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Quantitative $^{18}$F-FDG PET/CT to assess pulmonary inflammation in Chronic Obstructive Pulmonary Disease

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**Conflicts of Interest:**

IBW is funded by the British Heart Foundation and receives support from the Cambridge NIHR Comprehensive Biomedical Research Centre and Comprehensive Research Network. He has received grant support from GSK. JC is employed by Cambridge University Hospital NHS Foundation Trust and is obligated to spend 50% of his time on GSK clinical trial research via a
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Take home message

FDG PET/CT has potential utility to non-invasively evaluate pulmonary inflammation in COPD.

Pulmonary FDG uptake is increased in COPD patients, positively associated with systemic inflammatory markers and shows low interoccasion variability.

241 characters
Abstract

Rationale

Chronic obstructive pulmonary disease (COPD) and smoking are characterised by pulmonary inflammation. $^{18}$F-Fluorodeoxyglucose positron emission tomography/computed tomography (FDG PET/CT) imaging may improve knowledge of pulmonary inflammation in COPD patients and aid early development of novel therapies as an imaging biomarker.

Objectives

To evaluate pulmonary inflammation, assessed by FDG uptake in whole and regional lung in ‘usual’ (smoking-related)-COPD patients, alpha-1 antitrypsin deficiency ($\alpha_1$ATD)-COPD patients, smokers without COPD and never smokers using FDG PET/CT. Secondly, to explore cross-sectional associations between FDG PET/CT and systemic inflammatory markers in COPD patients and repeatability of the technique in COPD patients.

Methods

Data from two imaging studies were evaluated. Pulmonary FDG uptake was measured by Patlak graphical analysis in four subject groups: 84 COPD, 11 $\alpha_1$ATD-COPD patients, 12 smokers and 10 never-smokers. Within the COPD group, associations between nKi and systemic markers of inflammation were assessed. Repeatability was evaluated in 32 COPD patients comparing nKi values at baseline and 4-months follow up.

Results
COPD, α₁ATD patients and smokers had increased whole lung FDG uptake (nKi) compared to never-smokers (0.0037±0.001, 0.0040±0.001, 0.0040±0.001 versus 0.0028±0.001 ml/cm³/min⁻¹ respectively, p<0.05 for all). Similar results were observed in upper and middle lung regions. In COPD participants, plasma fibrinogen was associated with whole lung nKi, (β=0.30, p = 0.02) in multivariate analysis adjusted for current smoking, forced expiratory lung volume in 1 second % predicted, systemic neutrophils and C reactive protein levels. Mean percentage difference in nKi between the baseline and follow-up was 3.2%; and the within subject coefficient of variability was 7.7%.

Conclusions

FDG PET/CT has potential as a non-invasive tool to enable whole lung and regional quantification of FDG to assess smoking and COPD-related pulmonary inflammation.

ABSTRACT: 250 (limit)words

Keywords

Positron Emission Tomography Computed Tomography; Fluorodeoxyglucose F18; Lung; Inflammation; Pulmonary Disease, Chronic Obstructive; Biomarkers
Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a heterogenous condition characterised by persistent respiratory symptoms and airflow limitation due to airway and alveolar pathology [1]. Although the molecular origins of the disease have yet to be fully elucidated, it is known that tobacco smoking remains the main modifiable risk factor for the development of COPD and it is understood that tobacco smoke inhalation (or other toxic air particulates or gases) trigger an abnormal exuberant pulmonary inflammatory response, which may continue even when the noxious stimuli is removed. Persistent inflammation subsequently leads to irreversible tissue destruction and airway remodeling changes [2]. Inflammation is a driver of disease progression in COPD and is characterised by increased lymphocytes, macrophages and neutrophils within the lung [3]. Whilst Forced Expiratory Volume in 1 second (FEV₁), assessed by spirometry, is used to diagnose COPD, this measure does not relate well to pulmonary inflammation or indeed symptoms that patients experience [4]. Moreover, lung damage has often developed and progressed before the spirometric diagnostic threshold of COPD is reached [5]. Current treatment options for COPD remain limited, which may in part be due to the lack of biomarkers reflective of disease phenotypes, progression or severity [6].

¹⁸F-Fluorodeoxyglucose Positron Emission Tomography paired with computed tomography (FDG PET/CT) is a functional non-invasive imaging modality which enables in-vivo regional visualization and quantification of glucose metabolism to assess pulmonary inflammation [7]. To date, Jones et al showed that FDG uptake quantified by PET could distinguish COPD patients from healthy never-smokers and asthma sufferers [8]. Subramanian et al observed that in COPD patients, FDG uptake correlated with FEV₁ but found no difference in FDG uptake in alpha-1
antitrypsin PiZZ deficiency (α1ATD) patients with COPD (α1ATD-COPD) compared with healthy never-smoking controls [9].

Despite these encouraging results from small studies, it remains to be elucidated whether FDG PET has potential utility as a research tool to advance pathophysiological understanding and therapeutics development for COPD. Important questions that have not previously been addressed to support FDG PET’s utility in this domain, include the impact of smoking on pulmonary inflammation quantified by FDG PET uptake, repeatability of pulmonary FDG PET in COPD patients and the clinical relevance of FDG PET as a biomarker to evaluate pulmonary inflammation in COPD patients in relation to validated peripheral biomarkers of inflammation and computed tomography features of disease [10].

