Slow and fast lung compartments in cystic fibrosis measured by nitrogen multiple-breath washout

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Gustafsson PM, Robinson PD, Gilljam M, Lindblad A, Houltz BK. Slow and fast lung compartments in cystic fibrosis measured by nitrogen multiple-breath washout. J Appl Physiol 117: 720–729, 2014. First published July 18, 2014; doi:10.1152/japplphysiol.01274.2013.—Imaging studies describe significant ventilation defects across a wide range of cystic fibrosis (CF) related lung disease severity. These are unfortunately poorly reflected by phase III slope analysis—derived Scond and Sacin from multiple-breath washout (MBW). Methodology extending previous two-lung compartment model-based analysis is presented describing size and function of fast- and slow-ventilating lung compartments from nitrogen (N2) MBW and correlation to obstructive lung disease severity. In 37 CF subjects (forced expiratory volume in 1 s [FEV1] mean [SD] 84.8 [19.9]% predicted; abnormal lung clearance index [LCI] in 36/37, range 7.28–18.9) and 74 matched healthy controls, volume and specific ventilation of both fast and slowly ventilated lung compartments were derived from N2-based MBW with commercial equipment. In healthy controls lung emptying was characterized by a large compartment constituting 75.6 (8.4)% of functional residual capacity (FRC) with a specific ventilation (regional alveolar tidal volume/regional lung volume) of 13.9 (3.7)% and a small compartment with high specific ventilation (48.4 [15.7]%). In CF the slowly ventilated lung compartment constituted 51.9(9.1)% of FRC, with low specific ventilation of 5.3 (2.4)%. Specific ventilation of the slowly ventilated lung compartment showed stronger correlation with LCI (r2 = 0.70, P < 0.001) vs. Sacin (r2 = 0.44, P < 0.001) or Scond (no significant correlation). Overventilation of the fast lung compartment was no longer seen in severe CF lung disease. Magnitude and function of under- and overventilated lung volumes can be derived from routine N2 MBW in CF. Reported values agree with previous modelling-derived estimates of impaired ventilation and offer improved correlation to disease severity, compared with Sacin analysis.

TIDAL BREATHING INERT GAS multiple-breath washout (MBW) tests have been used for more than 60 years to assess the influence of airway disease on the uniformity of ventilation distribution in the lungs (10, 12, 13). There has been a recent explosion of interest in the technique, due to documented strong feasibility across wide age ranges, and ability to detect early lung disease, in important diseases such as cystic fibrosis (CF), even when spirometry is within normal limits (4, 21).

Technical advances have also had a considerable impact, facilitating accurate within-breath measurements of inert gas concentration in relation to expired flow and volume. Lung clearance index (LCI) is a global measure of the ventilation distribution inhomogeneity present and the most widely reported pediatric MBW index. Utility has also been demonstrated in adult CF (23, 24, 44). Strong correlation between LCI and structural changes of lung disease on computed tomography (CT) (22), together with demonstrated prognostic utility (6), and a high sensitivity to detect treatment response in small study numbers (1, 2) has enhanced interest further. However, the additional physiological insight we gain from LCI as a sole reported index is limited. Further insight appeared promising with analysis focused on the evolution of concentration-normalized phase III slopes (SnIII) of successive breaths through the MBW (termed SnIII analysis). Clinical indices termed Scond and Sacin were derived to reflect two mechanisms of ventilation distribution inhomogeneity generated within different zones of the lung (35, 47). Differing patterns of Scond and Sacin abnormality were subsequently described across important respiratory diseases (20, 46, 48). However, the modelling of these indices was based on healthy adult human lung data, and the integrity of this approach has recently been questioned in more severe disease (25, 26, 45).

The advances in imaging techniques have further enhanced our understanding of the pathophysiological processes in obstructive lung disease. Magnetic resonance imaging (MRI) studies using hyperpolarized noble inert gases such as the Helium isotope 3He, have shown large volumes of the lungs in CF subjects to be either very slowly ventilated or not ventilated at all (16, 43, 49). Regional specific ventilation measurements are also feasible with these imaging techniques (15). Heterogeneous airway obstruction not only produces slowly cleared subtended lung volumes, but also results in overventilated lung units served by patent airways, which are cleared of an inert marker gas long before the slow units. Underventilated lung volumes have therapeutic implications on delivery of nebulized or other inhaled medications to the targeted site of disease. Additionally, fast emptying compartments serve as dead space ventilation, which results in an overall increase in ventilatory demand. However, 3He MRI imaging is not widely feasible due to high cost and restricted access. It is also performed supine, in contrast to upright sitting position in standard lung function testing. Finally, only one breath of the tracer gas is inhaled, while MBW starts after equilibration of the marker gas in the lung. It would be highly desirable if comparable
information could be obtained from a more physiologically relevant and also more widely feasible test. Given the recent advances in equipment and availability of affordable validated commercial nitrogen (\(N_2\)) MBW equipment, MBW offers this potential (40).

