CLINICAL STUDY PROTOCOL

STRUCTURE AND FUNCTION MRI OF ASTHMA

PROTOCOL NO. ROB0037
Robarts Research Institute

Confidentiality Statement

The information contained in this document, especially unpublished data, is the property of Robarts Research Institute (or under its control), and is therefore provided to you in confidence for review by you, your staff and an applicable Institutional Review Committee. It is understood that this information will not be disclosed to others without written authorization from Robarts Research Institute, except to the extent necessary to obtain informed consent from those participants for whom the study may be implemented.

Title of Protocol:

STRUCTURE AND FUNCTION MRI OF ASTHMA

INVESTIGATOR(S):

Principal Investigators: Dr. Grace Parraga
Address: Robarts Research Institute
1151 Richmond St. N.
London, ON
N6A 5B7
Telephone: 519-931-5265
Email: gparraga@uwo.ca

Co-Investigator: Dr. David McCormack
Address: Dept. of Medicine
London Health Sciences Centre
Victoria Hospital
800 Commissioners Rd.
London, ON
N6A 5W9
Telephone: 519-667-6767
Email: david.mccormack@lhsc.on.ca

Name and address of Institutional Review Board/Ethics Review Committee:

Office of Human Research Ethics
Room 5150 Support Services Building
Western University
London, ON, N6G 1G9
Telephone Number: 519-661-3036
Fax: 519-850-2466
Email: wrem@uwo.ca

Grace Parraga	Scientist	RRI	519-931-5265
David McCormack	Respirologist	LHSC-VH	519-667-6767
Angela Wilson	Clinical Coordinator & Registered Respiratory Therapist (RRT)	RRI	519-931-5777 x 24197
Lauren Zelko	Regulatory & Data Quality Coordinator	RRI	519-931-5777 x 24197
Sam Tcherner	Graduate student	RRI	

Ali Mozaffaripour	Graduate student	RRI	

PROTOCOL SYNOPSIS FOR ROB0037

TITLE	STRUCTURE AND FUNCTION MRI OF ASTHMA
SPONSOR	Robarts Research Institute Western University 1151 Richmond St. N., London, ON, N6A 5B7
PROJECT PHASE	Exploratory Study
INDICATION	Asthma
OBJECTIVE	• Acquire ^{129}Xe MRI and optimize analysis methods to generate in vivo measurements • Evaluate ^{129}Xe MRI measurements over time and in response to therapy, relate them to airflow, dyspnea, airway remodeling and inflammation and validate using x-ray computed tomography (CT) and histology • Evaluate image analysis methods that generate ^{129}Xe MRI temporal lung function maps as image guidance/therapy planning tools for targeted asthma therapy • Develop ^{129}Xe MRI structure-function models computationally to understand mechanisms of localized therapy response • Evaluate oscillometry outcomes of the respiratory system as they correlate to lung function abnormalities detected using ^{129}Xe MRI • For the subset of patients identified as underperceivers using asthma control and quality of life questionnaires, we will acquire fMRI to determine if severe asthmatics with poor control exhibit baseline differences in brain structure and function compared to healthy volunteers, and if these alterations influence asthma symptom perception • To determine if differences in brain structure and function are related to lung function abnormalities quantified by pulmonary MRI • In healthy volunteers and the subset of asthmatic participants identified as underperceivers, to determine if differences exist in resting state brain connectivity pre- and post-Methacholine challenge • In the subset of asthmatic participants, to evaluate the differences in resting state brain connectivity compared to healthy volunteers post-Methacholine Challenge
STUDY DESIGN	Longitudinal Study
PLANNED TOTAL SAMPLE SIZE	200 Asthmatic participants & 30 Healthy Volunteers

PARTICIPANT SELECTION CRITERIA	Males and females, ages 18-85 years old that are either healthy volunteers or have a clinical diagnosis of Asthma.
TREATMENTS/MODALITY	MRI, fMRI, MDCT, spirometry, plethysmography, DL_{CO}, FOT, MBNW, FeNO, Borg Dyspnea Scale, MRC Dyspnea Scale, Quality of Life Questionnaires, Asthma Control Questionnaires, and St. George's Respiratory Questionnaire.
DOSAGES	There is no study medication evaluated. As part of the lung MRI, participants will inhale polarized ^{129}Xe and/or ^{3}He at a dosage of 5 ml/kg body weight diluted with helium-4 (^{4}He) gas to 1.0L. The polarized $^{129}Xe/^{4}He$ mixture will be inhaled in a single breath-hold. This is intended to fill the lungs with the gas, allowing for complete imaging. Participants will inhale the gas mixture up to five times at each imaging session.
ROUTE OF ADMINISTRATION	Oral inhalation directly into lungs from a Tedlar® plastic bag with an attached breathing tube/straw containing a stopcock on-off valve while inside MR magnet bore.
MAIN PARAMETERS OF: **-SAFETY** **-EFFICACY**	Heart rate and oxygen saturation will be monitored throughout the imaging session and recorded. Adverse events will be recorded. ^{129}Xe MRI ventilation image analysis (VDP) ^{129}Xe MRI diffusion-weighted image analysis (ADC) ^{129}Xe MRI dissolved phase spectroscopy analysis Pulmonary Function Testing MDCT image analysis
DURATION OF STUDY	Q1 2013 - Q4 2028

PROTOCOL SYNOPSIS FOR ROB0037

MAGNETIC RESONANCE IMAGING (MRI) PROCEDURE:

Participants will be screened for contraindications by the Study Coordinator and MR technologist. Prior to being made comfortable on the scanner bed in the magnet, participants will be asked to perform a 16-second breath-hold test using a dose bag containing medical grade nitrogen (N_2) with an identical volume to that used for xenon-129 (^{129}Xe). This maneuver ascertains their ability at that time to undergo administration of ^{129}Xe and/or ^{3}He gas and a 16-second breath-hold for the MR study. They will be placed in the 3T MR scanner with one of three ^{129}Xe chest coils fitted over their torso and chest. Hearing protection will be provided to each participant to muffle the noise produced by the gradient RF coils. A pulse oximeter lead will be placed on the participant's finger to monitor heart rate and oxygen saturation. MRI will be performed for up to a period of 30 minutes. All imaging will be performed in a "breath-hold" fashion. All participants will have supplemental oxygen provided via nasal cannula at a flow-rate of 2 liters per minute during the scanning process. There will be four breath-holds involving the hyperpolarized gases.

Up to four different types of pulmonary images will be acquired in the coronal plane during the single visit: 1) proton (^{1}H) thoracic cavity, 2) ^{129}Xe and/or ^{3}He static ventilation, 3) ^{129}Xe and/or ^{3}He diffusion-weighted, and 4) ^{129}Xe dissolved phase. Heart rate and oxygen saturation will be monitored throughout the imaging session.

For healthy volunteers and the subset of asthmatics who have been identified as underperceivers of dyspnea and exhibiting poor disease control using asthma control and quality of life questionnaires, resting state functional MRI (RS-fMRI) will also be performed pre- and post-methacholine challenge on the first visit only. Participants will be placed in the 3T MR scanner with a specialized coil fitted around their head. Hearing protection will be provided to each participant to muffle the noise produced by the gradient RF coils. A pulse oximeter lead will be placed on the participant's finger to monitor heart rate and oxygen saturation. MRI will be performed for up to a period of 20 minutes. All participants will have supplemental oxygen provided via nasal cannula at a flow-rate of 2 liters per minute during the scanning process. Participants will be placed in the supine position and will be asked to remain motionless during the scanning process. The scanning process will involve the acquisition of various images: an anatomical image, a blood oxygenation level dependent (BOLD) contrast image, and a diffusion tensor image (DTI). These images will provide information about neural activation and anatomical connections between brain areas. Brain fMRI and lung MRI using ^{129}Xe will be performed within five minutes of each other, and the order in which they are performed will be randomized for each participant.

Logs and records of gas exposure will be maintained for every volunteer in this study. Adverse events and pulse oximetry measurements during MRI will be recorded. If oxygen saturation falls to <80% continuously for ≥15 seconds, scanning will be discontinued, and the patient will be provided supplemental oxygen as necessary until oxygen saturation recovers to the patient's baseline value. This patient will then be discontinued from the study. Oxygen desaturation below 88% during any breath hold will be considered an adverse event.

PULMONARY FUNCTION TESTING

Full pulmonary function tests including spirometry, lung volumes and diffusing capacity of carbon monoxide (DLco) will be performed according to American Thoracic Society (ATS) guidelines using MedGraphics Elite Series, MedGraphics Corporation. St. Paul, MN USA and/or nDD EasyOne Spirometer, nDD Medical Technologies Inc. Andover, MA USA. All measurements will be performed in the Pulmonary Function Laboratory at Robarts Research Institute. Lung clearance index (LCI) will be derived from the multiple breath nitrogen washout (MBNW) test. Participants breathe normally into a mouthpiece connected to a device, the NDD Easy-One Pro Lab, for approximately 3-5 minutes. Fractional exhaled nitric oxide (FeNO) will be measured using the NIOX VERO® (Circassia Pharmaceuticals Inc., Morrisville, NC, USA).

FORCED OSCILLATION TECHNIQUE (FOT)

FOT will be performed at each visit using the tremoFlo™ airway oscillometry system (THORASYS Thoracic Medical Systems, Montreal, QC). FOT measures the mechanics of the respiratory system and is a method of evaluating lung function without patient effort, by superimposing a gentle multi-frequency airwave onto the patient's respiratory airflow. Patients breathe normally throughout the measurement sequence via a disposable mouthpiece. Measurements will be performed in the Pulmonary Function Laboratory at Robarts Research Institute and according to the recommended guidelines set by the

European Respiratory Society (ERS) (Oostveen et al., Eur Respir J 2003; 22: 1026–1041).

METHACHOLINE CHALLENGE

Methacholine Challenge and Salbutamol rescue: Methacholine Challenge will be performed in the Robarts Pulmonary Function Laboratory at Visit 1. According to the ATS guidelines (after recording baseline forced expiratory volume in 1 second (FEV_1)) up to and including the concentration of Methacholine required to decrease FEV_1 20% from baseline (PC20).

SPUTUM INDUCTION

Induced sputum will be collected at Visit 1, 1c and optional annual in-person visits. Sputum induction is a relatively non-invasive method to obtain sputum for cell or fluid phase inflammatory indices, culture or cytology. It is performed with an aerosol of normal or hypertonic saline generated by an ultrasonic nebulizer. Samples will be analysed by study staff at Robarts Research Institute. Analysis will begin within 2 hours of sputum collection, and samples will be kept in the locked sputum lab until analysis is complete, at which time samples will be destroyed.

MULTI-DETECTOR ROW COMPUTED TOMOGRAPHY (MDCT)

Thoracic low dose MDCT will be performed with the same inhalational breath-hold volume and maneuver (nitrogen (N_2) gas only) used for MRI. CT imaging will be performed using either an Aquilion ONE CT scanner (Canon Medical Systems USA, INC., Tustin, CA, USA) or a Lightspeed VCT CT scanner (General Electric, Waukesha WI, USA) at either Robarts Research Institute in London, Ontario or at University Hospital, located next door to Robarts Research Institute. Participants will be scanned in the supine position and during inspiration breath-hold from functional residual capacity (FRC) after inhaling 1L of N_2 gas as previously described in order to match CT and MRI breath-hold volumes and anatomy.

QUESTIONNAIRES

Participants with asthma will complete the Asthma Quality of Life Questionnaire with Standardised Activities (AQLQ(S)) (bi-weekly), and Asthma Control diary (weekly), and will upload to our website monthly using a confidential and password protected upload. The AQLQ(S) was developed to measure the functional problems (physical, emotional, social and occupational) that are most troublesome to adults (17-70 years) with asthma. Respiratory related quality of life will be assessed using the SGRQ. (Jones, P. W., et al. 1992) This tool is used to measure health-related quality of life.

The Modified Borg Dyspnea Scale and MRC Dyspnea scale will evaluate breathlessness at each clinic visit.

