ORIGINAL ARTICLE

Structure–function relationship in COPD revisited: an in vivo microscopy view

Borja G Cosío,[1,2] Hanaa Shafiek,[1,3] Federico Fiorentino,[1] Cristina Gómez,[4] Meritxell López,[1] Angel Rios,[1] Ana Kersul,[1] Bernardo Togores,[1] Joan Palmer,[5] Jaume Sauleda,[1,2] Alvar Agustí[2,6]

▶ Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/thoraxjnl-2013-204479).

[1]Department of Respiratory Medicine, Hospital Universitario Son Espases and Instituto de Investigación Sanitaria de Palma (IdISPa), Palma de Mallorca, Spain
[2]Ciber de Enfermedades Respiratorias (Ciberes), Bunyola, Spain
[3]Faculty of Medicine, Chest Diseases Department, Alexandria University, Alexandria, Egypt
[4]Department of Pathology, Hospital Universitario Son Espases, Palma de Mallorca, Spain
[5]Department of Radiology, Hospital Universitario Son Espases, Palma de Mallorca, Spain
[6]Thorax Institute, Hospital Clinic, IDIBAPS, Univ Barcelona and FISIB, Barcelona, Spain

Correspondence to
Dr Borja G Cosio, Department of Respiratory Medicine, Hospital Universitario Son Espases. Ctra. Valldemossa 79, Palma de Mallorca 07010, Spain; borja.cosio@ssib.es

Received 5 September 2013
Revised 7 January 2014
Accepted 12 January 2014
Published Online First 29 January 2014

ABSTRACT

Background Fibred confocal fluorescence microscopy (FCFM) is a novel technology that allows the in vivo assessment and quantification during bronchoscopy of the bronchial wall elastic fibre pattern, alveolar and vessel diameters and thickness of the elastic fibre in the alveolar wall.

Aims To relate these structural characteristics with lung function parameters in healthy subjects, smokers with normal spirometry and patients with chronic obstructive pulmonary disease (COPD).

Methods We performed FCFM in 20 never smokers, 20 smokers with normal spirometry and 23 patients with COPD who required bronchoscopy for clinical reasons. The bronchial wall elastic fibre pattern was classified as lamellar, loose and mixed pattern, and later confirmed pathologically. Airspace dimensions and extra-alveolar vessel diameters were measured. Lung function measurements and pulmonary CT scans were obtained in all participants.

Results Patients with COPD were characterised by a significantly higher prevalence of loose fibre bronchial deposition pattern and larger alveolar diameter which correlated inversely with several lung function parameters (forced expiratory volume in 1 s (FEV_1) , FEV_1/forced vital capacity ratio, maximum expiratory flow, carbon monoxide transfer factor and carbon monoxide transfer coefficient; $p<0.05$). Increased alveolar macrophages were demonstrated in active smokers with or without COPD.

Conclusions This is the first FCFM study to describe in vivo microscopic changes in the airways and alveoli of patients with COPD that are related to lung function impairment. These findings open the possibility of assessing the in vivo effects of therapeutic interventions for COPD in future studies.

Key messages

What is the key question?

- Are the relationships between the ex vivo structural changes and lung function that characterise Chronic Obstructive Pulmonary Disease (COPD) reproducible in vivo using a novel endoscopic microscopy view?

What is the bottom line?

- Fibered confocal fluorescence microscopy (FCFM) is a novel technology that allows the in vivo assessment and quantification during bronchoscopy of the bronchial wall elastic fiber pattern, alveolar and vessel diameters and thickness of elastic fiber in the alveolar wall.

Why to read on?

- This study shows that patients with COPD have specific structural in vivo abnormalities, such as a higher prevalence of the loose elastin deposition airway pattern and enlarged airspaces, which are significantly related to their lung function; given that FCFM was safe and well tolerated during routine bronchoscopy, these results pave the way for future in vivo structure-function interventional studies in COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a complex disease characterised by small airway fibrosis and parenchymal tissue destruction with loss of lung elastic recoil (emphysema) that leads to gas trapping and progressive airflow limitation,[1] as well as by central airway (chronic bronchitis) and pulmonary vasculature changes. These structure–function correlations in COPD were originally described in the late 1970s[2] and more recently confirmed using high-resolution CT.[3 4]

Fibred confocal fluorescence microscopy (FCFM) is a new technology that elicits real-time microscopic fluorescence imaging through a thin flexible mini-probe introduced into the 2 mm working channel of a flexible bronchoscope.[5] FCFM can provide an in vivo microscopic image of the elastin component of the bronchial wall and the acinus,[6–8] as well as the external sheath of the extra-alveolar microvessels.[9] Furthermore, FCFM can visualise alveolar macrophages because of their autofluorescent pigments.[10] Using FCFM, Thiberville *et al*[5 6] described in detail the bronchial wall and acini of both smokers and healthy non-smoker volunteers. More recently, Yick *et al*[11] used this technique to describe bronchial wall changes in asthma and observed novel relationships between the extracellular matrix of the airway wall and lung function in these patients. Although this technique is not able to explore the upper lobes due to the stiffness of the probe, it allows analysis of the elastic network in the small airways of the lower lobes in regions where the alveolar dimension can also be measured.

