Differences in regional air trapping in current smokers with normal spirometry

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ABSTRACT   We investigated regional air trapping on computed tomography in current smokers with normal spirometry. It was hypothesised that presence of regional air trapping may indicate a specific manifestation of smoking-related changes.

40 current smokers, 40 patients with chronic obstructive pulmonary disease (COPD), and 40 healthy never- smokers underwent computed tomography scans. Regional air trapping was assessed on end-expiratory scans and emphysema, micronodules and bronchial wall thickening on inspiratory scans. The ratio of expiratory and inspiratory mean lung attenuation (E/I) was calculated as a measure of static (fixed) air trapping.

Regional air trapping was present in 63% of current smokers, in 45% of never smokers and in 8% of COPD patients (p<0.001). Current smokers with and without regional air trapping had E/I ratio of 0.81 and 0.91, respectively (p<0.001). Forced expiratory volume in 1 s (FEV_1) was significantly higher and emphysema less frequent in current smokers with regional air trapping.

Current smokers with regional air trapping had higher FEV_1 and less emphysema on computed tomography. In contrast, current smokers without regional air trapping resembled COPD. Our results highlight heterogeneity among smokers with normal spirometry and may contribute to early detection of smoking related structural changes in the lungs.

Introduction

Repetitive exposure to cigarette smoke causes epithelial damage and an inflammatory response, which gradually leads to structural changes in the airways and lung parenchyma. These structural changes, "remodelling", are the hallmarks of chronic obstructive pulmonary disease (COPD). It is now generally accepted that the small airways with diameter less than 2 mm are the primary area for early obstruction [1, 2]. Small airways inflammation is considered to precede tissue destruction and development of emphysema [3]. Due to increased branching of the airways and subsequent expansion of the total cross-sectional lumen area, the small airways account for only 10–15% of the total airway resistance [4]. This area is sometimes referred as a "silent zone" because the disease cannot yet be measured by spirometry in an early stage and the patients do not experience significant clinical symptoms until substantial damage to the lungs has occurred [5].

The employment of computed tomography offers opportunity for early evaluation of subclinical, smoking-related pathological changes in the airways and lung parenchyma [6–8]. These early changes, distinguishable on inspiratory computed tomography scans as "tree in bud" and micronodules, gradually progress and lead to detachment of alveolar walls from the respiratory bronchiole to develop centrilobular emphysema [9]. Regional air trapping due to retention of air in the distal part of the respiratory tract appears, on expiratory computed tomography scans, as low attenuation patchy areas, which is regarded as a surrogate for small airways disease [10–12].

Quantification of pulmonary emphysema on computed tomography as volume of voxels below the threshold of –950 Hounsfield units is well established [13, 14]. The ratio of mean lung attenuation on paired expiratory and inspiratory scans has been considered to quantify air trapping and has been proposed as an indicator for small airways disease [15–17]. In COPD and asthma, the ratio has been shown to be increased [18, 19]. However, since the attenuation-based measurements are influenced by both hyperinflation and airways obstruction, visual assessment of regional air trapping on expiratory computed tomography scans may indicate a different finding [10].

This study was undertaken to compare visual assessments of early lungs lesions on computed tomography, with quantitative measurements and to signs of local and systemic inflammation. We hypothesised that a combination of quantitative lung attenuation measurements and visual assessment of morphological abnormality, i.e. regional air trapping on expiratory computed tomography scans and presence of micronodules, emphysema and bronchial wall thickening on inspiratory computed tomography, would reveal early pulmonary changes in smokers with normal spirometry. Furthermore, we performed bronchoscopy with bronchoalveolar lavage in order to characterise the inflammatory response in the distal airways, to reveal a potential link between imaging and smoking induced pathobiology.

Materials and methods

Subjects

The study was conducted as a part of the Karolinska COSMIC (COPD Smoking Proteomic) study, ClinicalTrials.gov identifier NCT02627872, [8, 20, 21]. Briefly, 45–65-year-old healthy never-smokers (n=40), Current smokers (hereafter referred as smokers) with normal spirometry (n=40) and COPD patients, Global Initiative for Chronic Obstructive Lung (GOLD) I and II (n=40) were included. Detailed demographic characteristics of the investigated groups have been published previously [8, 20] and a summary is shown in table 1. Of the COPD patients, 28 were current smokers and 12 were ex-smokers (smoking cessation >2 years). A medical examination and a standard chest radiograph were performed. Subjects with any significant medical condition, including asthma and allergy were not included. Inhaled or oral corticosteroids and exacerbation or airways infection during at least 3 months prior to inclusion were not allowed.

