Small Airways Dysfunction and Neutrophilic Inflammation in Bronchial Biopsies and BAL in COPD*

Thérèse S. Lapperre, MD; Luuk N. A. Willems, MD, PhD; Wim Timens, MD, PhD; Klaus F. Rabe, MD, PhD; Pieter S. Hiemstra, MSc, PhD; Dirkje S. Postma, MD, PhD; and Peter J. Sterk, MD, PhD; and the GLUCOLD Study Group†

Background: The single-breath N₂ test (sbN₂-test) is closely related to small airways pathology in resected lung specimens of smokers. We investigated whether uneven ventilation and airway closure are associated with specific markers of airway inflammation as obtained by bronchial biopsies, BAL, and induced sputum in patients with manifest COPD.

Methods: Fifty-one patients with stable COPD not receiving corticosteroids were examined in a cross-sectional study (43 men; mean [SD] age, 63 ± 8 years; exsmokers and smokers; median pack-years, 41 [interquartile range, 31 to 51 pack-years]). Postbronchodilator spirometry (FEV₁, 63 ± 8% of predicted) and sbN₂-test (slope of phase III [ΔN₂], closing capacity [CC]/total lung capacity [TLC] percentage of predicted) were performed. Inflammatory cell counts were assessed in bronchial biopsies, BAL (only in the first half of patients), and induced sputum. Neutrophil elastase (NE), secretory leukocyte proteinase inhibitor (SLPI), and interleukin-8 levels were determined in BAL fluid.

Results: ΔN₂ increased with subepithelial neutrophil numbers in bronchial biopsies (rs = 0.337, p = 0.017) and with NE levels (rs = 0.443, p = 0.039), NE/neutrophil ratio (rs = 0.575, p = 0.005) and SLPI levels (rs = 0.484, p = 0.022) in BAL. CC/TLC was associated with BAL neutrophil numbers (rs = 0.448, p = 0.048). The sbN₂-test was not associated with any other inflammatory cell type in BAL or biopsies, nor with inflammatory cell counts in sputum. Of importance, the correlations between ΔN₂ and BAL NE/neutrophil ratio, and between CC/TLC and BAL neutrophil numbers remained significant when adjusting for FEV₁ percentage of predicted.

Conclusions: The results of the sbN₂-test are associated with neutrophilic inflammation in bronchial biopsies and BAL in patients with COPD. Our findings support a role of neutrophilic inflammation in the pathogenesis of small airways dysfunction in COPD. (CHEST 2007; 131:53–59)

Key words: airway closure; COPD; induced sputum; neutrophil elastase; neutrophils; secretory leukocyte proteinase inhibitor; single-breath nitrogen washout test; uneven distribution of ventilation

Abbreviations: CC = closing capacity; CV = closing volume; GLUCOLD = Groningen Leiden Universities and Corticosteroids in Obstructive Lung Disease; GOLD = Global Initiative for Chronic Obstructive Lung Disease; IL = interleukin; IQR = interquartile range; IVC = inspiratory vital capacity; ΔN₂ = slope of phase III; NE = neutrophil elastase; RV = residual volume; sbN₂-test = single-breath N₂ test; SLPI = secretory leukocyte proteinase inhibitor; TLC = total lung capacity; VC = vital capacity

COPD is characterized by progressive airflow limitation that is not fully reversible.¹ The pathologic substrate of airflow limitation is predominantly located in the small airways and lung parenchyma.² The bronchi and bronchioles exhibit accumulation of inflammatory cells, mucus, and plasma exudates, which are accompanied with fibrosis and proliferation of smooth-muscle cells, resulting in airway wall thickening. Together with destruction of lung parenchyma, these multiple features are implicated in the development of irreversible narrowing of the airways.²,³

The pathologic changes in the periphery of the lung are reflected by functional impairment as mea-
sured by physiologic tests. Among these so-called “small airways tests,” the single-breath N<sub>2</sub> test (sbN<sub>2</sub>-test) has been used to evaluate the presence of uneven distribution of ventilation (slope of nitrogen alveolar plateau; phase III) and airway closure (phase IV). Phase III and phase IV reflect differences in time constants, which are dependent on both local resistances (small airways disease) and local compliances (emphysema). The sbN<sub>2</sub>-test is not specific for the periphery of the lung, but it has been validated against small airways pathology scores. Because of this landmark study, the sbN<sub>2</sub>-test is still one of the few physiologic measures that have been anchored to small airways pathology in smokers. Furthermore, the combination of spirometry and the sbN<sub>2</sub>-test has been suggested to be predictive of the annual decline of FEV<sub>1</sub> in smokers. Therefore, the sbN<sub>2</sub>-test might be a complementary noninvasive tool to monitor pathology in the periphery of the lungs in patients with COPD.

