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# Chronic bronchitis sub-phenotype within COPD: inflammation in sputum and biopsies

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**ABSTRACT: The presence of chronic bronchitis predicts a more rapid decline of forced expiratory volume in one second ($FEV_1$) in patients with chronic obstructive pulmonary disease (COPD). The hallmark of COPD is airway inflammation. It was hypothesised that COPD patients with chronic bronchitis are characterised by a distinct inflammatory cell profile, as measured in bronchial biopsies and sputum.**

**From 114 COPD patients (male/female ratio 99/15, mean±SD age 62±8 yrs, current smoking 63%, post-bronchodilator $FEV_1$ 63±9% predicted, no steroids), with and without chronic bronchitis, inflammatory cell counts in bronchial biopsies and induced sputum were measured. Analysis was carried out by logistic regression.**

**COPD patients with chronic bronchitis had lower eosinophil counts in biopsies and higher percentages of sputum eosinophils than patients without those symptoms, which remained after adjustment for smoking and sex. Patients with chronic bronchitis also showed higher percentages of macrophages and lower percentages of neutrophils in sputum, which could be explained by differences in smoking and sex.**

**It was concluded that chronic bronchitis reflects an inflammatory sub-phenotype among patients with chronic obstructive pulmonary disease. The present results indicate a preferential distribution of eosinophils towards the airway lumen in patients with chronic bronchitis. This may have implications for anti-inflammatory treatment of chronic obstructive pulmonary disease patients with chronic bronchitis.**

**KEYWORDS: Biopsies, chronic bronchitis, chronic mucus hypersecretion, chronic obstructive pulmonary disease, induced sputum, inflammation**

Chronic obstructive pulmonary disease (COPD) is a leading cause of death and disability worldwide [1]. COPD is characterised by progressive and not fully reversible airflow limitation, as measured by the forced expiratory volume in one second ($FEV_1$). 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 coexisting 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 $FEV_1$ [3]. However, subsequent findings suggested that chronic spu-

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tum 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 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 characterised predominantly by neutrophils, macrophages and CD8+ cells in the airways [10]. The role of excessive mucus production in the pathophysiology of 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, no differences have been observed in total mucin content of the surface epithelium between COPD patients with and without symptoms of chronic bronchitis [12]. Goblet cells in the surface epithelium are the main producers of the mucin MUC5AC, whereas MUC5B is a characteristic product of the submucosal glands [13]. 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 with asymptomatic non-COPD subjects [14]. 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 [15].

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

## METHODS

Detailed information about subjects and methodology has been published previously [16]. In brief, 114 patients with COPD were included for the Groningen Leiden Universities Chronic Obstructive Lung Disease (GLUCOLD) study. Patients were 45–75 yrs of age, current or ex-smokers with a history of $\geq$10 pack-yrs and respiratory symptoms. Post-bronchodilator FEV1 was >1.3 L and >20% predicted and less than the upper limit of the 90% confidence interval (CI) of the predicted FEV1 [17]. Post-bronchodilator FEV1/inspiratory vital capacity (IVC) ratio was <90% CI of the predicted FEV1/IVC ratio. Patients were clinically stable for >2 months before the measurements. They did not use a course of inhaled or oral corticosteroids during the 3 months prior to randomisation, or maintenance treatment with these drugs during the previous 6 months. The medical ethics committees of the Leiden University Medical Center (Leiden, the Netherlands) and the University Medical Center Groningen (Groningen, the Netherlands) approved the protocol, and patients provided written informed consent.

### *Design and definition of chronic bronchitis*

The present 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 $\geq$3 months per yr for >1 yr [5].

### *Pulmonary function tests*

Spirometry was performed according to international guidelines [18], using the reference values of QUANJER *et al.* [17]. Total lung capacity and residual volume were measured using a constant volume body plethysmograph [17]. Airway hyperresponsiveness was determined using the 2-min tidal breathing method [19] and expressed as the provocative concentration causing a 20% fall in FEV1.