ANALYSIS:

We will measure hyperpolarized noble gas MRI gas distribution after breath-hold using the ventilation defect percent (VDP), ^{129}Xe and/or ^{3}He MRI measurements of gas trapping apparent diffusion coefficient (ADC) and ^{129}Xe MRI dissolved phase spectroscopy measurements of pulmonary gas exchange. FOT measures of respiratory system resistance (Rrs) and reactance (Xrs) will be acquired following the experimental methods and recommended reporting set by the ERS. For the subset of patients performing resting state fMRI, BOLD contrast will be used to measure physiological changes in blood flow

and oxygen metabolism in the brain. The magnetic properties of oxygen-rich and oxygen-deficient blood provide a MR signal to indicate underlying neural activity changes. We will also acquire DTI from participants undergoing fMRI. DTI tracks the random motion of water in neural fibers to provide information about the orientation, magnitude, and direction of neural connections.

DURATION OF THE STUDY:

Study duration for each participant is up to 10 years (up to 10 face-to-face visits, 2 phone calls). A sub-set will also visit at 7 ±2 days and 14 ±2 days to evaluate reproducibility and for generation of temporal spatial maps. Each participant will spend approximately 1 ½ to 2 hours on site for each visit, including screening, full pulmonary function tests and MRI. There is a 24-hour follow up for adverse events after the end of the final visit.

TABLE OF CONTENTS

GLOSSARY OF ABBREVIATIONS

^{1}H	Proton
^{3}He	Helium-3
^{4}He	Helium-4
^{129}Xe	Xenon-129
ACQ	Asthma Control Questionnaire
ADC	Apparent Diffusion Coefficient
AE	Adverse Event
AQLQ(S)	Asthma Quality of Life Questionnaire
ATS	American Thoracic Society
CI	Confidence Interval
CV	Coefficient of Variation
DLCO	Diffusing Capacity of Lung for Carbon Monoxide
DS	Defect Score
DV	Defect Volume
ERS	European Respiratory Society
FeNO	Fractional Exhaled Nitric Oxide
FEV_1	Forced Expiratory Volume in one second
FOT	Forced Oscillation Technique
FRC	Functional Residual Capacity
FVC	Forced Vital Capacity
IC	Inspiratory Capacity
MDCT	Multi-Detector Row Computed Tomography
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
N_2	Nitrogen
RS-fMRI	Resting State Functional Magnetic Resonance Imaging
PFT	Pulmonary Function Test
PVV	Percentage Ventilation Volume
RBC	Red Blood Cell
Rrs	Respiratory System Resistance
SAE	Serious Adverse Event
SGRQ	St. George's Respiratory Questionnaire
SV	Slow Vital Capacity

TCV	Thoracic Cavity Volume
TGV	Thoracic Gas Volume
TLC	Total Lung Capacity
VDP	Ventilation Defect Percent
VDV	Ventilation Defect Volume
Xrs	Respiratory System Reactance

PART I: STUDY DESIGN AND CONDUCT

1. BACKGROUND AND RATIONALE

Asthma is commonly diagnosed and characterized using the forced expiratory volume in 1s (FEV_1) - a simple and inexpensive spirometry measurement of airflow obstruction. As a result, current and newly developed asthma therapies are mainly directed towards improvements in FEV_1. Unfortunately, FEV_1 measurements do not always capture peripheral airway dysfunction or provide information relevant to the heterogeneity of airway dysfunction, which in asthma may have profound consequences for the work of breathing and gas exchange. We also now realize that the specific airway abnormalities that may be directly responsible for asthma symptoms and exacerbations are most likely heterogeneously distributed in the lung [1] and this information cannot be easily evaluated using global airflow measurements. Because of this, we think that methods for measuring and mapping regional asthma structure-function are necessary before we can achieve the critical breakthroughs required for the development of new asthma therapies.

Despite decades of active research and the staggering and growing societal burden of asthma, current clinical tools cannot provide a non-invasive way to: 1) differentiate between diseased and normal airways in individual asthma participants, 2) identify regional structural and functional targets for asthma therapy, and, 3) measure regional asthma changes over time or in response to therapy. In other words, there is no way to measure regional lung abnormalities in asthma, making it difficult if not impossible to develop and test regional asthma therapies. We think that airway remodeling is mainly masked by global function measures and has real clinical impact on asthma symptoms, control and quality of life.

Hyperpolarized noble gas magnetic resonance imaging (MRI) of the lung is a relatively new imaging method that allows depiction of both lung function and morphology [2-7]. Hyperpolarized gases are a new class of MR contrast agent that, when inhaled, provide high temporal and spatial resolution MR images of the lung airspaces. Since no ionizing radiation is involved, hyperpolarized gas MR imaging is ideal for the evaluation of lung diseases. With hyperpolarized gases, the nuclear spins of the gas atoms are brought into alignment outside of the MR scanner via a process called optical pumping, yielding high polarizations and permitting the visualization of the lung airspaces with MR imaging despite the low physical density of the gas in the lung. Two non-radioactive, or stable isotopes of noble gases, helium-3 (^3He) and xenon-129 (^{129}Xe), can be hyperpolarized. Until recently, higher polarizations could be achieved with ^3He than with ^{129}Xe, so in humans, ^3He was more commonly used for hyperpolarized gas MR imaging of the lungs [8-13]. Recently, the technology has been developed to provide large quantities of highly polarized ^{129}Xe [14].

Several applications of noble gas MRI are in the process of development. Such techniques include diffusion-weighted and relaxation-weighted imaging. These techniques take advantage of the fact that the rate of loss of polarization is significantly influenced by the local concentration of molecular oxygen and the fact that the gas diffusion is governed by small airway space volumes. These data can be used to create maps of the lung reflecting regional ventilation/perfusion and micro-airway sizes [15]. Other data that can be obtained with noble gas MRI includes the volumes of ventilated and unventilated lungs that can then be analyzed to determine the homogeneity of gas distribution within the airspaces [16,17].

Furthermore, accurate perception of asthma symptoms is essential for motivating appropriate health management behavior, such as seeking timely medical and self-

treatment. “Underperceivers” are classified as asthmatics who do not experience the sensation of dyspnea during asthma flair ups [18,19]. Inaccurate symptom perception is a contributing factor to increased morbidity and mortality in asthma patients [18]. The blunted perception of asthma is poorly understood and may involve several neurological factors related to disease severity [20-22].

Few studies have used resting state functional magnetic resonance imaging (RS-fMRI) to assess the underlying neurological activities associated with symptom perception. fMRI uses a blood oxygenation level dependent (BOLD) signal to measure and track physiological changes in blood flow and oxygen metabolism in the brain [23]. In addition to BOLD signaling, Diffusion Tensor Imaging (DTI), another mechanism of fMRI, is implemented to track neural pathways and measure anatomical connections between brain areas [24]. This data can be used to identify brain biomarkers and alterations associated with asthma control and the under-perception of dyspnea sensation.

2. OBJECTIVES OF STUDY

- Acquire ^{129}Xe MRI and optimize analysis methods to generate *in vivo* measurements
- Evaluate ^{129}Xe MRI measurements over time and in response to therapy, relate them to airflow, dyspnea, airway remodeling and inflammation and validate using x-ray computed tomography (CT) and histology
- Evaluate image analysis methods that generate ^{129}Xe MRI temporal lung function maps as image guidance/therapy planning tools for targeted asthma therapy
- Develop ^{129}Xe MRI structure-function models computationally to understand mechanisms of localized therapy response
- Evaluate oscillometry outcomes of the respiratory system as they correlate to lung function abnormalities detected using ^{129}Xe MRI
- For the subset of patients identified as underperceivers using asthma control and quality of life questionnaires, we will acquire fMRI to determine if severe asthmatics with poor control exhibit baseline differences in brain structure and function compared to healthy volunteers, and if these alterations influence asthma symptom perception.
- To determine if differences in brain structure and function are related to lung function abnormalities quantified by pulmonary MRI
- In healthy volunteers and the subset of asthmatic participants identified as underperceivers, to determine if differences exist in resting state brain connectivity pre- and post-Methacholine challenge
- In the subset of asthmatic participants, to evaluate the differences in resting state brain connectivity compared to healthy volunteers post-Methacholine Challenge

3. HYPOTHESES

- Asthma lung functional abnormalities can be quantified using^{129}Xe MRI over 2 years correlate significantly and strongly with dyspnea, pulmonary function and sputum measurements as well as indices of asthma quality of life and control. In addition, MRI VDP measured annually over a decade reflects significantly worse remodeling or will improve in response to therapy in those participants who opt for an annual visit.
- ^{129}Xe MRI ventilation abnormalities are related to airway morphologies due to bronchospasm, airway structural remodeling and inflammation and these can be validated using CT and histology measurements of endobronchial biopsy samples;
- ^{129}Xe MRI temporal functional maps will show significant correlations with functional images acquired 1 year later.
- Asthma underperceivers with poor control will exhibit significant baseline differences in fMRI BOLD contrast and DTI signal data compared to healthy volunteers.

- Quantitative fMRI data will be significantly correlated to pulmonary MRI data that quantifies abnormalities in lung function.
- Healthy volunteers and asthma underperceivers will show significant differences in BOLD contrast after undertaking a Methacholine challenge
- BOLD contrast will be significantly different between asthmatics and healthy volunteers post Methacholine challenge

4. EXPERIMENTAL DESIGN AND METHODS

4.1. Overall Design and Control Methods

In 200 asthma patients, we propose to apply ^{129}Xe MR image acquisition and analysis methods in order to characterize and probe the relationship between lung structure and function using imaging.

The first 60 participants with asthma will also be asked to complete additional follow up visits at the clinic after 2, 4 and 78 weeks. These visits are detailed in the schedule below.

The 30 healthy volunteers will be asked to complete the same study procedures to allow for a comparison between the resting state brain activity as well as lung structure and function between those with and without asthma.

All participants will visit Robarts Research Institute according to the following schedule:

V1 **Baseline**	**V1a*** **BL+14±2d**	**V1b*** **BL+28±2d**	**V2 Telephone/** **optional** **In-person** **BL+52±2wk**	**V1c*** **BL+78±8wk**	**V3 Telephone/** **optional** **In-person** **BL+104±8wk**	**V4-10** **optional in-person annual visit**
^{129}Xe/^{3}He MRI	^{129}Xe/^{3}He MRI	^{129}Xe/^{3}He MRI	^{129}Xe/^{3}He MRI	^{129}Xe/^{3}He MRI	^{129}Xe/^{3}He MRI	^{129}Xe/^{3}He MRI
Full PFT	Full PFT	Full PFT	Full PFT	Full PFT	Full PFT	Full PFT
MCh	Meds/Exac	Meds/Exac	Meds/Exac	Meds/Exac	Meds/Exac	Meds/Exac
FOT	FOT	FOT	FOT	FOT	FOT	FOT
FeNO	FeNO	FeNO	FeNO	FeNO	FeNO	FeNO
BORG	BORG	BORG	BORG	BORG	BORG	BORG
MRC	MRC	MRC	MRC	MRC	MRC	MRC
sputum			sputum	sputum	sputum	sputum
CT						
RS-fMRI (if applicable)						

*Applicable to only the first 60 participants with asthma.

All participants with asthma will also complete bi-weekly and weekly quality of life questionnaires. Self-reported data will be uploaded by each participant monthly to our website using a confidential and password protected upload.

4.1.1. Methacholine Challenge

Methacholine Challenge will be performed for healthy volunteers and asthmatic participants in the Robarts Pulmonary Function Laboratory according to the American Thoracic Society Guidelines (ATS). After recording baseline FEV_1, participants will inhale Methacholine solutions up to and including the concentration of Methacholine required to decrease FEV_1 by 20% from baseline (PC20). After completion of dyspnea score and ^{3}He or ^{129}Xe at PC20, 400 µg of Salbutamol (4 puffs) will be administered followed by spirometry 20 minutes post-administration. Methacholine challenge will be performed at V1 baseline only.