The airway inflammation in COPD is characterized by influx of neutrophils and macrophages in the airway lumen, as well as elevated macrophage and T-lymphocyte and B-lymphocyte numbers in the airway wall. In addition, both interleukin (IL)-8 and neutrophil elastase (NE) are elevated in BAL fluid of smokers who acquire COPD. IL-8 is a main mediator of neutrophil chemotaxis in the airways, and NE is a neutrophil-derived serine protease that is able to cause structural changes in the lung, impairment of mucociliary clearance and host defense, and induction of mucous secretion. Secretory leukocyte proteinase inhibitor (SLPI) is a locally produced inhibitor of NE, and it has been suggested that SLPI plays a role in maintaining the protease-antiprotease balance in the lung, regulation of leukocyte function, host defense against infection, tissue repair, and matrix production.

Previous studies investigating the sbN<sub>2</sub>-test in smokers in relation to small airways disease have examined pathology scores, including scores of the degree of inflammatory cell infiltration in resected lung tissue or autopsy material without further characterization of the inflammatory profile because of lack of specific stainings at that time. In addition, measuring inflammation with less invasive tools, such as induced sputum and bronchoscopy with BAL and biopsies, has not been examined in relation to the sbN<sub>2</sub>-test in patients with COPD. Therefore, we postulated that small airways function in patients with COPD, as reflected by the sbN<sub>2</sub>-test, is associated with the inflammatory profile characteristic for COPD. To that end, we investigated the number of inflammatory cells in bronchial biopsies, BAL fluid, and induced sputum, together with the levels of NE, IL-8, and SLPI in BAL fluid in a cross-sectional study in 51 patients with COPD.

Materials and Methods

Subjects

Fifty-one patients with COPD, participating in the Groningen Leiden Universities and Corticosteroids in Obstructive Lung Disease (GLUCOLD) study were included in this study. The sbN<sub>2</sub>-test was performed in the Leiden center only. Patient characteristics and methods have been described in detail previously. In short, all patients had irreversible airflow limitation (postbronchodilator FEV<sub>1</sub> and FEV<sub>1</sub>/inspiratory vital capacity [IVC] < 90% confidence interval of predicted value [comparable with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stages II and III], FEV<sub>1</sub> ≥ 1.3 L and > 20% of predicted value) and respiratory symptoms. Patients with a history of asthma, α<sub>1</sub>-antitrypsin deficiency, other active lung disease, or receiving maintenance treatment with inhaled or oral steroids during the last 6 months were excluded from the study. They were allowed to use short-acting bronchodilators and were in clinical stable condition (no symptoms of respiratory tract infection for at least 2 weeks prior to the study, no course of oral or inhaled steroids during the last 3 months). Patients were ex-smokers with at least 10 pack-years of smoking history. The ethical committees of each center approved the study, and all patients gave written informed consent.

