

CHRONIC BRONCHITIS SUB-PHENOTYPE WITHIN COPD: INFLAMMATION IN SPUTUM AND BIOPSIES

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ABSTRACT:

Rationale: The presence of chronic bronchitis predicts more rapid decline of FEV₁ in patients with chronic obstructive pulmonary disease (COPD). The hallmark of COPD is airway inflammation. We hypothesized that COPD patients with chronic bronchitis are characterized by a distinct inflammatory cell profile as measured in bronchial biopsies and sputum.

Methods: From 114 COPD patients (M/F:99/15, age 62±8 yrs, current smoking 63%, post-bronchodilator FEV₁ 63±9% predicted, no steroids), with and without chronic bronchitis, inflammatory cell counts in bronchial biopsies and induced sputum were measured. Analysis was done by logistic regression.

Results: COPD patients with chronic bronchitis had lower eosinophil counts in biopsies (p=0.019) and higher percentages of sputum eosinophils (p=0.033) than patients without those symptoms, which remained after adjustment for smoking and gender (p=0.028, p=0.057, respectively). Patients with chronic bronchitis also showed higher percentages of macrophages (p=0.039) and lower percentages of neutrophils (p=0.049) in sputum, which could be explained by differences in smoking and gender.

Conclusion: We conclude that chronic bronchitis reflects an inflammatory sub-phenotype among patients with COPD. Our results indicate a preferential distribution of eosinophils towards the airway lumen in those with chronic bronchitis. This may

have implications for anti-inflammatory treatment of COPD patients with chronic bronchitis.

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Key words: chronic obstructive pulmonary disease, chronic bronchitis, chronic mucus hypersecretion, inflammation, biopsies, induced sputum

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a leading cause of death and disability worldwide [1]. COPD is characterized by progressive and not fully reversible airflow limitation, as measured by the forced expiratory volume in one second (FEV₁). The airflow limitation is associated with a chronic inflammatory response of the airways and lung parenchyma to noxious particles or gases, in particular tobacco smoking. Nonetheless there is increasing evidence that COPD is a heterogeneous disease and that different phenotypes contribute to a variable extent to the severity of the disease. On average 34 % of patients with COPD suffer from chronic cough and sputum expectoration [2]. However, it is still unclear whether these co-existing symptoms of chronic bronchitis among patients with COPD are relevant for the progression and treatment of COPD.

Early epidemiological studies in the 1980s did not observe an association between clinical symptoms of chronic bronchitis and the progression of disease in patients with mild COPD, as measured by the annual decline of FEV₁ [3]. However, subsequent findings suggested that chronic sputum expectoration is associated with a low FEV₁ in patients with alpha-1 antitrypsin deficiency [4], and a steeper decline in FEV₁ in population based studies (including patients with COPD) [5, 6]. In addition, chronic cough and sputum expectoration is associated with an increased risk in COPD-related mortality [7, 8]. These follow-up studies suggest that the presence of chronic bronchitis is not just an innocent bystander, but might contribute to, or is a reflection of the more rapid progression of COPD [9].

In COPD the inflammatory process is characterized predominantly by neutrophils, macrophages and CD8⁺ cells in the airways [10]. The role of excessive mucus production in the pathophysiology in COPD is still controversial. Chronic mucus hypersecretion *per se* is associated with distinct pathological features, such as persistent epithelial goblet cell hyperplasia and submucosal gland hypertrophy in the airways [11]. However, recently we observed in COPD patients no differences in total mucin content of the surface epithelium between those with and without symptoms of chronic bronchitis (Lapperre *et al*, submitted). Goblet cells in the surface epithelium are the main producers of the mucin MUC5AC, whereas MUC5B is a characteristic product of the submucosal glands [12]. COPD patients with chronic bronchitis have increased numbers of neutrophils in the epithelium and more neutrophils, macrophages and CD8⁺ cells in their bronchial glands as compared to asymptomatic non-COPD subjects [13]. This suggests that inflammatory cells and their mediators provide a major drive for mucus hypersecretion and subsequent symptoms of chronic bronchitis [11]. However, among patients with established COPD it is still unclear whether chronic bronchitis is featured by a distinct inflammatory cell profile in the airways. If so, the presence of chronic bronchitis in COPD may have therapeutic implications for current or future therapies [14].

Therefore, we tested the hypothesis that COPD patients with concurrent clinical symptoms of chronic bronchitis are characterized by a distinct inflammatory cell profile in the airways. This was addressed by measuring inflammatory cell counts in bronchial biopsies and induced sputum in well-characterized patients with COPD.