To cite: Cosío BG, Shafiek H, Fiorentino F, *et al*. *Thorax* 2014;**69**: 724–730.

We hypothesised that FCFM can provide real-time in vivo information of different structural changes that occur in COPD, and that these changes correlate with lung function abnormalities. Accordingly, this study sought to describe the FCFM changes of the bronchi and alveoli in patients with COPD compared with smoker and non-smoker controls and to correlate them with lung function changes.

METHODS

Study design

This was a prospective non-interventional controlled study that invited consecutive patients undergoing fibreoptic bronchoscopy for a clinical indication, mostly haemoptysis and/or lung mass on the chest x-ray or CT scan. The study was conducted at Son Espases University Hospital where the patients were recruited over 14 months ending in July 2013.

Study population

We studied 23 patients with a diagnosis of COPD according to the Global Initiative for Chronic Lung Disease (GOLD),[1] 20 smokers (according to the definition of the Centers for Disease Control and Prevention[12]) and 20 non-smoker controls, both with normal lung function. Those who had smoked at least 100 cigarettes in their entire life were considered as smokers, and those who smoked cigarettes every day or some days were considered to be current smokers.[12] Patients with a history of asthma, bronchiectasis or any suspicion of lung infection and/or patients with a contraindication for bronchoscopy according to international recommendations were excluded.[13] Patients with COPD were receiving treatment according to the GOLD recommendations.[1]

Characterisation of participants

All patients underwent clinical history (including age, gender and smoking history) and pulmonary function measurements including forced spirometry, static lung volumes and carbon monoxide transfer factor (TLCO) performed according to international guidelines.[14] Reference values were those of a Mediterranean population.[15]

CT scan analysis

Chest CT scans were also obtained in all patients in parallel with the study for clinical reasons. It was later re-analysed by an experienced radiologist involved in the study. The presence of emphysema was determined quantitatively as the percentage of low attenuation areas below −950 Hounsfield units (%LLA) using proprietary software (Philips Medical System), with 1% LLA as a threshold for the presence of emphysema as previously described,[16] and qualitatively using the so called 'total emphysema score' as previously described.[17 18]

Bronchoscopy

Fibreoptic bronchoscopy (Pentax, EB-1570 K, Tokyo, Japan) was performed under intravenous conscious sedation (midazolam 3–7 mg, alfentanil 500 μg). Nasal oxygen supply was provided if needed to maintain oxygen saturation ≥90% with complete monitoring of the patient during the procedure.

Fibred confocal fluorescence microscopy (FCFM)

FCFM was performed with the Cellvizio system F-400 (Cellvizio LUNG, Mauna Kea Technologies, Paris, France) with a laser wavelength 488 nm using a confocal mini-probe (AlveoFlex, Mauna Kea Technologies) as previously described by Thiberville *et al*.[6] Briefly, the confocal mini-probe (1.4 mm in diameter) was introduced through the working channel of the fibreoptic bronchoscope and advanced gently until reaching the lung alveoli (see below). Images of the bronchial wall of the main, lobar and distal bronchi as well as the alveolar space were continuously recorded for later off-line analysis. Images provided by the FCFM probes scan a 600 μm diameter surface with a lateral resolution of 5 μm and a depth below the bronchial surface of 0–50 μm. To compare FCFM images with pathological findings, a standard bronchial biopsy was taken in the same carina as the one where the image had been taken in a subgroup of participants, always in the second or third bronchial level (subsegmentary carina). Immediately after collection the biopsy specimen was fixed in 4% buffered formaldehyde and embedded in paraffin for later standard pathological analysis. At least three different bronchial and alveolar areas were explored in each patient. Because the stiffness of the probe made it difficult to explore adequately the upper lobes, all explored areas were in the lower lobes of the lung.

Image analysis

Morphometric analysis of the proximal bronchial wall and alveolar space was performed using the software provided by the FCFM manufacturer (MedViewer1.1.1; Mauna Kea Technologies) which allows quantification of the bronchial and alveolar wall fluorescence intensity, airspace dimensions (including alveoli and ducts), thickness of elastic fibre in the alveolar wall and extra-alveolar vessel diameter.[6 7] The distribution of elastic fibres in the bronchial wall was classified as lamellar, loose and mixed pattern according to its orientation, as previously described,[11] and it was later confirmed by pathological examination. Fluorescence intensity was quantified using the MedViewer Signal Quantification Toolbox as previously described,[6 7] and the ratio between the alveolar/bronchial wall fluorescence intensity (ABI) was calculated.[6]

Statistical analysis

The results are presented as mean±SD unless otherwise stated. Comparisons between groups were performed using the χ^2, Mann–Whitney or Kruskal–Wallis tests, as appropriate. The Spearman correlation coefficient test was used to investigate structure–function relationships of interest. MedCalc V.9.2.1.0 (Ostend, Belgium) was used for all analyses.

