ABSTRACT

Background The functional effects of abnormal diffusing capacity for carbon monoxide (DLCO) in ex-smokers without chronic obstructive pulmonary disease (COPD) are not well understood.

Objective We aimed to evaluate and compare well established clinical, physiological and emerging imaging measurements in ex-smokers with normal spirometry and abnormal DLCO with a group of ex-smokers with normal spirometry and DLCO and ex-smokers with Global Initiative for Chronic Obstructive Lung Disease (GOLD) stage I COPD.

Methods We enrolled 38 ex-smokers and 15 subjects with stage I COPD who underwent spirometry, plethysmography, St George’s Respiratory Questionnaire (SGRQ), 6 min Walk Test (6MWT), x-ray CT and hyperpolarised helium-3 (3He) MRI. The 6MWT distance (6MWD), SGRQ scores, 3He MRI apparent diffusion coefficients (ADC) and CT attenuation values below −950 HU (RA950) were evaluated.

Results Of 38 ex-smokers without COPD, 19 subjects had abnormal DLCO with significantly worse ADC (p=0.01), 6MWD (p=0.008) and SGRQ (p=0.01) but not RA950 (p=0.53) compared with 19 ex-smokers with normal DLCO. Stage I COPD subjects showed significantly worse ADC (p=0.02), RA950 (p=0.0008) and 6MWD (p=0.005), but not SGRQ (p=0.59) compared with subjects with abnormal DLCO. There was a significant correlation for 3He ADC with SGRQ (r=0.34, p=0.02) and 6MWD (r=−0.51, p=0.0002).

Conclusions In ex-smokers with normal spirometry and CT but abnormal DLCO, there were significantly worse symptoms, 6MWD and 3He ADC compared with ex-smokers with normal DLCO, providing evidence of the impact of mild or early stage emphysema and a better understanding of abnormal DLCO and hyperpolarised 3He MRI in ex-smokers without COPD.

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is characterised by chronic progressive expiratory flow limitation that develops as a result of the lung’s inflammatory response to inhaled toxic gases and particles, primarily from tobacco smoke. Airflow limitation is caused by both small airway disease (obstructive bronchiolitis) and parenchymal destruction (emphysema) but the relative contributions of these pathologies vary from person to person.

When COPD is suspected based on symptoms, such as dyspnoea, chronic cough or sputum production, and/or a history of exposure to risk factors, airflow limitation is measured using spirometry and severity is determined according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria. This approach, however, has been acknowledged to potentially result in an over diagnosis of COPD in the elderly, as well as under diagnosis of mild or early stage COPD.1

The COPDGene study recently reported low forced expiratory volume in 1 s (FEV₁) and normal FEV₁/forced vital capacity (FVC) in ex-smokers with significant symptoms and decreased 6 min Walk Distance (6MWD), and defined these patients as GOLD unclassified (GOLD-U). Until now, ex-smokers with GOLD-U or those with ‘non-obstructive’ or ‘pure’ emphysema without airflow limitation have been systematically excluded from COPD studies. With respect to non-obstructive emphysema, there have been a few case reports and pilot studies that described significant smoking history, severe symptoms and abnormal diffusing capacity for carbon monoxide (DLCO) in patients concomitant with normal expiratory airflow. A recent study also reported that otherwise normal asymptomatic smokers with abnormal DLCO showed evidence of endothelial microparticles in the circulation—a marker of early lung destruction associated with emphysema. Although abnormal DLCO in ex-smokers is a valuable marker of lung function impairment, even in the absence of airflow limitation, the relationship between DLCO with other functional markers (ie, symptoms and exercise limitation) is not well understood. We hypothesised that subjects with abnormal DLCO without airflow limitation would have imaging evidence of early or mild emphysema with measurable functional consequences.