METHODS

Detailed information about subjects and methodology has been published previously [15]. In brief, 114 patients with COPD were included for the Groningen Leiden Universities Chronic Obstructive Lung Disease (GLUCOLD) Study. Patients were 45-75 years, current or ex-smokers with a history of ≥ 10 pack-years and respiratory symptoms. Postbronchodilator forced expiratory volume in one second (FEV_1) was >1.3 l and $>20\%$ predicted and less than the upper limit of the 90% *confidence interval* of the predicted FEV_1 [16]. Postbronchodilator FEV_1/IVC ratio was below the 90% *confidence interval* of the predicted FEV_1/IVC ratio. Patients were clinically stable for more than 2 months before the measurements. They did not use a course of inhaled or oral corticosteroids during the past 3 months prior to randomization or maintenance treatment with these drugs during the past 6 months. Each centre's local medical ethics committee approved the protocol and patients provided written informed consent.

Design and definition of chronic bronchitis

This study represents cross-sectional data from the GLUCOLD Study and contained four visits. Chronic bronchitis was considered to be present when subjects reported daily cough and sputum production for at least 3 months a year, for more than one year [5].

Pulmonary function tests

Spirometry was performed, according to international guidelines [17], using the Quanjer reference values [16]. Total lung capacity (TLC) and residual volume (RV) were measured using a constant volume bodyplethysmograph [16]. Airway hyperresponsiveness was determined using the 2-minute tidal breathing method [18] and expressed as the provocative concentration causing a 20% fall in FEV_1 (PC_{20}).

Sputum induction and processing

Sputum was induced and processed according to a validated technique using the so called 'full sample' method [19]. After inhaling 200 μ g salbutamol the patients inhaled hypertonic sodium chloride aerosols (4.5 w/v %) during 3 periods of 5 min. Differential cell counts were expressed as a percentage of nucleated cells, excluding squamous cells. A sputum sample was considered adequate when the percentage squamous cells was less than 80%.

Bronchial biopsies

Fibreoptic bronchoscopy was performed using a standardized protocol and has been described in detail previously [20]. In brief, four paraffin embedded biopsies were cut in 4- μ m thick sections. Haematoxylin and eosin (H&E) staining was used for evaluation and selection of the two morphological best biopsies per patient. Specific antibodies against T-lymphocytes (CD3, DAKO, Glostrup, Denmark; CD4, Novocastra, Newcastle upon Tyne, UK; CD8, DAKO), macrophages (CD68, DAKO),

neutrophil elastase (NE, DAKO), mast cell tryptase (AA1, DAKO), plasma cells (CD138, IQ Products, Groningen, The Netherlands) and eosinophils (EG2, Pharmacia Diagnostics, Uppsala, Sweden) were used. Fully automated inflammatory cell counting procedures were performed according to previously described validated methods [21]. The number of sub-epithelial positively staining inflammatory cells was counted within the largest possible area of maximal 125 μm deep beneath the basement membrane, per biopsy section, and expressed as the mean number of cells/0.1 mm^2 of the two biopsies.

Statistical analysis

Data were presented as means and standard deviations or medians with interquartile ranges (IQR). The differences between patients with and without chronic bronchitis were analyzed using the unpaired Student's *t* tests for normally distributed continuous variables. Chi-square tests were used for categorical data. Non-normally distributed data were log transformed. Multiple logistic regression analysis was used to investigate the independent association between chronic bronchitis and inflammatory cells. In this model the dependent variable was the presence of chronic cough and sputum expectoration, whereas independent variables were bronchial and sputum inflammatory cells, with additional adjustment for smoking habits and gender. Differences at p-values <0.05 were considered to be statistically significant (tested 2-sided). We used SPSS 12.0 for all analyses.

RESULTS

The patient characteristics of COPD patients with and without chronic bronchitis are shown in Table 1. Data on the presence of chronic bronchitis were available for 113 out of 114 patients with COPD. All patients had moderate to severe COPD as based on an average postbronchodilator FEV₁ of 63±9% of predicted and most of them were current smoking, middle-aged males. A minority of patients was mildly reversible to salbutamol, as based on FEV₁ (% pred) post minus pre-salbutamol. 18 Patients (16%) showed a change in FEV₁ that was more than 12 % pred plus > 200 ml, whereas 9 out of these 18 patients had chronic bronchitis. COPD patients with and without chronic bronchitis exhibited a wide range of hyperresponsiveness to methacholine, were slightly hyperinflated and mildly impaired in CO-diffusion capacity per alveolar volume. Patients with chronic bronchitis were more likely to be current smokers than patients without these symptoms. Relatively more female patients reported chronic bronchitis. Other patient characteristics were similar between the two groups (Table 1).

