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Longitudinal Computed Tomography and Magnetic Resonance Imaging of COPD: Thoracic Imaging Network of Canada (TINCan) Study Objectives

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Abstract

Although the human and societal burden and cost of COPD is staggering, there are few clinical tools that provide earlier diagnoses or a means to regionally monitor disease in a way that might lead to improved therapies and outcomes. In acknowledgement of the current gaps in COPD therapy, the objective of the Thoracic Imaging Network of Canada (TINCan) is to improve COPD patient phenotyping through imaging, to provide methods and imaging-based intermediate endpoints for the development of new treatments, and to evaluate disease progression and patient-based outcomes in COPD patients and those at risk of COPD. Here we summarize and outline the TINCan study protocol and describe our objectives. TINCan is a prospective study that aims to identify and quantify novel COPD phenotypes from thoracic computed tomography (CT) and thoracic hyperpolarized noble gas magnetic resonance imaging (MRI) in 200 ex-smokers, 50 years of age or greater, including asymptomatic ex-smokers with normal pulmonary function and Global initiative for chronic Obstructive Lung Disease (GOLD) Unclassified (U) , and GOLD stages I-IV patients. Baseline and 2-year follow-up measurements will be acquired using spirometry, plethysmography, diffusing capacity of the lung for carbon monoxide (DL_{CO}), St. George's Respiratory Questionnaire (SGRQ), 6-minute walk test (6MWT), thoracic CT and hyperpolarized helium-3 (^{3}He) and xenon 129 (^{129}Xe) MRI. TINCan provides a unique opportunity to quantify and compare novel lung structure-function measurements and investigate their relationship with well-established clinical measurements and outcomes. Such intermediate endpoints of COPD may be used to stratify patients for personalized treatments and to develop new treatments to improve outcomes, a long-standing clinical goal.

Abbreviations: Thoracic Imaging Network of Canada, **TINCan**; computed tomography, **CT**; magnetic resonance imaging, **MRI**; Global initiative for chronic Obstructive Disease, **GOLD**; diffusing capacity of the lung for carbon monoxide, **DL_{CO}**; St. George's Respiratory Questionaire, **SGRQ**; 6-minute walk test, **6MWT**; helium-3, **^{3}He**; xenon-129, **^{129}Xe**; COPD Genetic Epidemiology, **COPDGene**; Global Chest Symptoms Questionaire, **GCSQ**; Capacity of Daily Living questionnaire, **CDLM**; Food Frequency Questionaire, **FFQ**; Community Healthy Activities Model Program for Seniors, **CHAMPS**; Short Form-36 version 2, **SF-36V2**; Hospital Anxiety and Depression Scale, **HADS**; Short Form of the Health and Labour Questionaire, **SF-HLQ**; COPD Assessment Test, **CAT**; cardiopulmonary exercise test, **CPET**; Centre for Epidemiological Studies of Depression, **CES-D**; Functional Assessment of Chronic Illness Therapy, **FACIT**; Canadian Chronic Obstructive Lung Disease study, **CanCOLD**; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints, **ECLIPSE**; Subpopulations and Intermediate Outcomes Measures in COPD, **SPIROMICS**; American Thoracic Society, **ATS**; European Respiratory Society, **ERS**; forced expiratory volume in 1 second, **FEV_1**; functional residual capacity, **FRC**; residual volume, **RV**; total lung capacity, **TLC**; percent of predicted value, **%pred**; nitrogen gas, **N_2**; lumen area, **LA**; airway wall area, **Aaw**; wall area percentages, **WA%**; internal perimeter of 10mm, **Pi_{10}**; Hounsfield units, **HU**; relative area with attenuation values below -950, **RA_{-950}**; low attenuation cluster, **LAC**; ventilation defect volume, **VDV**; ventilation defect percent, **VDP**; apparent diffusion coefficient, **ADC**; zero-inflated Poisson, **ZIP**

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Introduction

Chronic obstructive pulmonary disease (COPD) affects over 600 million people[1] and is the third leading cause of death worldwide.[2] Despite the huge societal, patient and health care system burden,[3,4] COPD remains largely underdiagnosed[5,6] and undertreated.[7] Although COPD is currently diagnosed and its severity graded by non-fully-reversible airflow limitation, there is tremendous heterogeneity in the underlying regional abnormalities within and across COPD severity categories.[8] However, until recently, COPD patient measurements have been dominated by airflow and physiological measurements (e.g. inert gas washout tests[9]), or invasive histological measurements and this has limited the scope of research related to regional COPD phenotypes and their effect on outcomes and disease pathogenesis.

Unlike spirometry and other measurements made at the mouth, pulmonary imaging provides quantitative, regional and independent measurements of the underlying disease contributions in COPD.[10,11] It is well-established that x-ray computed tomography (CT) provides a way to non-invasively measure the extent and regional distribution of emphysema and airway wall thickening in COPD[10,12-14] CT measurements also correlate significantly with mortality,[15] disease progression,[16-18] and exacerbation risk[19] and may identify suitable candidates for surgical intervention.[20-23] Complementary to this, pulmonary functional imaging using hyperpolarized noble gas (^{3}He and ^{129}Xe) magnetic resonance imaging (MRI)[24] provides unique and simultaneous microstructure and functional information of both the airways and parenchyma. MRI pulmonary structural and functional abnormalities are spatially reproducible over time[25-29] and correlate moderately with pulmonary function measurements.[11,30-34]

Unfortunately, unlike the case for many chronic diseases where *in vivo* imaging has been exploited to improve patient outcomes, the inclusion of imaging in the clinical workup and prospective study of COPD therapies remains very limited.[35, 36] Recently, however, large, prospective, multi-center cohort studies of COPD, such as the COPD Genetic Epidemiology (COPDGene)[37] the Canadian Chronic Obstructive Lung Disease (CanCOLD),[38] the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE)[39] and the Subpopulations and Intermediate Outcomes Measures in COPD (SPIROMICS)[40] studies as shown in Table 1, have included CT endpoints. Although these multi-center studies have provided a wealth of clinical and biological data, there are no longitudinal COPD studies that have incorporated emerging and non-radiation based imaging methods, such as provided by ^{1}H and hyperpolarized noble gas MRI. Emerging MRI approaches may provide complementary functional and microstructural information related to COPD disease heterogeneity and progression. Hence, the overarching objective of the Thoracic Imaging Network of Canada (TINCan) study is to determine quantitative *structural* and *functional* COPD imaging phenotypes that can be used to improve health outcomes in COPD patients. The 4 key objectives of TINCan are: 1) determine the significant cross-sectional univariate and multivariate relationships for ^{3}He with ^{129}Xe MRI and CT-derived phenotypes of airway and parenchymal abnormalities, 2) determine the longitudinal changes and relationships for ^{3}He MRI-derived phenotypes of airway and parenchymal abnormalities with other COPD measurements, 3) determine the associations