Multidetector CT and hyperpolarised helium-3 (³He) MRI have been used independently to measure emphysema and airways disease as distinct phenotypes in COPD. In particular, hyperpolarised ³He MRI apparent diffusion coefficients (ADC) provide a way to sensitively measure regional lung tissue destruction—the hallmark of emphysema. Abnormally elevated ³He ADC have previously been reported in asymptomatic smokers without COPD although the relationship between ³He MRI ADC in early disease with symptoms and other physiological measurements has never been reported and their functional impact is not known. To better understand the consequences of early or mild disease in ex-smokers, we have evaluated well established clinical, physiological as well as emerging imaging measurements in ex-smokers with normal spirometry but abnormal DLCO as well as ex-smokers with GOLD stage I COPD and those with normal spirometry and DLCO.

**MATERIALS AND METHODS**

**Study subjects**

All subjects provided written informed consent to the protocol approved by the local research ethics board and Health Canada, and the study was compliant with the Personal Information Protection and Electronic Documents Act (Canada) and the Health Insurance Portability and Accountability Act (USA). Ex-smokers were recruited from a local tertiary care centre and by advertisement. Thirty-eight subjects were enrolled who were ex-smokers without a diagnosis of COPD and 15 ex-smokers were enrolled with a previous diagnosis of GOLD stage I COPD, all of whom were 60–85 years of age, with a smoking history ≥10 pack-years. Subjects without a diagnosis of COPD had no history of previous chronic or current respiratory disease and were classified according to American Thoracic Society/European Respiratory Society recommendations on the approximate lower limits of normal for DLCO such that normal is defined as DLCO ≥75%pred and abnormal DLCO <75%pred.

Spirometry, plethysmography and other tests

Spirometry was performed using an EasyOne spirometer (Medizintechnik AG, Zurich, Switzerland) according to the American Thoracic Society guidelines. Lung volumes were measured using body plethysmography and DLCO was assessed using the attached gas analyser (MedGraphics Corporation, St Paul, Minnesota, USA). The St Georges Respiratory Questionnaire (SGRQ) was administered and a standard 6 min Walk Test (6MWT) was performed.

**Image acquisition**

MRI was performed on a whole body 3.0 T Discovery 750MR (General Electric Health Care, Milwaukie, Wisconsin, USA) MRI system. ³He gas was polarised to 30–40% (Helispin) and doses (5 ml/kg body weight) were administered in 1.0 l Teflar bags diluted with medical grade nitrogen (N2) (Linde, Ontario, Canada). ³He MRI diffusion weighted images were acquired using a fast gradient recalled echo sequence immediately following inhalation of the ³He/N2 gas mixture during breath hold conditions. Two interleaved images were acquired (14 s total data acquisition, repetition time (TR)/echo time (TE)/flip angle=7.6 ms/3.7 ms/8°, field of view (FOV)=40×40 cm, matrix 128×128, seven slices, 30 mm slice thickness, 0 gap), with and without additional diffusion sensitisation with b=1.6 s/cm² (gradient amplitude (G)=1.94 G/cm, rise and fall time=0.5 ms, gradient duration=0.46 ms, diffusion time=1.46 ms).

CT was performed on a 64 slice Lightspeed VCT scanner (General Electric Health Care) (64×0.625 mm, 120 kVp, 100 effective mA, tube rotation time=500 ms, pitch=1.0). A single spiral acquisition was acquired in breath hold after inhalation of 1.0 l of N2 from functional residual capacity. Reconstruction was performed (1.25 mm) using a standard convolution kernel.

To minimise the potential for differences in the levels of inspiration between ³He MRI and CT, extensive coaching was performed prior to the imaging sessions to ensure subjects could completely inspire the contents of the 1.0 l bag. The order of ³He MRI and CT acquisition was randomised for each subject.

**Image analysis**

Regions of signal void were quantified as the ³He ventilation defect per cent (VDP). ³He ADC maps were also generated as previously described. Regional differences in ADC were evaluated in the anterior–posterior (AP) direction. The AP gradient (APD) was the slope of the line of best fit that described the change in ADC as a function of distance (in cm). Analysis of CT was performed using the Pulmonary Workstation 2.0 (VIDA Diagnostics Inc, Coralville, Iowa, USA). Wall area per cent (WA%) was measured for the segmental and subsegmental airways and the relative area with attenuation values below −950 HU (RA950) was generated.