Bronchial inflammatory cell counts in COPD patients with and without chronic bronchitis.

Data on the number of bronchial inflammatory cells were available for 53 patients with and 59 patients without chronic bronchitis (Table 2). Patients with chronic bronchitis had significantly fewer eosinophils in biopsies than patients without chronic bronchitis (p=0.019, Figure 1). Logistic regression analysis confirmed this association. After adjustment for smoking and gender, for each doubling in bronchial eosinophils, there was a statistically significant lower chance of 16% of having chronic bronchitis (OR 0.84, 95% CI 0.72-0.98, p=0.028). Patients with chronic bronchitis tended to have fewer neutrophils in biopsies than patients without chronic bronchitis, but this difference was not statically significant (p=0.080). The remaining inflammatory parameters in bronchial biopsies were similar between the two groups.

Sputum inflammatory cells in COPD patients with and without chronic bronchitis.

Table 3 shows the numbers and percentages of inflammatory cells in induced sputum for COPD patients with and without chronic bronchitis. COPD patients with chronic bronchitis had significantly higher percentages of sputum eosinophils (p=0.033) than COPD patients without these symptoms (Figure 2a). After adjustment for smoking and gender, each doubling in sputum % eosinophils was borderline significantly associated with a 24% higher chance of having chronic bronchitis (OR=1.24, 95% CI 0.99-1.54, p=0.057). When using a sputum eosinophil percentage of > 3% as threshold, sputum eosinophils had a specificity of 87% in identifying patients with chronic bronchitis. COPD patients with chronic bronchitis had significantly higher percentages of macrophages (p=0.039, Figure 2b), and lower percentages of sputum neutrophils (p=0.049) than COPD patients without those symptoms. After adjustment for smoking and gender, these differences lost statistical significance (OR 1.45, 95% CI 0.94-2.24, p=0.097; OR=0.96, 95% CI 0.91-1.01, p=0.11, for macrophages and neutrophils respectively). No differences between patients with or without chronic bronchitis were found for percentages of lymphocytes and epithelial cells, or numbers of total cell counts, neutrophils, macrophages, lymphocytes, and epithelial cells in induced sputum.

Relation between sputum and bronchial inflammatory cell counts.

Percentages of eosinophils in sputum were positively associated with eosinophil counts in biopsies within the chronic bronchitis groups as well as within the group of patients without chronic bronchitis. Interestingly, the percentages of sputum eosinophils were doubled in patients with chronic bronchitis compared to patients without chronic bronchitis for a given number of eosinophils in the bronchial biopsies ($b=2.03$, $p=0.01$, Figure 3).

DISCUSSION

This study shows that chronic bronchitis reflects an inflammatory sub-phenotype among patients with moderate to severe COPD, that is characterized by a distinct inflammatory cell profile as measured in a large sample of induced sputum specimens and bronchial biopsies. More specific, clinical symptoms of chronic bronchitis in COPD are associated with a distinct distribution of bronchial and sputum eosinophils. In addition, patients with chronic bronchitis had higher percentages of macrophages and lower percentages of neutrophils in their sputum. The latter could be explained by differences in current smoking habits and gender distribution between the two groups. No significant differences were found between the two groups with regard to other inflammatory cells in biopsies or sputum. Taken together, clinical symptoms of chronic bronchitis in COPD appear to represent an inflammatory sub-phenotype, which may have implications in anti-inflammatory treatment in clinical practice.

This study demonstrates for the first time that chronic bronchitis among patients with manifest COPD is characterized by a partially distinct inflammatory cell profile of eosinophils, macrophages and neutrophils. Comparison between our findings on eosinophils and studies in literature is difficult as outlined below, because either chronic bronchitis within COPD was not addressed as a separate entity [13, 22] or different tissues were used [23]. Furthermore, in most of these studies lower numbers of patients were investigated [13, 23]. Therefore, our observation of a preferential distribution of eosinophils towards the airway lumen in COPD patients with chronic bronchitis extends a previous report, where no differences were observed in the number of eosinophils in the submucosa of the airway wall (resected lung tissue) from smokers with chronic bronchitis (COPD) as compared to non-smoking controls (non-COPD) [13]. Furthermore, we recently found that smokers with chronic bronchitis (COPD) have similar percentages of sputum eosinophils as compared to non-smoking controls (non-COPD) [22]. Together these studies and our own observations show that comparison of COPD patients with and without chronic bronchitis reveals different inflammatory sub-phenotypes within COPD, whereas comparison of COPD patients with chronic bronchitis and non-smoking controls may reflect the inflammatory process associated with the development of COPD.