### *Sputum induction and processing*

Sputum was induced and processed according to a validated technique using the so called ''full sample'' method [20]. After inhaling 200 μg salbutamol, patients inhaled hypertonic sodium chloride aerosols (4.5% weight/volume) during three 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 of squamous cells was <80%.

### *Bronchial biopsies*

Fibreoptic bronchoscopy was performed using a standardised protocol and has been described in detail previously [21]. In brief, four paraffin embedded biopsies were cut in 4-μm thick sections. Haematoxylin eosin staining was used for evaluation and selection of the two morphological best biopsies per patient. Specific antibodies against T-lymphocytes (CD3 and CD8: DAKO, Glostrup, Denmark; CD4: Novocastra, Newcastle-upon-Tyne, UK), macrophages (CD68; DAKO), neutrophil elastase (DAKO), mast cell tryptase (AA1; DAKO), plasma cells (CD138; IQ Products, Groningen, the Netherlands) and eosinophils (Pharmacia Diagnostics, Uppsala, Sweden) were used. Fully automated inflammatory cell-counting procedures were performed according to previously described validated methods [22]. 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 mean±SD or median (interquartile range). The differences between patients with and without chronic bronchitis were analysed using unpaired t-tests for normally distributed continuous variables. Chi-squared 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 the independent variables were bronchial and sputum inflammatory cells, with additional adjustment for smoking habits and sex. Differences at p-values <0.05 were considered to be statistically significant (tested two-sided).

**TABLE 1** Clinical characteristics of chronic obstructive pulmonary disease patients with and without chronic bronchitis (CB)

| | With CB | Without CB | p-value |
|---|---|---|---|
| **Subjects n** | 53 | 60 | |
| **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 smoker yes/no** | 40/13 | 31/29 | 0.009* |
| **Pack-yrs** | 42 (34–56) | 40 (28–53) | 0.12 |
| **Post-bronchodilator $FEV_1$# L** | 2.01±0.4 | 2.04±0.5 | 0.49 |
| **Post-bronchodilator $FEV_1$ % pred** | 63±8 | 63±10 | 0.54 |
| **Post-bronchodilator $FEV_1$/IVC %** | 49±8 | 47±9 | 0.34 |
| **Reversibility $FEV_1$ % pred** | 6.8±5 | 6.9±5 | 0.92 |
| **$PC_{20}$ methacholine¶ mg·mL$^{-1}$** | 0.59 (0.15–2.72) | 0.61 (0.17–2.07) | 0.89 |
| **RV/TLC %** | 48.6±10 | 47.8±7 | 0.61 |
| **$T_{L,CO}/V_A$ % pred** | 73.8±25 | 77.2±26 | 0.49 |

Data are presented as mean±SD or median (interquartile range (IQR)), unless otherwise stated. BMI: body mass index; $FEV_1$: forced expiratory volume in one second; % pred: % predicted; IVC: inspiratory vital capacity; $PC_{20}$: provocative concentration causing a 20% fall in $FEV_1$; RV: residual volume; TLC: total lung capacity; $T_{L,CO}$: transfer factor of the lung for carbon monoxide; $V_A$: alveolar volume. #: adjusted for length; ¶: geometric mean (IQR). *: $p<0.05$.

## RESULTS

The 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 mild-to-moderate COPD, as based on an average post-bronchodilator $FEV_1$ of 63±9% pred, and most of them were current smoking, middle-aged males. A minority of patients was mildly reversible to salbutamol, as based on $FEV_1$ (% pred) post- minus pre-salbutamol. A total of 18 (16%) patients showed a change in $FEV_1$ that was >12% pred and >200 mL, whereas nine 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 were mildly impaired in carbon monoxide 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 and 59 patients with and without chronic bronchitis, respectively (table 2). Patients with chronic bronchitis had significantly fewer eosinophils in biopsies than patients without chronic bronchitis ($p=0.019$; fig. 1). Logistic regression analysis confirmed this association. After adjustment for smoking and sex, there was a statistically significant lower chance (16%) of having chronic bronchitis for each doubling in bronchial eosinophils (odds ratio (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 patients without these symptoms (fig. 2a). After adjustment for smoking and sex, each doubling in the percentage of eosinophils in sputum 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

