CHRONIC OBSTRUCTIVE PULMONARY DISEASE

Relationship between peripheral airway dysfunction, airway obstruction, and neutrophilic inflammation in COPD


Background: Considerable research has been conducted into the nature of airway inflammation in chronic obstructive pulmonary disease (COPD) but the relationship between proximal airways inflammation and both dynamic collapse of the peripheral airways and HRCT determined emphysema severity remains unknown. A number of research tools have been combined to study smokers with a range of COPD severities classified according to the GOLD criteria.

Methods: Sixty-five subjects (11 healthy smokers, 44 smokers with stage 0–IV COPD, and 10 healthy nonsmokers) were assessed using lung function testing and HRCT scanning to quantify emphysema and peripheral airway dysfunction and sputum induction to measure airway inflammation.

Results: Expiratory HRCT measurements and the expiratory/inspiratory mean lung density ratio (both indicators of peripheral airway dysfunction) correlated more closely in smokers with the severity of airflow obstruction ($r = -0.64, p < 0.001$) than did inspiratory HRCT measurements (which reflect emphysema severity; $r = -0.45, p < 0.001$). Raised sputum neutrophil counts also correlated strongly in smokers with HRCT indicators of peripheral airway dysfunction ($r = 0.55, p < 0.001$) but did not correlate with HRCT indicators of the severity of emphysema.

Conclusions: This study suggests that peripheral airway dysfunction, assessed by expiratory HRCT measurements, is a determinant of COPD severity. Airway neutrophilia, a central feature of COPD, is closely associated with the severity of peripheral airway dysfunction in COPD but is not related to the overall severity of emphysema as measured by HRCT.

METHODS

The study was approved by the Southampton University and General Hospitals ethics committee. Volunteers were prospectively recruited for the purpose of the study and gave their written informed consent.

Clinical assessment and classification by disease severity

A detailed clinical history was taken and physical examination performed. Lung function testing consisted of spirometry, carried out according to the ATS guidelines, flow-volume loop with flow rate measurements at 50% expiration (MEF50), measurement of the ratio of residual volume to total lung capacity (RV/TLC), and carbon monoxide gas transfer coefficient (Kco) and factor (Tlco). Bronchodilator responsiveness to 400 µg salbutamol was assessed and post-bronchodilator spirometric values were recorded. Histamine bronchial provocation challenge was carried out as reported previously.

Based on clinical assessment and HRCT scanning, a total of 65 subjects were enrolled and classified into five groups according to the GOLD criteria: (1) healthy control subjects ($n = 10$) with normal lung function and no emphysema; (2) mild (GOLD 1) ($n = 10$) with mild airflow obstruction; (3) moderate (GOLD 2) ($n = 15$) with moderate airflow obstruction; (4) severe (GOLD 3) ($n = 15$) with severe airflow obstruction; and (5) very severe (GOLD 4) ($n = 15$) with very severe airflow obstruction.

Abbreviations: COPD, chronic obstructive pulmonary disease; E/I ratio, expiration/inspiration ratio; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; HRCT, high resolution computed tomography; Kco, lung carbon monoxide transfer coefficient; LAA, area of low attenuation; MEF, mid expiratory flow; MLD, mean lung density; RV, residual volume; TLC, total lung capacity; Tlco, carbon monoxide lung transfer factor.
HAUSANDARTH

HRCT

HRCT scanning was used for the evaluation of both emphysema and peripheral airways dysfunction. Scans (Hi-speed CTi scanner, General Electric) were performed on full inspiration and expiration at 10 mm intervals with a collimation (slice thickness) of 1 mm. The scanner was subject to a weekly quality assessment with a phantom check including uniformity, linearity, and noise. In addition there was a 3 monthly engineering check of spatial and contrast resolution and an annual medical physics check. Scanning voltage was 140 kV and current was 250 mA. Hard copy images were photographed at a window level of –650 HU and a window width of 1500 HU, as appropriate for viewing lung parenchyma. The scans were evaluated for the presence of emphysema both qualitatively by two radiologists independently of the remaining research and quantitatively by computerised density mask analysis. Two lung density parameters were measured: % area of low attenuation (% LAA) with low attenuation being defined as Hounsfield density units,9 and mean lung density (MLD)—and expiratory gas trapping were assessed radiologically by calculating the ratio of the MLD on expiration to MLD on inspiration (E/I ratio), as described in previous studies.15 16

Assessment of airway inflammation

Airway inflammation was assessed in induced sputum. Sputum induction was carried out as previously reported.21 Cytospins were made, coded, and differential cell counting was performed on 600 cells. Results are shown as absolute and relative counts (percentage of non-squamous inflammatory cells). In addition, sputum samples were sent for microbiological culture to allow exclusion of subjects with detectable bacterial infection of the airways.