We observed that COPD patients with chronic bronchitis had relatively higher percentages of macrophages and lower percentages of neutrophils in sputum, which was mainly due to differences in current smoking habits between the two groups. These associations with smoking are in line with results from Willemse *et al.* [22]. Remarkably, we found no differences with regard to T-lymphocytes, neutrophils, macrophages or mast cells in bronchial biopsies between COPD patients with and without chronic bronchitis. This extends the findings by Saetta *et al.*, where inflammatory cells in the submucosa were similar between subjects with chronic bronchitis (COPD) and non-smoking controls (non-COPD) [13]. However, again this may reflect the different populations and tissues examined.

To our knowledge, this is one of the largest studies using induced sputum as well as bronchial biopsies in well-characterized steroid naïve patients with COPD. Nonetheless, some limitations must be mentioned. There is overlap of data in biopsies and sputum between both groups. Yet, this is in line with results from other studies examining similar parameters in different and smaller groups of patients [13, 24]. More importantly, for a given number of eosinophils in the bronchial biopsies the percentages of sputum eosinophils were doubled in patients with chronic bronchitis

compared to patients without these symptoms. The presence of chronic bronchitis as based on clinical symptoms only may be biased due to different awareness by gender, through retrospective selection of the patients, or the influence of recurrent exacerbations. Our results, however, were corrected for gender, and it needs to be emphasized that symptoms of chronic bronchitis have been associated with hypersecretion of mucus from enlarged bronchial glands and inflammatory cells in resected lung tissue since the early 1950s [13, 25, 26]. Only 9 patients experienced exacerbations (symptoms plus prednisone) in the year prior to our study and we believe that the influence of exacerbations on the presence of chronic bronchitis was limited. Furthermore, chronic symptoms of cough and sputum production have been associated with a more rapid decline in FEV₁ and increased COPD-related mortality [5, 8]. Therefore, despite the fact that chronic bronchitis is indeed likely to be a continuum, the currently used definition is supported by clinical and pathological data.

It is possible that we did not investigate the right anatomic region when studying bronchial biopsies and that peripheral lung tissue is needed to investigate the total airway wall, and therefore allow the use of other parameters such as the Reid's index (i.e. the ratio of the thickness of the mucous gland layer to the thickness of the wall between the epithelium and cartilage). However, previous studies showed that patients with both chronic bronchitis and fixed airway obstruction had the same Reid's index compared to controls, whereas scores of inflammation were better associated with mucus hypersecretion [13, 26]. It may also be argued that the distribution of inflammatory cells obtained with different techniques (i.e. induced sputum and bronchial biopsies) is difficult to interpret. A previous study, however, showed fairly good agreement between the number of eosinophils in different compartments in the airways in patients with chronic bronchitis [27]. It is noteworthy that, although not significant, the differences in absolute numbers of total cells, eosinophils, macrophage and neutrophils in sputum between patients with and without chronic bronchitis demonstrated the same trend and magnitude as the differences in percentages between both groups. In addition, inflammatory cell number may not represent cell activity, which requires more in depth analysis. However this was beyond the scope of this study.

How can we interpret these results? Mucus hypersecretion, which is the hallmark of clinical chronic bronchitis, is the result of mucin production, secretion and clearance [28]. Inflammatory mediators such as neutrophil elastase, are important secretagogues for mucin producing cells. In COPD both cigarette smoke and neutrophil elastase are main determinants of not only mucin production and secretion but also of clearance, by impaired ciliary activity and dehydration of the airway surface layer [28]. Nevertheless, the present study shows that chronic bronchitis is related to eosinophils in biopsies and sputum. Increased numbers of eosinophils in sputum that have migrated through the epithelial layer may contribute to mucus hypersecretion through the action of TGF- α [29] or by stimulating degranulation of mucus producing cells through the release of inflammatory mediators, including cysteinyl leukotrienes (CysLTs) [30]. Therefore, our findings of a preferential distribution are in line with a role of eosinophils in mucus hypersecretion. This is further supported by other studies showing increased sputum eosinophils during COPD exacerbations [31] and a positive correlation between airway eosinophilia and increased sputum production in asthma [32]. A decrease in the number of eosinophils in the airway wall, especially around the glands

