LUNG CLEARANCE INDEX (LCI) IN DETECTION OF POST-TRANSPLANT BRONCHIOLITIS OBLITERANS SYNDROME

Online Supplement of Additional Methods and Results

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1. Lung Function Measurements

MBW_{SF6}

MBW_{SF6} was performed using an Innocor™ photo-acoustic gas analyser (Innovision, Odense, Denmark) and closed-circuit washin.

The closed-circuit rebreathing method, as previously described[1], involves breathing into and out of a sealed 3L bag filled with a mix of air, oxygen and the inert tracer gas (SF_6). This technique allows for a quicker and more efficient washin period than previously used open-circuit methods, due to the higher concentration of tracer gas taken into the lungs at the start of wash-in.

A double CO_2 scrubber was used to prevent a build-up of CO_2 from expired air within the circuit. Bag volume was set by the operator to be approximately the same as the subject's FRC before testing. This was adjusted up or down to ensure the right amount of test gas used, and to ensure wash-in was not prolonged.

Wash-in phase was started with the subject seated, mouthpiece in place and wearing a nose clip to prevent leaks of air at the nose. Subject was suitably distracted by watching a screen, and normal tidal breathing was established. In order to achieve a complete wash-in, the subject was instructed

to take six slow maximal inspirations at the start of wash-in. This increases total cumulative inspired volume and helps ensure poorly ventilated lung regions are filled at the start of the test.

Subjects are then instructed to continue with relaxed tidal breathing for the remainder of the wash-in period. Throughout this, inspired CO_2 levels ($FiCO_2$) are monitored to ensure this remains below 3% (usually <2%). Inspired O_2 (FiO_2) is maintained greater than 20%. Normal tidal breathing was continued until SF_6 signal was stable for at least 60s, at which point wash-in was deemed complete. Patient was then switched to breathing room air by the operator.

During washout, a fan is directed over the flow meter to blow away expired SF_6 and ensure that it is not re-inspired. Washout was continued through normal tidal breathing until the end tidal SF_6 concentration was less than $1/40^{th}$ of the starting concentration for three consecutive breaths, as identified by the device software.

Testing was continued until three technically acceptable tests were obtained. If an FRC measurement differed by >10% from the other two measurements, then an additional washout was performed. Tests were deemed technically unacceptable if patients could not achieve a stable FRC, or if a leak was identified on washout. Mean values of both FRC and LCI were used in analysis, from a minimum of at least two acceptable repeats.

Throughout the test, real-time visual feedback was provided, in which patients could observe their own inspired breath volume whilst completing washin/washout. This was provided alongside suitable distraction for the patient and meant a reproducible tidal volume was more easily attainable A tidal volume of around 500-1000ml was aimed for, adjusted according to height. Breathing pattern was continually monitored by the operator during washout, and the patient given feedback to avoid long pauses.

Overall testing duration was recorded for each subject by inspecting the time recorded for the washout file. This records all the time in seconds to complete the washin/washout manoeuvres, pauses between tests, and for the device to warm up (60 seconds). It does not however include time taken to explain the test and seat/position the patient, or time taken to clean apparatus afterwards.

Normal range of LCI

Healthy subjects were over the age of 5 years, were non-smokers or ex-smokers of >6months with less than 5 pack year smoking history, with no history of asthma or wheeze requiring any inhaler use in the last 12m. Additional exclusions included history of cardiac disease, pertussis, tuberculosis, or prematurity (<34 weeks). All participants provided assent, and parents and adult volunteers provided signed informed consent. This study was approved by the Lancaster Research Ethics