**Statistical methods**

A multivariate analysis of variance was performed using IBM SPSS Statistics V20.0 (SPSS Inc, Chicago, Illinois, USA). Univariate comparisons were performed using an unpaired two tailed t test, and Welch’s correction was used when the F test for equal variances was significant using GraphPad Prism V4.00. A Fisher’s exact test was performed for categorical variables. Linear regression (r²) and Pearson correlation coefficients (r) were used to determine correlations using GraphPad Prism V4.00. Results were considered significant when the probability of making a type I error was less than 5% (p<0.05).

**RESULTS**

We enrolled 53 ex-smokers, 38 subjects without a diagnosis of COPD and 15 subjects diagnosed with stage I COPD. Of the 38 ex-smokers without COPD, half had normal DLCO without airflow obstruction (ND, n=19) and the other half had...
normal DLCO without airflow obstruction (AD, n=19). Table 1 shows the subject demographics as well as pulmonary function, SGRQ, 6MWD, CT and 3He MRI measurements for all subjects categorised according to their spirometry and DLCO results.

Subjects with abnormal DLCO without airflow obstruction (AD) were not significantly different from ex-smokers with normal DLCO (ND) and stage I COPD subjects with respect to age, BMI, pack-years, years since smoking cessation, change in SpO2 after the 6MWT, CT WA% and 3He VDP. However, there were significantly more female AD subjects than ND (p=0.02) and stage I COPD (p=0.01) subjects.

Figure 1 shows the central coronal 3He MRI static ventilation image and 3He MRI ADC map for subjects with ND, AD and stage I COPD. As shown in table 1, AD subjects had a significantly worse 3He ADC (0.30±0.03 cm²/s; p=0.01), 6MWD (341±95 m; p=0.008) and SGRQ total score (29±21; p=0.01) compared with ND subjects, but there was no significant difference for RA950 (p=0.53). In comparison with stage I COPD, AD subjects had a significantly reduced 6MWD (341±95 m; p=0.005), FVC (93±12%pred; p=0.001), RA950 (1.6±1.1; p=0.0008) and ADC (0.30±0.03 cm²/s; p=0.02), and a significantly greater FEV1/FVC (80±7%; p<0.0001) and no significant difference for SGRQ total score (p=0.59).

Figure 2A shows the mean ADC on a slice by slice basis in the anterior to posterior direction for ND, AD and stage I COPD subjects. For AD ex-smokers, the ADC gradient in the anterior–posterior direction (ADC APG) was significantly different from COPD subjects. For ND, the ADC APG was significantly lower than for AD and stage I COPD (p=0.0002). Figure 2B shows the significant correlation between ADC APG and the 6MWD (r=0.51, p=0.0002).

Figure 3 shows the correlations between 3He ADC and CT RA950 with DLCO, SGRQ and 6MWD. There was a significant correlation between 3He ADC and DLCO (r=−0.55, p<0.0001) and SGRQ (r=−0.34, p=0.02) but not 6MWD (r=−0.17, p=0.24), and as shown in figure 2B, ADC APG was significantly correlated with 6MWD. RA950 was significantly correlated with DLCO (r=−0.31, p=0.03) but not SGRQ (r=0.24, p=0.10) or 6MWD (r=0.0013, p=0.99).

Table 1 Clinical, functional and radiographic measurements of asymptomatic ex-smokers with normal and abnormal diffusion capacity of the lung for carbon monoxide, compared with GOLD stage I chronic obstructive pulmonary disease

<table>
<thead>
<tr>
<th>Subject demographics</th>
<th>ND (n=19)</th>
<th>AD (n=19)</th>
<th>Stage I COPD (n=15)</th>
<th>Significance of difference (p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>71 (7)</td>
<td>74 (7)</td>
<td>77 (5)</td>
<td>0.09</td>
</tr>
<tr>
<td>No of women (n)</td>
<td>3</td>
<td>11</td>
<td>2</td>
<td>0.02</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.5 (3.4)</td>
<td>28.6 (4.0)</td>
<td>28.4 (4.0)</td>
<td>0.46</td>
</tr>
<tr>
<td>Pack-years</td>
<td>25 (12)</td>
<td>32 (23)</td>
<td>49 (36)</td>
<td>0.25</td>
</tr>
<tr>
<td>Time since quitting (years)</td>
<td>26 (9)</td>
<td>24 (14)</td>
<td>21 (14)</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Pulmonary function tests