4.1.2. MRI

Participants will be screened for contraindications by the MR technologist. Prior to being made comfortable on the scanner bed in the magnet, participants will be required to do a 16-second breath-hold test using a dose bag containing air similar to that used for xenon imaging. This maneuver ascertains their ability at that time to undergo administration of ^{129}Xe gas and a 16-second breath-hold for the MR study. They will be placed in the 3T MR scanner with one of three ^{129}Xe chest coils fitted over their torso and chest. Hearing protection will be provided to each participant to muffle the noise produced by the gradient RF coils. A pulse oximeter lead will be attached to all of the participants to monitor their heart rate and oxygen saturation. MRI will be performed for up to a period of 30 minutes. All participants will have supplemental oxygen provided via nasal cannula at a flow-rate of 2 liters per minute during the scanning process.

Once comfortable on the MRI scanner table, participants will undergo 1 MRI session containing acquisition of several images as follows:

Proton (^{1}H) imaging – participants will inhale a 1 litre gas mixture containing medical grade nitrogen (N_2). This is performed in order to match breath hold volumes and anatomy with ^{129}Xe images. Breath hold will be up to 16 seconds.

Static ventilation imaging – participants will inhale a 1 litre gas mixture containing ^{129}Xe and or ^{3}He (5 ml/kg body weight) mixed with helium-4 (^{4}He) from a 1 litre bag. Breath-hold will be up to 16 seconds.

Diffusion-weighted imaging – participants will inhale a 1 litre gas mixture containing ^{129}Xe and or ^{3}He (5 ml/kg body weight) mixed with ^{4}He from a 1 litre bag. Breath-hold will be up to 16 seconds.

Dissolved phase imaging – participants will inhale a 1 litre gas mixture containing ^{129}Xe mixed with ^{4}He from a 1 litre bag. Breath-hold will be up to 16 seconds.

Logs and records of ^{129}Xe and ^{3}He gas exposure will be maintained for every participant in this study. Adverse experiences and pulse oximetry measurements during MRI will be recorded. If oxygen saturation falls to <80% continuously for ≥15 seconds, scanning will be discontinued, and the patient will be provided supplemental oxygen as necessary until oxygen saturation recovers to the patient's baseline value. This patient will then be discontinued from the study.

Participants undergoing resting state fMRI (healthy volunteers and the subset of asthmatic participants identified as underperceivers) will also be placed in the 3T MR scanner with a 32-channel brain coil fitted around their head. Hearing protection will be provided to each participant to muffle the noise produced by the gradient RF coils. A pulse

oximeter lead will be attached to all of the participants to monitor their heart rate and oxygen saturation. MRI will be performed for up to a period of 20 minutes. All participants will have supplemental oxygen provided via nasal cannula at a flow-rate of 2 liters per minute during the scanning process. Participants will undergo fMRI on two occasions during the first visit, each containing acquisition of an anatomical image, a BOLD signal image, and a diffusion tensor image. The sessions will be performed before and after the methacholine challenge for baseline and induced asthma attack or dyspnea conditions, respectively. Outlined in the table below are the appropriate pulse sequences designed for resting state fMR image acquisition:

Brain fMRI and lung MRI using ^{129}Xe and/or ^{3}He will be performed within five minutes of each other at both baseline and post-methacholine, and the order in which they are performed will be randomized for each participant and remain the same for both time points.

MRI RS--fMRI Protocol 1 - GE MR 750 Discovery (3T)

Coil Selection: 32 Channel Head Coil

1. 3 Plane Localizer (2D FGRE)

TR (ms)	TE (ms)	TI (ms)	FLIP ANGLE	FOV (cm)	FREQ MATRIX (mm)	PHASE MATRIX (mm)	SLICE THICKNESS (mm)	SLICE GAP (mm)	NEX	BANDWIDTH (kHz)	FREQ DIRECTION	ASSET	
5	1.4	N/A	30	24	256	128	10	0	1	31.2	Unswap	N/A	**0:08**

2. Calibration Scan (3D FSPGR)

	0:06

3. Sagittal T1 (3D IR-FSPGR)

TR (ms)	TE (ms)	TI (ms)	FLIP ANGLE	FOV (cm)	FREQ MATRIX (mm)	PHASE MATRIX (mm)	SLICE THICKNESS (mm)	SLICE GAP (mm)	NEX	BANDWIDTH (kHz)	FREQ DIRECTION	ASSET	
7.4	3.1	400	11	25.6	256	256	1	0	1	31.25	S/I	2	**4:38**

4. Axial rsFMRI (Single Shot Multi-Phase EPI-GRE)

TR (ms)	TE (ms)	TI (ms)	FLIP ANGLE	FOV (cm)	FREQ MATRIX (mm)	PHASE MATRIX (mm)	SLICE THICKNESS (mm)	SLICE GAP (mm)	NEX	BANDWIDTH (kHz)	FREQ DIRECTION	ASSET	
2500	25	N/A	90	22.4	64	64	3	0.3	1	250	R/L	2	**6:00**

5. Axial DTI (Single Shot EPI-SE)

TR (ms)	TE (ms)	TI (ms)	FLIP ANGLE	FOV (cm)	FREQ MATRIX (mm)	PHASE MATRIX (mm)	SLICE THICKNESS (mm)	SLICE GAP (mm)	NEX	BANDWIDTH (kHz)	FREQ DIRECTION	ASSET	
11000	58.3	N/A	90	24	96	96	2.5	0	1	250	R/L	2	**8:48**

DIFFUSION TYPE	# OF DIRECTIONS	# OF T2 IMAGES
Tensor	41	6

TOTAL TIME (min:sec)	**19:40**

4.1.3. Pulmonary Function Tests

Full pulmonary function tests including spirometry (Appendix I), plethysmography and diffusing capacity of carbon monoxide (DL_{CO}) (Appendix II) will be performed on all participants according to ATS guidelines. MedGraphics Elite Series, MedGraphics Corporation. St. Paul, MN USA and/or nDD EasyOne Spirometer, nDD Medical Technologies Inc. Andover, MA USA will be used. All measurements will be performed in the Pulmonary Function Laboratory at Robarts Research Institute. Lung clearance index (LCI) is a marker of overall ventilation inhomogeneity within the lung derived from the multiple breath nitrogen washout (MBNW) test (Appendix III). Participants breathe normally into a mouthpiece connected to a device, the NDD EasyOne Pro Lab for approximately 3-5 minutes.

4.1.4. Forced Oscillation Technique (FOT)

FOT (Appendix IV) will be performed on all participants at each visit using the "tremoFlo™" Airwave Oscillometry System (THORASYS Thoracic Medical Systems, Montreal, QC). FOT is a method for measuring lung function without patient effort. FOT functions by superimposing a gentle multi-frequency airwave onto the patient's normal respiratory airflow while measures of airway impedance are acquired through the analysis of resulting pressure and flow signals. Participants breathe naturally throughout the measurement sequence, which comprises of at least 3 measurements of 16 seconds

each. Airwaves comprise of small amplitude pressure oscillations (<2 cm H2O peak to peak) well below the acceptable limits of 5 cmH2O, which are far below attainable physiological normal transpulmonary pressures. Airwaves are specifically designed for performance with carefully selected frequencies between 5 and 38 Hz, with a commonly used waveform including of 6, 11 and 19 Hz. These are experienced by the participants as very small flutters during breathing and are easily tolerated. Device verification will be conducted on each day of use to confirm calibration parameters and performance characteristics fall within expected values for a reference load.

4.1.5. Fractional Exhaled Nitric Oxide (FeNO)

FeNO (Appendix V) will be measured pre-bronchodilator using the NIOX VERO® (Circassia Pharmaceuticals Inc, Morrisville, NC, USA, License number 98844). Participants will be seated upright and given the mouthpiece of the NIOX VERO®. Participants will be instructed to breathe out fully, then bring the mouthpiece to their lips to create a seal around the mouthpiece. Participants are then asked to inhale deeply to total lung capacity, then exhale slowly and completely into the mouthpiece.

4.1.6. Multi-Detector Computed Tomography (MDCT)

Thoracic low dose MDCT will be performed with the same inhalation breath-hold volume and maneuver (N_2 gas only) used for MRI to obtain participant-specific high resolution images of lung anatomy (tissue structure and airway morphology). CT imaging will be performed using an Aquilion ONE CT scanner (Canon Medical Systems USA, INC., Tustin, CA, USA) or a Lightspeed VCT CT scanner (General Electric, Waukesha WI, USA) at either Robarts Research Institute in London, Ontario or at University Hospital, located next door to Robarts Research Institute. Participants will be scanned in the supine position and during inspiration breath-hold after inhaling 1L of N_2 in order to match CT and MRI breath-hold volumes and anatomy.

4.1.7. Sputum Induction

Induced sputum will be collected at Visit 1, 1c and optional annual in-person visits. Sputum induction is a relatively non-invasive method to obtain sputum for cell or fluid phase inflammatory indices, culture or cytology. It is performed with an aerosol of normal or hypertonic saline generated by an ultrasonic nebulizer. The test will be performed post-bronchodilator to reduce the risk of chest discomfort. Participants will inhale the nebulized saline for 7 minutes up to 3 times, and will attempt to cough up a sample of sputum after each nebulization. FEV_1 will be assessed after each nebulization, and the test will be stopped if FEV_1 falls by >20% or >0.2L. If FEV_1 falls by >20% or >0.2L, salbutamol will be given and FEV_1 will be monitored until it is within 5% of baseline. Samples will be analysed by study staff at Robarts Research Institute. Analysis will begin within 2 hours of sputum collection, and samples will be kept in the locked sputum lab until analysis is complete, at which time samples will be destroyed.

4.1.8. Instruments

Participants with asthma will complete self-assessments in the form of the Asthma Quality of Life Questionnaire with Standardised Activities (AQLQ(S)) (bi-weekly) and Asthma Control Questionnaire (Appendix VI and VII). Self-reported data will be uploaded by each participant with asthma monthly to our website using a confidential and password protected upload (https://apilab.ca/patients.html). Participants will also complete the modified Borg Dyspnea Scale (Appendix VIII) and MRC Dyspnea scale (Appendix IX) at each visit to evaluate breathlessness at baseline and after therapy. Participants will complete the St George's Respiratory Questionnaire (SGRQ) at the time of their visit (Appendix X).

4.2. Centers

This is a single center study conducted at Robarts Research Institute, The University of Western Ontario.

4.3. Number of Participants

200 Asthmatic male and female participants, as well as 30 healthy volunteers aged 18-85 will be used for this study.

5. PARTICIPANT SELECTION CRITERIA

Male and females aged 18-85 will be used for this study. Participants will be recruited through the Asthma Centre located at St. Joseph's Hospital as well as the site's established database of Asthma patients. The London City-Wide Respirology practice serves over 1000 asthma patients annually.

30 healthy volunteers will also be recruited. Volunteers will include males and females aged 18-85. Healthy volunteers will be recruited via flyer advertisement in the community.

30 participants with asthma will be recruited from Dr. Parameswaran Nair's practice and research program at the Firestone Institute, McMaster University, and will be included in our 200 asthmatic male and female participants.

5.1. Inclusion Criteria

- Participants male and female aged 18-85 with a clinical diagnosis of asthma
- Healthy participants male and female aged 18-85
- Asthmatic participants with smoking history ≤ 1 pack/year
- Participant understands the study procedures and is willing to participate in the study as indicated by signature on the informed consent
- Participant is judged to be in otherwise stable health on the basis of medical history
- Participant able to perform reproducible pulmonary function testing (i.e., the 3 best acceptable spirograms have FEV_1 forced vital capacity (FVC) values that do not vary more than 5% of the largest value or more than 100 ml, whichever is greater.)
- FEV1 >60% predicted

5.2. Exclusion Criteria

- Participant is, in the opinion of the investigator, mentally or legally incapacitated, preventing informed consent from being obtained, or cannot read or understand the written material
- Participant is unable to perform spirometry or plethysmography maneuvers
- Healthy participants with a previous clinical diagnosis of respiratory disease, psychological disorder(s) or head trauma

- For the subset of asthmatics identified as underperceivers and undergoing brain fMRI, participants with a previous clinical diagnosis of psychological disorder(s) or head trauma
- Participant has an implanted mechanically, electrically or magnetically activated device or any metal in their body, which cannot be removed, including but not limited to pacemakers, neurostimulators, biostimulators, implanted insulin pumps, aneurysm clips, bioprosthesis, artificial limb, metallic fragment or foreign body, shunt, surgical staples (including clips or metallic sutures and/or ear implants.) (At the discretion of the MRI Technologist/3T Manager).
- In the investigator's opinion, participant suffers from any physical, psychological or other condition(s) that might prevent performance of the MRI, such as severe claustrophobia.
- Patient is pregnant
- Healthy participants with a previous clinical diagnosis of a respiratory disease or a history of head trauma or psychological disorders that may affect the ability to participate or follow instructions during the MRI. The healthy volunteer has to be a never smoker.

