relation between the BEC and the COTE index in this thesis. Studies to confirm or disprove this, are still lacking. However, this index is used less frequently.

Vanfleteren et al. described that as many as 97.7% of COPD patients suffer from one or more comorbidities. [56] The comorbidities studied in our current study are mostly different from the thirteen listed in the previously mentioned study. Based on the GOLD comorbidities, in this study 91.25% of patients have one or more comorbidities. However, it is seen that arterial hypertension and renal impairment (estimated glomerular filtration rate <60ml/min) was reported about equally often: in 56% and 48%, and 21.25% and 22% of the patients in this study and the analyses of Vanfleteren et al. respectively. [56] Furthermore, obesity (BMI ≥30kg/m^2) was reported in 26.25% of the cases in our study and in 23% of the COPD patients in the previously mentioned study. [56] The comparisons between the prevalences of the comorbidities myocardial infarction (35% in this study and 9% in the literature, respectively), depression (17.5% and 16%, respectively) and anxiety (2.5% and 21%, respectively) should be interpreted cautiously as the predefined cut-offs were different. [56]

Osteoporosis was significantly more common in the study by Vanfleteren et al. (31% compared to 5% in this study), possibly reflecting an existing underdiagnosis of osteoporosis in primary care? [56]

The current study was, to the best of our knowledge, the first to examine the relation between the comorbidities described in the GOLD guidelines [3] and the BEC in COPD patients. As mentioned earlier, no significant association was found between the total number of GOLD comorbidities and the BEC. Noteworthy, this study uses real-world primary care data.

However, there are some limitations to this study. Most importantly, the small sample size, making it difficult to evaluate the relation between each type of comorbidity separately and the BEC. However, in previous studies it was noticed that different demographic characteristics such as age (some say higher age, others say lower age), BMI and smoking status, as well as the presence of other comorbidities are associated with higher BEC. [59–62] Hartl et al. described in the LEAD study, a positive association between COPD or asthma, metabolic syndrome, obesity, a positive skin prick test and BEC in the general population. [59] The meta-analysis of Benson and Hartl analysed 91 articles (that included patients from the general population, asthma or COPD patients, as well as control patients without asthma or COPD) and showed an association between several factors (adiposity, allergic rhinitis, elevated IgE, metabolic syndrome, asthma or COPD, male gender, ≤18 years, active smoking, and a positive skin prick test) and BEC. [62, 63] In an adult population (excluding COPD patients), chronic kidney disease, cancer, hypertension, hyperlipidaemia, diabetes mellitus, and cardiovascular diseases such as arrhythmia, heart failure, stroke or transient ischemic attack, coronary heart diseases, and cardiovascular disease deaths were considered comorbidities associated with higher BEC [60], where in Hartl et al. [59] for diabetes mellitus, CVD and hypertension no association was found. The pathophysiology behind the relation between BEC and higher cardiovascular risk remains uncertain. [60]

Hartl et al, also described the additive effect of the presence of several confounding factors on the BEC. [59] All these factors should be considered when interpreting BEC for use as a therapeutic decision tool. In addition, this study used only one BEC value for each patient. [59] The values were associated with some exclusion criteria, however. [59] Nevertheless, it remains a single moment in time and, as described more than once a single BEC value is not sufficient as a biomarker use due to their variability over time. [23, 51, 75] Knowing this, most studies also use only one baseline BEC value. Furthermore, the results of this study depend on the degree of accuracy of the EHRs. The assigned diseases of conditions were controlled and linked to ICPC (International Classification of Primary Care) and ICD

(International Classification of Diseases) codes as much as possible, which does not eliminate the fact that this can still conceive of interpretation bias.
In the future, other variables could be used as biomarkers, but this study focused only on the BEC. Increasing attention is being paid to the value of CRP or FeNO as a biomarker in COPD. However, Amaral et al. did not yet describe a relation between the previous two and non-respiratory diseases or comorbidities. Further research is needed to ascertain the relevance of these values as biomarkers in COPD. [50]

If the relation between BEC and comorbidities and/or indices such as CCI, COTE, but also BODE index will become clearer in future studies, the role of the general practitioner in this story will become very crucial. After all, general practitioners have an overview of the comorbidities of the COPD patient. Hence, their place in this whole story is indispensable. A clear overview and a good transfer of these comorbidities to the pneumologists and a more systematic determination of the BEC, can help in a better interpretation of the BEC in COPD patients, allowing it to be used as a biomarker in the therapeutic decision-making progress.

10. Conclusion

Several potential comorbidities as confounding factors for BEC have already been identified in different types of populations. However, in this exploratory study, no relation was found between the multidimensional predictive tools CCI and COTE index and the BEC in COPD patients, nor between the number of comorbidities described in the GOLD guideline and the BEC. Future and prospective multicentric research is necessary to better identify the influencing demographic factors, patient-specific characteristics, and concomitant comorbidities on patient BEC (over time) in stable COPD. In this way, the value of BEC as a natural biomarker in the therapeutic decision-making process in stable COPD patients, can be clarified and as such aid clinicians in primary, secondary, and tertiary healthcare.