| FEV1%pred | 107 (13) | 99 (12) | 95 (13) | 0.07 | 0.34 |
| FVC%pred | 98 (12) | 93 (12) | 108 (14) | 0.16 | 0.001 |
| FEV1/FVC | 80 (6) | 80 (7) | 63 (5) | 0.73 | <0.0001 |
| IC%pred | 112 (17) | 103 (22) | 103 (17) | 0.17 | 0.99 |
| RV%pred | 107 (17) | 71 (25) | 114 (29) | 0.58 | 0.45 |
| TLC%pred | 101 (10) | 101 (15) | 103 (19) | 0.96 | 0.12 |
| RV/TLC%pred | 101 (13) | 104 (16) | 103 (18) | 0.49 | 0.77 |

6MWT

| Pre 6MWT SpO2% | 97 (2) | 95 (2) | 95 (2) | 0.06 | 0.57 |
| Δ 6MWT SpO2% | 0 (2)* | 0 (2)* | −1 (3) | 0.55 | 0.25 |
| Distance (m) | 430 (99) | 341 (95) | 417 (41) | 0.008 | 0.005 |

SGRQ

| Symptoms | 18 (17)* | 36 (30)* | 36 (22) | 0.04 | 0.99 |
| Activity score | 19 (21)* | 41 (24) | 36 (25)* | 0.006 | 0.54 |
| Impact score | 6 (11)* | 17 (18) | 15 (13)* | 0.04 | 0.68 |
| Total score | 12 (14)* | 29 (21) * | 25 (17)* | 0.01 | 0.59 |

CT measurements

| RA950 | 1.36 (1.25) | 1.60 (1.06) | 5.50 (3.16) | 0.53 | 0.0008 |
| WA% | 57 (4) | 59 (2) | 58 (2) | 0.17 | 0.23 |

3He MRI measurements

| ADC (cm²/s) | 0.27 (0.03)* | 0.30 (0.03)* | 0.36 (0.08) | 0.01 | 0.02 |
| VDP (%) | 6 (3)* | 7 (4)* | 9 (5)* | 0.40 | 0.07 |

Values are mean (SD).

Missing values: SpO2 not recorded post-6MWT (n=1, normal DLCO; n=2, abnormal DLCO); incomplete SGRQ questionnaire (n=3, normal DLCO; n=1, abnormal DLCO; n=1, COPD); and image acquisition failures (n=1, normal DLCO; n=2, abnormal DLC0; n=1, COPD).

†n=18, †n=17, †n=14, §n=16.

AD, abnormal DLCO; ADC, apparent diffusion coefficient; BMI, body mass index; COPD, chronic obstructive pulmonary disease; DLCO, diffusion capacity of the lung for carbon monoxide; FEV1, forced expiratory volume in 1 s; FVC, force vital capacity; GOLD, Global Initiative for Chronic Obstructive Lung Disease; IC, inspiratory capacity; 6MWT, 6 min Walk Test; ND, normal DLCO; RA950, relative area with attenuation values below −950 HU; RV, residual volume; SGRQ, St George’s Respiratory Questionnaire; SpO2, peripheral oxygen saturation; TLC, total lung capacity; VDP, ventilation defect per cent; WA%, wall area per cent.
DISCUSSION
To better understand the relationship between lung structural markers, symptoms and physiological measurements in ex-smokers, we evaluated 53 ex-smokers, including 38 subjects who did not have a diagnosis of COPD and 15 subjects with stage I COPD, and observed the following. (1) Nineteen of 38 ex-smokers showed normal spirometry and CT but abnormal DLCO and 19/38 ex-smokers showed normal spirometry, CT and DLCO. (2) Subjects with abnormal DLCO had significantly worse 6MWD compared with stage I COPD ex-smokers and significantly worse $^3$He ADC, SGRQ and 6MWD compared with subjects with normal DLCO. (3) Subjects with abnormal DLCO had significantly smaller $^3$He MRI ADC AP gradients compared with subjects with normal DLCO.