MBW-based evaluation of the lung as differing compartments, emptying at different rates, were presented almost 60 years ago (28). This phenomenon is detectable in healthy lungs and exaggerated by nonuniform distribution of airway obstruction in disease, which results in increased ventilation inhomogeneity among several widely distributed smaller or larger lung units. These units can be effectively summarized into a very well ventilated compartment and a second very slowly ventilated compartment, constituting a two-compartment lung model. Early studies examining the characteristics of these compartments were based on averaged or mean inert gas concentrations during the MBW, due to the limitations of the technology present (19, 37). Subsequent research focus switched to the assessment of the degree of dead space ventilation in a given subject (7). Methods to calculate airway dead space volume (\(V_{Daw}\)) (18), corresponding to the volume of the conducting airways, were complemented by a measure of the Bohr dead space volume (\(V_{DBohr}\)) (17), which encompasses the \(V_{Daw}\) and the alveolar dead space volume (\(V_{DAlv}\)). Using \(N_2\) MBW, Arborelius (3) extended this work by calculating overall effective respiratory dead space volume, which he termed functional dead space volume (\(V_{DF}\)). More recent research has focused on global indices (e.g., LCI) and \(S_{\text{inh}}\) analysis and the utility, and relationships to, these alternate indices.

Addressing the functional consequences of under- and overventilated lung compartments in CF using \(N_2\) MBW has not been performed to date. The overall aim of this study was to demonstrate how this additional information can be easily derived from routine \(N_2\) MBW recordings. Two specific aims are addressed: first, to exploit the dual compartment pattern of simple semilog \(N_2\) volume MBW curves to calculate volumes and specific ventilation of the relatively underventilated and remaining overventilated lung compartments; second, to explore relationships between these novel indices and disease severity in CF, as reflected by LCI and \(S_{\text{inh}}\) analysis indices \(S_{\text{cond}}\) and \(S_{\text{acin}}\).

METHODS

CF subjects were recruited from the CF centers of the Queen Silvia Children’s Hospital and the Sahlgrenska University Hospital, Gothenburg, at the time of annual checkup and clinical stability. Healthy controls, matched for age and gender (at a ratio of 2:1), were recruited from an existing larger cohort, previously identified from the general population of Gothenburg using the Swedish population register. Healthy subjects were defined as individuals with no known lung disease, without evidence of recurrent respiratory symptoms, and a negative smoking history.

Lung function testing was performed at the Department of Clinical Physiology at the Sahlgrenska University Hospital/East, Gothenburg. Spirometry was performed using Jaeger Masterscreen (Care Fusion, Wurzburg, Germany) in accordance with current European Respiratory Society (ERS) and American Thoracic Society (ATS) standards (32). Spirometry data was expressed as percent predicted using published Caucasian reference equations (41).

MBW was performed prior to spirometry in all subjects. The commercial \(N_2\) MBW equipment used (ExhalyzerD N2, Eco Medics AG, Duerten, Switzerland) is based on indirect inert gas (\(N_2\)) calculation using carbon dioxide (\(CO_2\)) and oxygen (\(O_2\)) sensors and flow measurement from an ultrasound flow sensor. In each subject three technically acceptable \(N_2\) MBW tests were performed, in accordance with the recently published consensus statement (39). Validation of this device, as specified in this consensus statement, has also recently been performed (40). Further details on the device, quality control, and test protocol used are contained in APPENDIX. While the commercial software delivered with ExhalyzerD (Spiroware 3.1) was used for data recording, in-house software written with a commercial software package (TestPoint, Capital Equipment, MA) was used for off-line analysis. The basic algorithms in our custom TestPoint software are the same as those used in the commercial Spiroware software, and our custom software was also used for the recent device validation studies (40). Derivations of conventional \(N_2\) MBW parameters (LCI, \(S_{\text{acin}}\) \(\times V_T\) and \(S_{\text{cond}}\) \(\times V_T\), etc.) are described in APPENDIX. LCI, \(S_{\text{acin}}\) \(\times V_T\), and \(S_{\text{cond}}\) \(\times V_T\) data were compared with reference values calculated in the 2:1 matched control group.

In a semilog plot of exhaled volume of \(N_2\) for each breath (Fig. 1, closed circles), all subjects demonstrated a washout curve with two components. The latter component containing \(N_2\) from slowly ventilating lung compartments only, and the first component containing contributions of exhaled \(N_2\) from both fast and slowly ventilating lung compartments. A regression line containing the contributions of the slowly ventilated lung compartments only was then determined (Fig. 1, open triangles). This was plotted from the end of the washout extending backwards, with the proximal end of the regression line determined by the optimal strength of regression data fit (judged by the \(r^2\)-value). A semiautomated computer algorithm was used for this procedure (see APPENDIX). Back extrapolation of the slowly ventilated lung compartment regression line allowed its relative contributions to each breath in the initial phase to be calculated. Once subtracted, only the contributions of the fast lung compartment remained (Fig. 1, open squares).

