Multiple breath washout (MBW) testing using sulfur hexafluoride: reference values and influence of anthropometric parameters

Frederik Trinkmann, 1,2,3 Máté Maros, 1,4,5 Katharina Roth, 1 Arne Hermanns, 1 Julia Schäfer, 1 Joshua Gawlitza, 5 Joachim Saur, 1 Ibrahim Akin, 1,6 Martin Borggrefe, 1,6 Felix J F Herth, 2 Thomas Ganslandt 3

ABSTRACT

Background Multiple breath washout (MBW) using sulfur hexafluoride (SF 6 ) has the potential to reveal ventilation heterogeneity which is frequent in patients with obstructive lung disease and associated small airway dysfunction. However, reference data are scarce for this technique and mostly restricted to younger cohorts. We therefore set out to evaluate the influence of anthropometric parameters on SF 6 -MBW reference values in pulmonary healthy adults.

Methods We evaluated cross-sectional data from 100 pulmonary healthy never-smokers and smokers (mean 51 (SD 20), range 20–88 years). Lung clearance index (LCI), acinar (S acin) and conductive (S cond) ventilation heterogeneity were derived from triplicate SF 6 -MBW measurements. Global ventilation heterogeneity was calculated for the 2.5% (LCI 2.5) and 5% (LCI 5) stopping points. Upper limit of normal (ULN) was defined as the 95th percentile.

Results Age was the only meaningful parameter influencing SF 6 -MBW parameters, explaining 47% (CI 33% to 59%) of the variance in LCI, 32% (CI 18% to 47%) in S acin and 10% (CI 2% to 22%) in S cond. Mean LCI increases from 6.3 (ULN 7.4) to 8.8 (ULN 9.9) in subjects between 20 and 90 years. Smoking accounted for 2% (CI 0% to 8%) of the variability in LCI, 4% (CI 0% to 13%) in S acin and 3% (CI 0% to 13%) in S cond.

Conclusion SF 6 -MBW outcome parameters showed an age-dependent increase from early adulthood to old age. The effect was most pronounced for global and acinar ventilation heterogeneity and smaller for conductive ventilation heterogeneity. No influence of height, weight and sex was seen. Reference values can now be provided for all important SF 6 -MBW outcome parameters over the whole age range.

Trial registration number NCT04099225.

INTRODUCTION

Multiple breath washout (MBW) has the potential to reveal ventilation heterogeneity which is frequently present in patients with obstructive lung disease and small airway dysfunction. However, it is often missed by commonly used tests. 2,12 Spirometry was shown to be insensitive to changes in patients with asthma that can be made visible using MBW. 3,4 These changes are associated with symptoms, most notably dyspnoea, cough and phlegm in COPD. 5

Dyspnoea may be a direct expression of hyperinflation, 6 which was shown to be addressable therapeutically. 7 The impact of peripheral airways is yet not restricted to late stages. Instead small airway dysfunction occurs early in the natural course of the disease. In bronchial asthma, changes in small airways are also a hallmark and present in more than 90% of patients. Moreover, clinical subtypes of small airway disease were identified using different tests including MBW. 8 Lung clearance index (LCI) is the most frequently used MBW outcome parameter and refers to global ventilation heterogeneity. Phase III slopes represent local ventilation heterogeneity in the acinar (S acin) and conductive (S cond) airways. Open wash-in MBW using sulfur hexafluoride (SF 6 ) and mass spectrometry are considered the gold standard 9 while being associated with high costs and effort. Hence, nitrogen (N 2 ) has been frequently used as tracer gas despite suffering from several methodological drawbacks such as back diffusion, 10,11 measurement inaccuracies 12 and leaks. 13 Introduction of a photo-magneto-acoustic multigas analyser allowed the direct measurement of SF 6 concentrations with high accuracy. 14 Recently, this led to construction of a closed-circuit SF 6 -MBW set-up considerably facilitating application and reducing costs. 15 Meaningful differences in MBW outcome measures depending on the test gases have been described. 16,17 In general, N 2 -based...
systems reproducibly yield higher absolute LCI readings than SF₆-MBW. To date, much clinical data are available in patients with cystic fibrosis using N₂-based MBW systems. Reference data are scarce and mostly restricted to younger cohorts. Therefore, we set out to (1) evaluate the influence of anthropometric parameters on SF₆-MBW outcome measures and (2) generate reference values in pulmonary healthy controls.