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12. Conflict of interest

The author declares that there was no conflict of interest.

13. Abbreviations

ABPA	Allergic bronchopulmonary aspergillosis
AEP	Acute eosinophilic pneumonia
AIDS	Acquired immunodeficiency syndrome
ATS	American Thoracic Society
BEC	Blood eosinophil counts
BMI	Body mass index
BODE	Body mass index, airflow obstruction, dyspnea, exercise capacity
BODEx	Body mass index, airflow obstruction, dyspnea, exacerbations
CAT	COPD assessment test
CCI	Charlson Comorbidity Index
CEP	Chronic eosinophilic pneumonia
CHD	Coronary heart disease
CHF	Congestive heart failure
CKD	Chronic kidney disease
CODEX	Age-adjusted Charlson Comorbidity Index score, airflow obstruction, dyspnea, exacerbations
COPD	Chronic obstructive lung disease
COTE	COPD specific comorbidity test
CRD	Chronic respiratory disease
CRP	C-reactive protein
CRS	Chronic rhinosinusitis
CVA	Cerebrovascular accident
CVD	Cardiovascular disease
DM	Diabetes mellitus
EGPA	Eosinophilic granulomatosis with polyangiitis
EHR	Electronic health record
EMD	Elektronisch medisch dossier
ERS	European Respiratory Society
FeNO	Fraction of exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second
FVC	Forced vital capacity
GBD	Global burden of disease
GDPR	General data protection regulation
GERD	Gastroesophageal reflux disease
GLI	Global Lung Function Initiative
GM-CSF	Granulocyte-macrophage colony-stimulating factor
GMD	Globaal medisch dossier
GOLD	Global Initiative for Chronic Obstructive Lung Disease

GP	General practitioner
HES	Hypereosinophilic syndrome
HF	Heart failure
HIV	Human immunodeficiency virus
HOP	Hypereosinophilic obliterative bronchitis
ICD	International Classification of Diseases
ICHO	Interuniversitair Centrum voor Huisartsen Opleiding
ICPC	International Classification of Primary Care
ICS	Inhaled corticosteroids
IHD	Ischemic heart disease
IL	Interleukin
IQR	Interquartile range
LABA	Long-acting bèta$_2$-agonist
LAMA	Long-acting muscarinic antagonist
LEAD	Lung, hEart, sociAl, boDy
LIAR	Local Immunity And/or Remodelling/Repair
LLN	Lower limit of normal
M	Mean
Mdn	Median
M/F	Male/female
mMRC	modified Medical Research Council
MS	Metabolic syndrome
NHG	Nederlands Huisartsen Genootschap
OSAS	Obstructive sleep apnea syndrome
P	p-value
PAD	Peripheral arterial disease
ProAR	Program for Control of Asthma
PY	Pack-year
QOL	Quality of life
RCT	Randomized controlled trial
SABA	Short-acting bèta$_2$-agonist
SAMA	Short-acting muscarinic antagonist
SD	Standard deviation
SGRQ	St George's Respiratory Questionnaire
TI	Tiffeneau index
TIA	Transient ischemic attack
UZB	Universitair Ziekenhuis Brussel
VUB	Vrije Universiteit Brussel
WHO	World Health Organization
YLL	Years of life lost
µL	Microliter

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15. Appendix

15.1. ICD-10 codes in CCI

ICD-10 codes used in Charlson Comorbidity Index	
Comorbidity	**ICD-10 code**
Myocardial infarction	I21.x, I22.x, I25.2
Congestive heart failure	I09.9, I11.0, I13.0, I13.2, I25.5, I42.0, I42.5–I42.9, I43.x, I50.x, P29.0
Peripheral vascular disease	I70.x, I71.x, I73.1, I73.8, I73.9, I77.1, I79.0, I79.2, K55.1, K55.8, K55.9, Z95.8, Z95.9
Cerebrovascular disease	G45.x, G46.x, H34.0, I60.x - I69.x
Dementia	F00.x–F03.x, F05.1, G30.x, G31.1
Chronic pulmonary disease	I27.8, I27.9, J40.x–J47.x, J60.x–J67.x, J68.4, J70.1, J70.3
Rheumatic disease	M05.x, M06.x, M31.5, M32.x–M34.x, M35.1, M35.3, M36.0
Peptic ulcer disease	K25.x–K28.x
Mild liver disease	B18.x, K70.0–K70.3, K70.9, K71.3–K71.5, K71.7, K73.x, K74.x, K76.0, K76.2–K76.4, K76.8, K76.9, Z94.4
Diabetes without chronic complication	E10.0, E10.1, E10.6, E10.8, E10.9, E11.0, E11.1, E11.6, E11.8, E11.9, E12.0, E12.1, E12.6, E12.8, E12.9, E13.0, E13.1, E13.6, E13.8, E13.9, E14.0, E14.1, E14.6, E14.8, E14.9
Diabetes with chronic complication	E10.2–E10.5, E10.7, E11.2–E11.5, E11.7, E12.2–E12.5, E12.7, E13.2–E13.5, E13.7, E14.2–E14.5, E14.7
Hemiplegia or paraplegia	G04.1, G11.4, G80.1, G80.2, G81.x, G82.x, G83.0–G83.4, G83.9
Renal disease	I12.0, I13.1, N03.2–N03.7, N05.2–N05.7, N18.x, N19.x, N25.0, Z49.0–Z49.2, Z94.0, Z99.2
Any malignancy, including lymphoma and leukemia, except malignant neoplasm of skin	C00.x–C26.x, C30.x–C34.x, C37.x–C41.x, C43.x, C45.x–C58.x, C60.x–C76.x, C81.x–C85.x, C88.x, C90.x–C97.x
Moderate or severe liver disease	I85.0, I85.9, I86.4, I98.2, K70.4, K71.1, K72.1, K72.9, K76.5, K76.6, K76.7
Metastatic solid tumor	C77.x–C80.x
AIDS/HIV	B20.x–B22.x, B24.x