Design and sbN<sub>2</sub>-test

The study had a cross-sectional design including four visits. The sbN<sub>2</sub>-test was performed 15 min after administration of 400 μg of salbutamol per metered-dose inhaler connected to a spacer, in order to minimize contribution of smooth-muscle contraction. The measurement was performed using a dry rolling-seal spirometer (Spiroflow; Morgan; Kent, UK) filled with 100% oxygen and equipped with a N<sub>2</sub> meter (Morgan) connected to the mouthpiece allowing continuous sampling as described previously. During this test, seated patients performed a
slow full inspiratory and expiratory slow vital capacity (VC) maneuver at inspiratory and expiratory flow rates of approximately 0.5 L/s, which was controlled by a flow restrictor. The expiratory N₂ concentration was plotted against volume changes between total lung capacity (TLC) and residual volume (RV), and the slope of the nitrogen alveolar plateau (slope of phase III [ΔN₂]) was calculated by drawing the best-fit line through phase III of the expiratory volume concentration curve by a blinded observer. The first departure from this straight line was considered as indicative of airway closure, and closing volume (CV) [RV + CV] were calculated. This procedure has previously been validated in our laboratory, and the within-observer and between-observer reproducibility (determined by calculation of the intraclass correlation coefficient, Ri) of determining ΔN₂ (Ri = 0.940 and Ri = 0.995, respectively) and CV (Ri = 0.929 and Ri = 0.935, respectively) were high. Two measurements were performed, of which the one with the highest VC was selected for further analysis. The measurements were only accepted if the IVC and expiratory VC during the shN₂-test did not differ >15% or 0.5 L from each other. All volumes from the shN₂-test were corrected for body temperature and pressure, saturated with water vapor, and the parameters derived from the shN₂-test (ΔN₂, CV, CC) were expressed as percentage of predicted values.

Lung Function and Sputum Induction

Postbronchodilator spirometry, reversibility to 400 µg of salbutamol, body plethysmography, diffusing capacity, and sputum induction (full-sample method) were performed according to previously described methods.

Bronchoscopy and Biopsy Analysis

Fiberoptic bronchoscopy was performed using a previously described standardized protocol according to recent recommendations. Biopsy processing, staining, and analysis have also been described in detail previously. In short, 4-µm thick paraffin-embedded sections were stained using specific antibodies against T-lymphocytes (CD3, CD4, CD8), macrophages (CD68), NE, mast cell tryptase (AA1), eosinophils (EG2), and plasma cells (CD138). Digital images per coded biopsy section were prepared, and fully automated inflammatory cell counting procedures were performed according to previously described validated methods. The number of subepithelial 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 per 0.1 mm² of two tissue samples per patient.

BAL

We also performed BAL before biopsy samples were obtained. Because of ethical considerations, the BAL procedure was stopped during the course of the study because four patients had a serious adverse event that was considered to be possibly related to the BAL (pleural pain, fever, pneumonia, short-term cardiac ischemia). BAL was performed and processed according to previously described recommendations. First, 50 mL of NaCl 0.9% at 30°C was instilled, which was retrieved after 10 s of dwelling with gentle suction at ≤20 cm H₂O of pressure. This portion was not used for analysis. Thereafter, three times 50 mL of saline solution was instilled with dwelling times of 10 s. BAL processing and differential cell counts were performed analogous to the methods described for sputum processing, with the major exception that no dithiothreitol was used for homogenization. In addition, if required, lysis of RBC was performed before processing. A BAL sample was considered adequate when the amount of fluid instilled was at least 100 mL, and recovered BAL fluid was at least 10 mL (excluding the first portion). The levels of soluble NE, SLPI, and IL-8 were determined in BAL using enzyme-linked immunosorbent assay (IL-8; CLB; Amsterdam, the Netherlands; NE and SLPI enzyme-linked immunosorbent assays developed in our laboratory). For IL-8 measurements, BAL supernatant was first concentrated using filters (Centricon-3; Millipore; Bedford, MA).

Statistical Analysis

Mean values and SD were computed. When appropriate, variables were logarithmically transformed before statistical analysis and presented as median with interquartile range (IQR). Univariate correlations between postbronchodilator shN₂-test parameters (percentage of predicted) and inflammatory parameters were evaluated using Spearman rank correlation coefficient (rs). Multivariate linear regression analysis was used to adjust for FEV₁, to evaluate whether contribution of inflammation to small airways function was independent of the degree of airflow limitation. Statistical significance was assumed at p < 0.05.