Lung function

Forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC) were measured 20 min after bronchodilatation with two inhalations of 0.5 mg terbutalin (Bricanyl Turbuhaler; AstraZeneca, Södertälje, Sweden) using a spirometer (Vmax 229–6200 Legacy, Viasys, Yorba Linda, CA, USA). Total lung capacity and residual volume were measured by body plethysmography. Transfer factor of the lungs for carbon monoxide (T_{LCO}) was measured by the single-breath method. Lung function data, including standardised residuals, i.e. Z-scores are presented in tables 1 and 2. Lower limits of normal (LLN) for FEV_1/FVC, FVC and FEV_1 were calculated, using GLI-2012 references [22] and the equations of the European Community of Coal and Steel (ECCS) [23] were used calculating static lung volumes and T_{LCO}. All never smokers and smokers with normal spirometry had FEV_1/FVC >LLN. Two females and five males with COPD, had FEV_1/FVC >LLN. However, the ratio of FEV_1/FVC was <0.70 which was the inclusion criteria for the study. The other COPD patients had FEV_1/FVC <LLN.

TABLE 1 Demographic, lung function, quantitative and morphological computed tomography measurements for never smokers, smokers and COPD patients

	Never smokers	Smokers	COPD patients
Subjects n	40	40	40
Demographics and lung function			
Male/female %	50/50	50/50	50/50
Age years	57.0±7.0	54.0±6.2	59.2±5.3[+]
Body mass index kg·m^{-2}	25.9±3.7	24.3±3.1	25.3±4.1
Smoking history pack-years	NA	35.2±12	38.0±11
Current smoking	NA	17.8±7	16.4±6[¶]
FEV$_1$ % predicted	118±13	109±12[##]	79±12[§§§,+++]
FEV$_1$ Z-score	0.9 (−1.3–2.5)	0.2 (−1.1–1.9)[###]	−1.7 (−3.5−−0.5)[§§§,+++]
FEV$_1$/FVC	0.82	0.78	0.61[§§§,+++]
FEV$_1$/FVC Z-score	0.3 (−1.5–2.6)	−0.1 (−1.3–1.4)[##]	−2.3 (−4,2−−0.8)[§§§,+++]
TLC % predicted	106±10.7	107±11.1	108±14.6
TLC Z-score	0.4 (−1.0–2.3)	0.4± (−1.5–1.7)	0.5 (−1.4–2.4)
RV % predicted	102±25	113±23	138±34[+++,§§§]
RV Z-score	0.1 (1.0–2.6)[+++,§§§]	0.8 (−1.4–2.4)	0.8 (−0.7–2.7)
T_{LCO} % predicted	91±11	78±12[###]	67±13[+++,§§§]
T_{LCO} Z-score	−0.6 (−2.2–1.4)[+++,###]	−1.6 (−4.6–0.37)	−2.3 (−4.0–0.7)[+++,§§§]
Quantitative computed tomography			
Mean lung attenuation at expiration HU	−776±40	−756±45[#]	−809±39[+++,§§§]
Mean lung attenuation at inspiration HU	−874±20	−857±28[###,+++]	−876±17
E/I ratio	0.89±0.04	0.88±0.05	0.93±0.05[+++,§§§]
LA-950 at inspiration	27.4±6.2	21.3±6.6[###,+++]	27.6±6.8
Morphological (computed tomography)			
Emphysema	0 (0)	22 (55)[###]	32 (80)[§§§]
Regional air-trapping	18 (45)	25 (63)	3 (8)[+++,§§§]
Bronchial wall thickening	5 (12)	30 (75)[###]	32 (80)[§§§]
Nodules larger than 3 mm	15 (38)	21 (52)	23 (58)
Micronodules	0	23 (58)[###]	15 (38)[+,§§§]

Data are presented as mean±SD, mean (range) or n (%), unless otherwise stated. COPD: chronic obstructive pulmonary disease; FEV$_1$: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; T_{LCO}: transfer factor of the lung for carbon monoxide; HU: Hounsfield units; LA-950: percentage of lung volume with attenuation <−950 HU. Current smoking is defined as the number of cigarettes per day for the last 6 months and E/I ratio is the ratio between mean lung attenuation at expiration and inspiration. [¶]: only current smokers COPD; [#]: $p \leqslant 0.05$, [##]: $p \leqslant 0.01$ and [###]: $p \leqslant 0.001$ for comparison between never-smokers and smokers; [+]: $p \leqslant 0.05$, [++]: $p \leqslant 0.01$ and [+++]: $p \leqslant 0.001$ for comparison between smokers and COPD; and [§]: $p \leqslant 0.05$, [§§]: $p \leqslant 0.01$ and [§§§]: $p \leqslant 0.001$ for comparison between COPD and never smokers.