Table 11: ICD-10 codes to generate the Charlson Comorbidity Index (CCI). ICD: International Classification of Diseases; CCI: Charlson Comorbidity Index; AIDS: acquired immunodeficiency syndrome; HIV: human immunodeficiency virus.; Quan et al. 2005 [76]

15.2. Approval Ethics Committee UZ Brussel/VUB

Universitair Ziekenhuis Brussel

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JOHAN VANSINTEJAN
VAKGROEP HUISARTSENGENEESKUNDE EN

Brussels, 2022-01-18

EC number: EC-2021-354

ETHICS COMMITTEE – SINGLE OPINION

Concerning :
The relationship between blood eosinophils and comorbidities in COPD patients BUN: 1432021000643

During the meeting on 2021-12-22, the Medical Ethics Committee of UZ Brussel/VUB reviewed and approved the above mentioned project from the ethical, legal and medical science points of view.

Upon receipt of the adapted documents (see annex EC document list), the Ethics Committee decides that there are no objections to the study in its present form.

Please remark that the Green Light Procedure has to be completed before the study can be performed at UZ Brussel.

This approval is valid for the duration of the trial. The Committee wishes to obtain an annual trial status. Results of the trial should be transmitted to the Committee at the end of the trial (publication or summary if there is no publication planned). The Committee reminds the investigator of his/her personal responsibility for this project. The Committee does not take any responsibility for its favourable advice of the project. The Ethics Committee is organised and operates according to ICH-GCP and its applicable laws and regulations. The list of members is enclosed.

Kind regards,

A. VAN SCHAEREN

Chairman
Vice-Chairman

EC Document List

STUDY REGISTRATION

CV Johan Vansintejan

CV-Aline Beckwe´e-HAIO072021

GCP Aline Beckwee

Study team

Vansintejan certificaat GCP 1 aug 2021

Supporting departments

GDPR juli 2021

Study Protocol Aline version 1.2 dd 11/01/2022

Aanvraag EC ICHO Aline

Antwoordbrief20211207

Correspondence

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Meeting date: 2021-12-22
EC number: EC-2021-354

MEMBERS OF THE MEDICAL ETHICS COMMITTEE UZ BRUSSEL – VUB
(since 08-12-2021)

Name	Function	Gender
Prof. Dr. D. Michielsen, MD, PhD	CHAIRMAN Urology UZ BRUSSEL	M
Prof. A. Van Scharen, LLM	VICE- CHAIRMAN Legal Expert UZ Brussel	F
Dr. Y. Adriaenssens, MD	General Practitioner Mechelen	M
Dr. A. Bretz, MD	Medical Oncology /Radiotherapy UZ BRUSSEL	F
Prof. Dr. F. Cools, MD, PhD	Neonatology UZ BRUSSEL	M
Dr. Apr. P.-J. Cortoos, PhD	Pharmacist UZ BRUSSEL	M
Mr. P. De Knop	Patient representative External Member	M
Dr. P. Flamee, MD	Anesthesiology UZ BRUSSEL	M
Mrs. L. Goudman, PhD	Methodologist VUB	F
Prof. Dr. P. Lacor, MD	Internal Medicine UZ BRUSSEL	M
Prof. Dr. M. Moens, MD, PhD	Neurosurgery UZ BRUSSEL	M
Prof. Dr. C. Schotte, PhD	Clinical Psychology UZ BRUSSEL	M
Mrs. S. Pirlotte	Nurse UZ BRUSSEL	F
Mr. T. Van Sande	Nurse UZ BRUSSEL	M
Dr. K. Vergaelen, MD	Paediatric Intensive Care UZ BRUSSEL	F
Prof. Dr. I. Willekens, MD, PhD	Radiology UZ BRUSSEL	F

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Clinical Trial Center

Study Protocol

Study Title: The relationship between blood eosinophils and comorbidities in COPD patients
Study Acronym: EOSCOMOR
Phase of Development: not applicable
Protocol Number:
Protocol Version and Date: Version 1.2 11/01/2022
EudraCT Registry Number: not applicable
ClinicTrials.gov Registry Number: not applicable
Indication: masterthesis of Aline Beckwée (Huisarts in Opleiding, Vrije Universiteit Brussel)
Investigational product: not applicable
Sponsor: not applicable
Coordinating/Principal Investigator: Prof. Dr. Johan Vansintejan (Promotor of thesis student Dr. Aline Beckwée)

The information contained in this document is the property of the Sponsor/Coordinating Investigator and may not be reproduced, published or disclosed to others without written authorization of the Sponsor/Coordinating Investigator.