RESULTS

Study population

Table 1 summarises the main demographic and clinical characteristics of the participants. The patients with COPD were mostly men and were slightly older than the controls. Cumulative smoking exposure (pack-years) was higher in the patients with COPD than in the smoker controls, but the proportion of current smokers was higher in the latter (table 1). By design, lung function was normal in the controls whereas patients with COPD had moderate to severe airflow limitation and reduced TLCO (table 1). Eleven patients with COPD (48%) were on a scheduled combination of inhaled corticosteroids (ICS) and long-acting β_2 agonists (LABA), two patients (9%) were on LABA only and 12 were on long-acting muscarinic antagonists with or without LABA/ICS. Nineteen patients (82.6%) with COPD and eight patients (40%) had CT emphysematous changes with statistically significant differences between both groups, whereas none of the non-smokers had evidence of emphysema (table 1). The extent of emphysema was significantly higher in the patients with COPD than in the smoker

Table 1 Demographic, clinical, functional and radiological characteristics of participants

	COPD (n=23)	Smokers (n=20)	Non-smokers (n=20)	p Value
Age, years	64.8±9.7	54.6±10.5	61.2±18.2	0.023
Gender (M/F), n	21/2	13/7	7/13	0.0001
Smoking history				
Pack/year, median (IQR)	60 (40–75)	35 (30–50)	0	0.03*
Current smokers (%)	39%	85%	0	N/A
Lung function				
FEV_1/FVC	55.1±11.2	76.9±4.1	81.47±6.6	<0.0001
FEV_1%	67.3±17.6	94.8±21.4	96.5±17.1	0.0002
FVC%	92.4±19	98.1±22.4	96±18.3	0.548
TLCO %	58.6±13.5	78.4±18.6	81.4±18.7	0.002
KCO %	66.3±15	79.8±11	82.3±9.7	0.006
MEF %, median (IQR)	26 (20.3–38)	83 (69–96)	96 (88.5–103)	<0.0001
Chest CT scan				
Presence of emphysematous changes, n (%)	19 (82.6%)	8 (40%)	0	0.023*
Visual scoring of emphysema (tES)	1.76±1.3	0.5±0.52	0	0.001*
Quantitative scoring, median (IQR)	1.99 (0.4–5.65)	0.29 (0.16–0.43)	0	0.012*

*Mann–Whitney test.
FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; KCO, carbon monoxide transfer coefficient; MEF, maximum expiratory flow; tES, total emphysema score; TLCO, carbon monoxide lung transfer factor.

group both by visual scoring methods and quantitatively ($p=0.001$ and $p = 0.012$, respectively).

FCFM was performed in all participants without complications during or after the procedure. The duration of FCFM during the bronchoscopy ranged from 5 to 10 min in each participant. Indication for bronchoscopy on each patient is listed in the online supplementary table S1.

Patterns of bronchial wall elastin fibre distribution

As shown in figure 1, using FCFM we recognised the three previously described[11] patterns of bronchial wall fibre distribution (lamellar, loose and mixed patterns). The lamellar pattern was characterised by a linear and parallel bronchial thick fibre orientation (figure 1A), in the loose pattern the fibres were thin and non-specifically orientated (figure 1C) and the mixed pattern showed a combination of the two (figure 1E). These FCFM patterns were later confirmed pathologically in bronchial biopsies obtained from five patients with COPD, three smokers and three non-smokers (figure 1). The bronchial wall pattern could not be analysed because of poor image quality in one patient with COPD (4.3%), two smokers (10%) and one non-smoker (5%). With this caveat in mind, the lamellar and mixed patterns