Table 1. TINCan and Recent COPD Cohort Studies

		Measurements	Start/FU
Thoracic Imaging Network Canada ***TINCan***	N=200	**Questionnaires:** • SGRQ **Pulmonary Function Tests:** • Spirometry, plethysmography, 6MWT **Imaging:** • CT; FRC+1.0L (40mAs),[a] full inspiration (200mAs)[d] and full expiration (50mAs)[d] • MRI; ^{1}H, ^{3}He and ^{129}Xe MRI (FRC+1.0L),	2010/ 2yr
Canadian Cohort Obstructive Lung Disease ***CanCOLD***	N=1800	**Questionnaires[f]:** • Symptoms and disability (MRC, GCSQ, CDLM), dietary habits (FFQ), physical activity (CHAMPS), health status (SF-36V2), psychosocial (HADS), consequences of health problems for employment (SF-HLQ), COPD specific (CAT, SGRQ) **Pulmonary Function Tests:** • Spirometry, plethysmography, 6MWT, CPET **Imaging[e]:** • CT; full inspiration (40mAs) **Other:** Blood test	2009/ 18mo 3yr
Evaluation of COPD to Longitudinally Identify Predictive Surrogate Endpoints ***ECLIPSE***	N=2180	**Questionnaires:** • Standardized ATS epidemiology questionnaire,[a] depression questionnaire (CES-D),[g] fatigue questionnaire (FACIT),[g] health status (SGRQ, MRC, BODE) **Pulmonary Function Tests:** • Spirometry, plethysmography, impulse oscillometry, exhaled carbon monoxide, 6MWT **Imaging[e]:** • CT; full inspiration (40mAs) **Other:** Blood test, sputum, urine, exhaled breath condensate,[g] Exacerbation assessment	2005/ 1yr 2yr 3yr
Subpopulations and Intermediate Outcomes Measures in COPD Study ***SPIROMICS***	N=3200	**Questionnaires:** • SGRQ, MOT Short Form-12, CAT, MRC, sleep questionnaires, veterans specific activity questionnaire, HADS, FACIT, questionnaire for ease of cough and sputum clearance **Pulmonary Function Tests:** • Spirometry, exhaled carbon monoxide,[b] 6MWT **Imaging[c]:** • CT; full inspiration and full expiration (mAs adjusted based on BMI) **Other[b]:** Blood test, sputum, urine	2010/ 1yr 2yr 3yr
Genetic Epidemiology of COPD ***COPDGene***	N=10000	**Questionnaires:** • Standardized ATS epidemiology questionnaire, SGRQ **Pulmonary Function Tests:** • Spirometry, 6MWT **Imaging:** • CT; full inspiration (200mAs) and normal expiration (50mAs) **Other:** Blood test	2007/ 5yr

BODE index = body mass index, airflow obstruction, dyspnea and exercise capacity. [a]performed at baseline; [b]performed at year 1, 2 and 3; [c]performed at year 1 and 2 [d]performed at year 2; [e]performed at baseline and year 3; [f]performed at baseline, 18 months and year 3; [g]performed at year 3

between MRI and CT imaging phenotypes of COPD with exacerbations, and 4) determine whether MRI and CT phenotypes are predictors of longitudinal changes in pulmonary function measurements, lung volumes, symptoms and functional capacity. To our knowledge, this is the first such large patient group comparison of structure-function imaging with prospective cross-sectional and longitudinal components. This study was designed to be complementary to larger ongoing COPD investigations and to address some of the important information gaps that remain. Here we describe the study plan and aims for TINCan and the potential impact of this study to improve our understanding of COPD patient phenotypes.

Methods

Participants

As shown in the schedule of study assessments in Table 2, plans are to enroll 200 participants who will attend a baseline and a two-year follow-up visit. Imaging evaluations include thoracic CT and hyperpolarized ^{3}He MRI at baseline and follow-up imaging at year two using both ^{3}He and ^{129}Xe MRI as well as CT. Imaging will be performed at Robarts Research Institute (Ontario, Canada); MR image analysis will be performed at Robarts Research Institute and CT image analysis will be performed at Vancouver General Hospital (Vancouver, Canada). The goal is to recruit 200 ex-smokers with and without COPD between 50 and 80 years of age, including approximately 100 ex-smokers without airflow limitation and COPD Global initiative for chronic Obstructive Lung Disease (GOLD) stage Unclassified (U) and 100 ex-smokers with COPD GOLD stages I, II, III and IV. We previously demonstrated significant longitudinal changes in COPD ex-smokers in a two-year longitudinal pilot study and therefore, we limited our evaluation to ex-smokers. Ex-smokers had ceased smoking ≥1-year prior to the study visit but there was no maximum cut-off for when ex-smokers had ceased smoking. COPD was defined as post-bronchodilator forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio less than 0.70 according to the modified GOLD criteria.[1]