PROTOCOL SIGNATURE PAGE

Protocol Version and date: Version 1.2 11/01/2022

Protocol Title: The relationship between blood eosinophils and comorbidities in COPD patients

Sponsor: Not applicable

Principal Investigator: Prof. Dr. Johan Vansintejan, promotor of thesis student Dr. Aline Beckwée (Huisarts in Opleiding VUB)

I agree:

- to assume responsibility for the proper conduct of this study
- to conduct the study in compliance with this protocol and any future amendments
- not to implement any deviations from or changes to the protocol without prior review and written approval from the Ethics Committee, except where necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements)
- that I am aware of and will comply with the current good clinical practice (GCP) guidelines and ethical principles outlined in the Declaration of Helsinki
- to conduct the study in accordance with all applicable laws and regulations

Printed name	Signature	Date
Prof. Dr. Johan Vansintejan	DocuSigned by: Prof. Dr. Johan Vansintejan A0198C77C2A44EB...	12/01/2022

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2. Trial Registration/Protocol Summary

Information	
EudraCT number: Date of registration:	Not applicable
Clinical Trial Authorization (CTA) from FAMHP (FAGG) Date of approval:	Not applicable
ClinicalTrials.gov:	Not applicable
Official Title:	The relationship between blood eosinophils and comorbidities in COPD patients
Study Phase/Type:	Retrospective pooled analysis
Condition:	COPD
Objectives:	By means of a retrospective study, the relationship between the type and number of comorbidities and the blood eosinophil counts in stable COPD patients is examined.
Investigational Product:	Not applicable
Interventions:	Not applicable
Endpoints:	Not applicable
Study population:	COPD patients
Number of patients:	Estimation around 100-150 patiënts
Overview of study design:	Retrospective analysis
Statistical Considerations:	Descriptive and regression analysis
Sponsor:	Not applicable
Country(ies) of Recruitment:	Belgium, Flanders
Inclusion Criteria:	- Spirometry proven COPD patients. The spirometry report, with associated GOLD grade, must be available in the patient's electronic health record (EHR). - Availability of an accurate global medical record (globaal medisch dossier, GMD). - Availability of a blood analysis with a measurement of the absolute number of eosinophils.
Exclusion Criteria:	- Patients with concomitant asthma. - Patients younger than 18 years. - Blood analyses with the absolute number of eosinophils measured four weeks before to four weeks after following situations: hospitalisation for respiratory problems, respiratory tract infection, COPD exacerbation, systemic corticosteroid therapy (oral, intra-venous or parenteral) or antibiotic therapy. - Patients with an active malignancy.
Date of first enrolment:	After approval of the Ethics Committee UZ Brussels
Target sample size:	100-150 patients

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3. Protocol Version History

Version No.	Approval Date Lead EC	Release Date
1.0		

4. Sponsor/Coordinating Investigator Information

Coordinating Investigator: Dr. Aline Beckwée
Sponsor: not applicable
Principal Investigator: Prof. Dr. Johan Vansintejan (promotor of Dr. Aline Beckwée)
Co-investigators:
Dr. Aline Beckwée
Dr. Inès Van Rossem
Additional co-researchers: /
Statistician: Interfaculty Center Data Processing and Statistics (ICDS) – Biostatistics and Medical Informatics (BISI): Brussels Health Campus, Vrije Universiteit Brussel – Laarbeeklaan 103, 1090 Brussels (Jette)
Laboratory(ies): Not applicable
Pharmacy: Not applicable
Study Coordinator: Dr. Aline Beckwée
Study sites and co-investigators:
Group practice De nieuwe Baan, Cypriaan Verhavertstraat 111, 1500 Halle.
Two of the three general practitioners (Dr. Inès Van Rossem and Dr. Julie Vandevenne) are associated with the Department of General Medicine and Chronic Care VUB.

Group practice Merchtem, Oudstrijdersstraat 64-66, 1785 Merchtem (in application).
One of the five general practitioners (Dr. Julie Yap) is associated with the department of General and Family Medicine and Chronic Care VUB.

5. List of Abbreviations

BEC:	Blood eosinophil counts
BISI:	Biostatistics and Medical Informatics
BMI:	Body mass index
CAT:	COPD assessment test
CCI:	Charlson Comorbidity Index
CKD:	Chronic kidney disease
COPD:	Chronic obstructive lung disease
CRD:	Chronic respiratory disease
CVA:	Cerebrovascular accident
DM:	Diabetes mellitus
eID:	Elektronische identiteitskaart
EHR:	Electronic health record
FEV_1:	Forced expiratory volume in 1 second
FVC:	Forced vital capacity