In this study, we hypothesized that usual (smoking-related) COPD patients have increased pulmonary inflammation, as measured by FDG PET uptake, compared with α1ATD-COPD patients, chronic smokers (≥10 pack years) without COPD and healthy never-smokers. We sought to determine whole lung and regional FDG PET uptake in four subject groups and evaluate the impact of smoking on FDG PET uptake. Within the COPD group, we also assessed the association of pulmonary inflammation assessed by FDG uptake with peripheral markers of inflammation and explored FDG PET uptake stratified by high resolution computed tomography (HRCT) sub-types in COPD patients [10]. Finally, we evaluated the repeatability of FDG PET/CT to quantify FDG uptake in COPD patients at baseline and at 4 months.
Methods

Study Design

Data reported in this manuscript includes participants from two parallel FDG PET imaging studies: 1) An observational cross-sectional study (EVOLVE) (REC 13/EE/0165, UK CRN ID 1513), which included COPD patients with low fibrinogen levels (<2.8 g/L), $\alpha_1$ATD-COPD, both smokers and never-smokers without COPD. 2) The EVOLUTION clinical trial (NCT015410852), a double-blind, placebo-controlled phase 2a trial in COPD patients with baseline fibrinogen $\geq$ 2.8 g/L with baseline FDG PET/CT scans before intervention of Losmapimod (a p38 mitogen activated protein kinase inhibitor)/placebo with repeat imaging at 4-months follow-up. In this manuscript, baseline data from all trial participants is incorporated into the COPD group to create a large cohort of COPD patients for analyses. The trial’s placebo group was used to assess repeatability of FDGPET/CT data from baseline to 4-month follow-up. Methodology and results from both these studies have been published previously [11], [12]. Both studies recruited participants from two UK tertiary centres and received favourable opinions from the Cambridge South Research Ethics Committee (EVOLVE: REC 13/EE/0165, UK CRN ID 1513, EVOLUTION: 12/EE/0135). MHRA approval was obtained for the EVOLUTION clinical trial. Written informed consent was obtained from all participants and the studies were carried out in accordance with institutional guidelines and the Declaration of Helsinki.
**Participant groups:**

There were four participant groups included in the study. 1) Individuals clinically diagnosed with COPD as confirmed by post-bronchodilator spirometry FEV$_1$/forced vital capacity (FVC)<0.70, and a reported smoking history of $\geq$10 pack years smoked. 2) $\alpha_1$ATD-COPD patients (Pi ZZ phenotype), where clinical diagnosis had been confirmed and post-bronchodilator spirometry confirmed FEV$_1$/FVC <0.70. 3) Chronic smokers without COPD, defined as $\geq$10 year pack history, who smoked approximately $\geq$ 10 cigarettes per day in the preceding 12 months of study enrolment, with no clinical diagnosis of COPD, and spirometric values within the normal range (for both FEV$_1$ and FEV$_1$/FVC). 4) Never-smokers similarly with spirometric values in the normal range.

Both COPD and $\alpha_1$ATD-COPD participants had to be clinically stable, and free of exacerbations in the preceding 4 weeks before enrolment in the study. The PET acquisition protocol required a BMI in the range of 17-35 kg/m$^2$. Age and gender were prospectively matched across the four groups as closely as possible to enable cross-sectional analysis of the different subject groups.

**Lung Quantitative PET/CT Protocol**

Scans were performed at Cambridge (PET/CT unit, Addenbrookes Hospital) and London (Imanova Centre, Hammersmith). A General Electric Lightspeed VCT (Milwaukee, Wisconsin) scanner was used in Cambridge and a Siemens Biograph (Munich, Germany) scanner in London. All participants were imaged under a closely matched acquisition protocol.

Participants were required to fast for 6 hours prior to the scan and to avoid any strenuous exercise in the preceding 24 hours to limit muscle uptake of tracer. Blood glucose levels had to
be less than 11mmol/l to proceed with the scan. Participants were positioned comfortably on the scanner couch with arms down by sides.

In COPD and $\alpha_1$ATD patients, a HRCT scan was performed at maximum inspiration dependent on subject’s breath hold capability. In all participants, a non-contrast low dose CT scan covering one bed position centred on the lungs with the participant breathing freely was performed to enable attenuation-correction (AC) and anatomical co-registration of PET data. Following the CT scan, the PET scan was immediately commenced. A dose of approximately 240MBq $^{18}$F-Fluorodeoxyglucose (FDG) was injected at the start of the scan, followed by 10ml flush of normal saline. Dynamic data acquisition by list mode was acquired for 60 minutes from injection using the standard energy and coincidence timing window settings for the scanner and acquired in 23 dynamic frames.

**Lung Image Analysis**

Using Analyze 11.0 (AnalyzeDirect, Inc., Overland Park, KS, USA), the co-registered attenuation correction CT images were segmented using a semi-automated process into whole right and left lung, any large arteries or obvious airways were excluded. Next, the CT mask was down-sampled to match the PET resolution, and was further refined: (1) to exclude any holes where vessels were removed (2) a further 1cm$^3$ rim was removed from the edge of the fused mask to avoid motion artefact (3) to remove any areas of the mask proximal to the diaphragm with conspicuous motion artefact. The mask was then automatically divided into three regions of equal volumes along the transverse axis: upper, middle and lower.