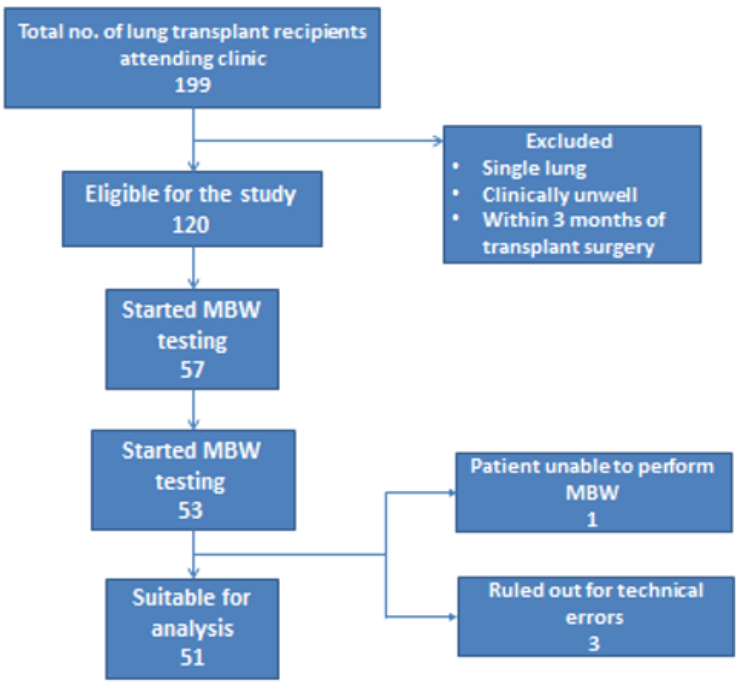
Committee (study reference 14/NW/1195). Multiple breath washout was performed using a closed circuit Innocor system. Analysis was carried out offline using a washout analysis package prepared in Igor Pro v6 (Wavemetrics Inc., Lake Oswego, OR, USA). This is based on the same washout analysis package already deployed in several other clinical studies and clinical trials. The final LCI and FRC measurements quoted are the average of at least two reproducible repeats. Repeats were excluded if there was evidence of leak, or in case of large differences seen in LCI or FRC measurements (>25% from median). Upper limit of normal (ULN) corresponding to the 97.5th centile (z-score 1.96) was used as recommended by the Global Lung Function Initiative[2].

160 healthy adults and children successfully completed LCI measurements (95%). Median age (range) was 13 (5-59) yrs. Mean (SD) LCI for the entire cohort was 6.13 (0.46), making the upper limit of normal LCI 7.0. There was no significant correlation between age and LCI (r^2=0.008, p=0.3). LCI was not significantly different between adults (age 18-59 years) and children (5-17 years) (mean difference=0.006, 95% CI 0.15 to -0.16, p=0.9)[3].

Spirometry

Routine spirometry, including maximum expiratory flow volume measurements, were performed on all patients at each study visit using SensorMedics VMAX® 22 system (Yorba Linda, California, USA). Tests were performed in triplicate with subjects sat in the seated position whilst wearing a nose clip, in accordance with the 2005 American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines[4].

Tests required a full inspiration to TLC, and then a sudden maximal forced expiration to RV, followed by a full maximal inspiration back to TLC for the inspiratory portion of the flow volume loop.



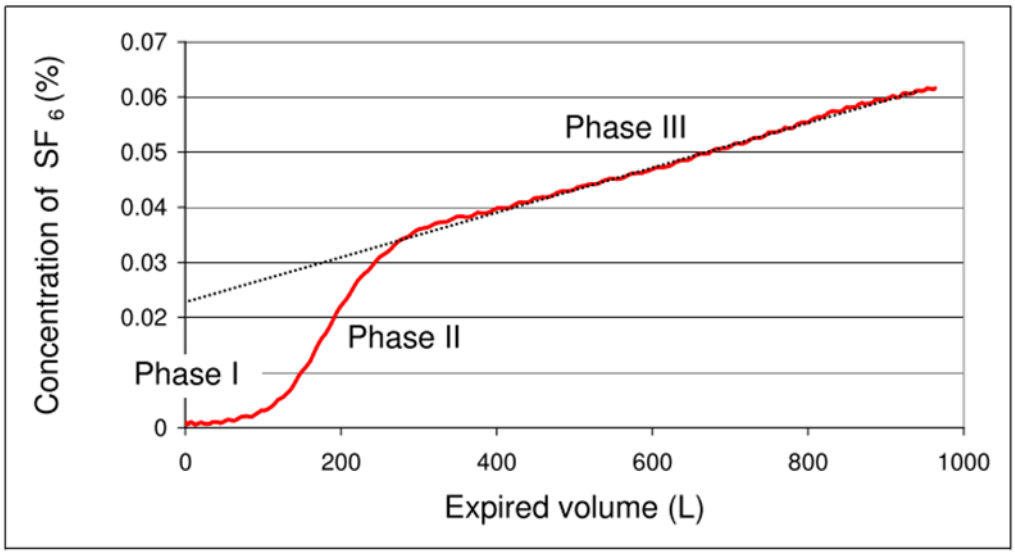
Predicted values for FEV_1 and FVC were not included as part of the analysis, as normal ranges become obsolete post-transplant. Baseline FEV_1 values for each subject were instead calculated using the two highest measurements obtained post-operatively; taken at least 3 weeks apart and made without the use of inhaled bronchodilator prior to testing, as defined by the ISHLT[5].