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GBD:	Global Burden of Disease
GERD:	Gastroesophageal reflux disease
GOLD:	Global Initiative for Chronic Obstructive Lung Disease
ICDS:	Interfaculty Center Data Processing and Statistics
ICHO:	Interuniversitair Centrum voor Huisartsen Opleiding
ICPC:	International Classification of Primary Care
ICS:	Inhaled corticosteroids
IHD:	Ischemic heart disease
LABA:	Long-acting bèta$_2$-agonist
LAMA:	Long-acting muscarinic antagonist
M/F:	Male/female
mMRC:	Modified Medical Research Council
MS:	Metabolic syndrome
OSAS:	Obstructive sleep apnea syndrome
PAD:	Peripheral arterial disease
PY:	Pack-year
RCT:	Randomized control trial
SABA:	Short-acting bèta$_2$-agonist
SAMA:	Short-acting muscarinic antagonist
TI:	Tiffeneau index
TIA:	Transient ischaemic attack
UZB:	Universitair Ziekenhuis Brussel
VUB:	Vrije Universiteit Brussel
WHO:	World Health Organization
YLL:	Years of life lost

6. Introduction

6.1 Overview of Disease Pathogenesis

GOLD (Global Initiative for Chronic Obstructive Lung Disease) defines chronic obstructive lung disease (COPD) as follows: "COPD is a common, preventable and treatable disease that is characterized 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)

Previously, neutrophilic white blood cells were identified as the main leader of the inflammation, whereas in asthma it was mainly caused by eosinophils. However, recently another identity has been described, the eosinophilic COPD. (2,3) The prevalence of eosinophilic inflammation is significant, yet an accurate percentage cannot be given, as the results of various prevalence studies vary enormously. (3) The meta-analysis and systematic review of Hong-Xia Wu et al, in which 19 trials with a total of more than 40,000 included COPD patients were analysed, showed that the prevalence of eosinophilic inflammation in this population ranged from 18.84% to 66.88% (average 54.95%). (3) In addition, an association was found between blood eosinophilic and pulmonary eosinophilic numbers. (2)

In patients (>40 years) with typical symptoms, recurrent lower respiratory tract infections and/or exposure to causally harmful substances, the diagnosis of COPD is made by spirometry. An irreversible airflow limitation can be confirmed when the Tiffeneau index (TI) or FEV_1/FVC (forced expiratory volume in 1 second / forced vital capacity) after bronchodilator use is less than 70%. (1)

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6.2 Epidemiology

Besides cardiovascular diseases, chronic respiratory diseases (CRD), including COPD, are major causes of morbidity worldwide. (4) The Global Burden of Disease Study (GBD) of 2015 found that COPD ranked seventh among the causes of years of life lost (YLLs). The same study described approximately 3.2 million deaths due to COPD. (5) The World Health Organization (WHO) predicts that COPD will be the third leading cause of death by 2030. (6) Over a 27-year period (between 1990 and 2017), the global number of patients with COPD increased by almost 50%. In contrast, the incidence decreased by 0.68%. In addition, the age-standardized prevalence rate is also falling. These phenomena can possibly be explained by the growing world population. (4) It is estimated that in Belgium some 700,000 people suffer from this lung disease, but only 400,000 of them have actually been diagnosed. (7) Previously, COPD was a disease that predominantly affected smoking men and to a minor extent women. However, recently, due to the increasing number of women smoking tobacco (mainly in developed countries), the gender distribution of COPD is thought to be roughly equal. (6, 8)

6.3 Current Treatments

Based on the FEV_1 (% of predicted), patients are classified into 4 GOLD grades, representative of the severity of their airflow limitation. The intensity of the symptoms reported by the patients and their disease experience are taken into account and classified, using 2 types of schemes: the modified Medical Research Council (mMRC) dyspnea scale and the COPD Assessment Test (CAT). Depending on the provided answers, patients are given a letter (A, B, C or D). This classification is the foundation for the therapeutic decision-making process. (fig. 1 and 2) Smoking cessation is the most important initial step in the therapeutic approach to COPD. Smoking cessation, with or without the help of pharmacotherapy, is the only intervention that favorably influences the course and prognosis of COPD. In addition, education, self-management and pulmonary rehabilitation improve the psychological and physical condition of the patient. Bronchodilatation, using long-acting (LA) (or short-acting, SA) bronchodilators, is the main pharmacological approach. This medication improves the symptoms and reduces the risk of exacerbations. Both anticholinergics (muscarinic antagonists, MA) and bèta$_2$-mimetics (bèta$_2$-agonists, BA) or their combination are used. (1)