Data analysis

Statistical analysis was performed using SPSS version 10.0. Normally distributed data (lung function and HRCT) were compared between multiple groups by ANOVA, followed by the Scheffe examination for individual comparisons. Cell counts in sputum were compared using the Kruskal-Wallis test followed by the Mann-Whitney test. Trend analysis for the clinical groups was performed using the Jonckheere-Terpstra test. Correlations were sought by Pearson’s and Spearman’s tests for normally and non-normally distributed data, respectively. Correction for multiple correlations was performed using the Bonferroni correction.

| Table 1 | Clinical characteristics and HRCT measurements performed on inspiration and expiration of the five subject groups |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Non-smokers    | Healthy smokers | Stage 0 COPD    | Stage 1 COPD    | Stage II–IV COPD | p value         |
| Age (years)    | 51 (7)         | 45 (12)         | 50 (7)          | 57 (7)          | 55 (7)          | (ANOVA)         |
| Pack years     | 0 (0)          | 40 (15)†        | 43 (19)†        | 44 (13)†        | 52 (14)†        | <0.001          |
| SGRQ (% post)  | 7.1 (5.0)      | 20.4 (16.1)†    | 27.2 (12.9)†    | 35.2 (19.7)†    | <0.001          |
| FEV1/FVC (%)   | 77 (5.4)†      | 50 (10)†        | 56 (14)†        | 69 (16)†        | 51 (12)†        | <0.001          |
| MEF25,50 (%)   | 85 (17.5)†     | 76 (22)†        | 66 (3)†         | 55 (12)†        | <0.001          |
| RV/TLC (%)     | 30 (3.5)†      | 35 (7.7)†       | 38 (5.2)†       | 49 (7.9)†       | <0.001          |
| Tco (%)        | 84 (12)†       | 63 (10)†        | 45 (18)†        | 56 (16)†        | <0.001          |
| Kco (%)        | 82 (12)†       | 61 (13)†        | 62 (19)†        | 64 (15)†        | <0.01           |
| Inspiratory MLD (HU) | 840 (27)       | 825 (23)        | 832 (23)        | 846 (25)        | <0.001          |
| Inspiratory %LAA | 7.0 (3.2)       | 5.8 (2.7)       | 12.6 (8.1)      | 11.4 (7.4)      | <0.01           |
| Expiratory MLD (HU) | 755 (44)†       | 824 (19)        | 852 (23)        | 846 (25)        | <0.001          |
| Expiratory %LAA | 3.3 (12)†       | 2.3 (1)†        | 7.1 (6.4)†      | 7.3 (6.8)†      | <0.01           |
| E/I ratio      | 0.89 (0.03)†   | 0.88 (0.04)†    | 0.89 (0.03)†    | 0.91 (0.02)†    | 0.94 (0.02)†    | <0.001          |

All data are shown as mean (SD). * † Homogenous Scheffe subsets.

SGRQ, St George’s Respiratory questionnaire; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; MEF, mid expiratory flow; Kco, lung carbon monoxide transfer coefficient; Tco, carbon monoxide lung transfer factor; MLD, mean lung density; LAA, area of low attenuation; E/I ratio, expiration/inspiration ratio.
carried out using the Holm method, which is a modification of the Bonferroni method but has higher power at the same type I error rate. Thus, if \( p_j \) is the p value corresponding to variable \( j \) and there are \( k \) variables (hypotheses) to test, the Holm method consists of multiplying the \( j \)-th smallest \( p \) value by \( k - j + 1 \). \( p \) values < 0.05 were regarded as significant.

**RESULTS**

The five subject groups were matched for age. Although the three COPD groups were matched for the number of pack years, smokers with stage II–IV COPD had a significantly greater pack year history than healthy smokers (table 1). The TLCO was significantly reduced in all three COPD groups compared with non-smokers and in patients with stage II–IV COPD compared with healthy smokers also. KCO measurements were lower in all smoking groups than in non-smokers but did not differ significantly between smoking groups. Using the Jonckheere-Terpstra test, a significant trend was observed for the TLco, but not the Kco, to fall with advancing disease stage (p < 0.001, table 1).