Calculations performed during this study are outlined below. The functional residual capacity (FRC) of the whole lung (FRC\(_{\text{total}}\)) was calculated from the sum of the measured exhaled \(N_2\) volumes contained in all breaths (\(V_{N2,\text{total}}\)) over the washout until 1/40\(^{th}\) of the initial end-tidal \(N_2\) concentration (Cet\(_{\text{N2,initial}}\)) (~78%), divided by the difference in initial and final (~1.95%) end-tidal \(N_2\) concentration (Cet\(_{\text{N2,final}}\)):

\[
\text{FRC}_{\text{total}} = \frac{V_{N2,\text{total}}}{(\text{Cet}_{\text{N2,initial}} - \text{Cet}_{\text{N2,final}})}
\]  

The regional FRCs of the fast (FRC\(_{\text{fast}}\)) and slow compartments (FRC\(_{\text{slow}}\)) were then derived as follows. A power function was applied to the regression line containing the log values of \(N_2\) volume and \(V_T\,\text{in}\,\%\). The difference in initial and final (~1.95%) end-tidal \(N_2\) concentration (Cet\(_{\text{N2,final}}\)):

\[
\text{FRC}_{\text{fast}} = \frac{V_{N2,\text{fast}}}{(\text{Cet}_{\text{N2,initial}} - \text{Cet}_{\text{N2,final}})}
\]

\[
\text{FRC}_{\text{slow}} = \frac{V_{N2,\text{slow}}}{(\text{Cet}_{\text{N2,initial}} - \text{Cet}_{\text{N2,final}} - 0)}
\]

For both the fast and slow compartment, Cet\(_{\text{N2,initial}}\) must be the same as for the lung as a whole, because these compartments are in equilibration before start of washout. The end-tidal \(N_2\) concentration was assumed to be 0.0% in the fast compartment when it has been cleared of its \(N_2\) content (i.e., fast compartment Cet\(_{\text{N2,final}}\) = 0.0%). The slow compartment Cet\(_{\text{N2,final}}\) was unknown, however, but must be markedly greater than the measured Cet\(_{\text{N2,final}}\) for the lung as a whole. Consequently, FRC\(_{\text{slow}}\) cannot be directly calculated, while FRC\(_{\text{fast}}\) can be calculated as outlined below:
**Fig. 1.** Multiple-breath washout (MBW) test plots of log values of measured volume of N₂ expired (ml) (closed circles) against breath number from two representative subjects: a healthy 19-year-old female (A) and a 19-year-old female cystic fibrosis (CF) subject (B). Open triangles represent the breath-by-breath contribution from the slowly ventilated lung compartment (based on its extrapolated regression line). Open squares represent the breath-by-breath contribution from the remaining fast emptying compartment (calculated by subtracting the slow compartment contribution from the overall lung emptying). The overall emptying curve of the healthy control appears almost monophasic but does contain two distinct components. In contrast, the overall emptying curve of the CF subject appears to be triphasic but can in fact be reconstructed from a slow and a fast linear semilog regression line. While the emptying rate of the fast compartment appears to be similar in the two subjects, the clearance of the slow compartment is markedly reduced in the CF subject with a high lung clearance index (LCI). For further details on calculation principles, see METHODS.

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**Restructuring Eq. 5,**

\[
\text{Cet}_{\text{N2,final,slow}} = \text{Cet}_{\text{N2,initial}} - \frac{V_{\text{N2,slow}}}{\text{FRC}_{\text{slow}}} \quad (6)
\]

As a quality control step, at this stage, the estimate of \(\text{Cet}_{\text{N2,final}}\) for the lung as a whole was calculated from \(\text{Cet}_{\text{N2,final,slow}}\), assuming no \(\text{N}_2\) was present in the fast compartment. This was then compared with measured overall \(\text{Cet}_{\text{N2,final}}\).

Estimated overall \(\text{Cet}_{\text{N2,final}}\) is given by:

\[
\text{Estimated overall } \text{Cet}_{\text{N2,final}} = \text{FRC}_{\text{slow}} \times \frac{\text{Cet}_{\text{N2,final,slow}}}{\text{FRC}_{\text{total}}} \quad (7)
\]

After determining the volume of the two compartments, the clearance rate of the two compartments was determined to allow for calculation of the respective alveolar tidal volumes \((V_{rT/A})\) and their specific ventilations \((rV'/V)\), i.e., regional \(V_{rT/A}\)/regional FRC.

The \(V_{\text{N2}}\) per breath from the fast component from breath number one onward was calculated from the overall \(V_{\text{N2}}\) per breath minus the calculated contributions from the slow compartment (see above). The exponential decay rates described by the semilog plots of \(V_{\text{N2}}\) per breath vs. breath number were then used to derive a dilution factor \(\omega\) for the two compartments as described in principle by Fowler et al. in 1952 (19):

\[
\text{FA}_n = \text{FA}_0 \times \omega^n, \quad (8)
\]

where \(\text{FA}_n\) is the fractional \(\text{N}_2\) concentration after \(n\) breaths, and \(\text{FA}_0\) is that at time zero. FA decreases with each breath by a factor \(\omega\).