**TABLE 2** Comparison of bronchial inflammatory cells in chronic obstructive pulmonary disease patients with and without chronic bronchitis (CB)#

| | With CB | Without CB | p-value |
|---|---|---|---|
| **Subjects n** | 53 | 59 | |
| **CD3+ lymphocytes** | 124 (71–182) | 121 (60–193) | 0.95 |
| **CD4+ lymphocytes** | 45 (24–72) | 48 (28–75) | 0.61 |
| **CD8+ lymphocytes** | 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** | 1.0 (0.25–2.5) | 2.0 (1.0–5.5) | 0.019* |
| **Neutrophils** | 3.0 (2.0–5.5) | 5.5 (2.0–8.5) | 0.080 |
| **Plasma cells** | 8.0 (3.5–15) | 9.0 (4.0–14.5) | 0.34 |
| **CD68+ cells** | 8.5 (4.3–11.8) | 10 (5.0–14) | 0.20 |
| **AA1+ cells** | 27 (21–34) | 26 (18–35) | 0.80 |

Data are presented as median (interquartile range) of bronchial inflammatory cells (per 0.1 $mm^2$ of sub-epithelial area), unless otherwise stated. EG2: eosinophils. #: data from one patient was excluded as the biopsy specimens were not adequate for analysis. *: $p<0.05$.

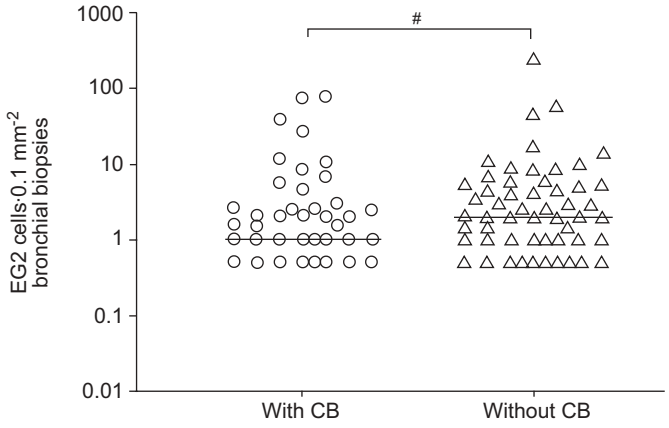


**FIGURE 1.** Number of eosinophils (EG2) in bronchial biopsies (per 0.1 $mm^2$ sub-epithelial area) in chronic obstructive pulmonary disease patients with and without chronic bronchitis (CB). The horizontal lines represent the median. #: p=0.019.

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; fig. 2b) and lower percentages of sputum neutrophils (p=0.049) than COPD patients without those symptoms. After adjustment for smoking and sex, these differences lost statistical significance (OR 1.45 (95% CI 0.94–2.24), p=0.097, and 0.96 (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.

### *Relationship 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 with patients without chronic bronchitis for a given number of eosinophils in the bronchial biopsies (b=2.03, p=0.01; fig. 3).

## DISCUSSION

The present study shows that chronic bronchitis reflects an inflammatory sub-phenotype among patients with mild-to-moderate COPD, which is characterised by a distinct inflammatory cell profile, as measured in a large sample of induced sputum specimens and bronchial biopsies. More specifically, 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 sex 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.

The present study demonstrates for the first time that chronic bronchitis among patients with manifest COPD is characterised by a partially distinct inflammatory cell profile of eosinophils, macrophages and neutrophils. Comparison between the present findings on eosinophils and studies in literature is difficult as outlined hereafter, since either chronic bronchitis within COPD was not addressed as a separate entity [14, 23] or different tissues were used [24]. Furthermore, in most of these studies lower numbers of patients were investigated [14, 24]. Therefore, the present observation of a preferential distribution of eosinophils towards the airway lumen in COPD patients with chronic bronchitis extends a previous report [14], 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 with nonsmoking non-COPD controls. Furthermore, it has recently been found that smokers with chronic bronchitis (COPD) have similar percentages of sputum eosinophils as compared with nonsmoking non-COPD controls [23]. Together, these studies and the present authors' 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 nonsmoking controls may reflect the inflammatory process associated with the development of COPD.