6. STUDY PROCEDURES

6.1. Screening Procedures for Study Entry

Eligibility for this study will be determined by the participant's clinical history, including respiratory disease and medication history. In addition, the investigator or designate shall discuss with each participant the nature of the study, its requirements and its restrictions, and an informed consent document will be signed. Each participant will be assigned a unique Study Number that will serve to identify participant study documentation. If a participant fails to qualify for enrollment, his/her Study Number may not be reused for another participant.

Participants performing fMRI will be either healthy volunteers or have been identified as having poor asthma control and demonstrate inaccurate perception of their asthma symptoms using asthma control and quality of life questionnaires. Specifically, these asthmatic participants are unable to sense sensations of dyspnea during asthma flair-ups. Participants who have suffered from a brain or head injury or participants that have diagnosed with an anxiety and depression disorder will be excluded from this part of the study.

6.2. Vital Signs

Vital signs will consist of sitting blood pressure, respiratory rate, heart rate, oral temperature, height and weight. Blood pressure will be taken using an appropriately sized cuff.

6.3. Pulmonary Function Tests

Full pulmonary function tests including spirometry (Appendix I), plethysmography, and DL_{CO} (Appendix II) will be performed according to ATS guidelines. MedGraphics Elite Series, MedGraphics Corporation. St. Paul, MN USA and/or nDD EasyOne Spirometer,

nDD Medical Technologies Inc. Andover, MA USA will be used. All measurements will be performed in the Pulmonary Function Laboratory at Robarts Research Institute.

LCI will be performed using the nDD Easy-One Pro Lab. Participants breathe normally into a mouthpiece connected to the device for 3-5 minutes.

6.4. FOT

FOT measures respiratory system function and will be performed according to European Respiratory Society (ERS) Guideline (Oostveen et al., Eur Respir J 2003; 22: 1026–1041) using a tremoFlo® C-100 airwave oscillometry system (Thorasys Thoracic Medical Systems Inc.; Montreal, QC, Canada, License number 92761). This is as follows; Participants breathe normally through a disposable standard antibacterial/antiviral filter as used in spirometry, and are in an upright and seated position. The participant's hands will support cheeks and a noseclip applied to the nose during the measurement. Three repeated measurements of 16 seconds each will then be acquired and measures of lung function evaluated using tremoFlo software. Since respiratory mechanics are slightly altered depending on supine or upright position, with an increase in respiratory system resistance (Rrs) and decrease in low frequency reactance (Xrs), in the supine position, FOT measurements will be obtained in the standard upright position for comparison with predicted values, but will then be obtained in the supine position to relate to the MRI data.

All measurements will be performed in the Pulmonary Function Laboratory at Robarts Research Institute (Appendix IV).

6.5. Fractional Exhaled Nitric Oxide (FeNO)

FeNO will be measured pre-bronchodilator using the NIOX VERO® (Circassia Pharmaceuticals Inc, Morrisville, NC, USA, License number 98844). Participants will be seated upright and given the mouthpiece of the NIOX VERO®. Participants will be instructed to breathe out fully, then bring the mouthpiece to their lips to create a seal around the mouthpiece. Participants are then asked to inhale deeply to total lung capacity, then exhale slowly and completely into the mouthpiece.

FeNO measurements will be performed at Robarts Research Institute in the Pulmonary Function Laboratory (Appendix V).

6.6. MDCT

Thoracic low dose MDCT will be performed with the same inhalation breath-hold volume and maneuver (N_2 only) used for MRI to obtain participant-specific high resolution images of lung anatomy (tissue structure and airway morphology). CT imaging will be performed using an Aquilion ONE CT scanner (Canon Medical Systems USA, INC., Tustin, CA, USA) or a Lightspeed VCT CT scanner (General Electric, Waukesha WI, USA) at either Robarts Research Institute in London, Ontario or at University Hospital, located next door to Robarts Research Institute. Participants will be scanned in the supine position and during inspiration breath-hold after inhaling 1L of N_2 in order to match CT and MRI breath-hold volumes and anatomy.

6.7. General MRI Testing Procedures

Participants will undergo MRI in the supine position. MRI localizing scans will be performed prior to administration of the hyperpolarized ^{129}Xe. One of three ^{129}Xe chest coils covering the entire thorax will be used. Participants will be instructed to take a deep

breath, exhale completely, then inhale the ^{129}Xe completely and hold their breath for 13-16 s. The chest coils which may be used are:

1. ^{129}Xe Radio Frequency Coil for Human Lung Imaging
2. CMRS Flexible Lung Coil (Clinical MR Solutions)
3. ^{129}Xe Lung Coil (RAPID Biomedical)

For the subset of participants undergoing fMRI acquisition, they will be placed in the supine position, and their head will be covered in an elliptical head coil (shown below). Participants will be instructed to remain motionless during the 20-minute scanning process.

Head Coil

7. STUDY PARAMETERS

7.1. Evaluation of Disease

7.1.1. Pulmonary Function Testing

The FEV_1 and FVC will be recorded according to ATS guidelines. Parameters to be assessed include but are not limited to: total lung capacity (TLC), functional residual capacity (FRC) and DLco. FRC measures the volume in the lungs when the muscles of respiration are relaxed. FOT measurements will be recorded according to ERS recommendations (Oostveen et al., Eur Respir J 2003; 22: 1026–1041). Input impedance is calculated as the ratio of pressure and flow signals and are given as Rrs and reactance Xrs at each oscillation frequency as well as comparison to predicted values. Additional outcomes that are provided from this measurement include tidal volume, respiratory rate, and resonant frequency.

Frequency dependence of Rrs is an index of ventilation heterogeneity, and low frequency reactance (6 Hz) can be used as an index of airway closure (Lutchen et al., 20xx) and we are investigating if this corresponds to the occurrence of ventilation defects. We will assess the frequency dependence by subtracting Rrs at 19 Hz from Rrs at 6 Hz. Furthermore, low frequency elastance at 6 Hz can be used as an index of airway closure indicative of the presence of low or near zero ventilation.

FeNO will be measured one time according to instructions from the machine which is set to perform to ATS guidelines. The FeNO value will be displayed on the machine and recorded in source documents, it is a valuable tool in the assessment of airway inflammation.

7.1.2. MRI

During the gas administration and breath hold, all participants will be monitored using pulse oximetry for potential desaturation events. In the research setting at Robarts Research Institute, ^{129}Xe gas is mixed with medical grade ^{4}He and administered in a bag to volunteers for a single breath-hold; thus, the gas mixture in the research volunteer lung is hypoxic (containing no oxygen). The effects and safety profile of a single breath of

hypoxic gas have been assessed in thousands of participants worldwide with excellent tolerability results. The safety risk to participants and research volunteers stemming from the use of ^{129}Xe gas contrast thus is directly related to: 1) the hypoxic nature of the gas mixture and not the actual content of the gas inhaled because the gas is chemically and physically inert and, 2) the length of the voluntary breath-hold (up to 16s maximum). With respect to the potential for oxygen desaturation events, at Robarts, participants are continuously monitored by pulse oximetry. Heart rate is also monitored using pulse oximetry. In addition, all participants will have oxygen nasal cannula in place during the MRI procedure. Supplemental oxygen will be administered at a flow rate of 2 L/m prior until immediately prior to administration of the hyperpolarized gas mixture. This "pre-oxygenation" procedure provides the lung with a greater amount of oxygen stored prior to breathing the single hypoxic breath, and minimizes any degree of desaturation that might occur. If a participant becomes uncomfortable during the maximum breath-hold of 16s they can breathe out the gas and subsequently breathe in room air and/or medical oxygen provided. Furthermore, in the event that oxygen saturation (monitored by pulse oximetry) during the MRI falls to below 88% for a period of > 10 seconds, all study participants will be administered an increase in supplemental oxygen. Moreover, research participants and volunteers may release the hypoxic breath-hold at any time and this effectively washes out the ^{129}Xe and N_2 immediately with room air and supplementary oxygen.

7.1.3. Hyperpolarized ^{129}Xe Contrast

For imaging, a gas contrast agent is used - hyperpolarized ^{129}Xe gas. The xenon component of a 99.25% medical grade ^{129}Xe; 0.75% medical grade N_2 mixture is polarized using a Xenon Hyperpolarizer Model 9800 (Polarean; XeniSpin, Durham NC, USA), until approximately 30% of the xenon is polarized. 5mL/kg (participant body weight) of this gas is mixed with medical grade ^{4}He to form a 1L volume and is dispensed immediately to the participant through a sample bag and straw while the participant is supine in the scanner. ^{129}Xe gas has been used in previous human studies at 0.5T, 1.5T and 3T with excellent safety and tolerability reported.

7.1.4. Hyperpolarized ^{3}He Contrast

For imaging, a gas contrast agent is used - hyperpolarized ^{3}He gas. The helium component of a 99.25% Medical grade ^{3}He; 0.75% Medical grade N_2 mixture is polarized using a GE polarizer IGI.9600, until approximately 30% of the Helium is polarized. 5mL/kg (participant body weight) of this gas is mixed with Medical Grade N_2 to form a 1L volume and is dispensed immediately to the participant through a sample bag and straw while the participant is supine in the scanner. ^{3}He gas has been used in previous human studies at 0.5T, 1.5T and 3T with excellent safety and tolerability reported.

7.1.5. fMRI BOLD Contrast Signal and DTI

For brain imaging, BOLD contrast will be used to measure physiological changes in blood flow and oxygen metabolism in the brain. The magnetic properties of oxygen-rich and oxygen-deficient blood provide a MR signal to indicate underlying neural activity changes.

DTI will be utilized to analyze and track neural pathways. The magnetic resonance signal is able to track the random motion of water molecules in white matter fiber tracts. The diffusion along the fibers provides information of the magnitude, orientation, and direction of cerebral connections.

7.2. Safety Parameters

7.2.1. General Safety

Adverse events (AEs) may occur in the course of the study within the screening or the follow-up period of 24 hr. Although there is no investigational drug or vaccine being administered, participants will be followed for all procedure-related serious adverse event (SAE) for 24 hours following each visit. Such events will be recorded at each examination in the study file.

Such events will be recorded at each examination on the AE Case Report Forms. An AE is defined as any unfavorable and unintended change in the structure, function, or chemistry of the body temporally associated with the test procedure, whether or not considered related to the test procedure. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a pre-existing condition which is temporally associated with the test procedure, is also an AE.

An investigator, who is a qualified physician, will evaluate all AEs as to:

Maximum intensity:

- Mild (awareness of sign or symptom, but easily tolerated);
- Moderate (discomfort enough to cause interference with usual activity);
- Severe (incapacitating with inability to work or do usual activity).

Seriousness:

An SAE is any AE that:

†Results in death; or

†Is life threatening (places the participant, in the view of the investigator, at immediate risk of death from the event as it occurred [Note: This does not include an AE that, had it occurred in a more severe form, might have caused death.]); or

†Results in a persistent or significant disability/incapacity (substantial disruption of one's ability to conduct normal life functions); or

†Results in or prolongs an existing inpatient hospitalization (hospitalization is defined as an inpatient admission, regardless of length of stay, even if the hospitalization is a precautionary measure for continued observation) (Note: hospitalization [including hospitalization for an elective procedure] for a pre-existing condition which has not worsened does not constitute am SAE.); or

†Is a congenital anomaly/birth defect (in offspring of participant taking the product regardless of time to diagnosis); or

Is a cancer; or

Is an overdose (whether accidental or intentional).

Other important medical events that may not result in death, not be life threatening, or not require hospitalization may be considered a SAE when, based upon appropriate medical judgment, the event may require medical or surgical intervention to prevent one of the outcomes listed previously (designated above by a †).