In the current setting this equation can be applied to the \(V_{\text{N2}}\) per breath:

\[
V_{\text{N2,n}} = V_{\text{N2,0}} \times \omega^n, \quad (9)
\]

Restructuring Eq. 9,

\[
\omega = n \sqrt{\frac{V_{\text{N2,0}}}{V_{\text{N2,n}}}} \quad (10)
\]

In practice, \(\omega\) is the ratio of \(V_{\text{N2,n}}\) from any breath divided by the \(V_{\text{N2,n}}\) from the previous breath, when these volumes have been derived from an exponential function (inverse log function) of the semilogarithmic plot of \(V_{\text{N2}}\) vs. breath number.

Darling et al. (14) described that \(\omega\) can be determined by the relationship between lung volume and alveolar ventilation:

\[
\omega = \frac{V_0}{(V_0 + VT - V_D)}, \quad (11)
\]

where, \(V_0\) is end-expiratory lung volume, \(V_T\) is overall tidal volume, and \(V_D\) represents the dead space volume of the breath, where \(V_T-V_D\) represents \(V_{rT/A}\).

In the current setting this relationship can be expressed as:

\[
\omega = \text{FRC}/(\text{FRC} + V_{rT/A}) \quad (12)
\]

Restructuring Eq. 11,

\[
V_{rT/A} = (\text{FRC}/\omega) - \text{FRC} \quad (13)
\]

This can then be applied on the fast and slow compartments, respectively, giving the regional \((r) V_{rT/A}:\)

\[
rV_{rT/A,\text{fast}} = (r\text{FRC}_{\text{fast}}/r\text{FA}_{\text{fast}}) - r\text{FRC}_{\text{fast}} \quad (14)
\]

\[
rV_{rT/A,\text{slow}} = (r\text{FRC}_{\text{slow}}/r\text{FA}_{\text{slow}}) - r\text{FRC}_{\text{slow}} \quad (15)
\]

Regional specific ventilation \((rV'/V)\) of each compartment can then be given:

\[
\frac{rV'}{V_{\text{fast}}} = \frac{rV_{rT/A,\text{fast}}}{r\text{FRC}_{\text{fast}}} \quad (16)
\]

\[
\frac{rV'}{V_{\text{slow}}} = \frac{rV_{rT/A,\text{slow}}}{r\text{FRC}_{\text{slow}}} \quad (17)
\]

Functional dead space volume \((V_{DF}/VT)\) % was calculated as outlined by Arborelius et al. (3). In their experiments, expired gas was collected through the MBW in Douglas bags and analyzed at the end of washout, rather than breath-by-breath as in modern day experiments. For a given washout, where the lungs empty as a single
As expected, MBW values were increased in CF compared with healthy control subjects (Table 2). LCI was abnormal in 36/37 CF subjects (97%, ULN 7.3, range 7.28–18.89). Only one subject had both a normal LCI and FEV1 (Fig. 2), while 25/37 subjects (68%) had a normal FEV1 but abnormal LCI. Scond × VT and Sacin × VT were abnormal in 36/37 (97%, ULN 0.023, 0.022–0.101) and 27/37 (73%, ULN 0.088, range 0.038–0.486) CF subjects, respectively. The relationships of Scond × VT and Sacin × VT to LCI in the CF cohort are shown in Fig. 3. Scond × VT appeared to increase early in disease severity but not in a linear way at higher LCI values, resulting in high correlation.

Results

Thirty-seven CF subjects (12 females) were recruited and all provided three technically acceptable MBW and spirometry data. Seventy-four age and gender matched healthy controls were selected (in a 2:1 ratio for each CF subject) for inclusion in the study as a healthy group for comparison. The demographic and spirometry data for healthy control and cystic fibrosis cohorts are shown in Table 1. Within the CF cohort, 10/37 (25%) were aged 11.0 to 17.8 years, 10/37 (27%) were pF508.del (median 15.5), and the remaining 27 subjects were 18.0 to 45.4 years old (median 24.4 years), 23/37 (62%) were pF508.del, 33/37 (89%) were pancreatic insufficient, and 16/37 (43%) were chronically colonized with Pseudomonas aeruginosa. CF subjects had significantly lower spirometry values (all P < 0.001) with FEV1 values predominantly in the mild to moderate range of obstructive lung disease (range 34.2–111.7% predicted; only four subjects had an FEV1 <60% predicted) (Table 1). No difference in nutritional status was detected between groups.