METHODS
Subjects
We evaluated cross-sectional data from pulmonary healthy never-smokers and smokers. Participants were included prospectively using convenience sampling. Subjects had normal lung function testing and no respiratory symptoms. Normal lung function required a combination of normal spirometry, whole-body plethysmography, impulse oscillometry and Krogh factor (KCO) in both qualitative and quantitative analyses. Details are given in the online supplemental appendix 1. The study was registered at ClinicalTrials.gov. Written informed consent was obtained from all participants prior to inclusion.

Study protocol
All subjects underwent three consecutive MBW tests in upright position followed by impulse oscillometry (MasterScreen IOS, CareFusion 234, Höchberg, Germany), spirometry and whole-body plethysmography (MasterScreen Body). Oscillometry was performed during tidal breathing. Parameters were averaged over multiple respiratory cycles within 20%. Transfer factor for carbon monoxide (TLCO) was derived from KCO and ventilated alveolar volume as measured in single breath technique. All lung function measurements were taken cross-sectionally at a single time point and independently assessed by two experienced investigators. Reference values for spirometry, whole-body plethysmography and transfer factors were derived from the revised 1993 version of the European Community for Steel and Coal (ECSC) equations. For impulse oscillometry, reference values as provided by Vogel and Smidt were used.

Multiple breath washout
A commercially available closed-circuit system (Innocor, PulmoTrace ApS, Glamsbjerg, Denmark) was used for MBW measurements. The device consists of a 3 L rebreathing bag filled with a mixture of room air and test gas (94% O₂, 1% SF₆, and 5% N₂O; PulmoTrace ApS) from a commercially available on-board gas cylinder (bolus fraction 20%). Subjects breathed through the device using a mouthpiece and wearing a nose clip. A scrubber was serially placed between the rebreathing bag and the patient to reduce carbon dioxide concentrations during rebreathing. A standard bag volume of 2.5 L was used. Adjustments were made upfront in steps of 0.25 to 0.5 L, if functional residual capacity (FRC) was known or suspected to considerably deviate. Likewise, adaptations became necessary during measurements with the subject emptying the bag early during rebreathing. Maximal slow inspirations up to the bag volume followed by slow expirations were performed for the first six breaths in order to achieve a complete wash-in. Inspiratory gas concentrations were closely monitored and held below 3% for carbon dioxide and above 18% for oxygen. The further manoeuvre was performed during relaxed tidal breathing. Washout was then started by switching between the bag and room air using a fast-operating pneumatic valve. This was automatically triggered at the end of expiration under the direction of the operator. We stopped the test when end tidal SF₆ had fallen below 1/40 of the starting concentration for three consecutive breaths. Detailed analysis and quality control were performed offline using proprietary software provided by the manufacturer (V8.0 beta 1 software). Mean FRC and LCI were derived from three consecutive washouts during tidal breathing. LCI is defined as the number of FRC turnovers to reduce tracer gas to a predefined level. This is referred to as stopping point and was calculated for the traditional 2.5% (LCI₅) as well as 5% (LCI₅) of the initial tracer gas concentration. Additionally, phase III slopes were analysed from multiple breaths yielding parameters of acinar (Sacin) and conductive (Scond) ventilation heterogeneity. We used a semiautomated approach plotting slopes against FRC turnovers. All breaths were visually analysed and excluded if no clear linear phase III portion could be identified. Scond was then derived by linear regression in the range between 1.5 and 6 turnovers. Sacin was calculated as the mean slope of the three first breaths minus Scond contribution. Only subjects with at least two technically acceptable MBW measurements based on slightly modified American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria (online supplemental appendix 1) were included in the final analysis.