The rate of FDG uptake, $K_i$, was evaluated using the widely established Patlak graphical technique [13] as pre-specified in the trial protocol [11]; $K_i$ was normalised ($nK_i$) as described in
previous studies [8] and used as a surrogate marker of pulmonary inflammation. Further information is provided in the online supplement (OLS). A lung segmentation of the FDG PET/CT scan image of a COPD patient in the study is shown in Figure 1.

**Perc 15**

In COPD and α₁ ATD patients, the Perc15 score calculated from the HRCT scan, as the 15th percentile of Hounsfield Units (HU) distribution (as described previously [14]) was used as a surrogate of emphysema severity.

**COPD CT image subtypes**

HRCT scans from usual COPD participants were visually analysed using the HOROS imaging platform to define the dominant CT-definable subtype of COPD according to the Fleischner classification system [10]. The Fleischner classification of COPD CT-definable subtypes includes main patterns of centrilobular emphysema, panlobular, paraseptal emphysema, airway disease and associated features. Scans were assessed for the predominant CT pattern by a radiology trainee with 3 years experience, following a period of initial training by a thoracic radiologist.

**Other Markers**

Blood samples were analysed for total white blood cell count (WCC), neutrophils, plasma fibrinogen (Klauss Method) and high sensitivity C-reactive protein (hsCRP) in NHS hospital laboratories using quality-controlled validated assays using in clinical practice. Spirometry was performed in accordance with ATS guidance [15]. For patients, up to 400mcg of salbutamol
inhaler was administered prior to assessment. For volunteers, no bronchodilator reversibility testing was performed.

The 6-minute walk distance (6MWD) test was performed in usual COPD participants according to the guidelines of the American Thoracic Society (ATS) with the exception that a practice test was not conducted [16].

**Statistical Analyses**

Data were analysed using SPSS software (version 23) and R version 3.0.0 for Microsoft Windows with R studio version 0.98.953. For cross-sectional analyses, never-smokers were the control group. Student’s unpaired t-test was used to compare imaging measures between the groups adjusted for multiple testing using the Bonferroni correction. Homogeneity of variance was assessed by Levene’s test and Shapiro-Wilks used to check for normality prior to performing statistical tests using. For unequal sample sizes, Welch’s t-test was used in cases of unequal variance. Pearson’s correlation coefficient and multivariate linear regression analysis were used to assess associations between variables, using mean values unless otherwise stated. Data were assessed for normality and log-transformed if necessary. A Bland-Altman plot was used to evaluate the repeatability of FDG-PET/CT in the placebo arm of the longitudinal EVOLUTION cohort and paired t-tests used to compare values at baseline and follow-up. P-values <0.05 were considered significant for all statistical analyses. All data are presented as mean ± standard deviation, percentages, or with 95% confidence intervals.
### Results

The demographics of the groups and main results are shown in Table 1. The total number of evaluable cross-sectional scans were 84 COPD patients, 11 α₁ATD-COPD patients, 12 chronic smokers without COPD and 10 never-smokers. Four participants were excluded due to excessive movement on the PET/CT scan which could not be corrected (one COPD patient, one α₁ATD-COPD, and two never-smokers). COPD patients and never-smokers were both older (68±8 years and 69±7 years) and had a higher BMI (25.9±3.9 and 26.6±2.6 kg/m²) than α₁ATD patients (62±8 years, 25.0±3.3 kg/m²) and smokers (62±6 years, 23.1±2.3 kg/m²), Table 1. There were a higher proportion of women in the α₁ATD-COPD and smokers without COPD groups, although this did not reach statistical significance. There was no difference in pack years smoked between COPD patients and smokers (43 ± 21 versus 37 ± 19 pack years, p = 0.36) but both groups smoked significantly more than α₁ATD-COPD patients (19 ± 11 pack years, p < 0.001 for both). Sixty-eight (81%) of COPD patients and 10 (91%) of α₁ATD patients used a combined long acting beta agonist/inhaled corticosteroid inhaler.

**Pulmonary FDG uptake (nKi)**

Whole lung, upper and middle lung nKi values were higher in COPD, α₁ATD-COPD and smokers without COPD compared with never-smokers, p<0.05, Table 1. There were no statistical differences in FDG uptake between COPD, α₁ATD, or smokers, or between all groups in the lower lung regions. A within-group comparison of regional nKi values in the upper, middle and lower lung revealed no significant differences in any of the subject groups (see Table 1 in OLS). The maximum within group difference observed was 0.49 ± 0.03 × 10⁻³ ml/cm³/min⁻¹, p=0.66, between the upper and lower lung zones in smokers.
Smoking

In the α₁ATD-COPD group, there was no difference in whole lung nKi between α₁ATD patients who were current smokers (n=2, 4.2 ± 0.1 × 10⁻³) versus ex-smokers (n=8, 3.9 ± 0.1 x 10⁻³, p=0.66). Grouping usual-COPD patients by smoking status showed that nKi was modestly elevated in COPD current smokers compared with COPD ex-smokers (n = 11 and 73; 4.2 ± 0.1 × 10⁻³ vs 3.7 ± 0.1 × 10⁻³, respectively; p = 0.047) despite no significant difference in FEV₁ between them (FEV₁ % predicted = 47 ± 18% vs. 50 ± 22% respectively). There was no difference in nKi in ex-smoker usual-COPD participants (3.7±0.1 x 10⁻³) compared with current smokers without COPD (4.0±0.1 x 10⁻³, p=0.10). Within the usual-COPD cohort, there was no correlation between the total pack years smoked and whole lung nKi (r=0.07, p=0.44). Stratifying whole lung nKi by quartiles of total pack years showed no statistical difference across groups, see OLS. Smoking intensity (average number of cigarettes smoked per day) was also not associated with nKi (r=0.13, p=0.71).