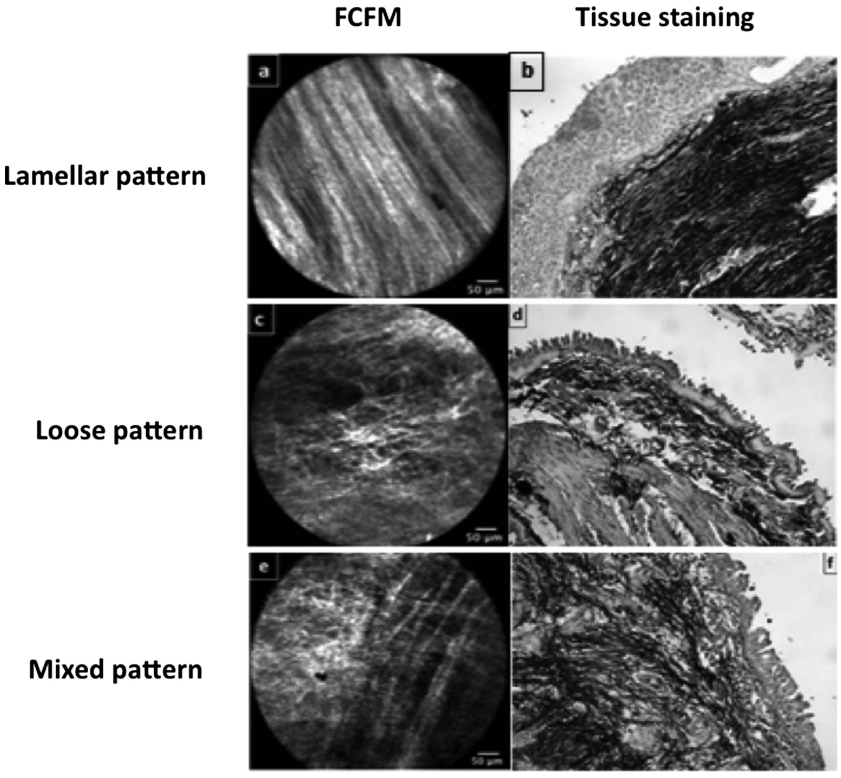

Figure 1 Morphological patterns of bronchial wall elastic fibre distribution by fibred confocal fluorescence microscopy (FCFM) and their corresponding pathological images (H&E stain followed by Elastica-van Gieson elastin fibres stain). For further explanations, see text.

Table 2 Relation between the different patterns of distribution of airway elastin fibres detected by FCFM (see figure 2) and lung function in the studied subjects

	Lamellar pattern	Mixed pattern	Loose pattern	p Value
TLCO, % reference	72.9±22.4	67.8±18	64.3±14.6	0.775
KCO, % reference	75.1±16.7	73.6±13.3	69±16.6	0.635
FEV_1, % reference	80.8±22.9	84.3±24.5	79.5±24.5	0.92
FEV_1/FVC, %	67.7±17.7	70.15±11.8	60±8.4	0.28

FCFM, fibred confocal fluorescence microscopy; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; KCO, carbon monoxide transfer coefficient; TLCO, carbon monoxide lung transfer factor.

accounted for the majority of available observations and were distributed relatively homogeneously between the groups, averaging 40–45% (figure 2). By contrast, the loose pattern was rare in non-smokers and smokers with normal lung function (5%), but its prevalence was more than three times higher (17%) in patients with COPD (figure 2). Lung function variables did not differ significantly according to bronchial elastin fibre pattern (table 2).

Alveolar measurements

Using FCFM, there is an abrupt transition between airways (figure 1) and alveolar space images (figure 3). Table 3 shows the alveolar diameter, alveolar elastic fibre thickness and extra-alveolar vessel diameter of each of the three groups studied. The alveolar diameter was significantly larger in the patients with COPD, but the alveolar elastic fibre thickness and ABI ratio were similar in the three groups. Extra-alveolar vessels tended to be smaller in patients with COPD, but the differences failed to reach statistical significance. In the patients with COPD, the drug treatment taken had no significant correlation with the extra-alveolar vessel diameter or with other FCFM measurements ($p>0.05$).

Alveolar macrophages

Alveolar macrophages were clearly visible (figure 3) in eight patients with COPD (35%) and in 14 smokers with normal spirometry (70%), all of them current smokers. By contrast, macrophages could not be identified in former smokers except for one never smoker control (5%).

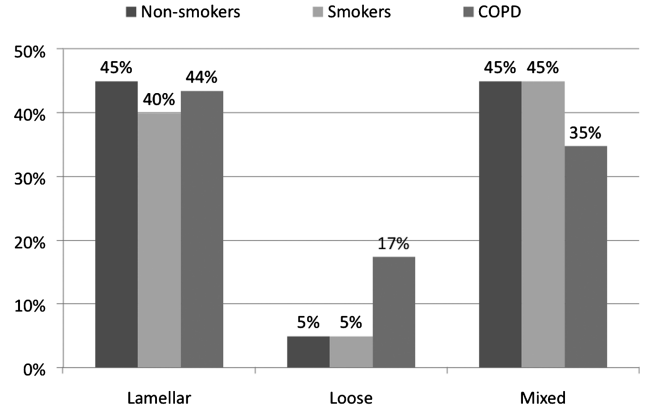

Figure 2 Relative frequency distribution of the three fibred confocal fluorescence microscopy (FCFM) patterns observed in the three groups of participants. For further explanations, see text.

Structure–function correlations

Figure 4 shows the structure–function correlations observed in all participants. The alveolar diameter was inversely related to measures of airflow limitation such as the forced expiratory volume in 1 s (FEV_1) both in absolute values and expressed as a percentage of reference ($r=-0.338$, $p=0.033$ and $r=-0.468$, $p=0.0027$, respectively), maximum expiratory flow (MEF) percentage of predicted ($r=-0.405$, $p=0.014$), the FEV_1/forced vital capacity (FVC) ratio ($r=-0.467$, $p=0.002$), as well as TLCO ($r=-0.393$, $p=0.034$). Additionally, we observed a significant direct correlation between the extra-alveolar vessel diameter and the carbon monoxide transfer coefficient (KCO) as a percentage of reference ($r=0.388$, $p=0.044$). Other FCFM morphometric parameters (such as the ABI ratio or alveolar elastic fibre thickness) were not related to lung function measures.

DISCUSSION

This is the first study to use FCFM to describe airway and alveolar abnormalities in patients with COPD in vivo and to relate them to lung function measurements. It also shows that this technique can be routinely performed during bronchoscopy with excellent tolerability and safety. Overall, these results pave the way for future studies investigating the in vivo effects of therapeutic interventions in COPD.