Duration:

Record the start and stop dates of the AE. If less than 1 day, indicate the appropriate length of time and units.

Action taken:

Did the AE cause the test procedure to be discontinued?

Relationship to test procedure:

The determination of the likelihood that the test procedure caused the AE will be provided by an investigator who is a qualified physician. The investigator's signed/dated initials on the source document or case report form, supporting the causality noted on the AE form, ensures that a medically qualified assessment of causality was done. This initialed document must be retained for the required regulatory time frame. The criteria below are intended as reference guidelines to assist the investigator in assessing the likelihood of a relationship between the test procedure and the AE based upon the available information.

The following components are to be used to assess this relationship; the greater the correlation with the components and their respective elements (in number and/or intensity), the more likely the test procedure caused the AE:

- Exposure:
  - Is there evidence there was an actual exposure to the test procedure?
- Time Course:
  - Did the AE follow in a reasonable temporal sequence from administration of the test procedure?
  - Is the time of onset of the AE compatible with a procedure-induced effect?
- Likely Cause:
  - Is the AE not reasonably explained by another etiology such as underlying disease, other procedure(s), or other host or environmental factors?

The assessment of relationship will be reported on the case report forms by an investigator who is a qualified physician according to his/her best clinical judgment, including consideration of the above elements. Use the following scale of criteria as guidance (not all criteria must be present to be indicative of a procedure relationship).

- Definitely related to test procedure:
  - There is evidence of exposure to the test procedure.
  - The temporal sequence of the AE onset relative to administration of the test procedure is reasonable.
  - The AE is more likely explained by the test procedure than by another cause.
  - The AE shows a pattern consistent with previous knowledge of the test procedure.
- Probably related to test procedure:
  - There is evidence of exposure to the test procedure.
  - The temporal sequence of the AE onset relative to administration of the test procedure is reasonable.
  - The AE is more likely explained by the test procedure than by another cause.

- Possibly related to test procedure:
  - There is evidence of exposure to the test procedure.
  - The temporal sequence of the AE onset relative to administration of the test procedure is reasonable.
  - The AE could have been due to another equally likely cause.
- Probably not related to test procedure:
  - There is evidence of exposure to the test procedure.
  - There is another more likely cause of the AE.
- Definitely not related to test procedure:
  - Did not receive the test procedure.

    OR
  - Temporal sequence of the AE onset relative to administration of the test procedure is not reasonable.

    OR
  - There is another obvious cause of the AE.

Any SAE, including death, related to the procedure, from the time the consent is signed through 24 hours thereafter, will be reported within 24 hours to the PI and UWO Health Sciences Research Ethics Board. All AEs will be assessed and evaluated 24 hours after study completion.

7.2.2. Immediately Reportable AEs

ANY CLINICAL AE OR ABNORMAL LABORATORY TEST VALUE THAT IS **SERIOUS** (INCLUDING DEATH, OVERDOSE OR CONGENITAL ANOMALY) OCCURRING DURING THE COURSE OF THE STUDY, IRRESPECTIVE OF THE TREATMENT RECEIVED BY THE PARTICIPANT, MUST BE REPORTED WITHIN **ONE** WORKING DAY OF OCCURRENCE.

An SAE is any experience that suggests a significant hazard, contraindication, side effect or precaution. With respect to human clinical experience, this includes any experience which

- is fatal or life-threatening;
- is permanently disabling, i.e., incapacitating or interfering with the ability to resume usual life patterns;
- requires in-patient hospitalization or prolongation of hospitalization;
- is a congenital anomaly, cancer; or
- is an overdose.

The term **severe** is a measure of **intensity**: thus, an SAE is not necessarily **serious**. For example, nausea of several hours' duration may be rated as severe, but may not be clinically serious.

An **overdose** is a deliberate or inadvertent administration of a treatment at a dose higher than that specified in the protocol and higher than known therapeutic doses. It must be reported irrespective of outcome even if toxic effects were not observed.

A **death** occurring during the study or which comes to the attention of the investigator within 4 weeks after stopping the treatment whether considered treatment-related or not, must be reported.

For all AEs, the following must be assessed and recorded on the AEs page of the Case Report Form: intensity, relationship to test substance, action taken regarding test substance, and outcome to date.

Such preliminary reports will be followed by detailed descriptions later, which will include copies of hospital case reports, autopsy reports and other documents when requested and applicable.

The Health Sciences Research Ethics Board at The University of Western Ontario will be notified of such an event in writing as soon as is practical.
SAEs related to the 129Xe Radio Frequency Coil for Human Lung Imaging, CMRS Flexible Lung Coil and/or 129Xe Lung Coil devices:
The qualified investigator is required to report SAEs to Health Canada and to the manufacturer and importer within 72 hours of discovery in cases in which the incident is related to failure of the device or a deterioration in its effectiveness, or an inadequacy in its labelling or in its directions for use and has led to the death or a serious deterioration in the state of health of a patient, user or other person, or could do so were it to recur.

Manufacturers and importers are required to provide a preliminary and a final report in respect of the incident within 10 days after they become aware of the incident, if 1) the incident has led to the death or a serious deterioration in the state of health of a patient, user or other person or 2) within 30 days after they become aware of the incident if the incident has not led to the death or a serious deterioration in the state of health of a patient, user or other person, but could do so were it to recur.

7.2.3. Premature Withdrawal/Discontinuation Criteria

Participants will be discontinued from further study participation if:

- A clinical AE occurs suggesting that:
    - The participant's health may be in jeopardy from continued participation in the study;

      OR
    - The participant is unable to complete the study procedures successfully for any reason
- Participant discloses they are pregnant.
- The participant withdraws from the study at any time.

Any AEs, present at the time of discontinuation/withdrawal, should be documented and followed until resolution.

8. IMAGE ANALYSIS AND STATISTICAL ANALYSIS PLAN

All images will be analyzed in a dedicated image visualization environment with room lighting levels equivalently established for all image analysis sessions.

1) Apparent Diffusion Coefficient (ADC) measurements and ADC maps
During a single breath-hold, two interleaved images are acquired – one without diffusion sensitization and the second image acquired with the presence of diffusion sensitization

through the application of the additional pulsed magnetic field gradient with pulse duration δ and maximum value **g**. The signal intensity of the second image is derived from

$$\mathbf{S = S_0 \exp(-b \cdot ADC)}$$

where $\mathbf{S_0}$ is a signal intensity of the first image, **ADC** is an apparent self-diffusion coefficient and $\mathbf{b} = \frac{2}{3}\gamma^2\delta^3\mathbf{g}^2$ (γ is a gyromagnetic ratio). Accordingly, the **ADC** value for each image pixel on a pixel by pixel basis or location can be calculated from the following equation:

$$\mathbf{ADC} = \frac{\ln\left(\frac{S_0}{S}\right)}{b}.$$

Diffusion weighted images from each visit will be reviewed and compared with the ^{1}H anatomical images. Mean, whole lung, and centre slice ADC (and standard deviation) will be calculated from the maps based on the formula above and recorded. ADC values will also be calculated for each slice and for six regions of interest to assess regional variation in this measure. ADC gradients in the superior-inferior and anterior-posterior directions will be calculated for each participant and visit.

2) Ventilation Defect Percent (VDP) and ^{1}H MRI thoracic cavity volume (TCV)
Ventilation defect volume (VDV), VDP, ventilation volume (VV) and percent ventilated volume (PVV) will be generated for whole lung and individual lung slices using manual or semi-automated segmentation. For ^{1}H thoracic cavity images, the TCV will be calculated by manual or semi-automated segmentation [29,30].

3) ^{129}Xe Dissolved phase spectroscopy measurements
^{129}Xe dissolved phase MRI data will be reconstructed using a re-gridding method. The following ratios will be determined by the area-under-the curve obtained from spectroscopy: red-blood-cell to alveolar membrane ratio (RBC:membrane); RBC to gas ratio (RBC:gas); and the membrane to gas ratio (membrane:gas). Spectroscopic signals will be used to reconstruct perfusion and alveolar membrane maps.

Seven-day, 14-day and 1-year reproducibility of ^{129}Xe MRI measurements will be determined using the coefficient of variation and intraclass correlation coefficients (ICC). The changes in regional VDP will be evaluated for all participants after 2 years pre- and post-salbutamol.

We will evaluate Pearson correlations for MRI measurements (VDP, TCV) with CT (WA%), spirometry and plethysmography measurements to determine whether structure relates to function (and *vice versa*). We will also evaluate the relationship between sputum, biopsy, CT and MRI measurements. Significant differences in Pearson correlations will be determined using 95% Confidence Intervals (CI). To evaluate agreement between measurements, Bland-Altman plots will be generated to show the differences between WA% and ^{3}He VDP with ^{129}Xe VDP.

Univariate and multivariate regression models will also be used and adjusted for confounders to assess correlations of imaging measurements with dyspnea score, and asthma control measures for a better understanding of the relationship between imaging and established/validated clinical measurements of asthma. We will also examine the relationship between clinically defined asthma severity and imaging. In each of these models, we will employ a forward stepwise selection modeling process to maximize the

efficiency, while maintaining the validity of the modeling process. We will select only those covariates with a p-value of 0.25 or less and include only those variables that have a p-value of 0.2 or less in the final model. From these models, we will determine the total R^2 values as well as partial R^2. The Holm-Bonferroni correction will adjust for multiple comparisons.

For participants undergoing fMRI, we will utilize an unpaired two tailed t-test to assess the differences of BOLD and DTI signal intensity between asthma underperceivers and healthy controls. Furthermore, we will assess Pearson correlations for fMRI measurements (BOLD signals and DTI data) with lung MRI and spirometry measurements. Spearman correlations will be used to analyze the relationship between fMRI measurements and Asthma Control Questionnaire (ACQ) scores. Significant differences in Pearson and Spearman correlations will be determined using 95% CI.

For healthy volunteers and asthmatic underperceivers undergoing fMRI, we will utilize a paired two tailed t-test to assess the within group differences of BOLD and DTI signal intensity before and after a methacholine challenge. Pearson correlations will be used to assess the relationship between quantitative fMRI data and spirometry measurements. Significant differences in Pearson correlations will be determined using 95% CI.

To demonstrate if differences exist in BOLD and DTI signal between asthmatics and healthy volunteers post methacholine challenge, we will utilize an unpaired two tailed t-test. Pearson correlations will be used to assess the relationship between quantitative fMRI data and spirometry measurements. Significant differences in Pearson correlations will be determined using 95% CI.

9. PREMATURE WITHDRAWAL OF PARTICIPANTS FROM STUDY

Participants may withdraw from the study at any time for any reason. Participants may be excluded from the study by the investigator due to failure of participant to comply with study procedures. In the event that a participant withdraws from the study voluntarily or is withdrawn by the investigator, it will be reported in the study file and will be documented appropriately. It will also be considered accordingly in the statistical analyses.

The participant will be discontinued from further study participation if:

- The participants oxygen saturation falls to <80% continuously for >15 seconds.
- A clinical adverse event occurs suggesting that:
    - The participant's health may be in jeopardy from continued participation in the study;

      OR
    - The participant is unable to complete the study procedures successfully.
- The participant withdraws voluntarily from the study at any time.

Participants who discontinue from the study for reasons unrelated to the study (e.g. personal reasons) will be replaced as required for the study to meet its objectives.