Computed tomography imaging

Multi-detector helical computed tomography examinations, without any contrast medium, were performed using a Siemens Somatom Sensation 16 slice CT (Siemens, Erlangen, Germany). Scans were performed in a supine position and covered the entire thorax. The scanning was performed with 16×0.75 mm collimation, 120 kV, rotation time 0.5 s and pitch 1.5. Dose modulation was used resulting in a mean of approximately 180 mA. Subjects were then guided to hold their breath at end-expiration and sequential scans were obtained with 10 mm intervals. Axial images with 1 mm thickness and 0.5 mm overlap were reconstructed with a medium-high spatial resolution algorithm (B60f) in a 512×512 matrix. The end-expiratory scans were reconstructed as single slices in a similar way.

Quantitative image analysis

As previously described [8], computed tomography images were analysed using a semiautomatic programme (Volume, Siemens, Erlangen Germany). Attenuation values between −300 and −1024 HU were considered to isolate the lung parenchyma from surrounding and mediastinal structures. Mean lung attenuation and lung volume were measured automatically on both expiratory and inspiratory scans. The ratio of expiratory and inspiratory mean lung attenuation was calculated and designated "E/I" [17, 24]. The volumes of pixels with lower attenuation than −950 HU were also calculated on inspiratory scans [13].

Morphological image analysis

All scans were assessed independently and blinded in random order by two experienced reviewers (S. Nyren and R. Karimi). In a few occasions, the final interpretations were achieved by consensus. Regional air trapping typically appears on end-expiratory scans as patchy areas with lower attenuation than the

TABLE 2 Comparison between two subgroups of smokers with and without regional air trapping on end-expiratory computed tomography

	Regional air trapping on end-expiratory scans		COPD current smokers
	Yes	No	
Subjects n	25	15	28
Demographics and lung function			
Male/female %	44/56	60/40	54/46
Age years	53±7.1	54±4.3	59±5.0[+++]
Body mass index kg·m^{-2}	25±2.7	24±3.8	24±3.8
Smoking history pack-years	33.2±11	38.5±14	40.9±10[+]
Current smoking	17.8±7	17.9±7	16.4±6
FEV_1 % predicted	113±10	105±14[##]	78±11[+++,§§§]
FEV_1 Z-score	0.4 (−1.1–1.9)	−0.1 (−1.1–1.7)[#]	−1.8 (−3.5−−0.6)[+++,§§§]
FEV_1/FVC	0.80	0.75[###]	0.61[+++,§§§]
FEV_1/FVC Z-score	0.1 (−1.0–1.4)	−0.5 (−1.3–1.1)[###]	−2.3 (−4.2−−0.8)[+++,§§§]
TLC % predicted	107±11.5	107±10.8	108±15.8
TLC Z-score	0.4 (−0.6–1.7)	0.4 (−1.5–1.5)	0.5 (−1.4–2.4)
RV % predicted	108±26	121±13	137±36[+++]
RV Z-score	0.7 (−0.3–2.9)	1.1 (−0.1–2.1)	0.8 (−0.7–2.7)
T_{LCO} % predicted	79±11	76±14	65±12[+++,§]
T_{LCO} Z-score	−1.5 (−2.7–0.4)	−1.7 (−4.6−−0.0)	−2.4 (−4.0–0.7)[+++]
Qualitative computed tomography			
E/I ratio	0.86±0.05	0.90±0.06[###]	0.93±0.03[+++]
Mean lung attenuation at expiration HU	−736±41	−787±33[###]	−806±34[+++]
Mean lung attenuation at inspiration HU	−852±31	−866±17	−871±16[++]
LA-950 at inspiration	20.0±7.1	23.6±5.2	25.2±5.2[++]
Morphological (computed tomography)			
Emphysema	11 (44)	12 (80)[#]	23 (82)[++]
Bronchial wall thickening	17 (68)	13 (86)	22 (79)
Micronodules	14 (56)	9 (60)	13 (46)