Table 1 Baseline characteristics

| Unit | Overall (N=100) | Never-smoker (n=68) | Smoker (n=32) |
|------|----------------|---------------------|--------------|
|      | Value          | Range               | Value        | Range       | Value          | Range       | P value | ES  |
| Age  | Years         | 51±20               | 20–88        | 49±21       | 20–88         | 54±16       | 22–81   | 0.22| 0.24 (0.19 to 0.66) |
| Height | cm             | 171±9               | 152–198      | 172±9       | 153–198       | 169±9       | 152–193 | 0.22| 0.26 (−0.16 to 0.69) |
| Weight | kg             | 76±17               | 45–132       | 75±16       | 45–132        | 79±20       | 51–120  | 0.33| 0.33 (−0.19 to 0.65) |
| BMI  | kg/m²         | 26.2±5.4            | 16.3–49.1    | 25.6±5.2    | 16.3–49.1     | 27.5±5.5    | 19.9–37.8| 0.11| 0.35 (−0.08 to 0.78) |

| Male | n (%)         | 54 (54)             | 37 (54)      | 17 (53)     | 1.0           | 0.03 (−0.39 to 0.45) |

Effect size (ES) as measured by Cohen’s d with 95% CI.
BMI, body mass index.

Trinkmann F, et al. Thorax 2021;76:380–386. doi:10.1136/thoraxjnl-2020-214717 381
Results

Baseline characteristics and lung function

Data were acquired in a total of 104 subjects. The prespecified exclusion criteria were met by four subjects, resulting in 100 subjects (54 men) undergoing final analysis. The baseline characteristics of 68 never-smokers and 32 smokers (5 active smokers, 27 former smokers) are summarised with between-group differences in Table 1. Lung function data are given in Table 2. Differences between smokers and never-smokers were only found for maximum expiratory flow at 25% (MEF_{25}) of FVC and S_{acin}. The overall CV was 3.2% for LCI_{2.5} and 3.3% for LCI_{5}. At least two valid phase III slope measurements could not be acquired in 10 subjects. These were slightly older (55±20 vs 50±20 years, p=0.46, ES=0.26, CI −0.40 to 0.91) as compared with subjects with successful measurements. Additionally, phase III slopes showed larger variability with a CV of 50% for Sacin and 124% for Scond. The mean washout duration was 104±37 s for LCI_{2.5} and 79±27 s for LCI_{5} (p<0.0001, ES=0.8, CI 0.5 to 1.1).

Influence of anthropometric parameters

Age was the only meaningful contributor to LCI and Sacin in the regularised model, whereas no association with Scond was found in the overall collective. In a non-penalised model, age explained 47% (CI 33% to 59%) of the variance in LCI, 32% (CI 18% to 47%) in Sacin and 10% (CI 2% to 22%) in Scond. Smoking accounted for 2% (CI 0% to 8%) of the variability in LCI, 4%
Further, it should be noted that a solid definition of normality during the first approximately 6 years of life and then remains rather stable after reaching around 115 cm. Hence, they proposed to use a constant ULN of 7.56 defined by the 97.5th percentile in subjects older than 6 years, which corresponds well to our model. No correlation between age and LCI was found when including younger collectives. With the normal range appearing to remain stable for the first four decades of life, a fixed ULN of 6.8 and 7.0 was proposed by Horsley et al. and Fuchs et al. In patients older than 40 years a larger dispersion was seen. Despite all of these approaches being based on SF₆, small differences may be partly explained by system set-ups and tracer gas concentrations. Considerably larger differences can be attributed to tracer gases themselves as compared with set-up. It is well known that N₂-MBW reproducibly yields higher absolute LCI readings than SF₆-MBW. Much of this difference can be explained by physiological properties and technical aspects associated with the test gases. Concerning local ventilation heterogeneity, age was found to explain 7% and 16% of the variability in S_{ac} and S_{cond} derived from N₂-MBW, respectively. Moreover, a significant influence of sex on S_{ac} as well as age on LCI was found. This corresponds to our data set where 10% of the variability in S_{cond} is explained by age. In contrast, contribution for LCI and S_{ac} is higher in our collective with 47% and 32%. Reference intervals for parameters of local ventilation heterogeneity using N₂ as tracer gas were higher than those found for SF₆ in our data at hand. Interestingly, mean LCI reference values were lower in their investigation than those for SF₆-MBW. This is contradictory to our findings and previously published data. While N₂ readings were found to be consistently higher in general as discussed above, Houlzt and coworkers could demonstrate higher mean predictions for LCI over a wide range between 7 and 70 years. Verbanck and coworkers found a 0.22-unit change in LCI per decade, while we could demonstrate an increasing rate with age between 0.2 and 0.5 units per decade. It should be noted that including smokers led to a 0.3 increase in mean LCI, corresponding to a 10-year effect of ageing. In addition to differences in test gases and set-ups, parts of these differences may be explained by the sequence of lung function tests. Potential airway dilatation may lead to distortion of tidal breathing techniques. Therefore, we conducted all examinations requiring tidal breathing strictly before forced manoeuvres. Taken together, either test gas requires separate reference data and individual values should not be interchangeably.