Previous studies

Cosio *et al*[2] were the first to describe in the late 1970s the structure–function relationships in smokers. This and other histological and experimental studies[19 20] firmly established that emphysema is associated with enlarged airspaces and narrower capillaries with loss of capillary density. Much more recently, Salaün *et al*[21] used FCFM in an animal model of elastase-induced emphysema and confirmed in vivo a significantly increased alveolar maximal diameter with larger intercapillary distance in emphysematous animals. Finally, Newton *et al*[22] used FCFM in 38 patients with different parenchymal lung diseases (and four healthy non-smokers) and reported increased alveolar diameter in those with emphysema.

Interpretation of results

The main results of this study show that: (1) patients with COPD tend to have a higher prevalence of the loose elastin deposition airway pattern (figures 1 and 2); (2) airspace dimension (including alveoli and ducts) is significantly larger (and the extra-alveolar vessel diameter tended to be smaller) in COPD (table 3); and (3) there are significant correlations between structure (by FCFM) and function (figure 4). These are discussed separately below.

Thiberville *et al*[7] described five different FCFM airway patterns in healthy subjects and demonstrated that they varied according to the generation of the bronchial tree imaged. Our samples and images were always captured from subsegmental bronchi. Yick *et al*[11] reported three different patterns of bronchial elastic fibres (later confirmed histologically) in patients with asthma and showed that the lamellar pattern was associated with lower FEV_1 values (percentage of reference). In our study we found that the loose pattern was more prevalent in patients with COPD than in controls (figure 2). This is in keeping with previous histological observations by Black *et al*[23] of elastic fibre destruction in the alveoli and the small airways in COPD. These structural abnormalities, however, were not related to any of the lung function parameters assessed (table 2), probably reflecting the fact that the central airways contribute less to functional

Table 3 Morphometric alveolar parameters by study group

	COPD (n=23)	Smokers (n=20)	Non-smokers (n=20)	p Value
Alveolar diameter, μm	322.4±45.8	282.2±42.2	267.8±54	0.002
Alveolar elastic fibre thickness, μm	12.7±2.2	13.6±3.3	12±2.0	0.293
Extra-alveolar vessel diameter, μm	91.1±16.1	102.1±22.5	102.1±26.7	0.077
ABI ratio, median (IQR)	0.85 (0.64–1.61)	1.2 (0.62–2.1)	0.78 (0.52–1.04)	0.164

ABI, alveolar/bronchial wall fluorescence intensity.

derangement in COPD than the periphery of the lung, as discussed below.

Our observation of increased alveolar size is also in keeping with previous pathological[2 19 20] and FCFM[21 22] studies in emphysema. Likewise, the narrower extra-alveolar vessels observed in COPD can also be explained by the stretching over the enlarged alveoli which characterises the emphysematous lung.[19] The observed variability in alveolar elastic fibre thickness and the ABI ratio is probably due to the intrinsic fluorescence variations of the elastic fibres among the subjects, either in the bronchial or alveolar walls.[6] Moreover, the variability in thickness of the alveolar elastic fibres in the COPD group could also be explained by the remodelling process accompanying this disease, previously pointed out by Vlahovic *et al*,[24] as both the loss and new synthesis of elastin fibres occurred in the emphysematous regions. Finally, the medications prescribed for the patients with COPD did not affect the morphological measurement. This could be due to reduced bronchial vascular reactivity in COPD and the insensitivity of the vasculature to the acute effect of inhalation therapy.[25 26]

The loss of elastic recoil and the destruction of alveolar attachments that maintain the patency of small airways are well-known determinants of airflow limitation in emphysema.[27 28] Recently, Yablonskiy *et al*[29] studied the morphological changes associated with emphysema using ^{3}He diffusion MRI; they found evidence of alveolar destruction in emphysematous lungs which correlated with diffusion defects. In keeping with this, we observed a significant correlation between the alveolar diameter (an in vivo surrogate marker of the extent of emphysema) and the severity of airflow limitation expressed by FEV_1 (%) and the FEV_1/FVC ratio. Of interest, too, was the observed inverse correlation between the TLCO and the vascular diameter, probably reflecting the well-established relationship between the former and the degree of emphysema present.[2] Furthermore, MEF (%), a measurement of small airway disease, was inversely correlated with alveolar diameter, which is consistent on the one hand with the previous explanation[27 28] and, on the other hand, with the findings of Black *et al*[23] that destruction of elastic fibres also involved the small airway in COPD leading to its obstruction.

Interestingly, our measurements of mean alveolar dimensions in patients with COPD, smokers and non-smokers (table 2) are consistent with measurements of mean linear intercept (Lm). Moreover, they fall well within the range of Lm reported in normal lungs using microCT,[30] which suggests that our measurements were obtained in regions of COPD lungs with very little emphysematous destruction, a fact that has been confirmed by CT scan analysis. This supports the hypothesis that bronchiolar destruction begins before the onset of emphysematous destruction in COPD.[30 31] The correlations between FCFM measurements and the extend of emphysema in both smokers and COPD patients are shown in the online supplementary tables S2 and S3.