As expected, MBW values were increased in CF compared with healthy control subjects (Table 2). LCI was abnormal in 36/37 CF subjects (97%, ULN 7.3, range 7.28–18.89). Only one subject had both a normal LCI and FEV1 (Fig. 2), while 25/37 subjects (68%) had a normal FEV1 but abnormal LCI. Scond × VT and Sacin × VT were abnormal in 36/37 (97%, ULN 0.023, 0.022–0.101) and 27/37 (73%, ULN 0.088, range 0.038–0.486) CF subjects, respectively. The relationships of Scond × VT and Sacin × VT to LCI in the CF cohort are shown in Fig. 3. Scond × VT appeared to increase early in disease severity but not in a linear way at higher LCI values, resulting in high correlation.
in low overall correlation to LCI \( (r^2 = 0.11, P = 0.049) \), compared with \( S_{\text{acinc}} \times V_T \), which became abnormal at higher LCI and showed high correlation to LCI \( (r^2 = 0.60, P < 0.0001) \). Log transformation of \( S_{\text{acinc}} \times V_T \) values did not strengthen this correlation \( (r^2 = 0.55, P < 0.0001) \).

As described in historical data, healthy control subjects also had two detectable lung compartments, one small very well ventilated compartment and one large with “normal” ventilation (Fig. 1A) (19, 28). Healthy subjects showed significant fit to the two-compartment model in all cases with overall mean (SD) \( r^2 \) of 0.997 (0.003), \( P < 0.001 \), with mean (SD) for \( r^2 \) CV% of 0.17 (0.15). Mean (SD) of \( r^2 \) for fast and slow regression lines were 0.981 (0.024) and 0.988 (0.007), with mean (SD) for CV% of \( r^2 \) values of 1.4 (1.7) and 0.40 (0.3), respectively. Mean (SD) of within-session SD for fast and slow compartments \( r^2 \) were 0.013 (0.016) and 0.004 (0.003), respectively. CF subjects showed strong fit to the two-compartment model in all cases, with overall mean (SD) \( r^2 \) of 0.961 (0.028), \( P < 0.001 \), and mean (SD) for \( r^2 \) values CV% of 1.65 (1.27). Mean (SD) of \( r^2 \) for fast and slow regression lines were 0.961 (0.044) and 0.913 (0.051), with mean (SD) for CV% of \( r^2 \) of 3.9 (4.8) and 4.4 (3.4), respectively. Mean (SD) of within-session SD for fast and slow compartments \( r^2 \) were 0.025 (0.041) and 0.039 (0.029), respectively.

Derived MBW results expressed as mean (SD) for healthy control and CF cohorts are given in Table 2. In CF, the measured volume of the slowly ventilated lung compartment varied from 34 to 67% of the full FRC (vs. 46 to 92% in healthy cohort, \( P < 0.001 \)). Mean (SD) within-session SD was 3.8 (2.0)% in CF vs. 4.9 (4.7)% in controls (nonsignificant). In the healthy cohort, regional alveolar tidal volume \( (V_{TA}) \) was similar between the two compartments with \( V_{TA} \), slow of mean (SD) 350 (106) ml and \( V_{TA} \), fast 366 (141) ml (nonsignificant). In CF significantly smaller \( V_{TA} \) was delivered to the slowly vs. the better ventilated compartment (82 [37] vs. 499 [161] ml \( [P < 0.001] \)). LCI increased as the relative volume of this slowly ventilated lung compartment (i.e., % of full FRC) increased \( (r^2 = 0.27, \text{low correlation, } P < 0.001 \text{ [Fig. 4A]}) \). A similar relationship with \( S_{\text{acinc}} \times V_T \) was observed \( (r^2 = 0.39, \text{moderate correlation, } P < 0.001; r^2 = 0.46, \text{after log transformation, } P < 0.001) \). No correlation was observed with \( S_{\text{cond}} \times V_T \) \( (r^2 = 0.01, P = 0.90 \text{ [Fig. 4B]}) \).

Specific ventilation of the slower compartment ranged between 1.8 and 13.1% in CF and was reduced compared with the healthy cohort (6.8 to 23.2%; \( P < 0.001 \)). Mean (SD) within-session SD was 0.6 (0.6) in CF, vs. 1.3 (1.2) in the healthy cohort. LCI increased as specific ventilation of the slowly ventilated lung compartment decreased \( (r^2 = 0.70, \text{very high correlation, } P < 0.001 \text{ [Fig. 5A]}) \). A similar but weaker relationship with \( S_{\text{acinc}} \times V_T \) was seen \( (r^2 = 0.44, \text{moderate correlation, } P < 0.001) \). No significant correlation was seen with \( S_{\text{cond}} \times V_T \) \( (r^2 = 0.11 \text{ [Fig. 5B]}) \).
A very strong curvilinear relationship was seen between \( V_D/F_T \) and LCI \( (r^2 = 0.96, \) very high correlation, after log transformation of LCI, \( P < 0.001 \) [Fig. 6]).

\( V_{D}/F_{T} \) (%) and specific ventilation of the slow compartment were very highly correlated in a linear manner to \( V_D/F_T \) \( (r^2 = 0.80, P < 0.001 \) [Fig. 7]). Curve index did not correlate with the relative volume of the slow compartment \( (r^2 = 0.00) \) but showed moderate correlation with the specific ventilation of the slow compartment \( (r^2 = 0.48, P < 0.001) \).