When interpreting our results, several aspects should be taken into consideration. For S_{ac} and MEF_{25}, significant differences were found between smokers and only never-smokers, respectively. For generating reference values, it would have been desirable to strictly include only never-smokers. However, this would have narrowed the age range investigated considerably as finding otherwise healthy, never-smoking septuagenarians and octogenarians is particularly difficult. We therefore applied an extremely strict definition for controls. This did not only include commonly used history, respiratory symptoms and spirometric findings. Additionally, we required parameters from whole-body plethysmography, oscilometry and gas transfer to be within the normal range for both never-smoking and smoking controls, respectively. As a consequence, smoking status explains a maximum of 4% of the variability in MBW parameters. Therefore, calculation of normal values itself is hardly affected by smoking status using our GAMLSS approach.

Further, it should be noted that a solid definition of normality is difficult especially in elderly patients. This is important in the context of the rather broad range of residual volume/total lung capacity (RV/TLC) (20%–77%) and TLCO (58%–134%)

Figure 1 LCI₂₅ normal values. Age-dependent plot of predicted values (solid line) with ULN and LLN. (A) Never-smokers (n=68) provide overall lower predictions as compared with (B) additionally including former or active smokers (n=100). Dashed lines and lighter grey represent a model-based extrapolation of actually measured cross-sectional data. LCI₂₅, lung clearance index at 2.5% stopping point; LLN, lower limit of normal; ULN, upper limit of normal.

(CI 0% to 13%) in S_{ac} and 3% (CI 0% to 13%) in S_{cond} respectively. We therefore calculated normal values with ULN and LLN corrected for age.

Reference values

Figure 1 gives age-dependent predictions for LCI₂₅ in the overall collective and never-smokers only. Likewise, predicted values for phase III slopes are given in figure 2. The underlying models are available for download from GitHub (https://github.com/ffrinkmann). A summary of calculated reference values per decade is given in table 3. Age-dependent predictions for LCI₂₅ in the overall collective and never-smokers can be found in online supplemental e-Figure 1.

**DISCUSSION**

We found that age is the only anthropometric parameter contributing to SF₆-MBW outcome measures. The effect was most pronounced for global and acinar ventilation heterogeneity and smaller for conductive ventilation heterogeneity. No influence of height, weight and sex was seen in our adult population. On this basis, we were able to generate age-corrected reference values for SF₆-MBW ranging from early adulthood to old age.