Data are presented as mean±SD, mean (range) or n (%), unless otherwise stated. COPD: chronic obstructive pulmonary disease; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; RV: residual volume; T_{LCO}: transfer factor of the lung for carbon monoxide; HU: Hounsfield units. Current smoking is defined as the number of cigarettes per day for the last 6 months and E/I ratio is the ratio between mean lung attenuation at expiration and inspiration. [#]: $p \leqslant 0.05$, [##]: $p \leqslant 0.01$ and [###]: $p \leqslant 0.001$ for comparison between smokers with regional air trapping and smokers without regional air trapping; [+]: $p \leqslant 0.05$, [++]: $p \leqslant 0.01$ and [+++]: $p \leqslant 0.001$ for comparison between COPD and smokers with regional air trapping; and [§]: $p \leqslant 0.05$ and [§§§]: $p \leqslant 0.001$ for comparison between COPD and smokers without regional air trapping.

surrounding tissues. In the current study, regional air trapping was considered present when at least three secondary lobules were involved (figure 1). Emphysema was evaluated on inspiratory scans dichotomously and considered to be present when >5% of the whole lung parenchyma was involved. Bronchial wall thickening was considered to be present when both central bronchi and peripheral wall thickening were present simultaneously. Micronodules were identified as ill-defined small (2–3 mm) parenchymal ground glass attenuation areas distinguishable on inspiratory CT scans (figure 2).
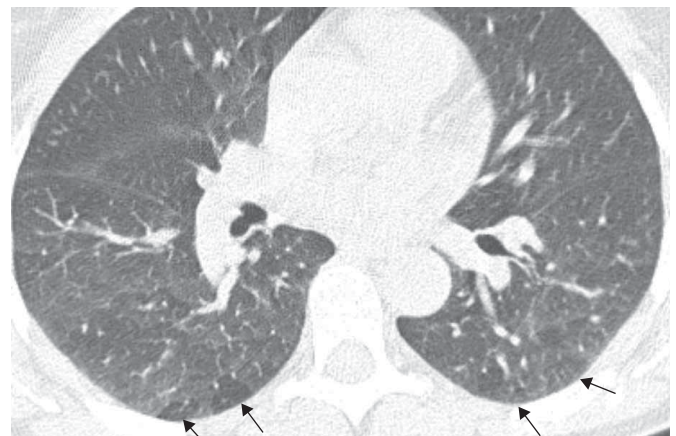

FIGURE 1 Expiratory thin-section computed tomography in a 50-year-old male smoker with normal lung function, demonstrating regional air trapping as patchy area of low attenuation located mainly at dependent area of the right and left lower lobes (arrows).

FIGURE 2 Axial inspiratory thin-section computed tomography of a 48-year-old female smoker with normal spirometry. At the level of the upper lobes micronodules can be visualised as indistinct and ill-defined ground glass attenuation. These are more abundant at the anterior and central part of left upper lobe. Bronchial wall thickening is also present.
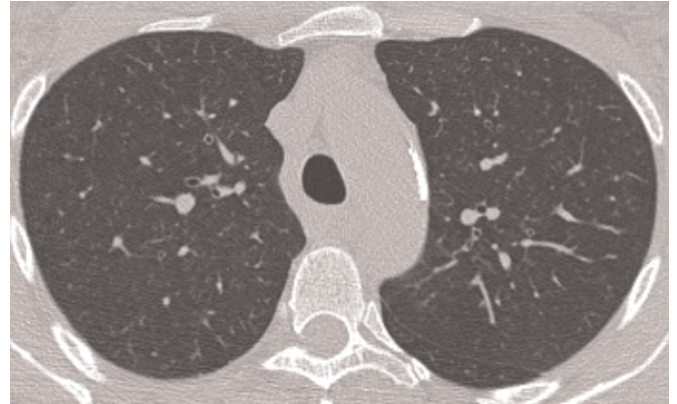
Bronchoscopy and bronchoalveolar lavage

Bronchoscopy with bronchoalveolar lavage (BAL) was performed in order to obtain cells from the distal airways. Bronchoscopy procedure and preparation of BAL cells were performed according to a standard procedure in our department, described in detail previously [25]. Immunofluorescent surface staining and flow cytometry were used to characterise the major lymphocyte subsets described in detail in previous study [21].