Association with peripheral inflammatory biomarkers and disease severity

In the COPD group, correlation between whole lung nKi and plasma fibrinogen (r = 0.40, p < 0.001), log₁₀hsCRP (r = 0.26, p =0.02), WCC (r = 0.24, p =0.03) and neutrophils (r= 0.28, p = 0.01) were observed, Table 2 . Upper and middle lung nKi also correlated with systemic inflammatory markers (p<0.05), but not for lower lung FDG uptake, Table 2. In regression analyses, plasma fibrinogen was associated with whole lung nKi independently of confounders including current smoking status, FEV₁ % predicted, neutrophil counts and hsCRP (see Table 3).
A modest inverse correlation was observed between FEV$_1$ (% predicted) ($r = -0.22$, $p = 0.04$) and whole lung nKi. However, no correlation was found between nKi and 6MWD ($-0.26$, $p = 0.15$) or whole lung emphysema assessed as Perc 15 ($r = -0.10$, $p = 0.42$), Table 2. The association of whole lung nKi with plasma fibrinogen was also assessed in the whole cohort ($r=0.39$, $p<0.001$), Figure 3.

**HRCT subtypes**

There were 74 evaluable HRCT scans in the COPD cohort. Table 4 summarises clinical information stratified by the visual subtypes defined according to Fleischner classification [10]. The majority of scans were defined as advanced destructive centrilobular emphysema followed by moderate or trace centrilobular emphysema. Three scans were classified as panlobular emphysema but given the small number of scans of this subtype, they were not included in analysis. No scans were characterised as paraseptal emphysema or airways disease as the predominant visual CT pattern or had associated features such as bronchiectasis identified. There were no significant differences in nKi values between the sub-classification of centrilobular emphysema identified, see Table 4.

**Repeatability**

We assessed repeatability of FDG PET in COPD patients using data from the placebo arm of the EVOLUTION clinical trial who had a follow-up scan that was available ($n=32$). The mean percentage difference in nKi between the baseline and follow-up was 3.2% and the within subject coefficient of variability was 7.7%, which is similar to previously reported values using FDG
and other tracers [18] (see Figure for the Bland-Altman plot). For further context of our between group differences observed, the mean percentage difference in nKi between never-smoking controls and COPD patients was +32% (2.8±0.1 x10^{-3} vs 3.7±0.1 x10^{-3}). Ten COPD patients who had an exacerbation in between the baseline and follow up scan, exhibited a slight increase in whole lung FDG uptake at follow up scan (baseline nKi= 3.6 ± 0.1 × 10^{-3}; follow-up nKi= 4.1 ± 0.1 × 10^{-3}, p = 0.05), despite the fact study participants had to be exacerbation free for 1 month prior to the follow up scan. 22 patients who did not experience an exacerbation had a baseline nKi = 3.8 ± 0.1 × 10^{-3} and follow-up scan of nKi= 3.7 ± 0.1 × 10^{-3}, p = 0.5, see Figure 5.

**Discussion**

This study sought to extend knowledge from prior small studies to evaluate whether FDG PET/CT is useful as a non-invasive tool to quantify pulmonary inflammation associated with COPD [8], [9]. We found that pulmonary FDG uptake measured by nKi, as a surrogate marker of pulmonary inflammation, was increased in both COPD and α1ATD-COPD individuals and in smokers with unobstructed spirometry when compared with never-smokers, but there was no difference in FDG uptake between COPD, α1ATD-COPD or smokers without COPD. Further, COPD individuals who were current smokers had increased inflammation compared with COPD ex-smokers. Additional findings of this study are the modest associations between pulmonary FDG uptake (especially in the upper lung regions) and peripheral inflammatory markers in
COPD individuals and the finding that FDG PET/CT imaging provides a reproducible, stable signal of FDG uptake measured by nKi, over 4 months follow up in COPD patients. A crude comparison of +32% percentage mean difference between groups compared with a 3.2% repeatability difference, shows it is of a magnitude 10 times higher. This supports the validity of our between-subject findings. Given these differences in nKi were observed despite the widespread use of inhaled corticosteroids in COPD patients, is also encouraging for this imaging’s modality utility to evaluate pulmonary inflammation.

We observed increased nKi in α1ATD-COPD patients versus never-smokers in this study, in contrast to findings reported by Subramanian et al who observed no difference between these two groups [9]. Our results are consistent with understanding the inflammatory pathological pathways underlying α1ATD, and highlight the high inflammatory burden of this condition, besides its well-recognized protease-antiprotease imbalance [19], [20]. Variation in scan acquisition protocol (for example Subramanian et al administered a lower FDG dose than used in this study [9]) may well account for the differences in data observed between studies.