Since 2019, the Global Initiative for Obstructive Lung Disease (GOLD) guideline uses the absolute number of blood eosinophils per microliter (BEC, blood eosinophil counts) in patients with COPD as a therapeutic marker to guide clinical decisions and to establish a personalized treatment approach. COPD patients belonging to group D may benefit from the addition of inhaled corticosteroids (ICS) to their treatment with a long-acting bronchodilator. Group D patients report a high symptom burden and have experienced two or more episodes of exacerbation in the last twelve months, an acute increase in the known respiratory symptoms, requiring medical intervention. (1) According to the randomized control trials (RCT), TRILOGY, TRINITY, TRIBUTE, IMPACT and ETHOS, a triple therapy, more specifically a combination of a LABA, a LAMA with an ICS would reduce the risk of exacerbations, improve quality of life, produce positive clinical effects compared to a bronchodilator monotherapy, dual bronchodilator therapy or ICS-bronchodilator combination therapy. (2, 9-13) Especially in patients with a blood count exceeding 100 and especially 300 eosinophils/µL (the so-called eosinophilic COPD patient), treatment with inhaled corticosteroids is recommended, whereas previously the decision about the possible start of this medication group was mainly based on the patient's exacerbation history, the severity of their symptoms and the presence or absence of a concomitant asthmatic disease. These cut-offs (<100, 100 to 300 and >300 eosinophils/microliter) were determined using data models that showed a continuous relationship between BEC and the effectiveness of ICS inhalation therapy in preventing exacerbations. Cases with BEC in the range of 100-300 eosinophils/microliter may possibly benefit from the addition of an ICS. (1)

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People with COPD often suffer from more than just a pulmonary disorder. Many of these patients have a wide range of co-occurring comorbidities. The prevalence of the individual comorbidities or of the clusters is not completely known and fluctuates between different studies. However, it does appear that the majority of COPD patients suffer from at least one comorbidity. A summarised list can be found in the GOLD guideline: cardiovascular diseases like heart failure, arrhythmias, ischaemic heart disease (IHD), hypertension and peripheral artery disease (PAD), gastroesophageal reflux disease (GERD), osteoporosis, metabolic syndrome (MS), diabetes mellitus (DM), bronchiectasis, obstructive sleep apnea (OSA), lung cancer, anxiety, depression and even cognitive impairment. These comorbidities and the general health status of the patient can influence the course of the pulmonary disease, the effectiveness of the treatment and the prognosis. Therefore, it is recommended that any comorbidities present be treated according to the guidelines for this specific condition. (1) The simultaneous presence of various comorbidities (clusters of comorbidities) often renders the formulation of an individual patient-specific therapeutic strategy very challenging.

6.4 Study Rationale and Purpose

The absolute number of eosinophils (BEC) is inter-individually variable over time. (14) Nevertheless, it was calculated that, when patients are divided into three groups (<100, 100 to 300 and >300 eosinophils/microliter) according to their eosinophil count, after 1 year almost 70% of the patients remained in their original group. (15) In addition, the BEC can be influenced by various factors, including different types of medication, the presence of parasitic infections, autoimmune diseases, hematological disorders and so on. (16) There is still insufficient knowledge about the stability and influencing factors such as patient characteristics or co-morbidities on BEC. For example, Hartl et al, described that in a general population the following potential confounders were significantly associated with a high BEC (>75th percentile): age ≤18 years, asthma or COPD, metabolic syndrome, male gender, positive skin prick test, active smokers and obesity. (17) Moreover, eosinophilic COPD seems to be more common in men, ex-smokers and patients with a higher BMI, whereas certain comorbidities such as heart failure, arterial hypertension and diabetes mellitus are less common in these eosinophilic COPD patients. (3) In addition, in a COPD cohort an association was found between higher eosinophil values (eos >2%) and increased age, male gender and severe concomitant asthma. In these patients, a lower prevalence of some comorbidities was observed (anemia and heart failure). (18)

Despite the remaining knowledge gaps, BEC is currently used for therapeutical decision making. Therefore, more needs to be learned about the possible influence of comorbidities on BEC. In this retrospective study, the comorbidities and most recent BEC of all spirometry confirmed COPD patients from two Flemish general practices will be incorporated. Based on these data we will look for the relationship between comorbidities, the number of comorbidities and the blood eosinophil values. In addition, the prevalence of the comorbidities of COPD (as described in the GOLD guideline of 2021) will be determined.

6.5 Rationale for Study Design

A retrospective, non-interventional study is used to estimate the relationship of BEC with the number of comorbidities. This study design allows us to first test the research question in a practically feasible way based on already available medical data from the elective medical records of COPD patients. Further prospective research will be subsequently required to validate the value of the results obtained.

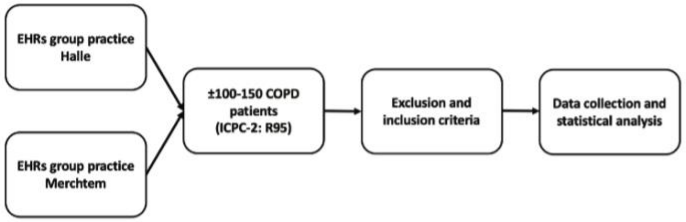
6.6 Rationale for Dose and Regimen/Schedule Selection

Not applicable for this non-interventional retrospective study.

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7. Study Schematic and Schedule of Activities

7.1 Study Schematic



7.2 Study Activities

The completion of the entire study (literature review, protocol description, ethics committee application, data collection and analysis) will take place from October 2020 to May 2023. Data collection can start after approval of the study by the ethics committee. For the collection of the data, we will go back in time to 01/01/2000 at the latest, to the date of approval. Data analysis on the collected data will take place from February 2022 to April 2023. Only data from the past will be collected.