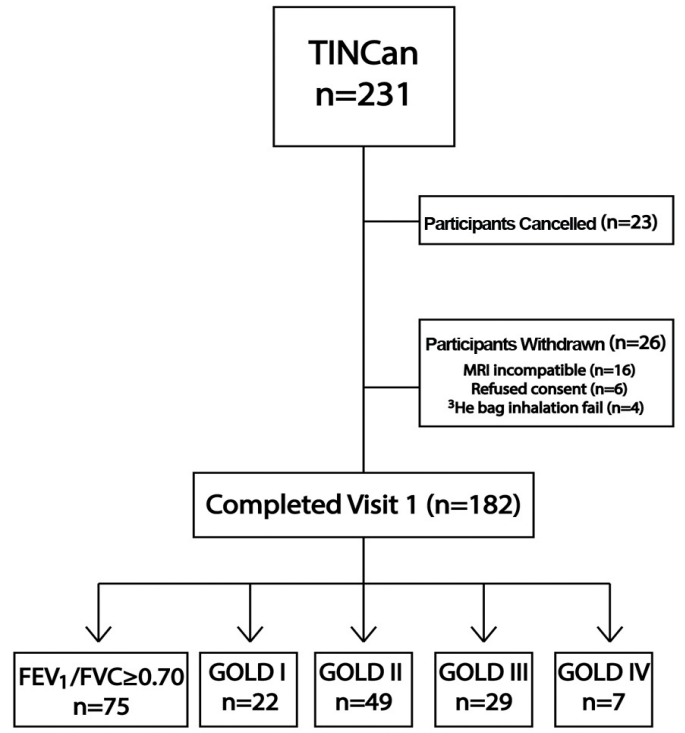
Recruitment and Retention

As shown in Figure 1, thus far, 231 potential participants have been screened for participation in the TINCan study; of those 231 individuals, 23 individuals cancelled prior to consent, 6 individuals refused consent, 16 individuals were excluded due to MRI incompatibilities and 4 individuals were excluded because they were unable to perform the ^{3}He gas inhalation and breath-hold. Thus far, a total of 182 participants have completed visit 1 and their demographic and pulmonary function measurements are provided in Table 3. The projected recruitment phase for TINCan is 2 years. An important goal of TINCan is to provide a better understanding of the early or mild subclinical disease, and therefore we plan to enroll a large number of ex-smokers

Table 2. Schedule of TINCan Study Assessments

Participants	Assessments	Initial Visit	2 Years
All Participants	Informed Consent	✓	✓
	Vital Signs, Height and Weight	✓	✓
	Blood Pressure	✓	✓
	Spirometry and Plethysmography	✓	✓
	SGRQ	✓	✓
	6MWT	✓	✓
	Borg Scale (Pre- and Post-6MWT)	✓	✓
	Exacerbations	✓	✓
	CT; FRC+1.0L	✓	-
	CT; Full Inspiration	-	✓
	CT; Full Expiration	-	✓
	^{1}H MRI	✓	✓
	^{3}He Static Ventilation MRI	✓	✓
	^{3}He Diffusion-weighted MRI	✓	✓
	^{129}Xe Static Ventilation MRI	-	✓
	^{129}Xe Diffusion-weighted MRI	-	✓
Select COPD Participants			
	^{129}Xe Static Ventilation MRI	✓	✓
	^{129}Xe Diffusion-weighted MRI	✓	✓

Figure 1. Consort Diagram of TINCan Participant Recruitment



For TINCan, 231 individuals were contacted and 208 individuals attended the baseline study visit. Of the 208 individuals who attended the baseline visit, 6 individuals refused consent, 16 individuals were excluded due to MRI incompatibilities and 4 individuals were excluded because they were unable to perform the ^{3}He gas inhalation and breath-hold. Complete visits, defined as one in which individuals were able to complete all study evaluations was performed for 182 individuals; 75 individuals did not have airflow limitation ($FEV_1/FVC \geq 0.70$) and 107 individuals had COPD defined using spirometry according to GOLD criteria.[1]

Table 3. Participant Demographics and Pulmonary Function Measurements for All TINCan Participants at Baseline

	$FEV_1/FVC>0.70$ (n=75)	GOLD I (n=22)	GOLD II (n=49)	GOLD III (n=29)	GOLD IV (n=7)
Age yrs (±SD)	69 (10)	75 (7)	69 (8)	70 (9)	68 (6)
Male n (%)	45 (51)	21 (95)	28 (57)	19 (66)	4 (57)
BMI (±SD)	28 (5)	28 (4)	26 (4)	27 (6)	25 (4)
Pack years (±SD)	23 (18)	48 (36)	51 (25)	58 (35)	53 (25)
6MWD m (±SD)	404 (92)	419 (47)	388 (89)	335 (90)	179 (144)
SGRQ Total Score (±SD)	27 (22)	20 (16)	38 (14)	55 (13)	61 (15)
FEV1%Pred (±SD)	94 (22)	95 (12)	63 (9)	39 (5)	25 (3)
FVC%Pred (±SD)	89 (19)	109 (12)	92 (15)	75 (15)	59 (11)
FEV1/FVC % (±SD)	79 (8)	86 (10)	52 (8)	39 (8)	32 (4)
RV%Pred (±SD)	112 (26)	123 (30)	144 (32)	199 (40)	214 (50)
TLC%Pred (±SD)	101 (14)	113 (13)	114 (17)	125 (18)	127 (17)
RV/TLC % (±SD)	43 (10)	107 (18)	125 (17)	60 (8)	64 (11)
DL_{CO} %Pred (±SD)	76 (21)	69 (19)	56 (16)	44 (19)	28 (17)

SD: Standard deviation

with normal pulmonary function. Thus far, a total of 75 ex-smokers without airflow limitation or GOLD U and 107 individuals with COPD, including GOLD stage I (n=22), GOLD stage II (n=49), GOLD stage III (n=29) and GOLD stage IV (n=7), have been enrolled.

Participant recruitment was primarily performed by local academic pulmonologists, advertisement in local newspapers, community newsletters and a local atherosclerosis prevention clinic. All participants will provide written informed consent to the protocol that was approved by a local research ethics board and Health Canada and compliant with the Health Insurance Portability and Accountability Act. Review and documented consent will be performed during the follow-up visit prior to participant evaluations. The primary responsibility of a full-time research coordinator (certified by the Association of Clinical Research Professionals) included management of recruitment and scheduling as well as participant retention activities. Based on our previous experience with a pilot two-year longitudinal study where the retention rate was 90%,[41] we expect similar participant retention over the two-year follow-up period.

Pulmonary Function and Other Measurements

All participants will undergo, at baseline and at the two-year follow-up, post-bronchodilator spirometry and plethysmography, the St George's Respiratory Questionnaire (SGRQ), used with permission,[42] and a 6-minute walk test (6MWT)[43,44] to measure the 6-minute walk distance (6MWD). Spirometry, plethysmography and diffusing capacity of the lung for carbon monoxide (DL_{CO}) will be measured using body plethysmography (MedGraphics Corporation, Minnesota, USA) with the attached Medgraphics gas analyzer according to American Thoracic Society (ATS)/European Respiratory Society (ERS) guidelines.[45-48] For all participants who complete the baseline study visit, the number of acute exacerbations requiring hospitalization or COPD-related hospitalizations will be reported using patient hospital records obtained with Cerner's PowerChart® (Cerner Corporation, Missouri, USA) up to 5 years prior to the initial participant baseline visit as well as during the follow-up period.