This current study included current smokers with spirometry in the normal predicted range. As far as we are aware, this is the first prospective pulmonary FDG PET/CT study in current smokers, although animal studies have demonstrated the pulmonary inflammatory effects of smoking using FDG PET/CT [21]. A previous study of PET imaging with 11CO labelled erythrocytes, found that smokers had increased pulmonary extra-vascular tissue density in comparison to never-smokers [22]. A possible explanation suggested by the authors, was that chronic pulmonary inflammation in smokers accounted for increased density, and this may outweigh any offsetting tissue loss due to emphysema that may be expected in smokers [20].
This is an important consideration in interpreting tissue density measurements within COPD patients and also in comparison to smokers without COPD and lends support to our study findings. We observed that smokers with normal spirometry values, had similar FDG uptake values compared with COPD patients and COPD individuals who were current smokers had higher FDG uptake than COPD ex-smokers. Although we observed no relationship with total pack years smoked, or smoking intensity, determined by number smoked per day, current smoking seems to be associated with higher FDG uptake determined by nKi. This suggests that smoking induces significant pulmonary inflammation and supports the pathophysiologic concept that the initiator of COPD is an inflammatory response to noxious inhaled stimuli [3]. In this study, we evaluated tobacco smoking although the effects of other air pollutants may be predicted to be similar. Further, our study highlights the importance of smoking cessation as an extremely critical public health need, given smokers without COPD had similar pulmonary FDG uptake values to COPD patients.

We noted regional differences in FDG uptake across subject groups, with higher nKi values in upper and middle lung regions in both COPD and \( \alpha_1 \)ATD-COPD patients as well as smokers without COPD compared with these lung regions in never-smokers. This is consistent with previously reported results [9] which showed a upper lung regional predilection for FDG uptake which correlated with COPD disease severity. There was no difference in nKi values in the lower lung across participant groups and we speculate that this was due to the motion of the diaphragm during the free-breathing acquisition of PET data. A further point of consideration is that emphysema predominantly affects the upper lobes; and therefore perhaps the number of patients with lower zone changes related to pulmonary inflammation evaluated by nKi, was too small to elicit differences across groups. Moreover, we observed no difference in nKi values across lung
regions within groups, if any regional differences within the lungs of individuals within a group exist, they may be too subtle to detect with this technique. We also evaluated the effect of gravity on pulmonary FDG uptake in the study by determining nKi values stratified by anterior and posterior distribution (data not shown) and observed no significant differences between them. This may be because similarly the technique is not sensitive enough to detect differences in blood flow due to gravity. However, nKi values were averaged across anterior-posterior direction to limit any potential effect.

We found modest correlations in COPD participants between nKi and peripheral inflammation markers. Given that we previously found no strong associations between systemic inflammation measures and vascular inflammation assessed by FDG PET/CT in the same cohort [7], these data support the hypothesis that increased systemic inflammatory markers in COPD patients likely derive from the lung. Plasma fibrinogen in particular had an independent association with nKi, and interestingly, in vitro studies suggest the pulmonary epithelium can be an extrahepatic source of fibrinogen in response to local inflammatory mediators [23], [24]. These data further support the relevance of plasma fibrinogen as an Food and Drug Administration and European Medicines Agency qualified drug development tool assessing risk for exacerbation and mortality in COPD [25]. Direct assessment of pulmonary inflammation via bronchoalveolar lavage or tissue biopsies may have yielded stronger correlations with pulmonary FDG uptake, although the invasive nature of these techniques, the patient related challenges of such invasive techniques to repeatability in an interventional drug study, and likely need for spatial consideration of where to sample from within the lungs are caveats to this consideration. Another interesting finding of our study which demonstrates the potential of FDG-PET as a drug development tool sensitive to
a change in COPD disease activity, was increased nKi in participants who had an exacerbation between the baseline and follow-up scan. This was evident despite a minimum one month period without exacerbation prior to the follow-up scan. Our data suggests a potential role for FDG-PET as a non-invasive tool to demonstrate response to novel anti-inflammatory therapy as proof of concept in COPD experimental studies and in other pulmonary diseases such as pulmonary fibrosis [26]. In-depth evaluation of FDG PET/CT to evaluation pulmonary inflammation in comparison to other biomarkers is more far-reaching than this discussion permits and each research technique or biomarker has their own benefits and limitations. However, for context, sputum eosinophils, a marker of specifically eosinophilic airway inflammation, showed in a very large cohort of COPD patients, differences in spirometry and some lung regions’ CT parameters between stratified levels of this biomarker, whereas peripheral eosinophils had little association with these markers of COPD severity. As far as we are aware, no study has compared sputum eosinophils in smokers without COPD vs COPD patients [27].

99mTc-DPTA (Technetium-99mTc-diethylenetriaminepentaacetic acid is an imaging biomarker that has been used to assess epithelial permeability and similarly showed significant differences in COPD patients and smokers without COPD vs controls [28].