Statistical analysis

Unpaired two-tailed t-tests were employed comparing normally distributed data between two groups. One way ANOVA with Bonferroni adjustment for multiple comparisons was utilised for univariate statistical testing between groups. The values were expressed as mean±SD. Weighted kappa scores and percentage of agreement for each computed tomography morphological finding were calculated between the two reviewers. Univariate linear regression was employed to evaluate relationship between E/I with lung function, age, body mass index (BMI) and smoking. Binary variables were analysed with logistic regression model. Statistical significances were defined as p-values <0.05. All statistical analysis were performed using Stata12 software (Stata Corp, College Station, TX, USA).

Results

Quantitative findings on computed tomography

Quantitative computed tomography indices are shown in table 1. Mean lung attenuation at expiration was significantly higher for smokers compared with never smokers. The mean±SD ratio of expiratory and inspiratory mean lung attenuation "static air trapping" (E/I), was significantly lower in smokers (0.88 ±0.05) and never smokers (0.89±0.04), compared with COPD patients (0.93±0.05) (p<0.001 for both compared with COPD), (figure 3). Percentage of lung volume at inspiration with voxels lower than
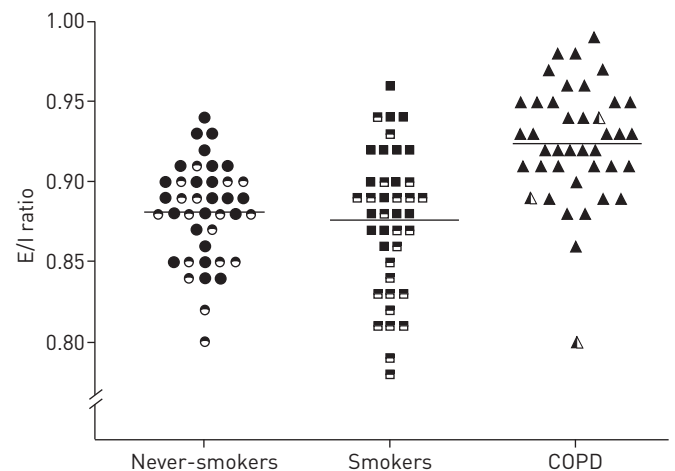

FIGURE 3 Ratio between mean lung attenuation at expiration and inspiration (E/I ratio) in never smokers, smokers and chronic obstructive pulmonary disease (COPD) patients. Half-filled symbols represent individuals with regional air trapping and filled symbols indicate individuals without regional air trapping. Note that smokers seems to be divided in two different subgroups based on the presence of regional air trapping while the never smokers are mixed homogenously.

−950 HU was significantly lower in smokers compared with both the never smokers and the COPD patients (p<0.001 for both). When the COPD patients were divided into current and ex-smokers, the latter group had higher percentage of lung volume at inspiration with voxels lower than −950 HU (33.2±6.2 versus 25.2±5.2) (p<0.001). There were no sex differences (data not shown).

Morphological findings on computed tomography

Morphological findings are illustrated in table 1. Regional air trapping was present in 63% of the smokers, in 45% of the never smokers and in 8% of the COPD patients (p<0.001 for both compared to COPD). The difference between smokers and never smokers was not statistically significant. Emphysema was not present in the never smokers but in 55% of the smokers and in 80% of the COPD patients; (p<0.001 for both compared to never smokers). Bronchial wall thickening was found in 75% of the smokers, in 12% of the never smokers and in 80% of COPD patients, (p<0.001 for both compared to never smokers). Micronodules were detected in 58% of the smokers which was significantly higher than COPD patients (38%; p<0.05). Micronodules were absent in never smokers. Solid nodules larger than 3 mm were found in all three groups without any significant difference (table 1).

Inter-observer agreement

Agreement between the two reviewers and the kappa score for regional air trapping was 83% and 0.66 in smokers and 80% and 0.60 in never smokers, respectively. The numbers of COPD with regional air trapping were too low to admit statistical calculations. Agreement and kappa score for presence of emphysema in smokers was 93% and 0.85 and in COPD 97% and 0.89; for micronodules, in smokers it was 75% and 0.5 and in COPD 86% and 0.68; and for bronchial wall thickening, in smokers was 55% and 0.17 and in COPD 71% and 0.37.

Comparison between smokers with or without regional air trapping

Smokers were divided in two groups according to presence (n=25) or absence (n=15) of regional air trapping on end expiratory computed tomography scans. This division was based on the hypothesis that smokers with and without regional air trapping may differ in their inflammatory response and other smoking-related changes.