**Appraisal of HRCT scans by radiologists**

None of the healthy non-smokers and, by definition, the healthy smokers had emphysema on HRCT scans as assessed by the radiologists. In contrast, 11/17 subjects with stage 0 COPD, 7/10 subjects with stage 1 COPD, and 11/17 with stage II–IV COPD were identified as having HRCT evidence of emphysema. The amount of emphysema in all 11 cases of stage 0 COPD was described as minimal (<5% of total lung area) while in stage I and II–IV disease the extent of emphysema ranged from minimal to severe (>30% of total lung area).

**Computerised density mask analysis of HRCT**

The comparison of %LAA and MLD by ANOVA (allowing for multiple comparisons) showed significant differences between the groups only when comparing expiratory HRCT measurements. Post hoc analysis using the Scheffe test showed no differences between non-smokers and any of the smoker groups for expiratory HRCT measurement (%LAA and MLD; table 1), while the E/I ratio was significantly higher in patients with stage II–IV COPD than in non-smokers, healthy smokers, and those with stage 0 COPD (table 1 and fig 1). Further comparison of %LAA on expiration showed a significant increase in both stages I and II–IV COPD compared with both healthy smokers and patients with stage 0 COPD (table 1). Moreover, MLD on expiration was significantly reduced in patients with stage II–IV COPD compared with healthy smokers and those with stage 0 COPD, and in stage I COPD compared with healthy smokers (table 1).

When smoking related trends in HRCT densitometry were analysed in the four smoking groups in isolation using the Jonckheere-Terpstra test, significant trends were observed for %LAA to rise and MLD to fall with advancing disease stage on both inspiration and expiration (p < 0.001 in all cases). There was also a significant trend for the E/I ratio to rise with advancing disease stage (p < 0.001).

**Correlations between HRCT and lung function**

Lung function correlated moderately strongly with HRCT parameters (table 2). The strongest correlations seen in smokers were between the E/I ratio on the one hand and airway obstruction (FEV1 % predicted; fig 2), mid expiratory flow (MEF50), and gas trapping (RV/TLC) on the other, with no correlation being seen with TLco or Kco. Strong and highly significant correlations were also observed between MLD and FEV1, MEF50, and RV/TLC but not either TLco or Kco. These correlations were stronger on expiration than inspiration. Both expiratory and inspiratory %LAA correlated weakly to moderately strongly with FEV1 and RV/TLC but also, albeit weakly, with TLco but not at all with Kco. These correlations were only marginally stronger on expiration.

**Assessment of airway inflammation**

No significant differences were observed between any of the groups with regard to the total numbers of cells in induced sputum. The only cell counts that were different between the groups were the relative counts of neutrophils and macrophages. Relative neutrophil counts were significantly higher in stage II–IV COPD than in all other groups (table 3). The relative macrophage counts followed a reciprocal pattern to the neutrophils, being significantly reduced in stage II–IV COPD compared with all the other groups. Despite the absence of significantly increased absolute neutrophil counts, significant trends were observed using the Jonckheere-Terpstra tests for both relative (p = 0.001) and absolute (p = 0.007) neutrophil counts to rise with advancing disease stage (table 3).

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**Table 2** Correlation in smokers between HRCT measurements performed on inspiration and expiration and lung function

<table>
<thead>
<tr>
<th>Functional variable</th>
<th>Correlation coefficient (r)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expiratory %LAA</td>
</tr>
<tr>
<td>FEV1 (%)</td>
<td>−0.52</td>
</tr>
<tr>
<td>RV/TLC (%)</td>
<td>0.47 (p &lt; 0.01)</td>
</tr>
<tr>
<td>MEF50 (%)</td>
<td>−0.51</td>
</tr>
<tr>
<td>TLco (%)</td>
<td>−0.42 (p &lt; 0.01)</td>
</tr>
<tr>
<td>Kco (%)</td>
<td>0.10 (p &lt; 0.05)</td>
</tr>
</tbody>
</table>

LAA, area of lung attenuation; MLD, mean lung density; FEV1, forced expiratory volume in 1 second; RV, residual volume; TLC, total lung capacity; MEF50, mid expiratory flow; TLco, carbon monoxide transfer factor; Kco, carbon monoxide transfer coefficient.

p < 0.001 unless otherwise stated.
Relative neutrophil counts correlated significantly with measurements of airway obstruction and gas trapping (FEV₁ % predicted ($r = -0.54$, $p < 0.001$), FEV₁/FVC ratio ($r = -0.52$, $p < 0.001$), RV/TLC ($r = 0.56$, $p < 0.001$)) and with the E/I ratio ($r = 0.55$, $p < 0.001$; fig 3). Relative neutrophil counts correlated more strongly with expiratory MLD ($r = -0.59$, $p < 0.001$) than with inspiratory MLD ($r = -0.42$, $p < 0.005$). No significant correlations were observed between relative neutrophil counts and either inspiratory %LAA or TLCO % predicted. Absolute sputum neutrophil counts correlated only weakly with the FEV₁/FVC ratio ($r = -0.36$, $p < 0.001$) and with the RV/TLC ($r = 0.3$, $p < 0.05$) but not with any other lung function or HRCT parameter. Like relative neutrophil counts, no significant correlations were observed between absolute neutrophil counts and either inspiratory %LAA or TLCO % predicted.