The observed \( C_{etN_2,final} \) was on average marginally lower than the estimated \( C_{etN_2,final} \) \( (\text{Eq. 7}) \) \( (\text{Mean [SD] 1.86 [0.05]% vs. 1.93 [0.20]%, } P = 0.031) \). Mean (SD) within-session SD of estimated \( C_{etN_2,final} \) was 0.24 (0.15), suggesting good quality of the modelling.

**DISCUSSION**

This study represents the first published effort to describe the quantitative and functional assessment of the slowly ventilated lung compartment based on \( N_2 \) MBW analysis in subjects with CF. The novel indices presented add to information provided by global measures of ventilation inhomogeneity, such as LCI. These indices also suggest that as CF lung disease severity increases, lung regions with marked underventilation are formed. This has not been shown before. The new indices show a strong relationship with LCI across disease severity, in contrast to \( S_{III} \)-based indices, particularly \( S_{cond} \times V_T \). Accuracy of our size estimates of the slowly ventilated lung compartment is suggested by good agreement with previous modelling and imaging studies (27, 44). Importantly, these easily derived new indices are potentially automatable from routine \( N_2 \) MBW tests.

Ventilation distribution inhomogeneity is a common finding in respiratory disease, especially in CF, where abnormal LCI values have been reported in 60% of preschoolers (42) and 95% of school aged children (5). \( S_{III} \) analysis is challenging in younger children, despite proposed \( V_T \) correction of \( S_{III} \) values (39). In infants, \( S_{III} \) analysis is further compromised by the relatively short phase III slope, due to the larger airway size to lung volume ratio and smaller amount of lung parenchyma present in this age group (38). For this reason we chose a predominantly adult cohort (but included some older pediatric subjects) where \( S_{III} \) analysis was highly feasible. Recent work has highlighted that the modelling, on which \( S_{III} \) analysis is based, may not hold in more severe disease (45). We describe the same problematic relationships of LCI and \( S_{III} \) parameters within our CF subjects reported previously in the adult CF literature (25). Poor correlation of \( S_{cond} \) was observed in more advanced disease (LCI values > 12), reaching an apparent “ceiling” or even decreasing as disease severity increased. In contrast, stronger correlation was observed with \( S_{acin} \) as LCI increased (Fig. 3). Imaging studies have shown that significant interregional differences in ventilation distribution occur in more severe lung disease (31). \( S_{cond} \) is proposed to reflect ventilation inhomogeneity arising due to changes in specific ventilation and sequencing among lung units sharing branch points proximal to the diffusion-convection front. However, \( S_{cond} \) failed to reflect this accurately in this setting, as discussed in recent literature (33, 45).

The indices we present extend the previous literature exploring information contained in semilog plot analysis, originally described almost 60 years ago (8, 9, 19, 28, 37). Comparison to other MBW indices based on semilog analysis highlights important limitations of currently reported indices. The initial rapidly declining portion of the semilog plot of end-tidal \( N_2 \) values used for curve index (44) and Slope ratio (11, 36) include contributions from both the fast and slow compartments. However, the corresponding plot of exhaled \( N_2 \) volume used for the current compartment analysis has been cleared of slow compartment contributions. This important modification may produce an improved index of interregional inhomogeneities. Currently used indices also fail to quantify the volume or specific ventilation of this impaired region, which constitute the main focus of the present study. Our proposed indices may provide insight into the underlying process that occurs with advancing disease severity in CF, as they display a more...
consistent correlation across disease severity than SnIII indices (Figs. 4 and 5). Importantly, these indices can be derived from modern tidal breathing MBW tests and offer potential for automated calculation, avoiding reliance on clear phase III slopes. Feasibility of calculation of these new indices may not be related to age; however, this needs to be confirmed in future studies. Intuitively, knowledge of the size and extent of the slowly ventilated lung compartment may inform the clinician of issues regarding possible efficacy of inhaled medication delivery to regions of disease, although regional imaging studies are required to further investigate what a given change (or lack of) in these indices represents.

Recent modelling work based on MBW or imaging data has suggested that these slowly ventilated lung regions contribute significantly to the ventilation inhomogeneity present in obstructive lung disease. Verbanck et al. (44) modelled MBW data to show that LCI values of \( \frac{V_F}{V_t} \) could be generated by the presence of a slowly ventilated lung compartment comprising 50% of the lung volume and receiving 10% of overall ventilation. Imaging work has described ventilation defects affecting on average 30% of the lung volume in a small group \( n = 5 \) of adult CF subjects (27). Our estimates are in agreement with this previous work in CF and also the early historical estimates of the relative magnitude of these two compartments in health (19, 37). In our study, a slowly ventilated lung compartment was found in all CF subjects and ranged from 34 to 67% of the full FRC, with a measured specific ventilation of the slow compartment ranging between 1.8 and 13.1%. Changes in curve index were driven by changes in specific ventilation of this slow compartment \( r^2 = 0.48, P < 0.001 \) rather than changes in its relative volume \( r^2 = 0.00, P = 0.93 \), suggesting utility in quantifying this functional component. In addition, our cross-sectional data provides potential insight into the overall functional response that occurs as this slowly ventilated lung compartment develops. As the slowly ventilated lung compartment increased across subjects within our cross-sectional CF cohort, there also appeared to be increased overventilation of the remaining lung units (data not shown). This overventilation was not present at greater lung disease severity (higher LCI values) (Fig. 8). This may reflect a smaller available volume of better functioning lung. Longitudinal studies are needed to confirm this hypothesis.