The two subgroups were similar regarding age, BMI, smoking history and current smoking status (table 2). Smokers without regional air trapping had significantly lower FEV_1 and FEV_1/FVC ratio (p<0.01 and p<0.001, respectively). They also had lower mean lung attenuation at expiration (p<0.001), a higher E/I ratio (0.90±0.060 versus 0.86±0.05; p<0.001), and more emphysema (80% versus 44%; p<0.05), compared with smokers with regional air trapping (figure 3 and table 2). Micronodules were found, in logistic regression model, more frequently in smokers with emphysema (odds ratio 6.8, 95% CI 1.7–27; p<0.01).

Regional air trapping in smokers: comparison with COPD

E/I ratio and the percentage of lung volume with attenuation <−950 HU (LA-950) at inspiration differed significantly between COPD and smokers with regional air trapping but not between COPD and smokers without regional air trapping (table 2). For morphological indices, emphysema was significantly more common in smokers without regional air trapping (82%) and in COPD (80%) compared with smokers with regional air trapping (44%).

Associations between BAL findings and computed tomography indices

The percentage of BAL fluid recovered was significantly (p<0.01) higher in smokers with regional air trapping (62.0±9.0%) compared both with smokers without regional air trapping (53±10%) and with COPD patients (45±12%), (table 3). The total cell yield in BAL was significantly higher in smokers with regional air trapping compared both to smokers without regional air trapping (p<0.01) and COPD (p<0.001). There were no differences regarding cell concentration and differential cell counts in BAL between the two smokers groups. The percentage of CD4^{+} T-lymphocytes was significantly lower (46±13% and 62±15%; p<0.01) and conversely, the percentage of CD8^{+} T-lymphocytes was significantly higher (45±13% and 29±15%; p<0.01) in smokers with bronchial wall thickening compared with those without (data not shown).

Correlations between static air trapping (E/I) and BAL findings

In a multivariate model, after correction for age, BMI and smoking, a negative correlation between E/I and neutrophil concentration in BAL in smokers was found (figure 4) (r=−0.47 and p<0.001). While mean lung attenuation at expiration was positively correlated to neutrophil concentration, (r=0.65 and p<0.001),

TABLE 3 Measurements of local and systemic inflammation on two subgroups of smokers, with and without regional air trapping, data on COPD current smokers are also presented

	Smokers with regional air trapping on expiratory scans		COPD current smokers
	Yes	No	
Subjects n	25	15	28
Male/female %	44/56	60/40	54/46
BAL recovery %	62.0±9.0	53±10[#]	45±12[+++]
Total cell yield ×10^6	89.2±48	66.6±27[#]	53.5±34[++]
BAL cell concentration 10^6·L^{-1}	578±303	519±164	493±255
BAL macrophages 10^6·L^{-1}	557±291	500±157	471±250
BAL lymphocytes 10^6·L^{-1}	14.0±11	14.3±13	13.1±9.0
BAL neutrophils 10^6·L^{-1}	5.3±6.2	3.5±3.1	4.7±4.1
BAL eosinophils 10^6·L^{-1}	1.5±3.5	1.4±2.4	3.5±9.7
Percentage of CD4$^+$ T-lymphocytes	54±17	44±18	50.0±22
Percentage of CD8$^+$ T-lymphocytes	37±16	48±18	43±23
Ratio of CD4$^+$ and CD8$^+$	2.2±2.5	1.5±2.1	2.1±2.3
White blood cell counts 10^9·L^{-1}	7.4±1.6	7.5±1.6	8.0±1.9
Serum high sensitive-CRP g·L^{-1}	2.1±2.1	1.6±1.0	3.1±3.0
Serum orosomucoid g·L^{-1}	0.83±0.20	0.71±0.12	3.8±4.0
Serum haptoglobin g·L^{-1}	1.3±0.53	1.0±0.40	1.5±0.45[§§]
Serum IgG g·L^{-1}	8.7±1.7	9.9±1.9[#]	9.4±1.7
Serum IgA g·L^{-1}	2.1±0.89	2.3±0.80	2.4±0.83

Data are presented as mean±SD, unless otherwise stated. COPD: chronic obstructive pulmonary disease; BAL: bronchoalveolar lavage; CRP: C-reactive protein. [#]: $p \leqslant 0.05$ for comparison between smokers with regional air trapping and smokers without; [++]: $p \leqslant 0.01$ and [+++]: $p \leqslant 0.001$ for comparison between COPD and smokers with regional air trapping; and [§§]: $p \leqslant 0.01$ for comparison between COPD and smokers without regional air trapping.

there were no correlations to any other cell types in BAL neither to inflammatory parameters in serum (data not shown).