8. Study Objectives and endpoints

8.1 Primary Objective

To describe the relationship between the BEC and the type and number of comorbidities, taking into account the GOLD proposed BEC thresholds.

8.2 Secondary Objectives

To describe the prevalence of the comorbidities of COPD as described in the GOLD guideline 2021.

8.3 Endpoints

Not applicable

9. Investigational Plan

9.1 Overall Study Design

Non-interventional, retrospective study.

9.2 Study Duration for Subjects

Not applicable.

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10. Selection of Subjects

10.1 Selection of Study Population

COPD patients from two general practices in Flemish Brabant (Flanders, Belgium):

- Group practice De nieuwe Baan, Cypriaan Verhavertstraat 111, 1500 Halle.
  Two of the three general practitioners (Dr. Inès Van Rossem and Dr. Julie Vandevenne) are associated with the Department of Family Medicine and Chronic Care VUB.

- Group practice Merchtem, Oudstrijdersstraat 64-66, 1785 Merchtem.
  One of the five general practitioners (Dr. Julie Yap) is associated with the department of Family Medicine and Chronic Care VUB.

The population size is estimated between 100 and 150 patients.

10.2 Inclusion Criteria

- Spirometry proven COPD patients. The spirometry report, with associated GOLD grade, must be available in the patient's electronic health record (EHR).
- Availability of an accurate global medical record (globaal medisch dossier, GMD).
- Availability of a blood analysis with a measurement of the eosinophils.

10.3 Exclusion Criteria

- Patients with concomitant asthma.
- Patients younger than 18 years.
- Blood analyses with the absolute number of eosinophils measured four weeks before to four weeks after following situations: hospitalisation for respiratory problems, respiratory tract infection, COPD exacerbation, systemic corticosteroid therapy (oral, intra-venous or parenteral) or antibiotic therapy.
- Active malignancy.

10.4 Contraception/Pregnancy Avoidance

Not applicable for this non-interventional retrospective study.

11. Screening and Randomization

11.1 Screening and Enrollment

Using the statistics module in the Electronic Health Record, all patients with COPD can be listed. COPD is a healthcare element and can be found in the medical history list. Using the ICPC-2 code for COPD, R95, a search can be performed.

11.2 Randomization

A randomization procedure is not applicable for this retrospective study.

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11.3 Blinding Procedures

A blinding procedure is not applicable for this retrospective study.

12. Interventions/Treatment

Not applicable for this non-interventional retrospective study.

13. Study Assessments and Procedures

Not applicable for this non-interventional retrospective study.

14. Safety Monitoring and Reporting

Not applicable for this non-interventional retrospective study.

15. Data Collection and Management

15.1 Monitoring

The function of data controller and data processor will both be assumed by the Aline Beckwée (and potentially) by the co-investigator or principal investigator.

15.2 Data Collection

An Excel database will be compiled with the following data acquired from the EHR of the selected COPD patients. The most recent available values will be used.

- Patient characteristics
  - Age (year)
  - Gender (M/F)
  - Height (m), weight (kg) and BMI (kg/m^2)
  - Ethnicity (Caucasian, North African, other African, Asian, South American)
- Smoking status
  - Never smoked/ current tobacco user/ ex-smoker
  - Number of packyears (PY)
- COPD
  Spirometry data of most recently available spirometry:
  - Post-bronchodilator Tiffeneau index (TI) = FEV_1/FVC
  - Spirometry GOLD grade (1-4) depending on the FEV_1 (% predicted)
- Blood analysis: latest number of white blood cells and eosinophils (cells/µL)
- Current treatment
  - Bronchodilator therapy: long-acting/short-acting, anticholinergics/$beta_2$-mimetics, combinations
  - Inhaled corticoids (ICS)
  - Other medication taken on daily basis
- The parameters for calculating the BODEx index
  - FEV_1 (% predicted)

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  - Exacerbation history
  - Modified Medical Research Council (mMRC) score (0-4)
  - BMI (kg/m^2)
- The parameters for calculating the Charlson Comorbidity Index (CCI)
  - Age (year)
  - The presence of the following antecedents
    - Chronic obstructive lung disease (COPD)
    - Congestive heart failure (CHF)
    - Ischaemic heart disease (IHD)
    - Peripheral arterial disease (PAD)
    - Cerebrovascular accident (CVA) or transient ischaemic attack (TIA)
    - Dementia (chronic cognitive deficit)
    - Connective tissue disease
    - Diabetes mellitus (none or diet-controlled/ complicated/ end-organ damage)
    - Liver disease (severe: cirrhosis and portal hypertension with variceal bleeding history/ moderate: cirrhosis and portal hypertension but no variceal bleeding history/ mild: chronic hepatitis or cirrhosis without portal hypertension)
    - Peptic ulcer disease
    - Hemiplegia
    - Leukemia
    - Lymphoma
    - AIDS
    - Chronic kidney disease (CKD) (severe: on dialysis, status post kidney transplant; moderate: creatinine >3mg/dl)
    - Solid tumor (none; localized; metastatic)
- The presence of the following comorbidities (described in the GOLD guideline)
  - Cardiovascular disorders: heart failure, ischaemic heart disease (IHD), arrhythmia, peripheral arterial disease (PAD), hypertension
  - Osteoporosis
  - Metabolic syndrome (MS)
  - Gastroesophageal reflux disease (GERD)
  - Bronchiectasis
  - Diabetes mellitus (DM)
  - Psychiatric disorders: depression and anxiety disorder
  - Obstructive sleep apnea syndrome (OSAS)

15.3 Database Management and Quality Control

The function of data controller and data processor will both be adopted by Aline Beckwée and the co-investigators.