may also contribute to mucus hypersecretion. Eosinophils are a major cellular source of transforming growth factor- β (TGF- β) [33] and Baraldo *et al* showed that impaired TGF- β signalling is associated with bronchial gland enlargement [34]. Therefore a lower number of eosinophils around the bronchial glands may lead to bronchial gland enlargement and a subsequent rise in mucus in the airway lumen, due to a decreased local TGF- β availability. Another explanation for our findings is that the same mechanism is involved in eosinophil recruitment into the airway lumen as well as in mucus hypersecretion. Th2 cytokines may be involved in such mechanisms since the expression of IL-4 and IL-13 is higher in patients with chronic bronchitis [35] and these cytokines are involved in the regulation of both eosinophil influx [36] and mucin production [37]. Based on the current information, we can not discriminate between these two explanations for the altered distribution of eosinophils in COPD patients with chronic bronchitis that we observed.

Our results also showed that patients with chronic bronchitis had higher percentages of macrophages in sputum, which was mainly explained by current smoking. This may indicate that sputum macrophages may act as an intermediary variable in the causal pathway of chronic bronchitis. Activated by cigarette smoke, macrophages might contribute to mucus hypersecretion directly via release of pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and indirectly by neutrophil-chemotactic factors such as leukotriene B4 (LTB4) and IL-8 [38]. One would expect neutrophils in bronchial biopsies to be related to the presence of chronic bronchitis. Neutrophil elastase is thought to stimulate both the release and production of mucin. The present study showed no differences in neutrophils between patients with and without chronic bronchitis. One explanation might be that the submucosal glands, that are thought to be responsible for the largest amount of mucus in the large airways [11], are more important in defining the sub-phenotype of chronic bronchitis.

What are the implications of this study? Previous studies showed that treatment with steroids may reduce numbers of sputum eosinophils in patients with COPD [39, 40], whereas sputum eosinophilia in COPD may be predictive of a clinical response to steroid treatment [41, 42]. Distinct inflammatory cell profiles may require different (anti-inflammatory) interventions. Therefore, this and other novel anti-inflammatory strategies [43] may need to be examined in COPD patients with and without chronic bronchitis.

We conclude that clinical symptoms of chronic bronchitis reflect a distinct sub-phenotype among patients with manifest COPD, as based on inflammatory cells in induced sputum and bronchial biopsies. Our results indicate a preferential distribution of eosinophils towards the lumen. This may have implications for current and future treatment strategies [40, 41, 42, 43] in COPD patients with clinical symptoms of chronic bronchitis.

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DECLARATION OF COMPETING INTEREST

HB, member of the Department of Epidemiology & Bioinformatics, WT, member of the Department of Pathology, NH, member of the Department of Pulmonology of the University Medical Center Groningen and JS, PS and PH, member of the Department of Pulmonology of the LUMC, are member of the Glucold Study group that has received a grant from GSK (UK and NL) as part of the sponsorship of this GLUCOLD study.

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ETHICS APPROVAL

The medical ethics committees of the Leiden University Medical Center and the University Medical Center Groningen approved the study.

FIGURE LEGENDS

Figure 1.

The number of eosinophils in bronchial biopsies (0.1 mm^{-2}) in COPD patients with and without chronic bronchitis.