RESULTS

Patient Characteristics

Table 1 shows the characteristics of the 51 patients who performed the shN₂-test after bronchodilation. The patients had moderate-to-severe COPD (GOLD stages II and III) based on a postbronchodilator FEV₁ of 63.3 ± 8.4% of predicted, and had a

<table>
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<th>Variables Data</th>
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<tr>
<td>General characteristics Male/female gender, No. 43/8 Age, yr 62.7 (8.1) Smoking history, pack-yr 41 (31-51) Current/not current smoker, No. 30/21 Lung function Postbronchodilator FEV₁, % predicted 63.3 (8.4) Postbronchodilator FEV₁/IVC, % 48.5 (8.5) ΔFEV₁, % predicted 7.4 (3.7) Kco, % predicted 73.5 (23.7) RV/TLC, % 48.2 (9.2) Postbronchodilator shN₂-test ΔN₂, % N₂/L 4.3 (2.9-6.6) CV, L 1.1 (0.45) CV/VC, % 25.6 (9.2) CC/TLC, % 62.5 (9.7) ΔCV, % predicted 314 (220-447) CC/VC, % predicted 114 (40.3) CC/TLC, % predicted 135 (19.7)</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SD) or median (IQR) unless otherwise indicated.
median smoking history of 41 pack-years (IQR, 31 to 51 pack-years). They demonstrated elevated values of \( \Delta N_2 \) and early airway closure (Table 1). In two patients, we were unable to determine a CV. Induced sputum and BAL cell counts are presented in Table 2, and bronchial inflammatory cell numbers are shown in Table 3. BAL was performed only in the first half of patients (n = 22).

**Relation Between Small Airways Function and Airway Inflammation**

Small airway parameters as assessed with the sbN\(_2\)-test were predominantly associated with neutrophilic inflammation in BAL (Fig 1). Postbronchodilator \( \Delta N_2 \) percentage of predicted increased with NE levels (median, 20.7 ng/mL; IQR, 7.5 to 36.4 ng/mL), NE/neutrophil number ratio, and SLPI levels (median, 110 ng/mL; IQR, 41.5 to 258 ng/mL) in BAL (\( rs = 0.443 \), \( p = 0.039 \); \( rs = 0.575 \), \( p = 0.005 \); and \( rs = 0.484 \), \( p = 0.022 \); respectively; Fig 1). However, \( \Delta N_2 \) percentage of predicted was not related with IL-8 levels (median, 64.5 pg/mL; IQR, 28.4 to 192 pg/mL) or other inflammatory cell counts in BAL. Furthermore, postbronchodilator CC/TLC percentage of predicted was also associated with BAL neutrophil numbers (\( rs = 0.448 \), \( p = 0.048 \); Fig 1). Postbronchodilator CVVC percentage of predicted was not related with any of the BAL inflammatory markers. Postbronchodilator FEV\(_1\) percentage of predicted was inversely associated with \( \Delta N_2 \) (\( R = -0.522 \), \( p < 0.001 \)). Interestingly, the correlations between \( \Delta N_2 \) and BAL NE/neutrophil ratio, and between CC/TLC and BAL neutrophil numbers remained significant when adjusting for FEV\(_1\) percentage of predicted (\( p = 0.008 \) and \( p = 0.041 \), respectively).

Bronchial biopsies showed associations with sbN\(_2\)-test that were largely consistent with those found in BAL. Again, postbronchodilator \( \Delta N_2 \) percentage of predicted was positively associated with subepithelial neutrophil numbers in bronchial biopsies (\( rs = 0.337 \), \( p = 0.017 \); Fig 2) but not with other bronchial inflammatory cell counts. Significance of this latter correlation was lost when adjusting for FEV\(_1\) percentage of predicted. The sbN\(_2\)-test was not significantly correlated with inflammatory cell counts in induced sputum.

**Table 2—Cell Counts in Induced Sputum and BAL***

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sputum</th>
<th>BAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>50</td>
<td>22</td>
</tr>
<tr>
<td>Volume recovered, mL</td>
<td>64.1 (26.8)</td>
<td></td>
</tr>
<tr>
<td>Recovery, %</td>
<td>44.2 (16.5)</td>
<td></td>
</tr>
<tr>
<td>Epithelial cells, %</td>
<td>1.3 (0.3–3.1)</td>
<td>1.9 (0.8–7.9)</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>68.9 (15.1)</td>
<td>3.5 (1.8–6.7)</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>23.7 (18.2–31.2)</td>
<td>80.5 (76.1–94.8)</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>1.0 (0.2–1.8)</td>
<td>0.2 (0.0–0.6)</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>1.9 (1.3–2.6)</td>
<td>2.6 (1.0–5.3)</td>
</tr>
<tr>
<td>Total cell count, ( \times 10^6/\text{mL} )</td>
<td>182 (101–333)</td>
<td>30.9 (13.7–66.4)</td>
</tr>
</tbody>
</table>

*Data are presented as mean (SD) or median (IQR) unless otherwise indicated.