10. LIST OF REFERENCES

1. Wheatley, A., L. Mathew, S. McKay, C. Lisckai, D. G. McCormack, and G. Parraga. *Reproducibility of Ventilation in asthma: Initial Findings of ventilation defect volume.* Proceedings of the International Society for Photonics and Instrumentation Engineers (SPIE): Medical Imaging 2008.
2 Black RD, Middleton HL, Cates GD, et al. In vivo He-3 MR images of guinea pig lungs. Radiology 1996; 199:867-870.
3. de Lange EE, Mugler JP, 3rd, Brookeman JR, et al. Lung air spaces: MR imaging evaluation with hyperpolarized ^{3}He gas. Radiology 1999; 210:851-857.
4. Kauczor H, Surkau R, Roberts T. MRI using hyperpolarized noble gases. Eur Radiol 1998; 8:820-827.
5. Altes TA, de Lange EE. Applications of hyperpolarized helium-3 gas magnetic resonance imaging in pediatric lung disease. Top Magn Reson Imaging 2003; 14:231-236.
6. Middleton H, Black RD, Saam B, et al. MR imaging with hyperpolarized ^{3}He gas. Magn Reson Med 1995; 33:271-275.
7. van Beek EJ, Wild JM, Kauczor HU, Schreiber W, Mugler JP, 3rd, de Lange EE. Functional MRI of the lung using hyperpolarized 3-helium gas. J Magn Reson Imaging 2004; 20:540-554.
8. Altes TA, Powers PL, Knight-Scott J, et al. Hyperpolarized 3He MR lung ventilation imaging in asthmatics: preliminary findings. J Magn Reson Imaging 2001; 13:378-384.
9. Donnelly LF, MacFall JR, McAdams HP, et al. Cystic fibrosis: combined hyperpolarized 3He-enhanced and conventional proton MR imaging in the lung--preliminary observations. Radiology 1999; 212:885-889.
10. Guenther D, Eberle B, Hast J, et al. (3)He MRI in healthy volunteers: preliminary correlation with smoking history and lung volumes. NMR Biomed 2000; 13:182-189.
11. Kauczor HU, Hofmann D, Kreitner KF, et al. Normal and abnormal pulmonary ventilation: visualization at hyperpolarized He-3 MR imaging. Radiology 1996; 201:564-568.
12. McAdams HP, Palmer SM, Donnelly LF, Charles HC, Tapson VF, MacFall JR. Hyperpolarized 3He-enhanced MR imaging of lung transplant recipients: preliminary results. AJR Am J Roentgenol 1999; 173:955-959.
13. Salerno M, Altes TA, Mugler JP, 3rd, Nakatsu M, Hatabu H, de Lange EE. Hyperpolarized noble gas MR imaging of the lung: potential clinical applications. Eur J Radiol 2001; 40:33-44.
14. Ruset IC, Ketel S, Hersman FW. Optical pumping system design for large production of hyperpolarized. Phys Rev Lett 2006; 96:053002
15. Patz S, Muradian I, Hrovat MI, et al. Human pulmonary imaging and spectroscopy with hyperpolarized ^{129}Xe at 0.2T. Acad Radiol 2008; 15:713-727.
16. Matsuoka S, Patz S, Albert M, et al. Hyperpolarized gas MR imaging of the lung: current status as a research tool. J Thorac Imaging 2009; 24:181-188
17. Patz S, Hersman FW, Muradian I, et al. Hyperpolarized ^{129}Xe MRI: A viable functional lung imaging modality? Eur J Radiol 2007; 64:335-344

18. Janssens T, Verleden G, De Peuter S, Van Diest I, Van den Bergh O. Inaccurate perception of asthma symptoms: a cognitive–affective framework and implications for asthma treatment. Clin Psychol Rev 2009; 29:317-27.

19. Steele AM, Meuret AE, Millard MW, Ritz T. Discrepancies between lung function and asthma control: asthma perception and association with demographics and anxiety. Allergy Asthma Proc 2012; 33:500-507.

20. Xiong X, Zhu H, Wang T, Ji Y. Altered intrinsic regional brain activity in female asthmatics with or without depressive symptoms: A resting-state functional magnetic resonance imaging study. J Asthma. 2016; 53:922-9.

21. Rosenkranz MA, Busse WW, Sheridan JF, Crisafi GM, Davidson RJ. Are there neurophenotypes for asthma? Functional brain imaging of the interaction between emotion and inflammation in asthma. PloS one. 2012; 7:e40921.

22. Von Leupoldt A, Brassen S, Baumann HJ, Klose H, Büchel C. Structural brain changes related to disease duration in patients with asthma. PloS one. 2011; 19: e23739.

23. Fransson P. Spontaneous low-frequency BOLD signal fluctuations: An fMRI investigation of the resting-state default mode of brain function hypothesis. Hum Brain Mapp. 2005; 26:15-29.

24. Alexander AL, Lee JE, Lazar M, Field AS. Diffusion tensor imaging of the brain. Neurotherapeutics. 2007; 4:316-29.

11. APPENDICES FOR PART I

Appendix I Guidelines for Spirometry Testing

Equipment Calibration

Calibration and maintenance logs must be maintained for the body plethysmograph and the hand-held spirometer. Calibration must be performed on days when there is a study visit or weekly, whichever is more often.

Procedures

In order to assure acceptable spirometry data with a sufficient level of accuracy and precision, the technician should coach each participant to perform maximal expiratory maneuvers with each measurement. The technician should pay special attention to ensure that the participant:

- Takes a full, deep breath (i.e., to total lung capacity) with nose clip in place (or nose pinched closed with thumb and forefinger) and has a tight seal with his/her lips around the mouthpiece.
- Blows out forcefully, without hesitation; and
- Continues exhalation with sufficient effort until all air is exhaled;
- Is coached continually during the maneuver by the technician (e.g., “BLOW OUT HARD...BLOW...BLOW...”) to assure maximum effort (but not to the point of syncope).

Maneuver Acceptability Criteria

A spirometry measurement will be judged acceptable when it conforms to ATS standards for acceptability. These criteria include:

- Adequate start of the test.
- Adequate expiratory time (at least 6 seconds).
- Adequate “time to” peak flow.
- No evidence of interruption by coughing, glottis closure, obstruction by tongue or false teeth.

The achievement of ATS reproducibility criteria will be the overall goal. This will be defined as: the best 2 acceptable spirograms have FEV_1 values that do not vary by more than 5% of the largest value or more than 100 mL, whichever is greater.
However, it is understood that reproducibility criteria in participants with respiratory disease is difficult to achieve. Thus, it is recognized that some participants, by giving maximal effort, become increasingly fatigued on each subsequent maneuver. In this case, fewer than 3 maneuvers are allowed; the first FEV_1 will be considered the best.

Reporting of Data

For the purpose of analysis, the largest FEV_1 and the largest FVC from the acceptable and reproducible maneuvers (regardless of whether they were obtained from the same curve or not) will be considered the true value for that series of measurements.
At the end of each spirometry test, a hardcopy printout of the data should be produced as described in the instrument manual. The original printout of the measurements will serve as the source documents for these data.

Appendix II Guidelines for Plethysmography

Equipment Calibration

Calibration and maintenance logs must be maintained for the body plethysmograph. Calibration must be performed on days when there is a study visit or weekly, whichever is more often.

Procedures

At each visit, the qualified investigator or other qualified site personnel will explain and demonstrate the test maneuver. The investigator or other site personnel will perform the procedure according to the manufacturer's guidelines. Lung volume measurements to be collected include but are not limited to: TLC and FRC. To determine the functional integrity of the alveolar-capillary membrane, a test to measure DL_{CO} will be performed.

Procedures for these measurements include the following:

- Participant will be seated upright and breathing movements should not be restricted by clothing.
- Participant will place the mouthpiece in their mouth and a nose clip on the nose.
- The participant will perform several tidal breaths to initiate the process and will be instructed to exhale slowly. At the end-exhalation the participant valve will close for start of inspiration.
- After the mouth shutter closes, the participant will be instructed to gently "pant" (approximately 50 to 60 mL of air). Pant frequency should be approximately 1 Hz. The cheeks and chin should be supported with both hands. This should be done without supporting the elbows or elevating the shoulders.
- The shutter should only be closed for about 2 seconds, to avoid undue participant discomfort.
- Lung volume measurements (inspiratory capacity (IC), slow vital capacity (SVC), etc.) may be measured immediately after the shutter is reopened and before coming off the mouthpiece. If the participant is too short of breath, the lung volume measurements may be performed after a brief rest period.
- At least 3 separate, acceptable maneuvers should be performed.
- The result from this effort is displayed on the computer screen displaying the pressure vs. time tracing.
- To measure DL_{CO}, the participant will perform several tidal breaths to initiate the process. Participant should then be instructed to exhale slowly and maximally to residual volume. At the end of the exhalation, the participant valve will be closed for the start of inspiration and the participant circuit will open to the source of gas. The participant will then inhale through the mouthpiece a special diffusion gas mixture containing neon, oxygen, nitrogen and carbon monoxide.
- The participant will hold his or her breath at TLC for 6 seconds. The participant will be instructed to relax against the closed valve for this period. When the valve opens, the participant will be instructed to exhale rapidly until the sample is collected. The test will then end and the participant can relax and remove the nose clips.

- The gas sample is collected automatically into the chromatograph for analysis. Once the results from this effort are displayed on the computer screen, the system is ready for additional efforts. At least 2 separate, acceptable maneuvers should be performed, allowing four minute interval between efforts. The results of the two efforts should agree within 10%.

Maneuver Acceptability Criteria

An acceptable thoracic gas volume (TGV) maneuver is defined as

- Proper panting technique is achieved as indicated by the tracing (the loop generated against a closed shutter should be closed or nearly so).
- Recorded pressure changes are within the calibrated pressure range of each transducer and that no leaks are evident.
- The entire tracing should be visible.
- Evidence of thermal equilibrium (i.e. tracings do not drift on the display or recording).
- If all data points lie within 5% of the mean TGV, these trials are considered "acceptable". Continue trials until at least 3 separate, acceptable trials are achieved. Up to a maximum of 8 trials should be performed for a given visit. NOTE: The mean TGV will be reported as the mean of all acceptable trials.

Multiple SVC measurement should be performed with the 2 largest measurements agreeing within 200 mL. The largest SVC measurement will be recorded. The mean values for IC should be recorded and the measurements should agree within 5% of the mean.

An acceptable DL_{CO} maneuver is defined as:

- The participant does not begin to inhale before the valving system is activated or the participant's inspired volume will not be measured correctly.
- If results of two efforts are within 10% these trials are considered "acceptable. A third effort should be attempted if the first two efforts are not reproducible.

Reporting of Data

At the end of each pulmonary function test, a hardcopy printout of the data should be produced as described in the instrument manual. The original printout of the measurements will serve as the source documents for these data.

Appendix III Guidelines for MBNW

Equipment Calibration

The machine will determine environmental conditions and auto-calibrate.

Procedures

In order to ensure acceptable MBNW data with a sufficient level of accuracy and precision, the technician should coach each participant. The technician should pay special attention to ensure that the participant:

- Has nose clip in place (or nose pinched closed with thumb and forefinger) and has a tight seal with his/her lips around the mouthpiece.

Maneuver Acceptability Criteria

A MBNW measurement will be judged acceptable when it conforms to ERS/ATS standards for acceptability. These criteria include:

- Adequate start of the test.
- Adequate SVC measurement.
- Regular breathing pattern throughout test.
- No evidence of interruption by coughing, glottis closure, obstruction by tongue or false teeth.

However, it is understood that reproducibility criteria in participants with respiratory disease is difficult to achieve. Thus, it is recognized that some participants, by giving maximal effort, become increasingly fatigued on each subsequent maneuver. In this case, fewer than 3 maneuvers are allowed; the first MBNW test will be considered the best.

Reporting of Data

For the purpose of analysis, the mean LCI from the acceptable and reproducible maneuvers will be considered the true value for that series of measurements.

At the end of each MBNW test, a hardcopy printout of the data should be produced as described in the instrument manual. The original printout of the measurements will serve as the source documents for these data.

Appendix IV Guidelines for FOT

Equipment Calibration

Device verification must be conducted on each day of use prior to initiating a Patient Test. The verification tests confirm that the calibrated parameters and performance characteristics fall within expected values for a reference load. The automated Test Load Calibration Wizard will guide the operator through the necessary sequence and displays a PASS/FAIL result for two verification tests: (1) Static Check, and (2) Frequency Sweep. Both verification tests must pass prior to measuring a patient. Successful verification results are valid for 24 hours.