The present authors 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.* [23]. Remarkably, no differences were found 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.* [14], where inflammatory cells in the submucosa were similar between subjects with chronic bronchitis (COPD) and nonsmoking non-COPD controls. However, again this may reflect the different populations and tissues examined.

To the present authors' knowledge, the present study is one of the largest using induced sputum as well as bronchial biopsies in well-characterised 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 [14, 25]. 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 with patients without these symptoms. The presence of chronic bronchitis, as based on clinical symptoms only, may be biased due to different awareness by sex, through retrospective selection of the patients, or the influence of recurrent exacerbations. The present results, however, were corrected for sex and it needs to be emphasised that symptoms

**TABLE 3** Comparison of nonsquamous sputum inflammatory cells in chronic obstructive pulmonary disease patients with and without chronic bronchitis (CB) #

| | With CB | Without CB | p-value |
|---|---|---|---|
| **Subjects n** | 50 | 55 | |
| **Absolute numbers** | | | |
| **$\times 10^4 \cdot mL^{-1}$** | | | |
| 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 |

Data are presented as median (interquartile range), unless otherwise stated. #: sputa from 106 patients were available for analysis. *: $p<0.05$

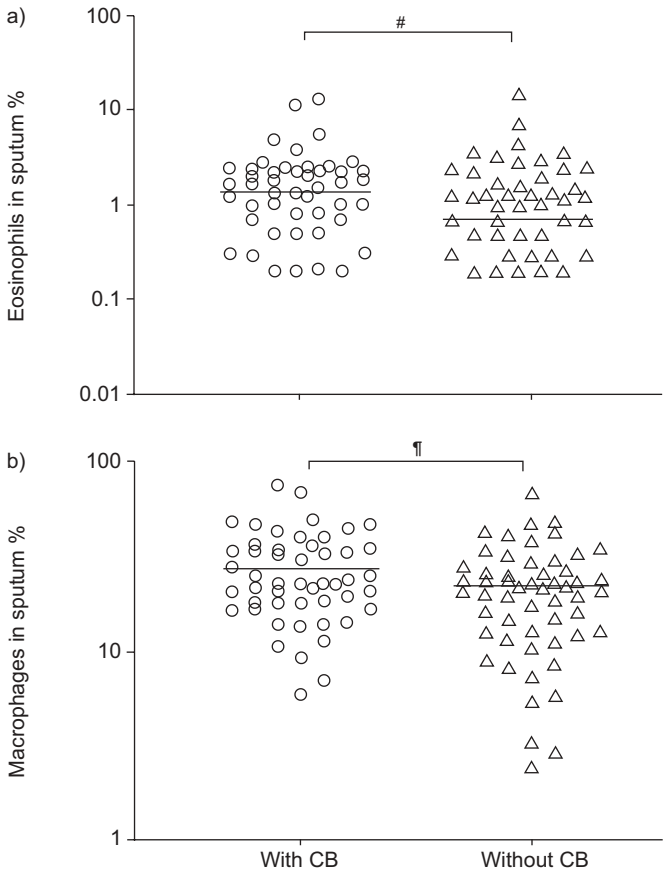


**FIGURE 2.** Percentages of a) eosinophils and b) macrophages in induced sputum in chronic obstructive pulmonary disease patients with and without chronic bronchitis (CB). #: $p=0.033$; ¶: $p=0.039$. The horizontal lines represent the median.