**Table 3—Bronchial Inflammatory Cell Counts (n = 50)**

<table>
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<tr>
<th>Cells</th>
<th>Median No./0.1 mm(^2) of Tissue (IQR)</th>
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<tbody>
<tr>
<td>CD3</td>
<td>141 (59–204)</td>
</tr>
<tr>
<td>CD4</td>
<td>51 (29–79)</td>
</tr>
<tr>
<td>CD8</td>
<td>19 (8.0–32)</td>
</tr>
<tr>
<td>NE (neutrophils)</td>
<td>4.0 (1.9–8.5)</td>
</tr>
<tr>
<td>CD68 (macrophages)</td>
<td>8.5 (5.4–12)</td>
</tr>
<tr>
<td>EG2 (eosinophils)</td>
<td>2.0 (1.0–6.3)</td>
</tr>
<tr>
<td>AA1 (mast cells)</td>
<td>26 (21–34)</td>
</tr>
<tr>
<td>CD138 (plasma cells)</td>
<td>8.5 (3.5–12)</td>
</tr>
</tbody>
</table>

**Discussion**

This study shows \( \Delta N_2 \) (nitrogen alveolar plateau) is associated with NE, NE/neutrophil ratio, and SLPI levels in BAL, as well as with neutrophil numbers in bronchial biopsies. In addition, CC appeared to be associated with neutrophil numbers in BAL. These relations were independent of the degree of airflow limitation. Our results indicate that uneven ventilation and airway closure in COPD indirectly reflect neutrophilic inflammation in the wall of large airways as well as in the lumen of peripheral airways and alveoli as mirrored by BAL.

To our knowledge, this is the first study in patients with COPD examining the relation between the sbN\(_2\)-test and airway inflammation in bronchial biopsies, BAL, and induced sputum. Multiple studies have focused on the relation between indexes of the sbN\(_2\)-test and small airways pathology, including inflammation scores, in resected lung tissue\(^7,20–32\) or autopsy material\(^21,24\). However, the type of inflammatory cells was not specified in these pathology studies. It has been demonstrated that peripheral airways dysfunction in COPD, as determined by quantitative high-resolution CT, is associated with sputum neutrophilia\(^3,7,20–24,37\). Our results extend these previous results\(^7,20–24,37\) by showing that neutrophilic inflammation in the airway wall of large airways, as well as in the lumen of peripheral airways and alveoli as reflected by BAL, correlates with uneven ventilation and airway closure as revealed by a relatively simple single-breath test in patients with COPD.

There are a few considerations when interpreting our results. First, the patients represented COPD...
patients of GOLD stages II and III. Because of ethical considerations, the BAL procedure was discontinued (ie, a few patients reported side effects in relation to the BAL), and therefore BAL was performed only in the first half of patients. Since this was not anticipated, it is unlikely that this introduced selection bias for the BAL results. Second, we used percentage of predicted values and postbronchodilator sbN2-tests for exploration of relations with inflammatory markers in order to prevent possible variability by age, sex, and smooth-muscle tone. Finally, we chose to use the sbN2-test to evaluate the presence of uneven distribution of ventilation (ΔN2) and airway closure (phase IV). Phase III and phase IV reflect differences in time constants, which are dependent on both local resistances (small airways disease) and local compliances (emphysema). It is known that besides small airways, other factors contribute to the outcomes of the sbN2-test, such as topographic distribution of ventilation. In addition, there is evidence that multiple breath washout curves can distinguish uneven ventilation in the conducting small airways from the acinar lung zones. However, the latter technique still lacks external validation by relating it to small airways pathology. Therefore, we gave preference to the sbN2-test based on its well-described relationship with small airways pathology in resected lung tissue.

How can the present results be interpreted? The association of the sbN2-test with neutrophil numbers in BAL and bronchial biopsies, and with NE and NE/neutrophil ratio supports a role for airway neutrophil accumulation and activation in the pathogenesis of small airways and/or alveolar dysfunction in COPD. Neutrophils migrate into the lung in response to the presence or release of chemotactants, such as IL-8. It is therefore tempting to...
speculate that accumulation of neutrophils in the airway wall of small airways also contributes to small airways dysfunction in COPD. However, we have recently demonstrated that the number of neutrophils in the lamina propria of the small airway is larger than in the lamina propria of the large airways in smokers. This suggests that the distribution of neutrophils along the tracheobronchial tree may not be uniform. This should be addressed in studies directly examining peripheral lung tissue.