Procedures

In order to ensure high quality and repeatable FOT data, the technician should follow a standard measurement sequence, allowing for an initial start-up time where the patient can familiarize his/herself with the device and oscillating airwaves. The recommended measurement sequence is as follows:

- Seat the patient in an upright position and ensure breathing is not restricted at the neck due to chin position or clothing. If required for research purposes, the measurements can also be done in the supine position, but the impedance values are then indicated as for the supine position.
- Connect a disposable, anti-viral/bacterial filter to the device. Ask the patient to block their nasal passage with a noseclip and place the mouthpiece in their mouth. Patients should gently rest their lips on the mouthpiece and avoid contacting the mouthpiece with any force with their teeth – avoid biting.
- Monitor the patient's normal breathing (no oscillating airwave) to ensure tidal breathing is even and stable. Typically, 5 to 10 seconds is sufficient. Coach the patient to support their cheeks and soft parts below the chin with both hands while breathing to prevent motion of the cheeks. This should be done without supporting the elbows or elevating the shoulders.
- Press either the left or right hand held button to activate an oscillating airwave and capture a recording. Allow the waveform to completely finish before removing the device from the patient's mouth. Typical waveforms run for 16 seconds.
- Provide the patient with a short break of 10 to 30 seconds, during which they may remove their mouth from the device and breathe naturally.
- Begin monitoring your patient once again and capture additional measurements.
- The results and repeatability are provided on the screen for brief evaluation after each measurement.
- Capture a minimum of 3-4 consistent measurements.
- The participant can come off the device at any time for any discomfort or if they need to cough or talk, but then the measurement will need to be repeated.

Acceptability Criteria

An FOT measurement and Patient Test result will be judged acceptable when they conform to the ERS guidelines for reproducibility and meet the following criteria:

Coherence of outcomes Rrs and Xrs at each frequency of interest (i.e. R_6, R_{19}, X_6, and X_{19}, where for example, R_6 means Rrs at 6 Hz) must exceed 90% for each measurement.

Coherence less than 90% can indicate that noise has contaminated the measurement. The coherence is indicated by the software.

- Coefficient of variation (CV) for Rrs and Xrs at each frequency of interests for the final array of successful measurements must NOT exceed 15%. CV is provided by the software.

It is acceptable to exclude whole or part measurements to achieve the above stated acceptability criteria. Exclusion may be conducted in two ways:

- Dataset Delimiting may be used to select portions of a measurement containing artefacts such as a cough, swallow or talking to be excluded from analysis.
- Whole measurements representing clear outliers and/or unsuccessful trials such as due to excessive coughing or coming off the device during measurement may be excluded by checking the 'Exclude' box on the measurement card in Results View.

The operator is recommended to ensure that averaged outcomes are calculated on a minimum of 3 satisfactory measurements.

Reporting of Data

For the purpose of analysis, the averaged results from only the acceptable and reproducible measurements will be considered valid for a Patient Test. Excluded datasets and/or reporting intervals will not be included in the final averaged values. At the end of each FOT Patient Test, a hardcopy printout of the data should be produced. The original printout of the test results will serve as the source documents for these data.

Appendix V FeNO

Equipment Calibration

Device quality must be performed on days when there is a study visit or weekly, whichever is more often.

Procedures

In order to obtain high quality FeNO values, participants should not eat, drink, or smoke an hour prior to testing. At each visit, the qualified investigator or other qualified site personnel will explain and demonstrate the test maneuver. This can be done using the "DEMO" feature of the machine for the participant.

Procedures for this measurement include the following:

- Participant will be seated upright and breathing movements should not be restricted by clothing.
- Participant will empty the lungs by fully exhaling.
- Participant will close their lips around the mouthpiece so that no air leakage occurs and should inhale deeply through the mouthpiece. The cloud on the display will move upward on the screen.
- Participant will exhale slowly through the mouthpiece while keeping the cloud within the limits as shown on the display listening and watching the screens for cues on the correct exhalation pressure.
- Participant will exhale until the cloud passes the flag as displayed on the screen.

The machine will analyze the sample and generate a result in approximately one minute.

Acceptability Criteria

An acceptable FeNO measurement is determined by the machine. The participant must be able to keep their exhalation pressure within the acceptable range and exhale until the cloud reaches the flag. Only one successful measurement should be completed.

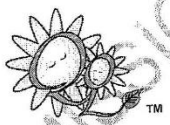
Reporting of Data

The FeNO result will be calculated and displayed on the machine. This result should be recorded on the participant source documents in the space provided.

Appendix VI AQLQ(S)

ASTHMA QUALITY OF LIFE QUESTIONNAIRE WITH STANDARDISED ACTIVITIES (AQLQ(S))

SELF-ADMINISTERED



For further information:

Elizabeth Juniper, MCSP, MSc
Professor
20 Marcuse Fields
Bosham, West Sussex
PO18 8NA, England
Telephone: +44 1243 572124
Fax: +44 1243 573680
E-mail: juniper@qoltech.co.uk
Web: http://www.qoltech.co.uk

Development and validation
supported by
GLAXO WELLCOME, INC

APRIL 2008

Modified September 2010
AQLQ(S)-SA North American English Version

ASTHMA QUALITY OF LIFE QUESTIONNAIRE (S) PATIENT ID: _____

SELF-ADMINISTERED DATE: _____

Please complete **all** questions by circling the number that best describes how you have been during the **last 2 weeks as a result of your asthma.**

HOW **LIMITED** HAVE YOU BEEN **DURING THE LAST 2 WEEKS** IN THESE ACTIVITIES **AS A RESULT OF YOUR ASTHMA**?

	Totally Limited	Extremely Limited	Very Limited	Moderate Limitation	Some Limitation	A Little Limitation	Not at all Limited
1. STRENUOUS ACTIVITIES (such as hurrying, exercising, running up stairs, sports)	1	2	3	4	5	6	7
2. MODERATE ACTIVITIES (such as walking, housework, gardening, shopping, climbing stairs)	1	2	3	4	5	6	7
3. SOCIAL ACTIVITIES (such as talking, playing with pets/children, visiting friends/relatives)	1	2	3	4	5	6	7
4. WORK-RELATED ACTIVITIES (tasks you have to do at work*)	1	2	3	4	5	6	7
*If you are not employed or self-employed, these should be tasks you have to do most days.							
5. SLEEPING	1	2	3	4	5	6	7

HOW MUCH **DISCOMFORT OR DISTRESS** HAVE YOU FELT **DURING THE LAST 2 WEEKS**?

	A Very Great Deal	A Great Deal	A Good Deal	Moderate Amount	Some	Very Little	None
6. How much discomfort or distress have you felt over the last 2 weeks as a result of CHEST TIGHTNESS?	1	2	3	4	5	6	7

Modified September 2010
AQLQ(S)-SA North American English Version

ASTHMA QUALITY OF LIFE QUESTIONNAIRE (S) PATIENT ID: _____

SELF-ADMINISTERED DATE: _____

IN GENERAL, **HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS** DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
7. Feel CONCERNED ABOUT HAVING ASTHMA?	1	2	3	4	5	6	7
8. Feel SHORT OF BREATH as a result of your asthma?	1	2	3	4	5	6	7
9. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO CIGARETTE SMOKE?	1	2	3	4	5	6	7
10. Experience a WHEEZE in your chest?	1	2	3	4	5	6	7
11. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF CIGARETTE SMOKE?	1	2	3	4	5	6	7

HOW MUCH **DISCOMFORT OR DISTRESS** HAVE YOU FELT **DURING THE LAST 2 WEEKS**?

	A Very Great Deal	A Great Deal	A Good Deal	Moderate Amount	Some	Very Little	None
12. How much discomfort or distress have you felt over the last 2 weeks as a result of COUGHING?	1	2	3	4	5	6	7

IN GENERAL, **HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS** DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
13. Feel FRUSTRATED as a result of your asthma?	1	2	3	4	5	6	7
14. Experience a feeling of CHEST HEAVINESS?	1	2	3	4	5	6	7

Modified September 2010
AQLQ(S)-SA North American English Version

ASTHMA QUALITY OF LIFE QUESTIONNAIRE (S) PATIENT ID: _____

SELF-ADMINISTERED DATE: _____

IN GENERAL, **HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS** DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
15. Feel CONCERNED ABOUT THE NEED TO USE MEDICATION for your asthma?	1	2	3	4	5	6	7
16. Feel the need to CLEAR YOUR THROAT?	1	2	3	4	5	6	7
17. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO DUST?	1	2	3	4	5	6	7
18. Experience DIFFICULTY BREATHING OUT as a result of your asthma?	1	2	3	4	5	6	7
19. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF DUST?	1	2	3	4	5	6	7
20. WAKE UP IN THE MORNING WITH ASTHMA SYMPTOMS?	1	2	3	4	5	6	7
21. Feel AFRAID OF NOT HAVING YOUR ASTHMA MEDICATION AVAILABLE?	1	2	3	4	5	6	7
22. Feel bothered by HEAVY BREATHING?	1	2	3	4	5	6	7
23. Experience asthma symptoms as a RESULT OF THE WEATHER OR AIR POLLUTION OUTSIDE?	1	2	3	4	5	6	7
24. Were you WOKEN AT NIGHT by your asthma?	1	2	3	4	5	6	7
25. AVOID OR LIMIT GOING OUTSIDE BECAUSE OF THE WEATHER OR AIR POLLUTION?	1	2	3	4	5	6	7

Modified September 2010
AQLQ(S)-SA North American English Version

ASTHMA QUALITY OF LIFE QUESTIONNAIRE (S) PATIENT ID: _____

SELF-ADMINISTERED DATE: _____

IN GENERAL, **HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS** DID YOU:

	All of the Time	Most of the Time	A Good Bit of the Time	Some of the Time	A Little of the Time	Hardly Any of the Time	None of the Time
26. Experience asthma symptoms as a RESULT OF BEING EXPOSED TO STRONG SMELLS OR PERFUME?	1	2	3	4	5	6	7
27. Feel AFRAID OF GETTING OUT OF BREATH?	1	2	3	4	5	6	7
28. Feel you had to AVOID A SITUATION OR ENVIRONMENT BECAUSE OF STRONG SMELLS OR PERFUME?	1	2	3	4	5	6	7
29. Has your asthma INTERFERED WITH GETTING A GOOD NIGHT'S SLEEP?	1	2	3	4	5	6	7
30. Have a feeling of FIGHTING FOR AIR?	1	2	3	4	5	6	7

HOW LIMITED HAVE YOU BEEN **DURING THE LAST 2 WEEKS**?

	Severely Limited Most Not Done	Very Limited	Moderately Limited Several Not Done	Slightly Limited	Very Slightly Limited Very Few Not Done	Hardly Limited At All	Not Limited Have Done All Activities
31. Think of the OVERALL RANGE OF ACTIVITIES that you would have liked to have done during the last 2 weeks. How much has your range of activities been limited by your asthma?	1	2	3	4	5	6	7

Modified September 2010
AQLQ(S)-SA North American English Version

ASTHMA QUALITY OF LIFE QUESTIONNAIRE (S) PATIENT ID: _____

SELF-ADMINISTERED DATE: _____

HOW LIMITED HAVE YOU BEEN **DURING THE LAST 2 WEEKS**?

	Totally Limited	Extremely Limited	Very Limited	Moderate Limitation	Some Limitation	A Little Limitation	Not at all Limited
32. Overall, among ALL THE ACTIVITIES that you have done during the last 2 weeks, how limited have you been by your asthma?	1	2	3	4	5	6	7

DOMAIN CODE:

Symptoms: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 29, 30
Activity Limitation: 1, 2, 3, 4, 5, 11, 19, 25, 28, 31, 32
Emotional Function: 7, 13, 15, 21, 27
Environmental Stimuli: 9, 17, 23, 26

Modified September 2010
AQLQ(S)-SA North American English Version

Appendix VII ACQ

ASTHMA CONTROL QUESTIONNAIRE

Please answer Questions 1–6.

Circle the number of the response that best describes how you have been during the past week.