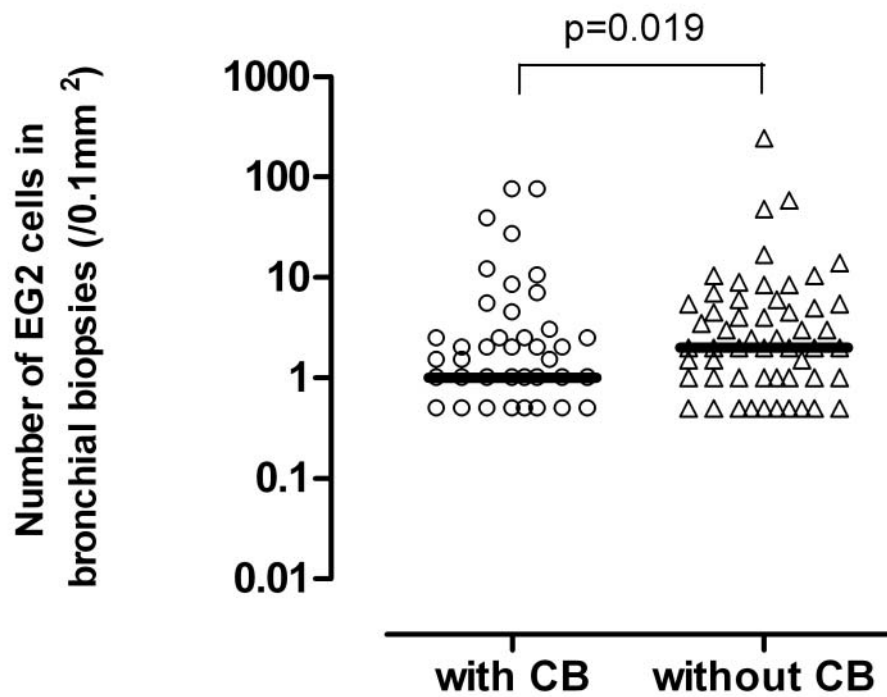


Figure 1

Figure 2.

The percentages of a) eosinophils and b) macrophages in induced sputum in COPD patients with and without chronic bronchitis.