Correlation between residual lung-volume on plethysmography with expiratory volume on computed tomography

Agreement between lung volume measured with computed tomography, at expiration, and residual volume measured with plethysmography is illustrated graphically in figure 5 for all subjects; correlation coefficient was 0.72 (p<0.001).
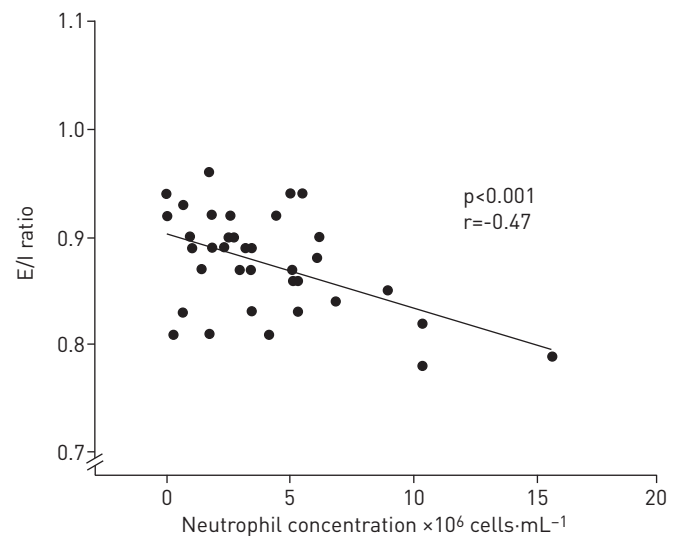

FIGURE 4 Correlation between neutrophil concentration (cells×10^6·L^{-1}) in broncho-alveolar lavage, and ratio between mean lung attenuation at expiration and inspiration (E/I-ratio; "static air trapping"), in smokers with normal spirometry.
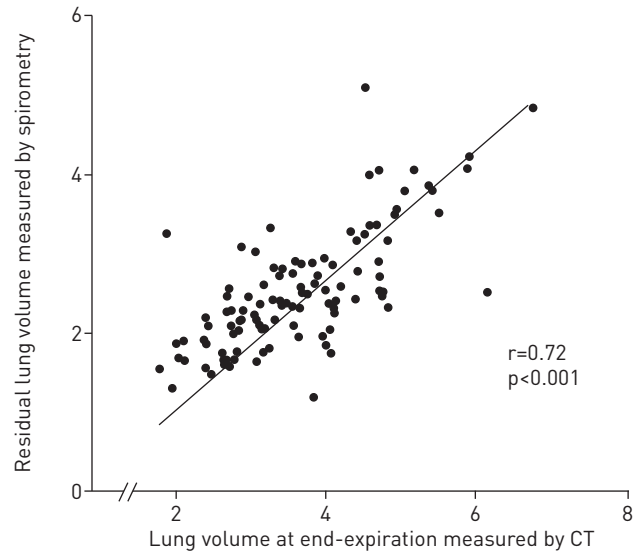
Comparison between COPD defined using fix ratio or LLN
Since seven of our COPD patients with FEV_1/FVC <0.7 had normal LLN, we also calculated our data excluding these individuals. The differences between our study groups did not change in any aspects whether we used the fixed ratio or LLN for definition of COPD in our material.

Discussion

In the present study, we demonstrate that morphological changes in smokers' lungs can be detected visually on inspiratory and end-expiratory computed tomography scans. We describe two populations of smokers, with and without regional air trapping, for whom the latter group had a lower FEV_1 and FEV_1/FVC, more emphysema, a lower BAL fluid recovery and a higher ratio of expiratory and inspiratory mean lung attenuation (E/I, "static air trapping").