CT Imaging

Thoracic CT will be acquired using a 64-slice (General Electric Health Care Milwaukee) CT system. Baseline CT images will be acquired with patients at inspiration breath-hold after inhalation of 1.0L medical grade nitrogen (N_2) from a 1.0 L Tedlar® bag (Jensen Inert Products, New Jersey, USA) from functional residual capacity (FRC) using the same imaging protocol as the ECLIPSE study[39] and the same lung volume used with an MRI. We use a Tedlar® bag filled with N_2 instead of room air for CT imaging because the hyperpolarized ^{3}He gas depolarizes when exposed to oxygen (O_2) and therefore, the gas dispensing apparatus must remain completely free of O2 in order to avoid the potential for cross-contamination. The baseline CT is acquired at the same lung volume as used with an MRI to enable image co-registration; CT-MRI co-registration will allow us to investigate the relationship between morphological CT airway measurements and hyperpolarized noble gas MRI static ventilation measurements. In order to evaluate regional pulmonary gas trapping, as well as to differentiate regions of gas trapping due to emphysematous tissue destruction from small airway disease using inspiratory to expiratory image registration,[49] a full inspiration and full expiration CT will be performed at the two-year follow-up using the same imaging protocol as the COPDGene[37] and CanCOLD[38] studies. Importantly, the CT images acquired at the two-year longitudinal follow-up will not be used to evaluate longitudinal measurement changes. Both baseline and longitudinal CT will be used for investigating relationships between MRI and CT measurements.

All CT images will be quantified using the Pulmonary Workstation 2.0 (VIDA Diagnostics, Inc., Iowa, USA). CT structural airway measurements will include: 1) lumen area (LA), 2) airway wall area (AAW) and 3) wall area percentage (WA%) measured for the segmental and subsegmental airways,[10] and, 4) the square root of airway wall thickness for airways with an internal perimeter of 10mm (Pi_{10}).[50] CT structural emphysema measurements will include: 1) the relative area with attenuation values below -950 Hounsfield units [HU] (RA_{950}),[13] and, 2) the low attenuation cluster (LAC) analysis of connected regions with CT densitometry values < -950 HU.[51-53] At the two-year follow-up, inspiratory and expiratory CT images will be co-registered to regionally identify normal lung, emphysema and air trapping as recently described.[49]

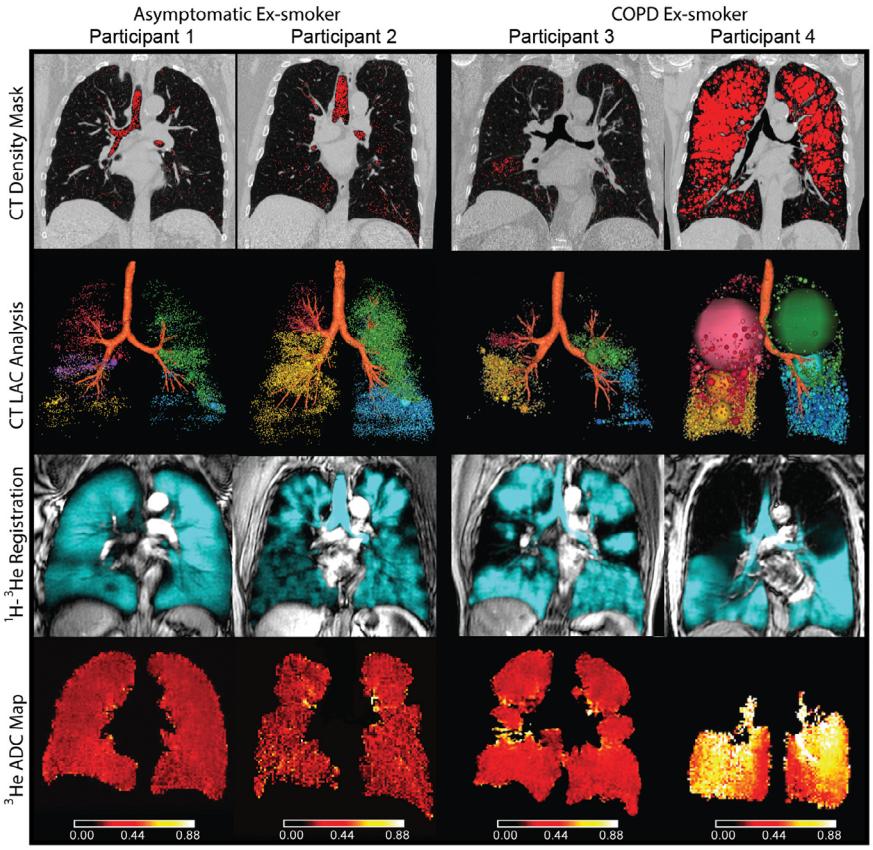
MR Imaging

Hyperpolarized ^{3}He MRI will be acquired using a 3.0 Tesla MR750 system (General Electric Health Care, Milwaukee, WI USA). Participants will undergo breath-hold imaging for acquisition of conventional ^{1}H MRI, ^{3}He static ventilation MRI and ^{3}He diffusion-weighted MRI. Conventional ^{1}H MRI will be acquired during 1.0L breath-hold of N_2 from a 1.0 L Tedlar® bag from FRC. ^{3}He static ventilation and diffusion weighted imaging will be performed following inhalation of a ^{3}He/N_2 gas mixture (^{3}He dose = 5 ml/kg body weight) from a 1.0 L Tedlar® bag from FRC as previously described.[25] At visit 2, a hyperpolarized ^{129}Xe MRI will also be acquired on the same system as 3He MRI using ^{129}Xe gas doses (50/50 ^{129}Xe/^{4}He) administered in 1.0L Tedlar® bags with ^{129}Xe MRI static ventilation and diffusion-weighted images acquired, as previously described.[34] There was not a commercially-available ^{129}Xe polarizer that was approved for human participants during baseline enrollment, and therefore, hyperpolarized ^{129}Xe was not performed at visit 1 for all participants. However, a small proof of concept study was performed in 10 TINCan COPD participants at visit 1 as previously described.[34]

All MR images will be evaluated using custom-built software generated using MATLAB R2007b (The Mathworks Inc., Massachusetts, USA). For ^{3}He and ^{129}Xe MRI static ventilation images, regions of signal void or ventilation defects will be quantified as the ratio of the ventilation defect volume (VDV) to the thoracic cavity volume (segmented from ^{1}H MRI) to generate the whole lung ventilation defect percentage (VDP)[11,54]; ^{3}He and ^{129}Xe apparent diffusion coefficient (ADC) maps will also be generated from diffusion-weighted images using custom built software (MATLAB R2007b) .[55]

Figure 2 shows for 2 representative asymptomatic ex-smokers with normal pulmonary function and 2 representative COPD participants at baseline, the CT density mask highlighting areas with CT densitometry values less than -950 HU, CT low attenuation clusters registered to the 3D reconstruction of the CT airway tree for measurement of airway dimensions. The ^{1}H and ^{3}He MRI co-registered central coronal slices (^{3}He shown in blue) are also shown to highlight ^{3}He MRI ventilation defects, and the ^{3}He MRI ADC maps are provided as evidence of both ventilation and morphological abnormalities in these individuals.