We were surprised that half of the ex-smokers without COPD showed abnormal DLCO and significantly worse $^3$He ADC, but
with normal CT, which, based on previous studies, was an unexpected result. Although we were not able to confirm significant disease other than emphysema that could account for these findings, we note that a previous evaluation of 10 younger asymptomatic smokers (mean age=47 years, range=23–73) showed that three of five subjects aged 60 years or older also reported DLCO<75%pred. In ex-smokers, abnormal DLCO is thought to reflect diminished lung surface area available for gas exchange although DLCO also reflects the volume of blood in the pulmonary capillaries and thickness of the alveolar capillary membrane, related to bronchiectasis and interstitial lung disease. Abnormally low DLCO is also consistent with pulmonary vascular disease, and such patients exhibit normal spirometry, dyspnoea on exertion and a decline in oxygen saturation with exertion. In the current study, AD subjects did not show reduced oxygen saturation during the 6MWT nor did they report a history of pulmonary vascular disease, so there was no evidence to support the notion that pulmonary vascular disease was responsible for the abnormal exercise performance and dyspnoea observed here. Although DLCO is a very sensitive marker of emphysema in smokers, reproducibility can be low, and in some cases, low to moderate correlations have been reported between DLCO and pathological assessments of emphysema.

Previous work by Woods and Hogg compared 3He ADC with histology measurements of emphysema in explanted lungs and showed that ADC values could be used to distinguish normal from emphysematous lung tissue with greater precision than the mean linear intercept measurement from histology samples. Another previous study in COPD showed that while 3He ADC correlated significantly with CT measurements (ie, RA950), stronger correlations were observed for 3He ADC and DLCO than for RA950 and DLCO. In asymptomatic smokers, 3He ADC was shown to correlate with DLCO but there was no significant correlation between DLCO and CT RA950. Finally, abnormally elevated 3He ADC values were previously observed in never smokers exposed to significant second hand-smoke compared with never smokers with no such exposure. Taken together, these previous findings support the observation here that elevated 3He ADC in ex-smokers with abnormal DLCO may reflect mild emphysema not detected by CT. Our observations are also consistent with previous reports and the identification of mild emphysema using histology that was not predicted using preoperative CT. While we cannot rule out the presence of small airways disease in subjects with AD, there was no significant difference between the AD and ND subjects for 3He VDP and CT WA%, both of which provide estimates of airways disease. Taken together, these results suggest that 3He ADC is sensitive to very mild emphysema in subjects with abnormal DLCO who have no CT evidence of airways disease or emphysema.

Concomitantly with significantly elevated 3He ADC, we observed significantly worse 6MWD in AD compared with COPD and ND ex-smokers. This is an important finding and the first to provide evidence of a relationship between 3He MRI ADC reflective of early or mild emphysema and exercise capacity. It is also important to note that the ratio of female/male ex-smokers with AD was 11/8 (1.4), and for ND this ratio was 3/16 (0.2). Although the current study was not powered to evaluate sex differences, previous evidence suggests that female sex is significantly associated with early onset COPD. However, previous studies have also shown that emphysema dominates in men compared with women, whereas here the sex ratio was reversed. We note that imaging was performed at a fixed volume and because there were more women in the AD group (who potentially had smaller lungs), we investigated the relationship between lung size and 3He ADC and observed no correlation for 3He ADC with height (r=-0.36, p=0.18), total lung capacity (r=0.33, p=0.21) or thoracic cavity volume (r=-0.20, p=0.45). Therefore, the elevated ADC in the AD subjects observed here was not related to lung size and cannot explain the preponderance of female subjects in the AD subgroup. Consistent with our findings, the 6MWD in COPD was also previously shown to be lower for FEV1 matched women versus men.

We took advantage of the fact that 3He MRI diffusion weighted images were acquired in the supine position and...
measured compression of the dependent lung due to gravity. Several sites have reported smaller $^3$He ADC in the dependent lung (or posterior slices) relative to the non-dependent lung,\textsuperscript{25 44 45} likely due to gravitational compression of the parenchyma. In COPD subjects,\textsuperscript{25 44} this anterior to posterior difference is significantly smaller and this is thought to be due to regional gas trapping that counteracts gravitational compression of the dependent regions. Here we observed that these gradients were significantly smaller in AD subjects compared with ND subjects, suggesting that regional gas trapping was greater in the AD subgroup.