Finally, numerous previous studies have reported increased macrophage numbers in bronchoalveolar lavage and surgical lung specimens in smokers.[32 33] Our results confirm these observations and others using FCFM,[7] since alveolar macrophages were easily detectable in vivo in current smokers, with or without COPD (figure 3). This is probably due to their high content of tobacco tar which acts as an exogenous fluorophore.[10] It is unclear from our data why one non-smoker subject had visible alveolar macrophages using FCFM, but environmental exposures such as passive smoking might have contributed.[34]

Clinical implications

Our study is a pilot descriptive study without direct clinical implications, but at least two can be envisaged. First, it shows that FCFM can be used safely and with excellent tolerability during routine bronchoscopic examination. Second, and more importantly, it opens a new way potentially to assess

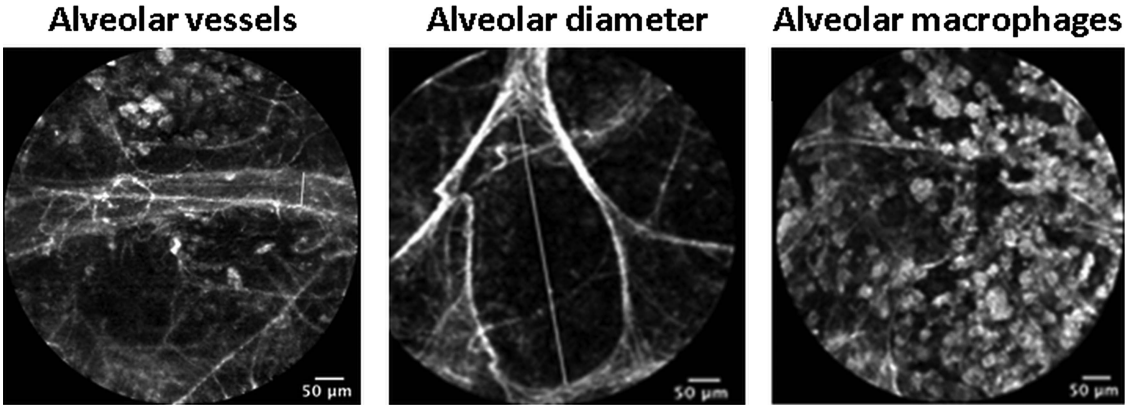

Figure 3 Fibred confocal fluorescence microscopy (FCFM) images of the alveolar space obtained in patients with chronic obstructive pulmonary disease. For further explanations, see text.