Figure 1: Consort diagram for the study cohort. Recruitment from the pool of 120 was limited by time available.

2. Phase III slope analysis

During MBW, each expired breath can be divided into three distinct phases (figure 2). Phase III, the alveolar plateau, represents mixed alveolar gas[6]. Analysis of the evolution of the concentration-normalised phase III slope (S_{nIII}) during MBW has been proposed as a method to separate the contributions of convective gas mixing in the conductive airways from the interaction between diffusion and conduction in the acinar regions of the lung[7]. Both diffusive and convective ventilation heterogeneity are present in the normal lung (e.g. gravity dependent variations in ventilation distribution[8]), but are affected to varying extents by different disease processes. These can be separated by looking at how the phase III slope of each breath changes over the course of a washout. In a perfectly mixed lung, there is no phase III slope (i.e. all alveolar tracer concentrations are the same) and this does not change during washout. In real life, there is diffusive gas mixing heterogeneity that causes a fixed offset in the slope signal. There is also convective (i.e. flow, or airway-dependent) heterogeneity that will lead to increasing differences between best and least well ventilated lung compartments as washout progresses, and a corresponding increase in phase III slope.

The mean expired gas concentration is applied to each S_{III} to give the concentration normalised

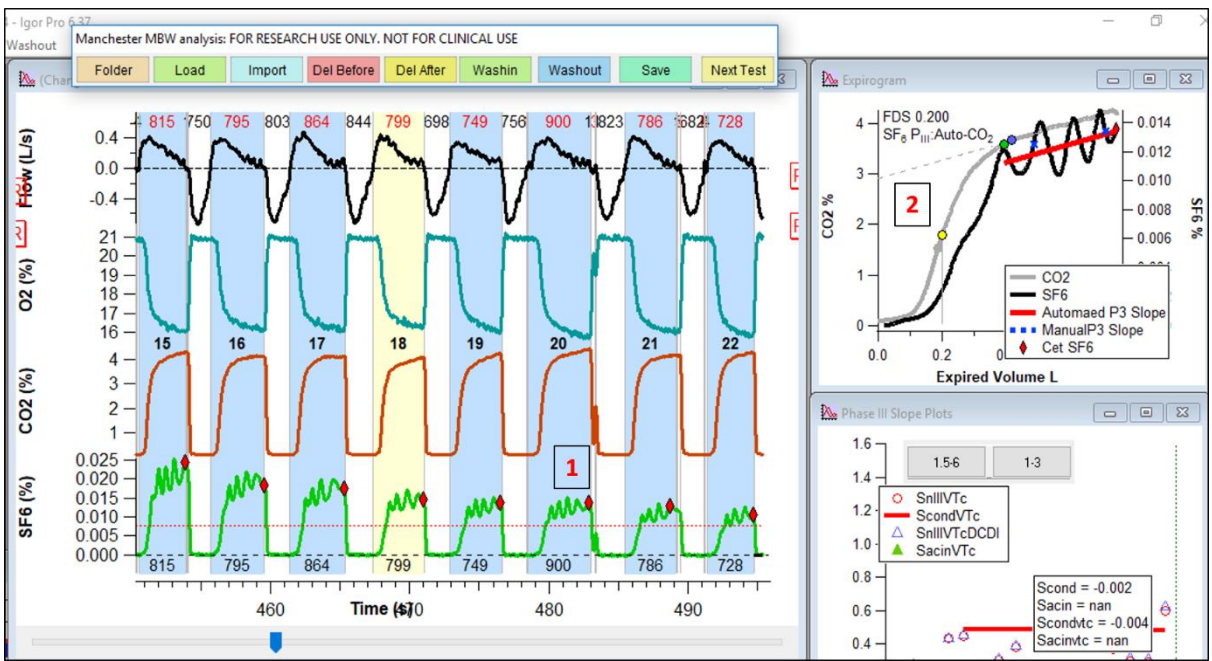


phase III slope, termed S_{nIII}. This is to allow breath by breath comparison and to account for individual variation amongst breaths, as well as to allow for the progressive decrease in the absolute gradient of the slope as the concentration falls.