Figure 2. CT and ^{3}He MRI for Four Representative TINCan Participants



For 4 representative participants (2 asymptomatic ex-smokers without COPD and 2 COPD ex-smokers) the CT density mask highlighting areas with CT densitometry values below -950 HU, CT LAC of connected regions of the lung with CT densitometry values below -950 HU registered to the 3D reconstruction of the airway tree for measurement of airway dimensions, ^{1}H and ^{3}He MRI registration (^{3}He shown in blue) and ^{3}He ADC maps are shown.

Imaging evidence of disease is apparent in some asymptomatic ex-smokers without COPD, as shown for Participant 2 (72 year-old male, FEV_1=122%$_{pred}$, FEV_1/FVC=80%, DL_{CO}=73%$_{pred}$, RA_{950}=4%, VDP=17%, ADC=0.25cm^2/s) but not Participant 1 (70 year-old male, FEV_1=101%$_{pred}$, FEV_1/FVC=75%, DL_{CO}=113%$_{pred}$, RA_{950}=1%, VDP=4%, ADC=0.26cm^2/s).

For the COPD ex-smokers, significantly greater emphysema extent is demonstrated by the highlighted areas on the CT density mask and bright ADC maps in Participant 4 (76 year-old female, FEV_1=79%$_{pred}$, FEV_1/FVC=64%, DL_{CO}=63%$_{pred}$, RA_{950}=33%, VDP=36%, ADC=0.63cm^2/s), while a lesser extent is observed for Participant 3 (76 year-old female, FEV_1=52%$_{pred}$, FEV_1/FVC=42%, DL_{CO}=18%$_{pred}$, RA_{950}=1%, VDP=17%, ADC=0.31cm^2/s).

Data and Statistical Analysis Plan

There are 4 key objectives for the TINCan study and these are enabled by the statistical analysis plan. First, we will determine the significant cross-sectional univariate and multivariate relationships for ^{3}He with ^{129}Xe MRI and CT-derived phenotypes of airway and parenchymal abnormalities. Pearson correlation coefficients and linear regression will be performed to determine the univariate relationships for baseline ^{3}He and ^{129}Xe gas distribution measurements (regional and whole lung VDV, VDP) with CT airway disease measurements and all other participant measurements. Multivariate linear regression models will also be constructed for predicting SGRQ total score and 6MWD using CT (Pi10, RA_{950}) and ^{3}He MRI (VDP, ADC) phenotype measurements, adjusted for sex, BMI, FEV_1, and DL_{CO}.

Second, we will determine the longitudinal changes and relationships for ^{3}He MRI-derived phenotypes of airway and parenchymal abnormalities with other COPD measurements (SGRQ, 6MWD, spirometry, plethysmography and DL_{CO}). We will determine the significant longitudinal changes for ^{3}He MRI measurements using paired two-tailed t-tests. Pearson correlation coefficients and linear regression analyses will be performed to determine the relationships between the change in ^{3}He MRI measurements with the longitudinal change in physiological measures (FEV_1, FEV_1/FVC, RV, DL_{CO}), functional capacity (6MWD) and quality of life (SGRQ) measurements. We previously performed and reported the results of a pilot longitudinal study in 15 ex-smokers with COPD that demonstrated statistically significant ^{3}He ADC and VDP worsening in 2 years.[41] Based on this pilot study, with 100 COPD participants enrolled thus far and a longitudinal retention rate of 90% expected, this study has sufficient power to detect significant longitudinal changes for ^{3}He MRI measurements (α=.05, ß=0.20 and power=.80).

Third, the associations between imaging phenotypes of COPD with exacerbations will be evaluated. To accomplish this, a zero-inflated Poisson (ZIP) model will be used to determine the relationship between selected variables with COPD exacerbations, including hospitalization and death. The variables that will be included in the regression include: age, sex, BMI, smoking status, FEV_1, DL_{CO}, RV, SGRQ, 6MWD, CT RA_{950}, CT WA%, ^{3}He MRI VDP, and ^{3}He MRI ADC. We have recently evaluated the association between imaging phenotypes of COPD with the number of hospitalizations and demonstrated that over a 5-year period, 58 hospitalizations occurred.[56] We

also reported that ^{3}He VDP was a significant predictor of hospitalization due to COPD exacerbation.[56] Therefore, based on this study we are powered to detect statistically significant associations between ^{3}He MRI measurements with all COPD acute exacerbations, including those that resulted in treatment, emergency department visits and hospitalizations, as well as COPD-related mortality.

Finally, we will determine whether MRI and CT phenotypes are predictors of longitudinal changes in pulmonary function measurements, lung volumes, symptoms and functional capacity. Using mixed linear regression analyses adjusted for cofounders, we will determine which baseline imaging phenotype measurements have the strongest relationship with the longitudinal changes in health status (SGRQ), functional capacity (6MWD) and FEV_1. Cofounders will include age, sex, smoking status, BMI, and baseline FEV_1. In a similar manner we will also use hierarchical clustering models (Principal Component analysis) and generate icicle plots (showing cases as rows and number of clusters as columns) and dendograms (or hierarchical tree diagrams). Principal component analysis using hierarchical clustering is appropriate for smaller samples (typically < 250) and we will generate a dataset for all evaluable participants with ^{3}He and ^{129}Xe MRI structural and functional variables as well as CT-derived variables. In this way, we can attempt to determine which clusters of imaging and other measurements are the predictors of longitudinal progression and outcomes.

Discussion

In COPD, the current gold standard approach for evaluating disease severity, disease progression and treatment response relies on lung function measurements that provide little information about the underlying morphological, anatomical and functional abnormalities that result in symptoms, airflow limitation and adverse outcomes. To address this critical gap in knowledge, TINCan was developed and implemented. The major anticipated value of TINCan is an enhanced understanding of COPD-imaging phenotypes, and their relationship to well-established measurements of COPD and subclinical disease and disease progression. In addition, we hypothesize that non-invasive COPD-imaging phenotypes directly reflect the underlying abnormalities in COPD and these can be used as intermediate endpoints in the development of new treatment approaches.