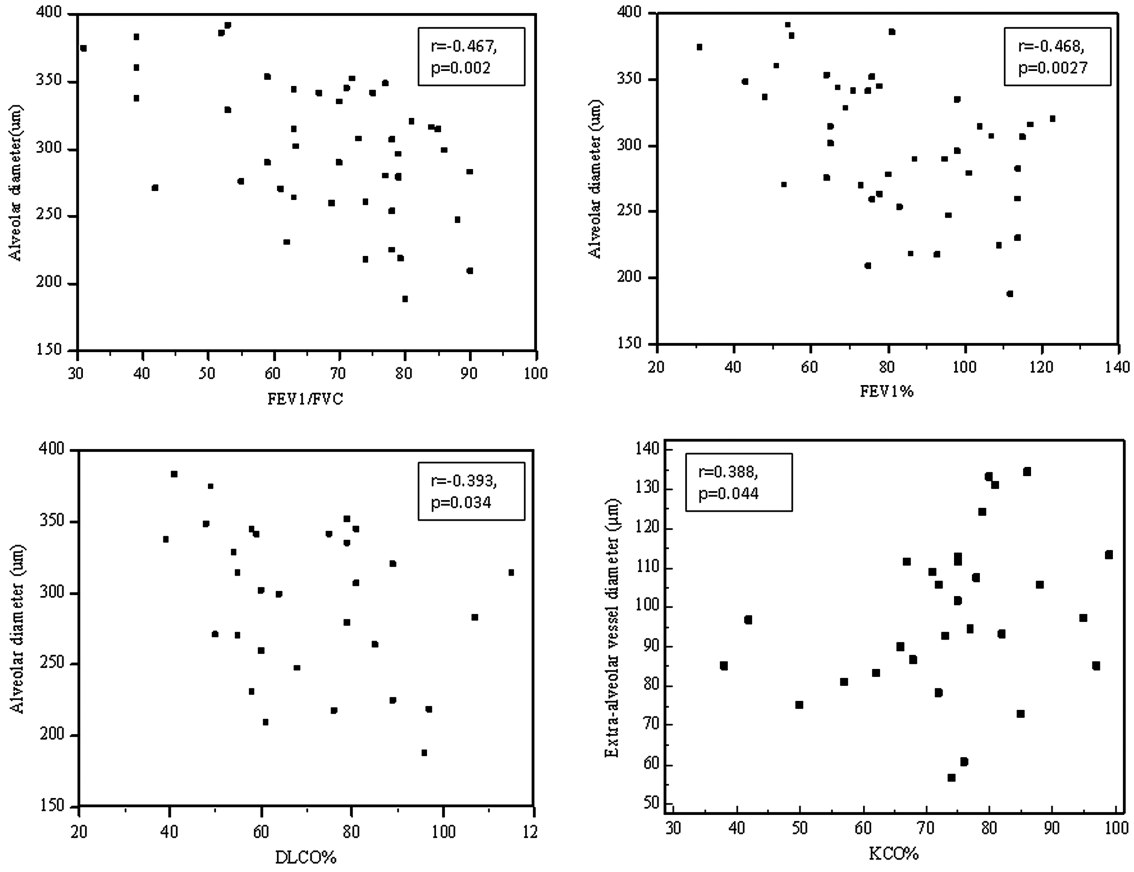

Figure 4 Correlations between fibred confocal fluorescence microscopy (FCFM) morphometric measurements and several lung function parameters. For further explanations, see text. FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; K_{CO}, carbon monoxide transfer coefficient; T_{LCO}, carbon monoxide lung transfer factor.

microscopically the impact of therapeutic interventions in the lung of patients with COPD in vivo which, of course, requires further research. Further, we assume that FCFM, as a minimally invasive tool, could be used to validate the novel microstructural biomarkers of emphysema found with hyperpolarised gas diffusion MRI[29 35] in future studies.

Study limitations

The present study has some limitations. First, we used different mini-probes with potentially different fluorescent properties in different participants. To address this issue we expressed the results as the ABI ratio following previous recommendations.[6] Second, measurement of the different parameters was performed on captured videos taken during spontaneous breathing, so alveolar diameter measurements were the average of several respiratory cycles, also as previously published.[6] Third, owing to the stiffness of the mini-probe used, we were not able to reach the upper lung lobes so only the lower lobes were studied with FCFM; this may not reflect the maximal extent of emphysematous changes which is known to predominate in the upper lung zones. The fact that control groups were studied in a similar way partly addresses this limitation.

CONCLUSIONS

COPD is characterised by microscopic changes in the airways and parenchyma that can be detected in vivo by FCFM and that relate to clinically relevant measurements of lung function. These findings open new possibilities for the in vivo assessment of therapeutic interventions in COPD.

Acknowledgements The authors thank the participants for their willingness to contribute to medical research and the nursing staff of our institution (Maria Angels Noguera and Rocío Martinez) for the excellent technical contribution. BGC also would like to thank Professor Manuel Cosio and Professor Marina Saetta for their useful comments and encouraging support on life and research. The authors would also like to thank one of the co-authors, Dr Bernado Togores, for his long life dedicated to lung physiology and teaching.

Contributors BGC and AK: designed the protocol, acquired and analysed the data and prepared the manuscript. HS, FF and JS: data acquisition and analysis. CG: pathological analysis. ML and AR: patient recruitment and sample processing. BT: lung function tests; JP: analysis of emphysema in CT scans; AA: data analysis and manuscript preparation.

Funding The study was supported in part by grants from the Spanish Society of Respiratory Medicine (Separ), Conselleria de Economia and Conselleria de Salud de las Islas Baleares.

Competing interests HS was granted by the University of Alexandria and Ministry of Higher Education of Egypt as member of ParOwn (the Partnership and Ownership initiative).

Patient consent Obtained.

Ethics approval The study protocol was approved by the local Ethics Committee (number IB1097/08).

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Vestbo J, Hurd SS, Agusti AG, *et al*. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2013;187:347–65.
2 Cosio M, Ghezzo H, Hogg JC, *et al*. The relations between structural changes in small airways and pulmonary-function tests. *N Engl J Med* 1978;298:1277–81.