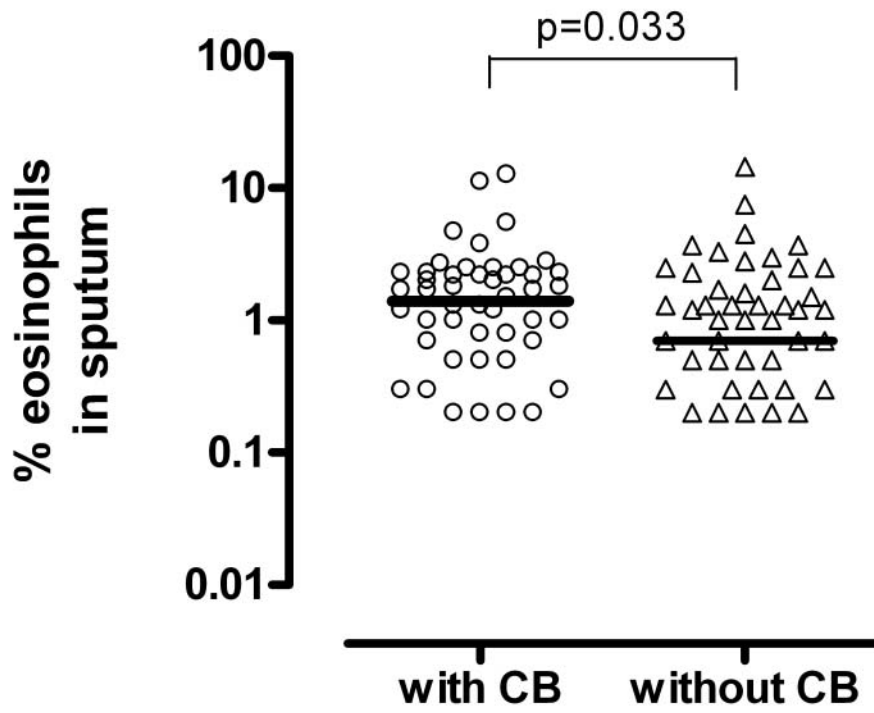


Figure 2a

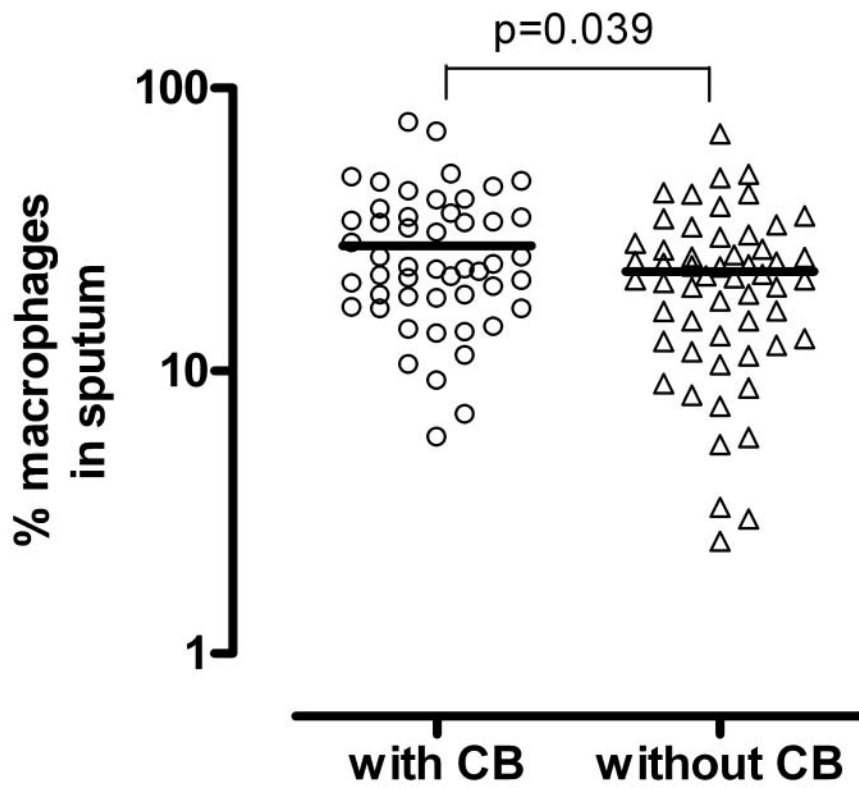


Figure 2b

Figure 3. Relationship between the percentages of eosinophils in induced sputum (*y-axis*) and eosinophils (0.1 mm^{-2}) in bronchial biopsies (*x-axis*) in COPD patients with and without chronic bronchitis.

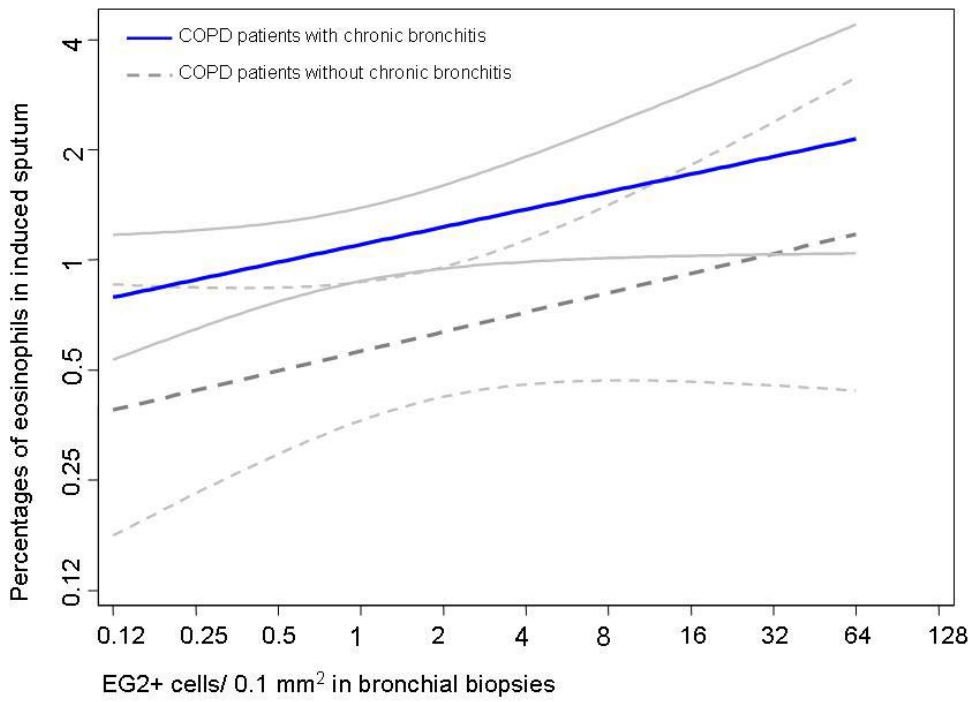


Figure 3

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Table 1. Clinical characteristics of COPD patients with and without chronic bronchitis

	COPD patients		
	with chronic bronchitis (n=53)	without chronic bronchitis (n=60)	p-value
Age, yrs	61±8	62±7	0.28
Male / female	42 / 11	56 / 4	0.028*
BMI	25.1±4	25.3±4	0.87
Current smoking, yes/no	40 / 13	31 / 29	0.009*
Pack-years [median, IQR]	42 [34-56]	40 [28-53]	0.12
Postbr. FEV ₁ , L [#]	2.01±0.4	2.04±0.5	0.49
Postbr. FEV ₁ , % pred	63±8	63±10	0.54
Postbr. FEV ₁ /IVC, %	49±8	47±9	0.34
Reversibility FEV ₁ , % pred	6.8±5	6.9±5	0.92
PC ₂₀ methacholine, mg/ml [†]	0.59 [0.15-2.72]	0.61 [0.17-2.07]	0.89
RV/TLC, %	48.6±10	47.8±7	0.61
TLCO/VA, % pred	73.8±25	77.2±26	0.49

Footnote table 1.