One important technical goal for TINCan is to provide tools and methods that streamline image analysis workflow to accelerate the translational potential of pulmonary functional MRIs. Therefore, we focused on developing image segmentation and registration tools to quantify measurements with high precision for detecting small measurement changes in treatment or longitudinal studies. Towards this goal, we developed a multi-step, semi-automated ^{3}He-^{1}H registration and segmentation method[57] and demonstrated that it provides excellent inter-/intra-observer precision and good agreement with expert observers' manual measurements. With the development of image analysis tools underway, we can apply these tools to the TINCan cohort and be confident that the longitudinal changes measured are likely due to disease changes. Another important goal of TINCan is to provide a better understanding of the early or mild subclinical disease observed in ex-smokers with normal spirometry. We recently demonstrated that abnormal DL_{CO} in ex-smokers without COPD was related to worse health status, functional capacity and elevated ^{3}He ADC consistent with early emphysema revealed by ^{3}He MRI but not CT.[58] At the 2-year follow-up, we will determine whether such smokers with ^{3}He MRI evidence of early or mild emphysema (but without airflow limitation) show evidence of progressive disease.

For a small group of participants we have incorporated hyperpolarized ^{129}Xe MRI as part of the baseline visit to test hypotheses related to potential differences in ^{129}Xe and ^{3}He MRI ventilation. To better understand any potential differences between ^{3}He and ^{129}Xe MRI gas distribution, we quantitatively compared ^{3}He and ^{129}Xe MRI in COPD participants and observed that ^{129}Xe VDP was significantly greater than ^{3}He VDP.[34] We also observed that the regions of decreased ^{129}Xe ventilation were spatially and significantly correlated with regions of emphysema.[33] Taken together, these important findings suggest that ^{129}Xe gas may be more sensitive to changes in both the airways and parenchyma in COPD, and therefore may be more sensitive than ^{3}He for detecting changes at the 2-year follow-up.

The important next step of the TINCan cohort is to complete enrollment and then follow-up with all participants in order to evaluate longitudinal changes. We will identify whether MRI phenotypes

detect significant disease progression, how changes in MR imaging-derived measures relate to changes in physiologic and health outcome measurements, and determine which baseline imaging phenotypes can predict declines in quality of life, functional capacity and physiologic measurements. We will also determine whether imaging phenotypes can independently identify individuals with more frequent exacerbations and more rapid declines in lung function.

In contrast to other cohort studies of COPD,[37-40] TINCan's novelty stems from its incorporation of emerging non-radiation-based imaging measurements in addition to conventional CT. These emerging imaging methods have the potential to provide complementary quantitative information regarding subclinical and clinical COPD pathophysiologies that may eventually allow for more personalized treatment. Another strength of TINCan is the inclusion of individuals often excluded from COPD cohort studies. For example, TINCan includes a relatively large group of ex-smokers without COPD. Identifying imaging phenotypes that can characterize these unique pathologies non-invasively and evaluate their relationships with disease progression and exacerbations is an important goal for the TINCan study.

In conclusion, here we describe the largest prospective, longitudinal pulmonary structure-function imaging study of COPD planned to-date using emerging MRI and CT imaging methods. Upon completion we will have a better understanding of the relationships between imaging phenotypes with well-established lung function and health status measurements, as well as outcomes, in a well-defined and relatively large group of ex-smokers. Finally, once complete, the resulting validated imaging measurements may be considered as intermediate endpoints for future COPD treatment studies to better target therapy, a long-standing research and clinical goal for COPD.