Our large dataset of COPD participants with HRCT and FDG PET/CT data also enabled stratification of FDG uptake values by visual subtypes of CT imaging, which has not be evaluated before. The majority of scans were centrilobular predominant pattern with advanced destructive emphysema and no scans were classified as predominant airway disease. We did not observe any differences in FDG uptake across the CT defined subtypes of COPD observed in this study, which is likely to be expected given that although a large FDG PET/CT study, the
HRCT scans are predominantly of the same visual subtype or phenotype (i.e. centrilobular emphysema). Previous studies have found that CT defined COPD subtypes exhibit differences in mortality and disease progression [29], [30]. Although there was an indication that severe forms of centrilobular emphysema had higher nKi values than milder forms, the FDG signal may have been affected by differences in air and pulmonary blood volume between these different severities of emphysema. Further study of the relationship between CT classification and FDG uptake in patients with defined phenotypes of airways disease versus emphysema may be helpful. HRCT scans were not performed in smokers without COPD, but this may have enabled exploration of FDG uptake with visual CT subtypes in this group compared with COPD patients.

The strengths of this study include the large COPD group, which enabled robust evaluation of associations with inflammatory markers and the ability to assess the reproducibility of the technique, which is similar to previously reported values using FDG [17] and other tracers [18] and is reassuring for future research studies in COPD patients. Nevertheless, there were limitations. Smoking status was self-reported and α1ATD-COPD patients were studied at one centre only. CT-AC scans were acquired under free breathing, which may lead to inaccuracies when quantifying PET data and FDG-PET scans were analysed using the Patlak Graphical approach; which has been shown to be influenced by blood and air volume [31], future work will be required to determine the most accurate PET measure of inflammation. Indeed standardised uptake value (SUV) which is a measure of FDG uptake often used in clinical practice, is highly influenced by blood and air volume [7]. Normalised Patlak analysis is the most widely established method to measure pulmonary FDG uptake in research studies because it uses dynamic data rather than a single time-point and is therefore generally considered more accurate.
than SUV. A further point of consideration is that exposure to ionizing radiation will limit widespread use of FDG-PET/CT imaging in clinical trials requiring measurement of inflammation at multiple time-points.

In summary, patients with COPD, $\alpha_{1}$ATD-COPD and current smokers without COPD, have increased levels of pulmonary FDG uptake quantified by nKi from FDG-PET/CT imaging compared with never-smokers. nKi as a surrogate marker of pulmonary inflammation is a stable and reproducible parameter at 4 months in COPD patients, is associated with current smoking, has modest correlations with peripheral systemic inflammatory markers including plasma fibrinogen and may reflect COPD disease activity defined by exacerbations. Further work is needed to elucidate reasons for regional differences in FDG uptake across subject groups and determine if meaningful COPD phenotypes can be defined by FDG-PET, although exposure to ionizing radiation will limit the study sample size of such studies. This study supports advancing research into pulmonary FDG-PET as a non-invasive tool to evaluate visualization and quantification of pulmonary inflammation in modest size, precision medicine experimental studies.

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### Table 1: Demographics, spirometry, image data

| Group                        | COPD n=84 | α1ATD n=11 | Smokers n=12 | Never smokers n=10 |
|------------------------------|-----------|------------|--------------|--------------------|
| **Demographics**             |           |            |              |                    |
| Age (years)                  | 68±8      | 62±8*      | 62±6*        | 69±7               |
| Gender (% male)              | 67        | 73         | 58           | 83                 |
| BMI (kg/m²)                  | 25.9±3.9  | 25.0±3.3   | 23.1±2.3*    | 26.6±2.6           |
| Current smoker n (%)         | 11 (13)***| 2 (17)***  | 12 (100)***  | 0                  |
| Pack years smoked            | 45±25***  | 19±11***   | 37±19***     | 0                  |
| LABA/LAMA/ICS n (%)          | 68 (81)   | 10 (91)    |              |                    |
| **Lung function**            |           |            |              |                    |
| FEV₁ (l)                     | 1.37±0.6***| 1.47±0.4***| 2.84±0.56    | 2.88±0.6           |
| FEV₁ % predicted             | 51±20***  | 45±16***   | 95±17        | 100±15             |
| FEV₁/FVC                     | 0.45±0.15 | 0.36±0.10  | 0.79±0.08    | 0.77±0.06          |
| **Laboratory data**          |           |            |              |                    |
| Fibrinogen (g/l)             | 3.4±0.7*  | 3.1±0.6    | 2.8±0.6      | 2.7±0.5            |
| hsCRP (mg/l)                 | 5.2±7.0*  | 3.3±2.3*   | 2.1±1.4      | 1.2±0.6            |
| White cell count (x10⁹/l)    | 6.54±1.83 | 7.01±2.72  | 7.28±2.02    | 5.84±1.31          |
| Neutrophils (x10⁹/l)         | 4.43±3.6  | 4.68±2.47  | 4.53±1.45    | 3.63±1.11          |
| **Pulmonary Image Data**     |           |            |              |                    |
| Whole lung (WL)              |           |            |              |                    |
| nKi (ml/cm³/min)             | 0.0037±0.001* | 0.0040±0.001* | 0.0040±0.001* | 0.0028±0.001*     |
| Perc15 (HU)                  | -889±54*** | -942±28*** | -942±28***   |                    |
| Upper lung (UL)              |           |            |              |                    |
| nKi (ml/cm³/min)             | 0.0040±0.001** | 0.0038±0.001* | 0.0044±0.00**  | 0.0027±0.001**    |
| Perc15 (HU)                  | -884±61*   | -922±34*** | -922±34***   |                    |
| Middle lung (ML)             |           |            |              |                    |
| nKi (ml/cm³/min)             | 0.0037±0.001** | 0.0042±0.001** | 0.0041±0.001** | 0.0027±0.001**    |
| Perc15 (HU)                  | -889±52*   | -943±29*** | -943±29***   |                    |
| Lower lung (LL)              |           |            |              |                    |
| nKi (ml/cm³/min)             | 0.0036±0.001 | 0.0038±0.001 | 0.0040±0.001 | 0.0032±0.001      |
| Perc15 (HU)                  | -880±49**  | -952±26*** | -952±26***   |                    |