1. On average, during the past week, how often were you woken by your asthma during the night?

0 Never

1 Hardly ever

2 A few times

3 Several times

4 Many times

5 A great many times

6 Unable to sleep because of asthma

2. On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?

0 No symptoms

1 Very mild symptoms

2 Mild symptoms

3 Moderate symptoms

4 Quite severe symptoms

5 Severe symptoms

6 Very severe symptoms

3. In general, during the past week, how limited were you in your activities because of your asthma?

0 Not limited at all

1 Very slightly limited

2 Slightly limited

3 Moderately limited

4 Very limited

5 Extremely limited

6 Totally limited

4. In general, during the past week, how much shortness of breath did you experience because of your asthma?

0 None

1 A very little

2 A little

3 A moderate amount

4 Quite a lot

5 A great deal

6 A very great deal

5. In general, during the past week, how much of the time did you wheeze?

0 Not at all

1 Hardly any of the time

2 A little of the time

3 A moderate amount of the time

4 A lot of the time

5 Most of the time

6 All the time

6. On average, during the past week, how many puffs of short-acting bronchodilator (e.g., Ventolin) have you used each day?

0 None

1 1–2 puffs most days

2 3–4 puffs most days

3 5–8 puffs most days

4 9–12 puffs most days

5 13–16 puffs most days

6 More than 16 puffs most days

To be completed by a member of the clinic staff

7. FEV1 prebronchodilator: 0 > 95% predicted

1 95–90%

FEV1% predicted: 2 89–80%

3 79–70%

FEV1% predicted: 4 69–60%

Appendix VIII Modified Borg Scale

MODIFIED BORG SCALE (Breathlessness) Worksheet

How breathless are you? (✓ one box below)	
❒ 0	Nothing At All
❒ 0.5	Very, Very Slight (Just Noticeable)
❒ 1	Very Slight
❒ 2	Slight
❒ 3	Moderate
❒ 4	Somewhat Severe
❒ 5	Severe
❒ 6	
❒ 7	Very Severe
❒ 8	
❒ 9	Very, Very Severe (Almost Maximal)
❒ 10	Maximal

Appendix IX MMRC Dyspnea Score

	Description
0	I only get breathless with strenuous exercise.
1	I am short of breath when hurrying on the level or up a slight hill.
2	I walk slower than people of the same age on the level because of breathlessness or have to stop for breath when walking at my own pace on the level.
3	I stop for breath after walking 100 meters (108 yards) or after a few minutes on the level.
4	I am too breathless to leave the house.

Appendix X SGRQ

Particiapnt ID:
Initials:
Visit Date:

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE
ORIGINAL ENGLISH VERSION

ST. GEORGE'S RESPIRATORY QUESTIONNAIRE (SGRQ)

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problems, rather than what the doctors and nurses think your problems are.

Please read the instructions carefully and ask if you do not understand anything. Do not spend too long deciding about your answers.

Before completing the rest of the questionnaire:

Please tick in one box to show how you describe your current health:

Very good	Good	Fair	Poor	Very poor
☐	☐	☐	☐	☐

P.W. Jones, PhD FRCP
Professor of Respiratory Medicine,
St. George's University of London,
Jenner Wing,
Cranmer Terrace,
London SW17 ORE, UK.

Tel. +44 (0) 20 8725 5371
Fax +44 (0) 20 8725 5955

UK/ English (original) version 1

continued…

f:\institut\outdep\projects\gsk1881\questionf\final versions\sgrqorig.doc 14/03/03

St. George's Respiratory Questionnaire
PART 1

Questions about how much chest trouble you have had over the past 3 months.

Please tick (✓) *one box for each question*:

	most days a week	several days a week	a few days a month	only with chest infections	not at all
1. Over the past 3 months, I have coughed:	☐	☐	☐	☐	☐
2. Over the past 3 months, I have brought up phlegm (sputum):	☐	☐	☐	☐	☐
3. Over the past 3 months, I have had shortness of breath:	☐	☐	☐	☐	☐
4. Over the past 3 months, I have had attacks of wheezing:	☐	☐	☐	☐	☐

5. During the past 3 months how many severe or very unpleasant attacks of chest trouble have you had?

Please tick (✓) *one*:

- more than 3 attacks ☐
- 3 attacks ☐
- 2 attacks ☐
- 1 attack ☐
- no attacks ☐

6. How long did the worst attack of chest trouble last?
(Go to question 7 if you had no severe attacks)

Please tick (✓) *one*:

- a week or more ☐
- 3 or more days ☐
- 1 or 2 days ☐
- less than a day ☐

7. Over the past 3 months, in an average week, how many good days (with little chest trouble) have you had?

Please tick (✓) *one*:

- No good days ☐
- 1 or 2 good days ☐
- 3 or 4 good days ☐
- nearly every day is good ☐
- every day is good ☐

8. If you have a wheeze, is it worse in the morning?

Please tick (✓) *one*:

- No ☐
- Yes ☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 1

How would you describe your chest condition?

Please tick (✓) *one*:

- The most important problem I have ☐
- Causes me quite a lot of problems ☐
- Causes me a few problems ☐
- Causes no problem ☐

If you have ever had paid employment.

Please tick (✓) *one*:

- My chest trouble made me stop work altogether ☐
- My chest trouble interferes with my work or made me change my work ☐
- My chest trouble does not affect my work ☐

Section 2

Questions about what activities usually make you feel breathless <u>these days</u>.

Please tick (✓) in ***each box*** that applies to you ***these days***:

	True	False
Sitting or lying still	☐	☐
Getting washed or dressed	☐	☐
Walking around the home	☐	☐
Walking outside on the level	☐	☐
Walking up a flight of stairs	☐	☐
Walking up hills	☐	☐
Playing sports or games	☐	☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 3

Some more questions about your cough and breathlessness <u>these days</u>.

	Please tick (✓) in *each box* that applies to you *these days*: True	False
My cough hurts	☐	☐
My cough makes me tired	☐	☐
I am breathless when I talk	☐	☐
I am breathless when I bend over	☐	☐
My cough or breathing disturbs my sleep	☐	☐
I get exhausted easily	☐	☐

Section 4

Questions about other effects that your chest trouble may have on you <u>these days</u>.

	Please tick (✓) in *each box* that applies to you *these days*: True	False
My cough or breathing is embarrassing in public	☐	☐
My chest trouble is a nuisance to my family, friends or neighbours	☐	☐
I get afraid or panic when I cannot get my breath	☐	☐
I feel that I am not in control of my chest problem	☐	☐
I do not expect my chest to get any better	☐	☐
I have become frail or an invalid because of my chest	☐	☐
Exercise is not safe for me	☐	☐
Everything seems too much of an effort	☐	☐

Section 5

Questions about your medication, if you are receiving no medication go straight to section 6.

	Please tick (✓) in *each box* that applies to you *these days*: True	False
My medication does not help me very much	☐	☐
I get embarrassed using my medication in public	☐	☐
I have unpleasant side effects from my medication	☐	☐
My medication interferes with my life a lot	☐	☐

continued…

St. George's Respiratory Questionnaire
PART 2

Section 6

These are questions about how your activities might be affected by your breathing.

	Please tick (✓) in *each box* that applies to you *because of your breathing*: True	False
I take a long time to get washed or dressed	☐	☐
I cannot take a bath or shower, or I take a long time	☐	☐
I walk slower than other people, or I stop for rests	☐	☐
Jobs such as housework take a long time, or I have to stop for rests	☐	☐
If I walk up one flight of stairs, I have to go slowly or stop	☐	☐
If I hurry or walk fast, I have to stop or slow down	☐	☐
My breathing makes it difficult to do things such as walk up hills, carrying things up stairs, light gardening such as weeding, dance, play bowls or play golf	☐	☐
My breathing makes it difficult to do things such as carry heavy loads, dig the garden or shovel snow, jog or walk at 5 miles per hour, play tennis or swim	☐	☐
My breathing makes it difficult to do things such as very heavy manual work, run, cycle, swim fast or play competitive sports	☐	☐

Section 7

We would like to know how your chest <u>usually</u> affects your daily life.

	Please tick (✓) in *each box* that applies to you *because of your chest trouble*: True	False
I cannot play sports or games	☐	☐
I cannot go out for entertainment or recreation	☐	☐
I cannot go out of the house to do the shopping	☐	☐
I cannot do housework	☐	☐
I cannot move far from my bed or chair	☐	☐

continued…

St. George's Respiratory Questionnaire

Here is a list of other activities that your chest trouble may prevent you doing. (You do not have to tick these, they are just to remind you of ways in which your breathlessness may affect you):

Going for walks or walking the dog

Doing things at home or in the garden

Sexual intercourse

Going out to church, pub, club or place of entertainment

Going out in bad weather or into smoky rooms

Visiting family or friends or playing with children

Please write in any other important activities that your chest trouble may stop you doing:

.....

.....

.....

.....

Now would you tick in the box (one only) which you think best describes how your chest affects you:

It does not stop me doing anything I would like to do ☐

It stops me doing one or two things I would like to do ☐

It stops me doing most of the things I would like to do ☐

It stops me doing everything I would like to do ☐

Thank you for filling in this questionnaire. Before you finish would you please check to see that you have answered all the questions.

PART II: GENERAL STUDY ADMINISTRATION

1. ETHICAL ASPECTS

1.1. Declaration of Helsinki

The investigator will ensure that this study is conducted in full conformance with the principles of the "Declaration of Helsinki" (as amended in Tokyo, Venice and Hong Kong) or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. For studies conducted in the USA, the investigator will ensure that the basic principles of "Good Clinical Practice" as outlined in CFR 312, subpart D, "Responsibilities of Sponsors and Investigators", are adhered to.

1.2. Informed Consent

It is the responsibility of the investigator to obtain written informed consent from each participant participating in this study, after adequate explanation of the aims, methods, anticipated benefits, and potential hazards of the study. For participants not qualified to give legal consent, written consent must be obtained from the parent or legal guardian.

The investigator must also explain that the participants are completely free to refuse to enter the study or to withdraw from it at any time, for any reason. The study file documentation for this study contains a section for documenting informed participant consent.

1.3. Institutional Review Boards

It is the understanding that this protocol (and any modifications) as well as appropriate consent procedures, will be reviewed and approved by the UWO Health Sciences Research Ethics Board. This board operates in accordance with the current Federal Regulations. Approval from the committee must be obtained before starting the study, and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval.

2. CONDITIONS FOR MODIFYING (AMENDING) THE PROTOCOL OR TERMINATING THE STUDY

Protocol modifications to ongoing studies which could potentially adversely affect the safety of participating participants or which alter the scope of the investigation, the scientific quality of the study, the experimental design, dosages, duration of therapy, assessment variables, the number of participants treated or participant selection criteria, must be made only after consultation with the Institutional Review Board.

All protocol modifications must be reviewed and approved by the appropriate Institutional Review Board in accordance with local requirements, before the changes can be implemented. Modifications which eliminate an apparent immediate hazard to participants do not require pre–approval by the Institutional Review Board.

The investigator reserves the right to terminate the study at any time. Should this be necessary, the procedures will be arranged on an individual study basis after review and consultation by both parties. In terminating the study, Robarts and the investigator will assure that adequate consideration is given to the protection of the participant's interests.

3. STUDY DOCUMENTATION, CRF'S AND RECORD KEEPING

3.1. Investigators Files / Retention of Documents

The investigator must maintain adequate records to enable the conduct of the study to be fully documented.

Copies of protocols, originals of test result reports, correspondence, records of informed consent and other documents pertaining to the conduct of the study must be kept on file by the investigator for a period of time specified by local law for the preservation of participant documents.

3.2. Inspections

The investigator should understand that source documents for this trial should be made available to the appropriately qualified personnel or to health authority inspectors after appropriate notification. The verification of the data may be by direct inspection of source documents (where permitted by law) or through an interview technique.

3.3. Study Documentation

For each participant enrolled, a study file must be started and source documents created. This also applies to records for those participants who fail to complete the study. If a participant withdraws from the study, the reason must be noted in the study notes. If a participant is withdrawn from the study because of a study related adverse event, thorough efforts should be made to clearly document the outcome.

All forms should be typed or filled out using a black ball–point pen, and must be legible. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the individual making the change.

4. CONFIDENTIALITY OF TRIAL DOCUMENTS AND PARTICIPANT RECORDS

The investigator must ensure that the participant's anonymity will be maintained and that their identities will be protected from unauthorized parties. On study documents which leave the Clinical Imaging Research Laboratories, participants should **not** be identified by their names, but by an identification code. The investigator should keep a participant enrollment log showing codes, names and addresses.

5. PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

The results of this study will be published or presented at scientific meetings.