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Declaration of Interest

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References

1. Global initiative for chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. http://www.goldcopd.org/. Updated January 2014. Accessed July 28, 2014.
2. World Health Organization. The global burden of disease: 2004 update. Geneva: WHO Press; 2008.
3. Mittmann N, Kuramoto L, Seung SJ, Haddon JM, Bradley-Kennedy C, FitzGerald JM. The cost of moderate and severe COPD exacerbations to the Canadian healthcare system. *Respir Med* 2008;102(3):413-421. doi: http://dx.doi.org/10.1016/j.rmed.2007.10.010
4. Canadian Institute for Health Information. Health Indicators 2008. Ottawa: (Ottawa: CIHI, 2008) 2008.
5. Buist AS, McBurnie MA, Vollmer WM, et al. International variation in the prevalence of COPD (the BOLD Study): a population-based prevalence study. *Lancet*. 2007;370(9589):741-750. doi: http://dx.doi.org/10.1016/S0140-6736(07)61377-4
6. Halbert RJ, Natoli JL, Gano A, Badamgarav E, Buist AS, Mannino DM. Global burden of COPD: systematic review and meta-analysis. *Eur Respir J*. 2006;28(3):523-532. doi: http://dx.doi.org/10.1183/09031936.06.00124605
7. Bednarek M, Maciejewski J, Wozniak M, Kuca P, Zielinski J. Prevalence, severity and underdiagnosis of COPD in the primary care setting. *Thorax*. 2008;63(5):402-407. doi: http://dx.doi.org/10.1136/thx.2007.085456
8. Hogg JC. Pathophysiology of airflow limitation in chronic obstructive pulmonary disease. *Lancet*. 2004;364(9435):709-721. doi: http://dx.doi.org/10.1016/S0140-6736(04)16900-6
9. Robinson PD, Latzin P, Verbanck S, et al. Consensus statement for inert gas washout measurement using multiple- and single-breath tests. *Eur Respir Rev*. 2013;41(3):507-522. doi: http://dx.doi.org/10.1183/09031936.00069712
10. Nakano Y, Muro S, Sakai H, et al. Computed tomographic measurements of airway dimensions and emphysema in smokers. Correlation with lung function. *Am J Respir Crit Care Med*. 2000;162(3 Pt 1):1102-1108. doi:http://dx.doi.org/10.1164/ajrccm.162.3.9907120
11. Mathew L, Kirby M, Etemad-Rezai R, Wheatley A, McCormack DG, Parraga G. Hyperpolarized ^{3}He magnetic resonance imaging: preliminary evaluation of phenotyping potential in chronic obstructive pulmonary disease. *Eur J Radiol*. 2011;79(1):140-146. doi: http://dx.doi.org/10.1016/j.ejrad.2009.10.028
12. Gevenois PA, De M, V, De VP, Zanen J, Yernault JC. Comparison of computed density and macroscopic morphometry in pulmonary emphysema. *Am J Respir Crit Care Med*. 1995;152(2):653-657. doi: http://dx.doi.org/10.1164/ajrccm.152.2.7633722
13. Gevenois PA, P. DV, De Maertelaer V, et al. Comparison of computed density and microscopic morphometry in pulmonary emphysema. *Am J Respir Crit Care Med*. 1996;154(1):187-192. doi: http://dx.doi.org/10.1164/ajrccm.154.1.8680679
14. Turner MO, Mayo JR, Muller NL, Schulzer M, FitzGerald JM. The value of thoracic computed tomography scans in clinical diagnosis: a prospective study. *Can Respir J*. 2006;13(6):311-316.
15. Johannessen A, Skorge TD, Bottai M, et al. Mortality by level of emphysema and airway wall thickness. *Am J Respir Crit Care Med*. 2013;187(6):602-608. doi: http://dx.doi.org/10.1164/rccm.201209-1722OC
16. Vestbo J, Edwards LD, Scanlon PD, et al. Changes in forced expiratory volume in 1 second over time in COPD. *N Engl J Med*. 2011;365(13):1184-1192. doi: http://dx.doi.org/10.1056/NEJMoa1105482
17. Nishimura M, Makita H, Nagai K, et al. Annual change in pulmonary function and clinical phenotype in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2012;185(1):44-52. doi: http://dx.doi.org/10.1164/rccm.201106-0992OC
18. Coxson HO, Dirksen A, Edwards LD, et al. The presence and progression of emphysema in COPD as determined by CT scanning and biomarker expression: a prospective analysis from the ECLIPSE study. *Lancet Respir Med*. 2013;1(2):129-136. doi: http://dx.doi.org/10.1016/S2213-2600(13)70006-7
19. Han MK, Kazerooni EA, Lynch DA, et al. Chronic obstructive pulmonary disease exacerbations in the COPDGene study: associated radiologic phenotypes. *Radiology*. 2011;261(1):274-282. doi: http://dx.doi.org/10.1148/radiol.11110173
20. Fishman A, Martinez F, Naunheim K, et al. A randomized trial comparing lung-volume-reduction surgery with medical therapy for severe emphysema. *N Engl J Med*. 2003;348(21):2059-2073. doi: http://dx.doi.org/10.1056/NEJMoa030287
21. Rogers RM, Coxson HO, Sciurba FC, Keenan RJ, Whittall KP, Hogg JC. Preoperative severity of emphysema predictive of improvement after lung volume reduction surgery: use of CT morphometry. *Chest*. 2000;118(5):1240-1247. doi: http://dx.doi.org/10.1378/chest.118.5.1240
22. Coxson HO, Nasute Fauerbach PV, Storness-Bliss C, et al. Computed tomography assessment of lung volume changes after bronchial valve treatment. *Eur Respir J*. 2008;32(6):1443-1450. doi: http://dx.doi.org/10.1183/09031936.00056008
23. Sciurba FC, Ernst A, Herth FJ, et al. A randomized study of endobronchial valves for advanced emphysema. *N Engl J Med*. 2010;363(13):1233-1244. doi: http://dx.doi.org/10.1056/NEJMoa0900928
24. Albert MS, Cates GD, Driehuys B, et al. Biological magnetic resonance imaging using laser-polarized ^{129}Xe. *Nature*. 1994;370(6486):199-201. doi: http://dx.doi.org/10.1038/370199a0
25. Parraga G, Ouriadov A, Evans A, et al. Hyperpolarized 3He ventilation defects and apparent diffusion coefficients in chronic obstructive pulmonary disease: preliminary results at 3.0 Tesla. *Invest Radiol*. 2007;42(6):384-391. doi: http://dx.doi.org/10.1097/01.rli.0000262571.81771.66
26. Mathew L, Evans A, Ouriadov A, et al. Hyperpolarized ^{3}He magnetic resonance imaging of chronic obstructive pulmonary disease: reproducibility at 3.0 tesla. *Acad Radiol*. 2008;15(10):1298-1311. doi: http://dx.doi.org/10.1016/j.acra.2008.04.019

27. Parraga G, Mathew L, Etemad-Rezai R, McCormack DG, Santyr GE. Hyperpolarized ^{3}He magnetic resonance imaging of ventilation defects in healthy elderly volunteers: initial findings at 3.0 Tesla. *Acad Radiol*. 2008;15(6):776-785. doi: http://dx.doi.org/10.1016/j.acra.2008.03.003

28. Morbach AE, Gast KK, Schmiedeskamp J, et al. Diffusion-weighted MRI of the lung with hyperpolarized helium-3: a study of reproducibility. *J Magn Reson Imaging*. 2005;21(6):765-774. doi: http://dx.doi.org/10.1002/jmri.20300

29. Diaz S, Casselbrant I, Piitulainen E, et al. Hyperpolarized 3He apparent diffusion coefficient MRI of the lung: reproducibility and volume dependency in healthy volunteers and patients with emphysema. *J Magn Reson Imaging*. 2008;27(4):763-770. doi: http://dx.doi.org/10.1002/jmri.21212

30. Kaushik SS, Cleveland ZI, Cofer GP, et al. Diffusion-weighted hyperpolarized ^{129}Xe MRI in healthy volunteers and subjects with chronic obstructive pulmonary disease. *Magn Reson Med*. 2011;65(4):1154-1165. doi: http://dx.doi.org/10.1002/mrm.22697

31. Salerno M, de Lange EE, Altes TA, Truwit JD, Brookeman JR, Mugler JP, III. Emphysema: hyperpolarized helium 3 diffusion MR imaging of the lungs compared with spirometric indexes--initial experience. *Radiology*. 2002;222(1):252-260. doi: http://dx.doi.org/10.1148/radiol.2221001834

32. Diaz S, Casselbrant I, Piitulainen E, et al. Validity of apparent diffusion coefficient hyperpolarized ^{3}He-MRI using MSCT and pulmonary function tests as references. *Eur J Radiol*. 2009;71(2):257-263. doi: http://dx.doi.org/10.1016/j.ejrad.2008.04.013

33. Kirby M, Svenningsen S, Kanhere N, et al. Pulmonary ventilation visualized using hyperpolarized helium-3 and xenon-129 magnetic resonance imaging: differences in COPD and relationship to emphysema. *J Appl Physiol*. (1985). 2013;114(6):707-715. doi: http://dx.doi.org/10.1152/japplphysiol.01206.2012