*COPD = Chronic Obstructive Pulmonary Disease, α1ATD = Alpha-1 anti-trypsin deficiency, HU = Hounsfield units, FEV₁ = Forced Expiratory Volume in 1 second, FVC=forced vital capacity, hsCRP = high sensitivity C-reactive Protein, WCC = White Cell Count. ***p<0.001, **p<0.01, *p<0.05 significant difference compared to never-smokers. LABA=inhaled long acting beta agonist, LAMA=long acting muscarinic antagonist, ICS=inhaled corticosteroid*
Table 2: Correlations between rate of $^{18}$F-FDG uptake classified by lung regions in participants with COPD.

| Variable (x)                | Upper Lung | Middle Lung | Lower Lung |
|-----------------------------|------------|-------------|------------|
|                             | r          | sig         | r          | sig         | r           | sig         |
| Fibrinogen (g/L)            | 0.42       | <0.001      | 0.38       | <0.001      | 0.16        | 0.16        |
| Log$_{10}$hsCRP (mg/L)      | 0.31       | 0.005       | 0.31       | 0.005       | 0.09        | 0.41        |
| WCC ($x 10^9$/L)            | 0.24       | 0.04        | 0.25       | 0.03        | 0.17        | 0.14        |
| Neutrophils ($x 10^9$/L)    | 0.31       | 0.005       | 0.29       | 0.009       | 0.19        | 0.10        |
| FEV$_1$ (L)                 | -0.33      | 0.003       | -0.18      | 0.10        | 0.02        | 0.89        |
| FEV$_1$ % predicted          | -0.26      | 0.02        | -0.16      | 0.17        | -0.04       | 0.74        |
| 6MWD (m)                    | 0.28       | 0.01        | -0.25      | 0.03        | 0.08        | 0.51        |
| Perc 15 score (HU)          | -0.35      | 0.004       | -0.11      | 0.38        | -0.04       | 0.76        |

Pearsons bivariate test used. Strength of correlation determined by $r$ and significance by $p$ value.

WCC = White Cell Count, hsCRP = high sensitivity C-reactive Protein, FEV$_1$ = Forced Expiratory Volume in 1 second, 6MWD = Six Minute Walk Distance
Table 3: Variables associated with whole lung pulmonary FDG uptake in participants with COPD assessed by regression analysis.

| Variable (x)                  | Standardised Beta | 95% CI of Beta | Significance |
|-------------------------------|-------------------|----------------|--------------|
| Fibrinogen (g/L)              | 0.30              | 0.00, 0.01     | 0.02         |
| Current smoking (yes/no)      | 0.20              | -0.02, 0.01    | 0.07         |
| FEV1 % predicted              | -0.12             | 0.00, 0.00     | 0.37         |
| Neutrophils (x10^9/l)         | 0.10              | 0.00, 0.00     | 0.41         |
| Log_{10}hsCRP                 | 0.02              | -0.01, 0.01    | 0.90         |

Dependent variable = whole lung FDG uptake (nK_i [ml/cm³/min⁻¹]). Multivariable model, \( R = 0.48, R^2 = 0.23, \) adjusted \( R^2 = 0.17.\)

\( hsCRP = \) high sensitivity C-reactive Protein, \( FEV_1 = \) Forced Expiratory Volume in 1 second.
Table 4: Clinical and imaging measurements for COPD subtypes defined by CT*.

| ADE          | Confluent | Moderate | Mild | Trace | P-value^ |
|--------------|-----------|----------|------|-------|----------|
| **Number (%)** | n=22 | n=9 | n=10 | n=15 | n=15 |
|              | (30%) | (12%) | (14%) | (20%) | (20%) |
| **FEV1**     | 0.99±0.30 | 1.05±0.46 | 1.5±0.73 | 1.4±0.46 | 1.8±0.52 | <0.001 |
| **FVC**      | 3.1±0.78 | 3.1±0.70 | 3.0±1.2 | 2.7±0.75 | 3.1±0.74 | ns |
| **Smoker (%)** | 10 | 11 | 20 | 11 | 7 |
| **Smoking yrs** | 42±6 | 44±13 | 39±12 | 35±11 | 35±13 | ns |
| **6WMD (m)** | 343±104 | 335±145 | 375±98 | 377±81 | 478±115 | <0.05 |
| **Perc15**   | -949±41 | -918±18 | -890±35 | -865±24 | -833±32 | <0.001 |
| **nKi**      | 4.0±1.1 | 4.0±1.1 | 3.9±1.1 | 3.5±1.1 | 3.5±0.9 | ns |

*Only centrilobular emphysema subtypes are analysed. N=74 HRCT COPD scans analysed. 3 (4%) of scans were panlobular emphysema so not included in analysis. No scans were identified as paraseptal emphysema, airway disease or associated features noted.