3 Boschetto P, Quintavalle S, Zeni E, *et al*. Association between markers of emphysema and more severe chronic obstructive pulmonary disease. *Thorax* 2006;61:1037–42.
4 Orlandi I, Moroni C, Camiciottoli G, *et al*. Chronic obstructive pulmonary disease: thin-section CT measurement of airway wall thickness and lung attenuation. *Radiology* 2005;234:604–10.
5 Thiberville L, Salaun M, Lachkar S, *et al*. Confocal fluorescence endomicroscopy of the human airways. *Proc Am Thorac Soc* 2009;6:444–9.
6 Thiberville L, Salaun M, Lachkar S, *et al*. Human in vivo fluorescence microimaging of the alveolar ducts and sacs during bronchoscopy. *Eur Respir J* 2009;33: 974–85.
7 Thiberville L, Moreno-Swirc S, Vercauteren T, *et al*. In vivo imaging of the bronchial wall microstructure using fibered confocal fluorescence microscopy. *Am J Respir Crit Care Med* 2007;175:22–31.
8 Bourg-Heckly G, Thiberville L, Vever-Bizet C, *et al*. In vivo endoscopic autofluorescence microspectro-imaging of bronchi and alveoli. *Proc SPIE* 2008;6851.
9 Weibel ER, Hsia CC, Ochs M. How much is there really? Why stereology is essential in lung morphometry. *J Appl Physiol* 2007;102:459–67.
10 Pauly JL, Allison EM, Hurley EL, *et al*. Fluorescent human lung macrophages analyzed by spectral confocal laser scanning microscopy and multispectral cytometry. *Microsc Res Tech* 2005;67:79–89.
11 Yick CY, von der Thusen JH, Bel EH, *et al*. In vivo imaging of the airway wall in asthma: fibered confocal fluorescence microscopy in relation to histology and lung function. *Respir Res* 2011;12:85.
12 US Centers for Disease Control and Prevention. Health behaviors of adults: United States, 2005–2007. Vital and Health Statistics Series 10, Number 245, Appendix II, 2010: 80.
13 Anon. Guidelines for fiberoptic bronchoscopy in adults. American Thoracic Society. Medical Section of the American Lung Association. *Am Rev Respir Dis* 1987;136:1066.
14 Laszlo G. Standardisation of lung function testing: helpful guidance from the ATS/ERS Task Force. *Thorax* 2006;61:744–6.
15 Roca J, Sanchis J, Agusti-Vidal A, *et al*. Spirometric reference values from a Mediterranean population. *Bull Eur Physiopathol Respir* 1986;22:217–24.
16 Grydeland TB, Dirksen A, Coxson HO, *et al*. Quantitative computed tomography: emphysema and airway wall thickness by sex, age and smoking. *Eur Respir J* 2009;34:858–65.
17 Bergin C, Muller N, Nichols DM, *et al*. The diagnosis of emphysema. A computed tomographic-pathologic correlation. *Am Rev Respir Dis* 1986;133:541–6.
18 Park KJ, Bergin CJ, Clausen JL. Quantitation of emphysema with three-dimensional CT densitometry: comparison with two-dimensional analysis, visual emphysema scores, and pulmonary function test results. *Radiology* 1999;211:541–7.
19 Yamato H, Sun JP, Churg A, *et al*. Cigarette smoke-induced emphysema in guinea pigs is associated with diffusely decreased capillary density and capillary narrowing. *Lab Invest* 1996;75:211–19.
20 Schraufnagel DE, Schmid A. Capillary structure in elastase-induced emphysema. *Am J Pathol* 1988;130:126–35.
21 Salaün M, Modzelewski M, Marie JP, *et al*. In vivo assessment of the pulmonary microcirculation in elastase-induced emphysema using probe-based confocal fluorescence microscopy. *IntraVital* 2012;1:122–31.
22 Newton RC, Kemp SV, Yang GZ, *et al*. Imaging parenchymal lung diseases with confocal endomicroscopy. *Respir Med* 2012;106:127–37.
23 Black PN, Ching PS, Beaumont B, *et al*. Changes in elastic fibres in the small airways and alveoli in COPD. *Eur Respir J* 2008;31:998–1004.
24 Vlahovic G, Russell ML, Mercer RR, *et al*. Cellular and connective tissue changes in alveolar septal walls in emphysema. *Am J Respir Crit Care Med* 1999;160:2086–92.
25 Paredi P, Barnes PJ. The airway vasculature: recent advances and clinical implications. *Thorax* 2009;64:444–50.
26 Paredi P, Ward S, Cramer D, *et al*. Normal bronchial blood flow in COPD is unaffected by inhaled corticosteroids and correlates with exhaled nitric oxide. *Chest* 2007;131:1075–81.
27 Timmins SC, Diba C, Farrow CE, *et al*. The relationship between airflow obstruction, emphysema extent, and small airways function in COPD. *Chest* 2012;142: 312–19.
28 Saetta M, Finkelstein R, Cosio MG. Morphological and cellular basis for airflow limitation in smokers. *Eur Respir J* 1994;7:1505–15.
29 Yablonskiy DA, Sukstanskii AL, Woods JC, *et al*. Quantification of lung microstructure with hyperpolarized 3He diffusion MRI. *J Appl Physiol* 2009;107:1258–65.
30 McDonough JE, Yuan R, Suzuki M, *et al*. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. *N Engl J Med* 2011;365: 1567–75.
31 Galban CJ, Han MK, Boes JL, *et al*. Computed tomography-based biomarker provides unique signature for diagnosis of COPD phenotypes and disease progression. *Nat Med* 2012;18:1711–15.
32 Wallace WA, Gillooly M, Lamb D. Intra-alveolar macrophage numbers in current smokers and non-smokers: a morphometric study of tissue sections. *Thorax* 1992;47:437–40.
33 Warr GA, Martin RR. Chemotactic responsiveness of human alveolar macrophages: effects of cigarette smoking. *Infect Immun* 1974;9:769–71.
34 Gordon SB, Read RC. Macrophage defences against respiratory tract infections. *Br Med Bull* 2002;61:45–61.
35 Quirk JD, Lutey BA, Gierada DS, *et al*. In vivo detection of acinar microstructural changes in early emphysema with (3)He lung morphometry. *Radiology* 2011;260:866–74.