Our results extend the data available for paediatric cohorts and are in accordance with previous findings. Lum and coworkers could demonstrate that LCI decreases with height during the first approximately 6 years of life and then remains rather stable after reaching around 115 cm. Hence, they proposed to use a constant ULN of 7.56 defined by the 97.5th percentile in subjects older than 6 years, which corresponds well to our model. No correlation between age and LCI was found when including younger collectives. With the normal range appearing to remain stable for the first four decades of life, a fixed ULN of 6.8 and 7.0 was proposed by Horsley et al. and Fuchs et al. In patients older than 40 years a larger dispersion was seen. Despite all of these approaches being based on SF₆, small differences may be partly explained by system set-ups and tracer gas concentrations. Considerably larger differences can be attributed to tracer gases themselves as compared with set-up. It is well known that N₂-MBW reproducibly yields higher absolute LCI readings than SF₆-MBW. Much of this difference can be explained by physiological properties and technical aspects associated with the test gases. Concerning local ventilation heterogeneity, age was found to explain 7% and 16% of the variability in S_{ac} and S_{cond} derived from N₂-MBW, respectively. Moreover, a significant influence of sex on S_{ac} as well as age on LCI was found. This corresponds to our data set where 10% of the variability in S_{cond} is explained by age. In contrast, contribution for LCI and S_{ac} is higher in our collective with 47% and 32%. Reference intervals for parameters of local ventilation heterogeneity using N₂ as tracer gas were higher than those found for SF₆ in our data at hand. Interestingly, mean LCI reference values were lower in their investigation than those for SF₆-MBW. This is contradictory to our findings and previously published data. While N₂ readings were found to be consistently higher in general as discussed above, Houlzt and coworkers could demonstrate higher mean predictions for LCI over a wide range between 7 and 70 years. Verbanck and coworkers found a 0.22-unit change in LCI per decade, while we could demonstrate an increasing rate with age between 0.2 and 0.5 units per decade. It should be noted that including smokers led to a 0.3 increase in mean LCI, corresponding to a 10-year effect of ageing. In addition to differences in test gases and set-ups, parts of these differences may be explained by the sequence of lung function tests. Potential airway dilatation may lead to distortion of tidal breathing techniques. Therefore, we conducted all examinations requiring tidal breathing strictly before forced manoeuvres. Taken together, either test gas requires separate reference data and individual values should not be interchangeably.

When interpreting our results, several aspects should be taken into consideration. For S_{ac} and MEF_{25}, significant differences were found between smokers and only never-smokers, respectively. For generating reference values, it would have been desirable to strictly include only never-smokers. However, this would have narrowed the age range investigated considerably as finding otherwise healthy, never-smoking septuagenarians and octogenarians is particularly difficult. We therefore applied an extremely strict definition for controls. This did not only include commonly used history, respiratory symptoms and spirometric findings. Additionally, we required parameters from whole-body plethysmography, oscilometry and gas transfer to be within the normal range for both never-smoking and smoking controls, respectively. As a consequence, smoking status explains a maximum of 4% of the variability in MBW parameters. Therefore, calculation of normal values itself is hardly affected by smoking status using our GAMLSS approach.

Further, it should be noted that a solid definition of normality is difficult especially in elderly patients. This is important in the context of the rather broad range of residual volume/total lung capacity (RV/TLC) (20%–77%) and TLCO (58%–134%)}
of predicted) found in our study. An increased RV/TLC as well as differences between TLCO and K CO are suggestive of ventilation heterogeneity and air-trapping. However, most of the values at the upper end (RV/TLC) or lower end (TLCO) were found in subjects well above 70 years of age. While reference data for body plethysmography are scarce in this age group, none of the subjects reported respiratory symptoms nor showed abnormal values in standard diagnostics. Moreover, we did not find disproportional deviations for ventilation heterogeneity, peripheral resistance or elastic properties. Taken together, this lets us assume that ageing effects within the physiological range rather than unrecognised disease can be considered responsible for these changes.

Although age was not associated with S cond in the regularised model, we decided to also provide age-corrected reference values since it explains as much as 10% of the variance. S cond is primarily subject to heterogeneity in pressure volume characteristics and bronchomotor tone of conductive airways. In contrast, S acin is heavily depending on branching asymmetry as well as parallel variability of the acini. This does not only explain differences between subjects, diseases or risk factors. Also, the different behaviour of ageing on S acin and S cond found in our investigation may be attributable to these factors. This is substantiated by previous findings. Considerable effects of ageing were described on airflow dynamics in a computational model. These correspond to ventilation defects found on imaging in otherwise healthy elderly subjects. However, no longitudinal data are currently available for functional tests and should therefore be subject of further investigation.