Due to limited resolution of computed tomography to depict small airways with diameter less than 2 mm, some authors [6, 11, 12] have evaluated air trapping and micronodules on computed tomography as signs of small airways disease. Previous studies based on visual assessments of air trapping on expiratory computed tomography have demonstrated a negative correlation between presence of regional air trapping and lung function in asthma [11, 12, 26]. Recent studies have implemented several quantitative methods for evaluation of air trapping [10, 17]. We applied a frequently used method, the ratio between mean lung attenuation at expiration and inspiration (E/I) [10, 15, 17] combined with visual assessment of regional air trapping. Interestingly, our results show a reciprocal relation between regional air trapping and E/I, ("static air trapping") in smokers. Thus, the subgroup of smokers with regional air trapping had lower E/I, a higher FEV_1 and FEV_1/FVC, less emphysema and a higher BAL fluid recovery. The other smoking group, without regional air trapping, had higher E/I, resembling the COPD patients, with a reduction in FEV_1/FVC and FEV_1, lower BAL-fluid recovery.

The ratio between mean lung attenuation at expiration and inspiration (E/I), "static air trapping" is influenced by lung parenchymal destruction and airways obstruction; both jeopardising the subject's ability to expire properly. Smokers with a better preserved lung parenchyma should therefore be able to empty their lungs more properly; enhancing appearance of a typical patchy pattern of regional air trapping on end-expiratory computed tomography. Higher lung density on expiratory computed tomography, less emphysema, less airways obstruction and higher BAL fluid recovery in this group of smokers, despite of equal smoking burden, age and BMI, are findings indicating better preserved lung parenchyma [27]. They might respond to cigarette smoke in a different way, or they are in an earlier stage of disease development, *i.e.* predominantly small airways disease prior to parenchymal destruction [9]. Regional air trapping was present only in three COPD patients, which indicates a widespread presence of static air trapping due to emphysema and airways obstruction in these patients.

Regional air trapping was detected in 45% of the healthy never smokers which is in line with previous studies [28, 29]. Air trapping in the healthy never smoking lungs might be a physiological phenomenon related to ventilation and perfusion and should be an area for future research. Urban air pollutions may also trigger subtle small airways inflammation, without obvious clinical significance. Nevertheless, regional

air trapping in never smokers in the present study was an isolated occurrence without any correlation to any other measured variables.

Neutrophils and macrophages are the first line of recruited cells to the airways and are abundant in BAL fluid or surgical resected tissue obtained from smokers [1, 25, 30]. Due to the ability of neutrophils to produce elastase and other proteinases with tissue destructing potentials, neutrophils are considered as the major culprit cells causing emphysema [1, 31]. However, some data suggest a preservation of alveolar walls in the presence of neutrophils rather than pure destruction [32]. Our results show a weak but statistically significant negative correlation between E/I and BAL-neutrophils which may indicate that smokers with regional air trapping have more neutrophils in their lungs and can, simultaneously, resist parenchymal destruction. Presence of a less aggressive neutrophil phenotype and better ability to tissue repair in this group may be a possible explanation. However, this association should be interpreted with caution since it is only seen in smokers with normal spirometry and relies on a few subjects with higher neutrophil concentration.

REMY-JARDIN et al. [6] described micronodules in 27% and emphysema in 20% of smokers. These proportions are lower than in our study. Higher prevalence of micronodules and emphysema in the present study might be explained by a higher age and smoking burden. In a longitudinal follow up of the same smokers, the authors found that micronodules were replaced by emphysema [33]. These micronodules, likely representing inflammatory lesions, may thus be a precursor for discrete centrilobular emphysema. Interestingly, the number of subjects with micronodules was much lower in COPD than in smokers, suggesting that the micronodules may have been replaced by emphysema at this stage.

This study has some limitations. The inspiratory and expiratory computed tomography scans were not spirometry gated which may have influenced our measurements. However, we found a correlation of 0.72 between residual-volume measured with plethysmography and lung volumes measured with expiratory computed tomography which indicates an adequate level of expiration (figure 5). Furthermore, we did not use a scoring system for morphological changes that allows assessing degree and severity of air trapping and emphysema. However, we deliberately avoided a more complicated scoring system to reduce the impact of inter-observer variability and excluded trivial air trapping and emphysema.

Using quantitative and morphological computed tomography and BAL, we demonstrate that smokers with normal spirometry can be divided into two different groups in an early preclinical stage. Smokers with regional air trapping had predominantly neutrophilic inflammation and a higher FEV_1 and FEV_1/FVC with less emphysema and lower E/I. These smokers might be less susceptible to parenchymal destruction or investigated at an earlier stage of smoking-related lung injury. Our results highlight heterogeneity among smokers with normal lung function and may be of help for early detection of smoking related structural changes in the lungs.

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