^Overall comparison across 5 sub-types

ADE = Advanced destructive emphysema, FEV1 = Forced Expiratory Volume in 1 second, FVC = Forced Vital Capacity, 6MWD = Six Minute Walk Distance.
Figure Legends

Figure 1: Pulmonary FDG PET/CT fused image of participant in the study.

Figure 2: Rate of FDG uptake measured using nKi as a marker of whole lung pulmonary inflammation in COPD groups, smokers and never-smokers. Individual values are shown along with median, first and third quartile values. A statistical difference was observed between COPD patients, α₁ATD patients and smokers versus never-smokers as a control group. COPD = Chronic Obstructive Pulmonary Disease, α₁ATD = Alpha-1-anti-trypsin deficiency, Smokers, never-smokers.

Figure 3: Scatterplot of plasma fibrinogen and whole lung nKi values for the whole cohort. Pearson correlation between 111 cases with both fibrinogen and nKi available, r=0.391, p<0.001.

Figure 4: Bland Altman plot of the rate of FDG uptake (nKi). These data were obtained from the baseline and follow-up FDG scans of COPD participants in the placebo arm. The interval between scans was approximately 4 months. Horizontal lines show the mean difference and upper and lower limits (±1.96 standard deviation) of the mean difference between paired baseline and 4 month values.

Figure 5: A) nKi values at baseline and 4 months for participants without an exacerbation in between these time points (n=22). B) nKi values at baseline and 4 months for participants that experienced an exacerbation (n=10).
Figure 2
Figure 3

The scatter plot shows the relationship between fibrinogen concentration (g/L) and nkit (ml/cm3/min-1). The data points are categorized into different groups: smokers, A1ATD-COPD, COPD, and never-smokers. The line of best fit is represented by the equation y = 1.51E-3 + 6.87E-4x, with an R² value of 0.133 for the linear relationship.
Figure 4

![Graph showing scatter plot with difference in nK_i (ml cm^{-3} min^{-1}) against mean nK_i (ml cm^{-3} min^{-1}).]
Figure 5

A

B
Online Supplement for

Quantitative $^{18}$F-FDG PET/CT to assess pulmonary inflammation in Chronic Obstructive Pulmonary Disease

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**Methods:**

**Acquisition**

All subjects were scanned at two sites: Cambridge University Hospitals NHS Foundation Trust using a GE690 PET/CT scanner or at Invicro, Hammersmith using a Siemens Biograph TruePoint 6 PET-CT scanner with closely matched protocols. Participants were told to fast for 6 hours prior to the scan, a blood glucose test was performed and if $>11\text{mmol}$ participants were rescheduled. Prior to the PET scan participants underwent an CT Attenuation Correction (CT-AC) scan; this was acquired under normal breathing. $^{18}\text{F-FDG}$ was administered intravenously in the antecubital vein; the target administered activity was 240MBq. Venous blood samples were drawn from the contralateral arm at 12 time points for the POB correction (at 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 45 and 60 minutes). PET data was acquired under list mode and binned into 23 frames (8x15s, 3x60s, 5x120s, 5x300s, 2x600s); histograms were reconstructed at either 2mm nominal slice thickness using DIFT (Siemens) or 3.27mm nominal slice thickness using 3D FBP (GE). Corrections for attenuation, deadtime, decay and scatter were incorporated. The DICOM files were converted into a single 4D Nifti file for analysis.

**Lung Image Analysis-further information**

To determine the net trapping, or rate of FDG tracer uptake (defined by $K_i$), time activity curves (TACs) of the different lung regions of interest (whole lung, upper, middle and lower (each average of right and left) were obtained and transformed by Patlak graphical analysis. The rate of FDG uptake was quantified using the normalised Patlak graphical technique, $nK_i$, as described previously [3], [10]; where $K_i$ is divided by the intercept of the Patlak plot. The intercept represents the steady state partition coefficient of the tracer between tissue and
plasma within the region of interest. nKi values were averaged in anterior-posterior direction to limit any potential effect of gravity.

Results

Table S1

Comparison of nKi values across regional lung volumes within subject groups.

|        | Upper lung | Middle lung | Lower lung | P-value* |
|--------|------------|-------------|------------|----------|
| COPD   | 3.9 ± 1.3  | 3.6 ± 1.2   | 3.5 ± 1.4  | 0.107    |
| α1ATD-COPD | 3.8 ± 1.1  | 4.2 ± 1.1   | 3.8 ± 1.7  | 0.699    |
| Smokers| 4.5 ± 1.3  | 4.3 ± 1.4   | 4.0 ± 1.3  | 0.685    |
| Never-smokers | 2.7 ± 0.8  | 2.7 ± 0.6   | 3.3 ± 1.4  | 0.264    |

Values are reported as \( \times 10^{-3}\) ml/cm\(^3\)/min\(^{-1}\). *overall comparison for upper, middle and lower lung within subject groups.
**Figure S1** Comparison of nKi values across total pack years smoked quartiles within the usual-COPD group

Box plots starting from left ≤ 25 years, 26 ≤ 41 years, 42 ≤ 60 years, 61 ≤ 110 years. No statistical difference in nKi between quartiles (p>0.1).