Finally, we showed that $^3$He ADC was significantly correlated with SGRQ and that $^3$He ADC APGs were significantly correlated with the 6MWD. The significant relationships between $^3$He ADC with respiratory symptoms and exercise capacity suggest that in early emphysema, symptomatic changes can go unnoticed in older patients even when standardised tests report significant changes in health related quality of life and exercise capacity. While elevated $^3$He ADC in asymptomatic ex-smokers was previously described,\textsuperscript{14 15} the imaging to exercise capacity and imaging to symptoms correlations observed here in very early emphysema are novel findings. The unexpected finding of $^3$He ADC AP gradient correlations with 6MWD also provides more evidence about the role of mild emphysema and regional gas trapping that may together lead to exercise limitation even in early disease. AD ex-smokers also reported a SGRQ that was not significantly different from the stage I COPD ex-smokers, and worse than ND subjects, which supports previous reports of compromised health related quality of life\textsuperscript{46} and reduced work capacity in very early disease.\textsuperscript{47}

This study was limited by the relatively small number of subjects evaluated, although we note that this is the single largest prospective study that directly compared CT, symptoms, exercise capacity and $^3$He MRI in ex-smokers with and without airflow obstruction. We admit that we were surprised to find such a large proportion of asymptomatic ex-smokers without airflow limitation and abnormal DLCO in this study. This finding raises the important question of whether this subgroup is...
atypical or perhaps this is a unique finding because ‘asymptomatic’ ex-smokers are rarely administered the SGRQ or the 6MWT. Importantly, the selection criteria, manner and location for subject recruitment are those we have previously used for the recruitment of older ex-smokers, and typical of other studies. It is possible that in this unique subgroup, patients were less likely to recognise and report symptoms. Our results certainly raise many intriguing questions regarding whether these subjects are unusual or whether we have simply uncovered a group of older ex-smokers with both unrecognised mild emphysema and functional limitations.

In summary, we evaluated 38 ex-smokers without airflow limitation and 15 ex-smokers with COPD. In the absence of spirometry or CT abnormalities, half of the ex-smokers without COPD showed abnormal DL\textsubscript{CO} and abnormally elevated \textsuperscript{3}He ADC, consistent with early or mild emphysema. These subjects had significantly and markedly worse 6MWD and SGRQ compared with ex-smokers with normal ADC and DL\textsubscript{CO}, and worse 6MWD than subjects with COPD. These findings provide a better understanding of abnormal DL\textsubscript{CO} in ex-smokers without COPD.

Acknowledgements We thank S McKay and S Halko for clinical coordination and clinical database management, and T Szekeres for MRI of research volunteers.

Contributors MK was responsible for acquisition of the data, data analysis and interpretation, and drafting, final revisions and final approval of the manuscript. AO was responsible for acquisition of the data, and revision and final approval of the manuscript. SS was responsible for acquisition of the data, and revision and final approval of the manuscript. HOC was responsible for conception and design, data interpretation, final revisions to the manuscript and final approval of the manuscript. NAMP was responsible for conception and design, data interpretation, final revisions to the manuscript and final approval of the manuscript. GP, the principal investigator, was responsible for conception and design, data acquisition and analysis plan and interpretation, drafting, final revisions and final approval of the manuscript, as well as guarantor of the integrity of the data. GP was also responsible for good clinical practice.

Funding MK and SS gratefully acknowledge scholarship support from the Natural Sciences and Engineering Research Council (NSERC, Canada) and GP gratefully acknowledges support from a Canadian Institutes of Health Research (CIHR) New Investigator Award. Ongoing research funding from the CIHR Team grant CIHR# 97687 is gratefully acknowledged.

Competing interests None.

Ethics approval The study was approved by the local research ethics board and Health Canada, and the study was compliant with the Personal Information Protection and Electronic Documents Act (Canada) and the Health Insurance Portability and Accountability Act (USA).

Provenance and peer review Not commissioned; externally peer reviewed.

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