**DISCUSSION**

We have investigated the relationship in COPD between proximal airway inflammation, as judged by induced sputum, and quantitative HRCT measurements of peripheral airway dysfunction and emphysema. The study shows that peripheral airway dysfunction is significantly associated with airflow limitation in mild and moderate COPD, although it does not establish whether this occurs due to small airway remodelling or loss of elastic recoil caused by emphysema. As shown previously, sputum neutrophil counts were significantly related to COPD severity. However, the results show for the first time that neutrophil counts are significantly associated with HRCT indices of peripheral airways dysfunction but not with the severity of emphysema.

HRCT is a useful non-invasive tool to assess emphysema. Its interpretation depends on whether scans are taken on inspiration or expiration. Thus, %LAA correlates better with emphysematous destruction of the lungs on inspiration while both MLD and %LAA correlate more closely with functional impairment (airflow obstruction and gas transfer) when analysed during expiration and CT scanning on expiration differentiates better between COPD subjects and healthy controls. Calculation of the MLD E/I ratio allows indirect evaluation of gas trapping and small airway dysfunction. These differences have been explained by the fact that expiratory, but not inspiratory, CT scanning also reflects peripheral airway dysfunction that causes air trapping. By contributing to both airway obstruction and gas trapping, peripheral airway dysfunction is believed to provide an explanation for the stronger correlation between spirometric and expiratory HRCT parameters.

The current study shows that both expiratory HRCT measurements and the MLD E/I ratio correlate more closely with lung function impairment and are more effective in distinguishing smokers with and without airflow obstruction than either inspiratory HRCT, TLCO or KCO. Although the

**Table 3** Relative (percentage of non-squamous cells) and absolute (total number of cells x 10⁶ expectorated over 20 minutes) neutrophil and macrophage counts in induced sputum

<table>
<thead>
<tr>
<th></th>
<th>Non-smokers</th>
<th>Healthy smokers</th>
<th>Stage 0 COPD</th>
<th>Stage I COPD</th>
<th>Stage II–IV COPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total inflammatory cells</td>
<td>1.6 (0.9–6)</td>
<td>1.65 (0.8–24.7)</td>
<td>2.0 (0.95–7.5)</td>
<td>2.8 (1.2–5.1)</td>
<td>2.6 (1.3–10.6)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>37.6 (16.1–47.6)</td>
<td>46.0 (30.7–67.9)</td>
<td>50.9 (25.1–66.9)</td>
<td>53.9 (26.9–70.7)</td>
<td>67.8 (44.6–78.1)</td>
</tr>
<tr>
<td>Neutrophils (x 10⁶)</td>
<td>776 (103–2774)</td>
<td>546 (217–4358)</td>
<td>830 (233–3692)</td>
<td>1387 (482–1986)</td>
<td>1416 (789–6485)</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>55.1 (41.9–75.1)</td>
<td>49.5 (24.5–68.9)</td>
<td>42.4 (30–70.4)</td>
<td>43.4 (17.6–59.2)</td>
<td>27.5 (10.1–41.5)</td>
</tr>
<tr>
<td>Macrophages (x 10⁶)</td>
<td>618 (335–3330)</td>
<td>513 (91–4195)</td>
<td>871 (298–2840)</td>
<td>806 (298–2646)</td>
<td>722 (203–1941)</td>
</tr>
</tbody>
</table>

Data are expressed as median (range).

*p < 0.05 vs all other groups.

![Figure 3](http://thorax.bmj.com/)

**Figure 3** Relationship between relative sputum neutrophil counts and lung function measurements of (A) airflow obstruction (FEV₁ % predicted: $r = -0.54$, $p < 0.001$), (B) gas trapping (RV/TLC: $r = 0.56$, $p < 0.001$) and (C) the expiratory/inspiratory (E/I) ratio ($r = 0.54$, $p < 0.001$).
TLC0, which may be affected by peripheral airways obstruction, displayed a significant downward trend with advancing disease stage and was associated with %LAA, the Kco, which is more closely related to diffusion impairment, was statistically similar in smokers with and without COPD. The results support the view that small airway dysfunction is a major determinant of reduced lung function in mild to moderate COPD, and a greater predictor of functional impairment than the percentage volume of emphysema as measured by inspiratory %LAA in the current patients.