15.4 Statistical Considerations and Data Analysis

For the statistical analyses, the investigators will be assisted by the Interfaculty Center Data Processing and Statistics (ICDS) of the Biostatistics and Medical Informatics (BISI) research group of the VUB.
In this study, descriptive statistics and regression techniques will be used. Statistical analyses will be conducted using SPSS® (IBM®).

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16. Ethical Considerations

16.1 Ethical conduct of the study

16.1.1 Declaration of Helsinki

The study will be conducted according to the standards of Good Clinical Practice and the Declaration of Helsinki.

16.1.2 Ethics Committee

An application is submitted to the ethics committee of the UZ Brussel. The study will not start until the researchers have obtained their approval.

16.2 Informed Consent

Since this is a retrospective study based on already existing data, informed consent is not required.

16.3 Patient and Study Data Protection

The database (Exel-files) as well as the folder containing the Excel file will be password protected. This database will be located on the personal VUB-desktop (Virtual MINF) of Aline Beckwée. This desktop is also secured with a personal password. The laptop on which Aline Beckwée will consult the desktop is also protected by a personal password and fingerprint recognition.
Statistical analyses will be carried out using SPSS® (IBM®). This computer program is located on the personal VUB-desktop (Virtual MINF) of Aline Beckwée.

16.4 Subject Identification

This study may involve a relatively small study group. The included patient characteristics or antecedents of the study group will be collected and grouped together into a large pool per patient characteristic or comorbidity (expressed as an absolute number of a defined characteristic). No individual cases with combinations of characteristics will be presented in this study. In addition, the specific comorbidity will not be discussed, but the general condition will be used (e.g. atrial fibrillation will be classified under arrhythmia). Furthermore, the original patient data will be completely pseudonymised using a specific patient study number. Aline Beckwée will perform this pseudonymisation and is the only person who will have a conversion list. In this way the confidentiality of the patients can be assured by the researchers.

16.5 Conflict of Interest

The investigators certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

17. Finance and Insurance

Not applicable for this non-interventional retrospective study.

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18. Reporting and Dissemination

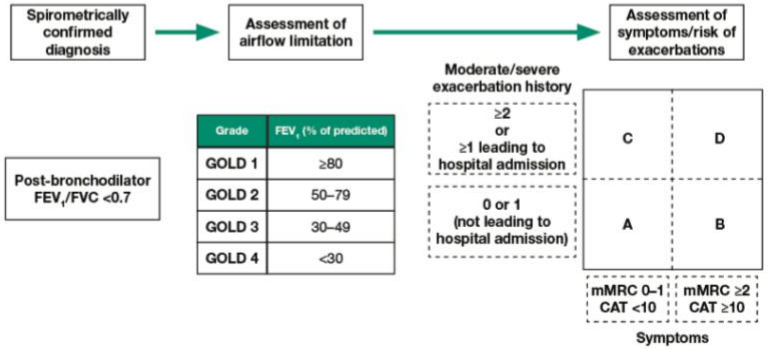
This retrospective study is an academic study in the context of completing a master after master degree in Family Medicine. This master's thesis will be submitted to the ICHO (Interuniversitair Centrum voor Huisartsen Opleiding).

If an article on this study is written by one or more of the investigators, they will all have all the publication rights.

19. Conflict of interest statement

The investigators certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

20. Tables and Figures



FEV_1=forced expiratory volume in the first second; FVC=forced vital capacity; mMRC=modified Medical Research Council dyspnoea questionaire; CAT=COPD assessment test.

Figure 1. GOLD COPD ABCDE classification scheme based on FEV_1 (% predicted), symptoms and/or exacerbation risk.

≥2 moderate exacerbations or ≥1 leading to hospitalisation	**Group C** LAMA	**Group D** LAMA or LAMA + LABA[A] or ICS + LABA[B] [A]Consider if highly symptomatic (e.g. CAT >20) [B]Consider if eos ≥300
0 or 1 moderate exacerbations (not leading to hospital admission)	**Group A** A Bronchodilator	**Group B** A Long Acting Bronchodilator (LABA or LAMA)
	mMRC 0–1 CAT <10	mMRC ≥2 CAT ≥10

LAMA=long-acting muscarinic receptor antagonists; LABA=long-acting beta$_2$ agonist; ICS=inhaled corticosteroids; CAT=COPD assessment test; COPD=chronic obstructive pulmonary disease; eos=blood eosinophil count in cells per microlitre; mMRC=modified Medical Research Council dyspnoea questionnaire.

Figure 2. Initial pharmacological treatment depending on patients' level of symptoms (assessed using either CAT or mMRC) and the number and severity of exacerbations in the last year.

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