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 [14, 26, 27]. Only nine patients experienced exacerbations (symptoms plus prednisone) in the year prior to the present study and it is believed 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 $FEV_1$ 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 the present authors did not investigate the right anatomic region when studying bronchial biopsies and that peripheral lung tissue is needed to investigate the total airway wall, therefore allowing 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 with controls, whereas scores of inflammation were better associated with mucus hypersecretion [14, 27]. 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 [28], however, showed fairly good agreement between the number of eosinophils in different compartments in the airways in patients with chronic bronchitis. 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 numbers may not represent cell activity, an aspect that requires more in-depth analysis. However, this was beyond the scope of the present study.

How can these results be interpreted? Mucus hypersecretion, which is the hallmark of clinical chronic bronchitis, is the result of mucin production, secretion and clearance [29]. 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 [29]. 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 transforming growth factor (TGF)-$\alpha$ [30] or by stimulating degranulation of mucus-producing cells through the release of inflammatory mediators, including cysteinyl leukotrienes [31]. Therefore, the present 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 [32] and a positive correlation between airway eosinophilia

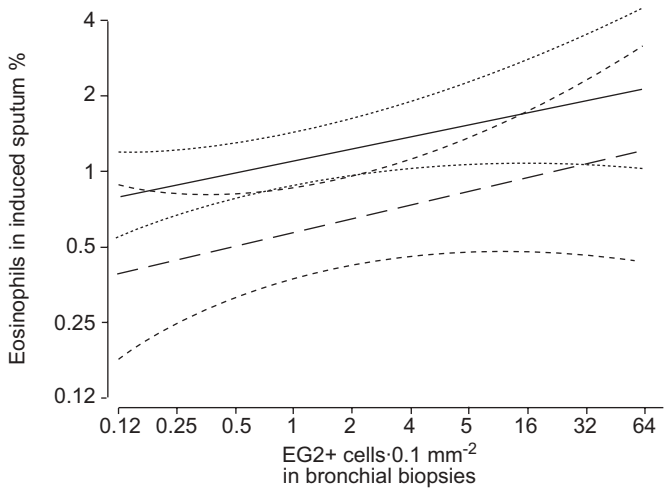


**FIGURE 3.** Relationship between the percentages of eosinophils in induced sputum and eosinophils (per 0.1 $mm^2$ sub-epithelial area) in bronchial biopsies of chronic obstructive pulmonary disease patients with (——) and without (– – – –) chronic bronchitis. ·····: 95% confidence intervals (CIs) of ——; ------: 95% CIs of – – –.

and increased sputum production in asthma [33]. 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 TGF-β [34] and BARALDO *et al.* [35] showed that impaired TGF-β signalling is associated with bronchial gland enlargement. 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 the present findings is that the same mechanism is involved in eosinophil recruitment into the airway lumen as well as in mucus hypersecretion. T-helper cell type 2 cytokines may be involved in such mechanisms since the expression of interleukin (IL)-4 and -13 is higher in patients with chronic bronchitis [36], and these cytokines are involved in the regulation of both eosinophil influx [37] and mucin production [38]. Based on the current information, it is not possible to discriminate between these two explanations for the altered distribution of eosinophils observed in COPD patients with chronic bronchitis.

The present 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 IL-1β, and indirectly by neutrophil-chemotactic factors, such as leukotriene $B_4$ and IL-8 [39]. Neutrophils in bronchial biopsies would be expected 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, 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 the present study? Previous studies showed that treatment with steroids may reduce numbers of sputum eosinophils in patients with COPD [40, 41], whereas sputum eosinophilia in COPD may be predictive of a clinical response to steroid treatment [42, 43]. Distinct inflammatory cell profiles may require different (anti-inflammatory) interventions. Therefore, this and other novel anti-inflammatory strategies [44] may need to be examined in COPD patients with and without chronic bronchitis.

It can be concluded that clinical symptoms of chronic bronchitis reflect a distinct sub-phenotype among patients with manifest chronic obstructive pulmonary disease, as based on inflammatory cells in induced sputum and bronchial biopsies. The present results indicate a preferential distribution of eosinophils towards the lumen. This may have implications for current and future treatment strategies [41–44] in chronic obstructive pulmonary disease patients with clinical symptoms of chronic bronchitis.

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