Whether peripheral airways dysfunction is principally determined by emphysematous destruction of the lung or small airways remodelling cannot be determined by HRCT scanning because it is not as sensitive as histological analysis of lung tissue and may not be sensitive enough to detect early or mild emphysematous changes that can be seen by pathological examination. Even histological assessment has limitations; peripheral airway instability and loss of elastic recoil linked to destruction of alveolar attachments may be present in smokers without emphysema being detected even by pathological examination. Thus, subjects with significant airflow limitation may have more parenchymal destruction than can be appreciated by HRCT but which, nonetheless, is contributing to peripheral airway dysfunction.

An important objective of this study was to relate sputum inflammatory cells to airflow limitation and the HRCT indicators of air trapping and emphysema. The finding of raised relative sputum neutrophil counts in COPD is consistent with other studies. Importantly, the counts correlated strongly with measurements of peripheral airway obstruction and gas trapping (RV/TLC ratio), MLD on expiration and the E/I ratio but, interestingly, no correlation was observed with either inspiratory %LAA or TLC0, both indicators of emphysema. Only subjects with moderate to severe COPD had significantly raised neutrophil counts. In keeping with these results, it has previously been shown that, although smokers with very severe emphysema have prominent neutrophilic inflammation in the alveolar walls and air spaces, smokers with mild emphysema without airflow obstruction have neither BAL nor alveolar neutrophilia. Our results therefore suggest that relative sputum neutrophil counts in smokers are of much greater clinical relevance as an indicator of peripheral airways dysfunction than of emphysema detected by HRCT scanning. We speculate that increased relative sputum neutrophil counts reflect the more distal neutrophilic inflammation that is associated with bronchiolitis and/or destruction of peripheral airway alveolar attachments. To what degree, however, the results may be affected by chronic bacterial colonisation of the airways is uncertain. Although the study only included subjects without either a history of lower respiratory tract infection or evidence of bacterial infection as determined by microbiological culture, undetected bacterial airway colonisation in some smokers remains an important potential influence on neutrophil counts. Finally, when neutrophilic inflammation was assessed in terms of absolute counts, the differences between COPD and healthy subjects did not achieve significance although there was also a significant trend for counts to increase with advancing disease stage. This observation is in contrast to previous studies. The counts correlated only weakly with airflow obstruction and not at all with any other lung function or HRCT parameter, suggesting that they are of limited value as indicators of COPD severity.

In conclusion, this study provides evidence that peripheral airways dysfunction is a key determinant of COPD severity and that airway lumen neutrophilia is associated with this dysfunction but is not associated with the overall extent of emphysema as determined by quantitative HRCT. These findings suggest that the study of proximal airway secretions is of limited value in identifying the pathological determinants of emphysema severity.

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REFERENCES


No difference in mortality between low and high PEEP

Positive end expiratory pressure (PEEP) during ventilation improves arterial oxygenation by recruiting alveoli, but it may cause circulatory depression and increase airway pressure and lung volumes resulting in ventilator induced lung injury from over-distension.

In acute lung injury and acute respiratory distress syndrome (ARDS), mechanical ventilation is critical for the survival of the majority of patients. Most patients with acute lung injury and ARDS have been treated with PEEP values of 5–12 cm H\(_2\)O. In this study, 549 patients with acute lung injury and ARDS were randomised to receive either high PEEP (13.2 (3.5) cm H\(_2\)O) or lower PEEP (8.3 (3.2) cm H\(_2\)O). All patients received a tidal volume goal of 6 ml/kg predicted body weight and an inspiratory plateau pressure of 30 cm H\(_2\)O or less. Blood samples reflecting mechanisms of lung inflammation and injury (plasma interleukin-6, surfactant protein D, and intercellular adhesion molecule 1) were obtained before randomisation (day 0) and on day 3. The mortality rate from any cause was 24.9% in the lower PEEP group and 27.5% in the higher PEEP group. There were no changes in plasma levels of biological markers of inflammation and lung injury in either group.

The authors conclude that, in patients with acute lung injury and ARDS who receive mechanical ventilation with a tidal volume goal of 6 ml/kg predicted body weight and an end inspiratory plateau pressure limit of 30 cm H\(_2\)O, clinical outcomes are similar whether lower or higher PEEP levels are used.

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