The previous focus of work by Arborelius et al. (3) on the role of the respiratory dead space suggested that functional dead space measures derived from \( N_2 \) MBW should increase as ventilation distribution inhomogeneity increases. Our data confirms the strong curvilinear relationship between LCI and functional respiratory dead space volume \( V_D/V_t \) (%) (Fig. 6) initially described by Arborelius et al. The data presented in our manuscript extends this earlier work by describing a more linear relationship between increasing \( V_D/V_t \) (%) and reduced specific ventilation of this slow compartment than is evident with LCI (Fig. 7 and Fig. 5, respectively). This suggests that \( V_D/V_t \) %, as a global index of ventilation distribution inhomogeneity, should be considered as a reported outcome, particularly in severe disease. This ongoing decrease in specific
ventilation to an increasing volume of slowly emptying lung drives the increase seen in $S_{\text{acm}}$ in our cohort.

There are several limitations of the underlying assumptions on which the new indices are based. The two-compartment model is obviously a simplification of the widely differing specific ventilations among thousands of lung units with different mechanical properties (i.e., regional compliance and resistance). The label “two-compartment model” may be misleading, as it is in reality a mathematical two-component model. Nevertheless, the model provides additional insights into the lung pathophysiology in CF, which may help the clinician understand the disease process. Another limitation is that the current modelling work does not take into account $N_2$ dissolved in blood and other tissue, which will diffuse into the alveoli when pure $O_2$ is breathed. This, however, is thought to contribute only to a small proportion of the expired $N_2$ over the first minutes of washout (29) and has been shown not to affect $S_{\text{HH}}$ data significantly (11). Last, lung units with fast turnover and units more slowly ventilated have a common dead space volume in the central airways, a fact that the present model does not consider. When well-ventilated lung units have been washed out of most or all their alveolar $N_2$, alveoli in poorly ventilated lung units will continue to deliver $N_2$ to the expirate. The common dead space volume will speed up the washout of the slow compartments and delay clearance of the fast (30).

Despite all shortcomings and limitations of the current model, the relatively good agreement between the observed and calculated overall final end-tidal $N_2$ concentrations suggest that it delivers fairly representative and robust measures. Several aspects of the indices reported here, and the hypotheses generated, remain to be addressed in future studies. These studies will need to validate the accuracy of these new measures against functional imaging-based measurements, feasibility in younger age groups, and assess the repeatability and clinical utility of the new indices generated. The CF cohort studied here included mainly subjects with either normal FEV1 values or mild obstructive disease. The integrity of the relationships between the novel indices, LCI and $V_T/F/V_T$ in moderate and severe obstructive lung disease, has not been shown, but it should be noted that the utility of MBW is felt to lie predominantly in mild disease due to the increasing test duration in more severely obstructed subjects. The chosen cohort did not affect our ability to replicate the issues previously described with $S_{\text{HH}}$ indices and advanced lung disease. This also limits our ability to discuss utility in very early CF disease with this data. Because the calculation of global indices of inhomogeneity, $S_{\text{HH}}$ data, and compartment indices were undertaken in one sequence, there was no blinding to severity of disease.

In conclusion, additional mechanistic data can be derived from $N_2$ MBW tests to provide both quantitative and functional information about the slowly ventilating lung compartments in CF lung disease. These presented indices were strong drivers of the observed change in global ventilation heterogeneity (LCI) in our cohort of CF subjects and showed a more consistent relationship across disease severity than the previously proposed $S_{\text{HH}}$ analysis variables. These indices offer useful additional information to indices such as LCI and the curve index, and we would advocate their inclusion to existing reported indices as they provide a more complete picture of the impact of the underlying disease process. Importantly, these indices offer potential for automated calculation. Future studies are required to validate measurement accuracy and stability and explore clinical utility, but good agreement with values generated by previous modelling studies is encouraging.

**APPENDIX**

Quality control performed at the time of MBW testing.