34. Kirby M, Svenningsen S, Owrangi A, et al. Hyperpolarized ^{3}He and ^{129}Xe MR imaging in healthy volunteers and patients with chronic obstructive pulmonary disease. *Radiology*. 2012;265(2):600-610. doi: http://dx.doi.org/10.1148/radiol.12120485

35. Dirksen A, Piitulainen E, Parr DG, et al. Exploring the role of CT densitometry: a randomised study of augmentation therapy in alpha1-antitrypsin deficiency. *Eur Respir J*. 2009;33(6):1345-1353. doi: http://dx.doi.org/10.1183/09031936.00159408

36. Dirksen A, Dijkman JH, Madsen F, et al. A randomized clinical trial of alpha(1)-antitrypsin augmentation therapy. *Am J Respir Crit Care Med*. 1999;160(5 Pt 1):1468-1472. doi: http://dx.doi.org/10.1164/ajrccm.160.5.9901055

37. Regan EA, Hokanson JE, Murphy JR, et al. Genetic epidemiology of COPD (COPDGene) study design. *COPD*. 2010;7(1):32-43. doi: http://dx.doi.org/10.3109/15412550903499522

38. Bourbeau J, Saad N. COPD: Success needs implementing a participative care model and early treatment? [French] *Rev Mal Respir*. 2013;30(2):93-94. doi: http://dx.doi.org/10.1016/j.rmr.2012.07.003

39. Vestbo J, Anderson W, Coxson HO, et al. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). *Eur Respir Rev*. 2008;31(4):869-873. doi: http://dx.doi.org/10.1183/09031936.00111707

40. Couper D, LaVange LM, Han M, et al. Design of the Subpopulations and Intermediate Outcomes in COPD Study (SPIROMICS). *Thorax*. 2014;69(5):491-494. doi: http://dx.doi.org/10.1136/thoraxjnl-2013-203897

41. Kirby M, Mathew L, Wheatley A, Santyr GE, McCormack DG, Parraga G. Chronic obstructive pulmonary disease: longitudinal hyperpolarized (3)He MR imaging. *Radiology*. 2010;256(1):280-289. doi: http://dx.doi.org/10.1148/radiol.10091937

42. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. *Am Rev Respir Dis*. 1992;145(6):1321-1327. doi: http://dx.doi.org/10.1164/ajrccm/145.6.1321

43. Crapo RO, Casaburi R, Coates AL, et al. ATS statement: Guidelines for the six-minute walk test. *Am J Respr Crit Care Med*. 2002;166(1):111-117. doi: http://dx.doi.org/10.1164/ajrccm.166.1.at1102

44. Enright PL. The six-minute walk test. *Respir Care*. 2003;48(8):783-785.

45. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319-338. doi: http://dx.doi.org/10.1183/09031936.05.00034805

46. Macintyre N, Crapo RO, Viegi G, et al. Standardisation of the single-breath determination of carbon monoxide uptake in the lung. *Eur Respir J*. 2005;26(4):720-735. doi: http://dx.doi.org/10.1183/09031936.05.00034905

47. Wanger J, Clausen JL, Coates A, et al. Standardisation of the measurement of lung volumes. *Eur Respir J*. 2005;26(3):511-522. doi: http://dx.doi.org/10.1183/09031936.05.00035005org/10.1183/09031936.00159408

48. Coates AL, Graham BL, McFadden RG, et al. Spirometry in primary care. *Can Respir J*. 2013;20(1):13-22.

49. 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(11):1711-1715. doi: http://dx.doi.org/10.1038/nm.2971

50. Nakano Y, Wong JC, de Jong PA, et al. The prediction of small airway dimensions using computed tomography. *Am J Respir Crit Care Med*. 2005;171(2):142-146. doi: http://dx.doi.org/10.1164/rccm.200407-874OC

51. Mishima M, Hirai T, Itoh H, et al. Complexity of terminal airspace geometry assessed by lung computed tomography in normal subjects and patients with chronic obstructive pulmonary disease. *Proc Natl Acad Sci U S A*. 1999;96(16):8829-8834. doi: http://dx.doi.org/10.1073/pnas.96.16.8829

52. Gietema HA, Muller NL, Fauerbach PV, et al. Quantifying the extent of emphysema: factors associated with radiologists' estimations and quantitative indices of emphysema severity using the ECLIPSE cohort. *Acad Radiol*. 2011;18(6):661-671. doi: http://dx.doi.org/10.1016/j.acra.2011.01.011

53. Coxson HO, Whittall KP, Nakano Y, et al. Selection of patients for lung volume reduction surgery using a power law analysis of the computed tomographic scan. *Thorax*. 2003;58(6):510-514. doi: http://dx.doi.org/10.1136/thorax.58.6.510

54. Woodhouse N, Wild JM, Paley MN, et al. Combined helium-3/proton magnetic resonance imaging measurement of ventilated lung volumes in smokers compared to never-smokers. *J Magn Reson Imaging*. 2005;21(4):365-369. doi: http://dx.doi.org/10.1002/jmri.20290

55. Kirby M, Heydarian M, Wheatley A, McCormack DG, Parraga G. Evaluating bronchodilator effects in chronic obstructive pulmonary disease using diffusion-weighted hyperpolarized helium-3 magnetic resonance imaging. *J Appl Physiol*. 2012;112(4):651-657. doi: http://dx.doi.org/10.1152/japplphysiol.01295.2011

56. Kirby M, Pike D, Coxson HO, McCormack DG, Parraga G. Hyperpolarized He ventilation defects used to predict pulmonary exacerbations in mild to moderate chronic obstructive pulmonary disease. *Radiology*. 2014:140161. doi: http://dx.doi.org/10.1148/radiol.14140161

57. Kirby M, Heydarian M, Svenningsen S, et al. Hyperpolarized ^{3}He magnetic resonance functional imaging semiautomated segmentation. *Acad Radiol*. 2012;19(2):141-152. doi: http://dx.doi.org/10.1016/j.acra.2011.10.007

58. Kirby M, Owrangi A, Svenningsen S, et al. On the role of abnormal DL(CO) in ex-smokers without airflow limitation: symptoms, exercise capacity and hyperpolarised helium-3 MRI. *Thorax*. 2013;68(8):752-759. doi: http://dx.doi.org/10.1136/thoraxjnl-2012-203108