The recruited neutrophils in the airways can release NE, which is able to cause tissue destruction but also plays a role in mucus hypersecretion. Consequently, NE may contribute to loss of alveolar attachments and/or mucus hypersecretion, which both may lead to small airways narrowing and according to inhomogeneous distribution of ventilation and early airway closure in patients with COPD. It has been shown previously in our laboratory that numbers of SLPI-containing epithelial cells increase with more severe small airways disease and destruction of alveolar attachments, which may possibly be part of the defense against inflammatory and destructive processes in small airways. This may explain the observed association between small airways function and SLPI levels in BAL in the present study. Furthermore, the lack of an association between small airways function and inflammatory cells in induced sputum may not be unexpected because induced sputum is likely to represent a different compartment than BAL and bronchial biopsies in COPD, presumed to originate from large airways. Finally, lymphocytes, especially CD8 T-cells, have been found to play a role in the pathogenesis of COPD. In the present study, we did not observe a relation between these cells and the sbN2-test. This may be due to nonuniform distribution of inflammatory cells along the tracheobronchial tree, even though CD8 cell numbers do not appear to be different between large and small airways in smokers.

What is the clinical relevance of our results? It appears from our results that the sbN2-test is a noninvasive tool, complementary to spirometry, that is associated with neutrophilic airway inflammation in COPD. It has been observed that the sbN2-test contributes to prediction of the decline in FEV1 in patients with established airflow limitation. Therefore, we may speculate that neutrophilic inflammation is involved in progression of COPD by contributing to small airways and/or alveolar pathology. Consequently, monitoring the sbN2-test might add to the management of patients with COPD.

In conclusion, uneven ventilation and airway closure in patients with stable COPD are associated with neutrophil numbers in bronchial biopsies and BAL, and with NE and its local inhibitor SLPI in BAL. These findings suggest a role for neutrophilic inflammation in small airways dysfunction in COPD.

APPENDIX

The GLUCOLD study group consists of the following: H.F. Kauffman, D. de Reus, Department of Allergology; H.M. Boezen, D.F. Jansen, J. Vonk, Department of Epidemiology and Statistics; M.D.W. Barentsen, W. Timens, M. Zeinstra-Smit, Department of Pathology; A.J. Luteijn, T. van der Molen, G. ter Veen, Department of General Practice; M.M.E. Gosman, N.H.T. ten Hacken, H.A.M. Kerstjens, M.S. van Maaren, D.S. Postma, C.A. Veltman, A. Verbokkem, I. Verhage, H.K. Vink-Kloosters, Department of Pulmonology: University of Groningen and University Hospital of Groningen, Groningen, the Netherlands; J.B. Snoeek-Strob band, H. Thia liens, Department of General Practice; J.K. Sont, Department of Medical Decision Making; I. Bajema, Department of Pathology; J. Gast-Strookman, P.S. Hiemstra, K. Janssen, T.S. Lappere, A.C. van der Linden, K.F. Rabe, A. van Schadewijk, J. Smit-Bakker, P.J. Sterk, J. Stolk, A.C.J.A. Tijdé, H. van der Veen, R. Verhoosel, M.M.E. Wijffels, and L.N.A. Willems, Department of Pulmonology: Leiden University Medical Center, Leiden, the Netherlands; and T. Manaud, University of Sao Paulo, Sao Paulo, Brazil.

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REFERENCES

11 Traves SL, Culpitt SV, Russell RE, et al. Increased levels of
the chemokines GROx and MCP-1 in sputum samples from patients with COPD. Thorax 2002; 57:590–595
18 Stockley RA. Neutrophil and protease/antiprotease imbalance. Am J Respir Crit Care Med 1999; 160:S49–S52
26 Lapperre TS, Postma DS, Gosman MME, et al. Relation between duration of smoking cessation and bronchial inflammation in COPD. Thorax 2006; 61:115–121
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