All gas and flow signals in the Exhalyzer D were sampled at 200 Hz. Calibrations of the ultrasonic flow meter, $O_2$ and $CO_2$ analyzers, and gas and flow signals synchronization were performed, in accordance with manufacturer’s recommendations, prior to each subject test session. All test subjects wore a nose clip and breathed via a trimmed silicon mouthpiece (to minimize equipment-related dead space volume) attached to a bacterial filter (Air Safety Ltd, Morecambe, UK) in line with the recording system. The set 3 dead space reducer (Ecomedics AG, Duernten, Switzerland) was used to reduce the equipment dead space volume. The measured pre-gas-sampling point external dead space volume of the system was 35 ml and the post-gas-sampling point dead space volume was 22 ml. This is ±2 ml/kg body wt for all but one of the subjects tested (99.8%). $N_2$ MBW was performed in the upright sitting position during relaxed tidal breathing. On-line tidal volume monitoring was performed by the operator such that the phase III slope of each breath (reflecting alveolar gas concentration) constituted approximately half the breath. A minimum interval of the preceding washout time was observed between tests to ensure resting end-tidal $N_2$ concentration values had returned to normal levels prior to the next test.

**Derivation of reported conventional $N_2$ MBW parameters.**

This information is provided in keeping with the recommendation of the recent consensus statement (39). FRC derivation is outlined in the main manuscript. Lung volume turnover values (TO) were calculated for each breath over the washout as the cumulative expired volume (CEV) of the expired gas at the point of the lips divided by FRC. The CEV of each breath was corrected for total equipment-related dead space volume. LCI was calculated as TO value of the first of three consecutive breaths where end-tidal $N_2$ concentration had fallen below 1/40th of starting end-tidal $N_2$ concentration.

The phase III slope of each breath was determined from 50 to 95% of the inhaled volume of air by linear regression over the slowly rising portion of the breath containing alveolar gas. By visual inspection, this was taken to avoid influence of the gas from the bronchial phase (phase II) or the fast rising phase IV, occurring due to airway closures. The concentration-normalized phase III slope ($S_{\text{HH}}$) was
calculated by dividing the phase III slope by the mean N2 concentration over the slope. Before calculation of S\textsubscript{cond} and S\textsubscript{acin}, Sn\textsubscript{II} values were further normalized by multiplying them by their respective tidal volume (Sn\textsubscript{II} \times V\textsubscript{T}), i.e., simulating a V\textsubscript{T} of 1000 ml to account for differences in size between subjects and for variations in V\textsubscript{T} during the washout. Data from the three MBW recordings were averaged before S\textsubscript{cond} and S\textsubscript{acin} calculation (39). S\textsubscript{cond} was calculated by linear regression from a plot of Sn\textsubscript{III} vs. TO as the increase of Sn\textsubscript{III} from 1.5 to 6 TO. S\textsubscript{acin} was calculated as the first breath Sn\textsubscript{III} minus the S\textsubscript{cond} contribution to Sn\textsubscript{III}.

Off-line analysis of MBW tests.

The off-line data analysis in the TestPoint software started with a synchronization of the CO\textsubscript{2} and O2 signals to the flow signal for each recording. The delay time of the gas signals was defined as the time between the moment when the post-gas-sampling dead space volume (measured to be 22 ml) had been inhaled until the 50% deflection point of the CO\textsubscript{2} and O2 signals, respectively. The median lag time value for all breaths over the three recordings was applied. Typically lag times varied less than 10 ms for both gases in each of the two recording devices over the 18 mo period that recordings were done. After synchronization of CO\textsubscript{2} and O2 signals, the N2 concentration was calculated N2\% = 100\% - O2\% - CO2\% - Ar\%, where Argon (Ar) was assumed to form a fixed portion of the N2 signal (39).

Analysis of the MBW recordings involved visual check of the quality of each breath and correction of default estimation of phase III slopes (e.g., error due to cardiogenic oscillations).

Determination of the fast and slowly ventilated lung compartments.

A semiautomatic TestPoint algorithm was used to decide the optimal starting point for the regression line delineating the sole contribution from the slowly ventilated lung compartments to the overall semilog washout curve (Fig. 1, closed circles). This was performed by scanning in increasing segments of 0.05 (i.e., 5\% of the total number of breaths) from 90\% of the washout back to 10\% of the washout. The peak r\textsuperscript{2} value obtained was used to define the starting point of this regression line. The operator then reviewed this visually and confirmed the starting point. Back extrapolation of this regression line to the first breath of the washout was performed to calculate the contribution of these slowly ventilated lung regions across the full washout (Fig. 1, open triangles). Once subtracted, only the contributions of the fast lung compartments remained (Fig. 1, open squares).

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: P.M.G., P.R., M.G., A.L., and B.K.H. conception and design of research; P.M.G. and B.K.H. performed experiments; P.M.G., P.R., and B.K.H. analyzed data; P.M.G., P.R., M.G., A.L., and B.K.H. interpreted results of experiments; P.M.G. and P.R. prepared figures; P.M.G. and P.R. drafted manuscript; P.M.G., P.R., M.G., A.L., and B.K.H. edited and revised manuscript; P.M.G., P.R., M.G., A.L., and B.K.H. approved final version of manuscript.

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