Clinical characteristics of COPD patients with and without chronic bronchitis. Values are mean±standard deviation (SD) or [†]for PC₂₀ methacholine geometric mean [interquartile range (IQR)], unless stated otherwise; Definition of abbreviations: postbr. = postbronchodilator; FEV₁ = forced expiratory volume in one second; % pred = percentage of predicted value; FEV₁/IVC = forced expiratory volume in one second/inspiratory vital capacity; PC₂₀ methacholine = the provocative concentration of methacholine that causes a decrease in FEV₁ of 20%; RV/TLC = residual volume/total lung capacity; *p<0.05, [#]adjusted for length.

Table 2. Comparison of bronchial inflammatory cells in COPD patients with and without chronic bronchitis[#].

	COPD patients		p-value
	with chronic bronchitis (n=53)	without chronic bronchitis (n=59)	
CD3+ lymphocytes (/0.1 mm ²)	124 [71-182]	121 [60-193]	0.95
CD4+ lymphocytes (/0.1 mm ²)	45 [24-72]	48 [28-75]	0.61
CD8+ lymphocytes (/0.1 mm ²)	18 [11-33]	23 [9.0-42]	0.48
CD4/CD8, %	2.04 [1.2-4.5]	2.08 [1.2-3.8]	0.96
CD8/CD3, %	0.16 [0.11-0.34]	0.20 [0.11-0.31]	1.00
CD4/CD3, %	0.37 [0.26-0.63]	0.43 [0.25-0.74]	0.51
EG2+ cells (/0.1 mm ²)	1.0 [0.25-2.5]	2.0 [1.0-5.5]	0.019*
Neutrophils biopt (/0.1 mm ²)	3.0 [2.0-5.5]	5.5 [2.0-8.5]	0.080
Plasma cells (/0.1 mm ²)	8.0 [3.5-15]	9.0 [4.0-14.5]	0.34
CD68+ cells (/0.1 mm ²)	8.5 [4.3-11.8]	10 [5.0-14]	0.20
AA1+ cells (/0.1 mm ²)	27 [21-34]	26 [18-35]	0.80

Footnote table 2.

The number of bronchial inflammatory cells (per 0.1 mm² sub-epithelial area) from COPD patients with and without chronic bronchitis. Data are presented as median [IQR]. *p=<0.05. [#]Data from one patient was excluded, because biopsy specimens were not adequate for analysis.

Table 3. Comparison of sputum inflammatory cells in COPD patients with and without chronic bronchitis #.

	COPD patients		p-value
	with chronic bronchitis (n=50)	without chronic bronchitis (n=55)	
Absolute numbers (10⁴/ml)			
Total cell count	135.0 [78.6-283.9]	149.1 [73.8-313.0]	0.54
Neutrophils	90.0 [46.1-204.6]	110.0 [56.3-231.0]	0.31
Macrophages	32.6 [18.8-64.8]	29.6 [13.0-59.3]	0.45
Lymphocytes	2.2 [1.0-5.6]	2.1 [1.0-7.2]	0.94
Eosinophils	1.7 [0.48-4.9]	1.1 [0.2-3.3]	0.13
Epithelial cells	1.26 [0.69-3.39]	1.37 [0.43-3.82]	0.40
Basophils	0 [0-0]	0 [0-0]	-
Percentages			
Neutrophils	69.9 [55.0-97.5]	73.8 [65.8-83.7]	0.046*
Macrophages	23.0 [17.7-35.1]	21.3 [12.7-28.3]	0.039*
Lymphocytes	1.8 [1.2-2.4]	1.7 [0.8-2.2]	0.47
Eosinophils	1.4 [0.5-2.3]	0.7 [0.2-1.7]	0.033*
Epithelial cells	1.0 [0.5-2.3]	1.3 [0.2-2.3]	0.32

Footnote table 3.

The number and percentage of non-squamous sputum inflammatory cells from COPD patients with and without chronic bronchitis. Data are presented as median [IQR].

*p=<0.05. #Sputa from 106 patients were evaluable for analysis.