Figure 2: S_{nIII} analysis of a single breath during washout. Phase I represents anatomical dead space, Phase II represents where gas in the dead space begins to mix with alveolar gas, and Phase III (alveolar plateau) represents gas mixing at the alveolar level.

S_{cond} and S_{acin} are indices of deranged ventilation derived from the phase III slope that represent ventilation inhomogeneity in the conductive and acinar airways zones respectively. These two indices were previously described by Verbanck et al, based upon mathematical models of lung gas mixing and supporting clinical studies[7]. S_{cond} is derived from the increase in the concentration-normalised phase III slope over the middle portion of the washout (between lung volume turnovers 1.5 and 6). This represents the rate that alveolar slope changes during washout. To get the contribution of convention-dependent inhomogeneity for the total S_{nIII} for a breath, S_{cond} is multiplied by the TO number[9]. This contribution is subtracted from the total S_{nIII} of the first breath to give a measure of the offset in phase III slope, known as S_{acin}.

Analysis of washout data was performed offline, using the Manchester MBW analysis package on Igor Pro v6 (WaveMetrics Inc., OR, USA). Innocor™ files containing tests were transferred onto a separate computer and converted to text files for analysis. The analysis software displays both washin and washout across all tests for that subject. Flow in litres per second (L/S), O_2, CO_2 and SF_6 concentrations (%) over time in seconds (s) are also shown (figure 3). Breath volume is displayed along the bottom of each breath and is derived from integration of flow. All tests were inspected for validity, ensuring no leaks on the trace and a complete washin. S_{nIII} analysis was performed in a separate window to the main washout screen. The phase III slope is automatically detected by the



software and is based on linear regression of the gas concentration over the alveolar plateau, during the final part of the breath. This could be manually adjusted or deleted if inaccurate or appeared to be an outlier. The software automatically calculates the S_{nIII} for each breath, plots these against lung turnover number for that breath, and performs linear regression of the included data points to calculate S_{cond} and derive S_{acin}.

Figure 3: MBW_{SF6} showing cardiogenic oscillations during washout. Severe oscillations can be observed on washout trace (1) and phase III slope analysis (2) screens

3. Cardiogenic oscillations

These have been previously reported in other conditions, and several explanations have been suggested. Dahlstrom, Murphy and Roos first described the finding in 1954; the term is used to refer to the heart-synchronous fluctuation in gas concentration, flow or pressure within the lungs[10]. The full origin and mechanisms of what causes these are not yet fully understood but are widely accepted to be caused by direct pulsatile cardiac waves that push gas molecules within the airways. This, in turn causes further mixing of the gas within the anatomical dead space and alveolar gas, therefore causing small changes in gas concentration [11]. In the current study, these were observed in 27 patients (53%) and were seen in some cases to have a significant impact on S_{nIII} analysis and end-point identification due to fluctuations in end-expiratory gas concentration, therefore potentially affecting LCI value.

In the current study, cardiogenic oscillations were categorised into three groups according to the judgement of the operator (table 1). Excluding those patients with severe and moderate cardiac oscillations from the LCI analysis reduced patient numbers significantly, especially in the 'no BOS' and 'BOS 0p' subgroups. However, overall the trend of increasing LCI with worsening BOS stage was still significant (p= 0.0015).

Table 1: Categories of severity of cardiogenic oscillations on MBW_{SF6}

Category	Definition	n
Mild	Small oscillations visible, but phase III slope	19

	easily identifiable and clear endpoint visible	
Moderate	No accurate phase III analysis possible but clear endpoint still visible	1
Severe	No clear endpoint, regardless of phase III slope	7

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