In contrast to the high intratest and intertest variability of traditional parameters of small airway obstruction such as MEF, LCI was shown to be highly reproducible. Variability of phase III slopes is larger as compared with the CV of up to 34% shown for MEF. Clinical implications of these have not been conclusively investigated to date. Nevertheless, impairment of S acin in patients with pulmonary hypertension allows to assume that early changes can already be detected and tracked today. In smokers with normal spirometry, changes in S cond were found and improvements to normal remained 12 months after smoking cessation. These findings are supported by data in patients with cystic fibrosis and adults with asthma. S cond and S acin may not represent all ventilation heterogeneities covered by LCI. Vice versa, LCI may not represent all ventilation heterogeneities covered by LCI. However, we decided to also provide age-corrected reference values since it explains as much as 10% of the variance.
covered by either $S_{\text{cond}}$ or $S_{\text{acet}}$. Therefore, a combined interpretation of parameters representing global and local ventilation heterogeneity seems promising. In contrast, MEF-derived parameters were shown to not contribute usefully to clinical decision making.

Total test duration is important in MBW testing. In general, washout is time-critical in $N_2$-MBW, whereas wash-in and costs are relevant factors in $SF_6$-MBW. Shorter washout durations are therefore an advantage of $SF_6$, allowing successful measurements also in patients with severe obstructive lung disease. Nevertheless, it should be noted that requiring a wash-in phase may neutralise some of the advantages of $SF_6$. For both tracer gases, several adaptions to the measurement protocols have been proposed, including reduction of total measurements or earlier cut-offs for terminating the washout. Moreover, closed-circuit $SF_6$-MBW was shown to effectively reduce wash-in times. We therefore calculated reference values for an earlier 5% stopping point reducing washout times considerably by 24%. Previously, an additional 34% reduction of total test time was achieved when only conducting two technically acceptable measurements. Together these adaptions may increase success rates in general. However, it should be noted that a comparative evaluation of clinical endpoints and diagnostic performance is warranted especially concerning abbreviated washout.

Our data considerably extend the current knowledge in adult $SF_6$-MBW testing. Reference values can now be provided over the whole age range. For the first time in adult MBW testing, this is not only restricted to the most common parameter LCI, but also includes phase III slopes $S_{\text{ana}}$ and $S_{\text{acet}}$. While extrapolations of our model fit well to available data for subjects older than 6 years, normative data for phase III parameters that can be used for comparison are overall scarce. We were able to acquire local ventilation heterogeneity parameters in a considerable number of subjects but also encountered some difficulties. Analysis was not successful in 10 subjects. This was most often due to either not having sufficient phase III to compute slopes or an intermittently irregular breathing pattern. Together with the overall variability of these analyses, further research is warranted especially concerning clinical outcome studies. Our findings were acquired exclusively in white European adults sharing the lack of different ethnicities as a major drawback of most cohorts. Nevertheless, our model provides a sound basis for calculation of normal values for both research settings and clinical routine. Finally, two methodological considerations should also be taken into account. First, reference values for conventional lung function tests were derived from ECSC equations. Second, we used commercially available gas cylinders. These also contain $N_2O$, which is identical to the mixture used for cardiac output determination while differing in bolus fraction. Although $N_2O$ is dispensable for MBW measurements, we were not able to use an $N_2O$-free mixture due to lack of availability.

CONCLUSIONS

$SF_6$-MBW parameters showed an age-dependent increase from early adulthood to old age. The effect was most pronounced for global and acinar ventilation heterogeneity. A smaller yet still considerable influence was seen for conductive ventilation heterogeneity. No influence of other anthropometric parameters such as height, weight and sex was found. Together with previous findings, reference values for LCI can now be provided over the whole age range as well as for an earlier 5% stopping point. Moreover, our data are not restricted to global ventilation heterogeneity but also comprise the parameters of local ventilation heterogeneities $S_{\text{ana}}$ and $S_{\text{acet}}$.

Author affiliations

1 First Department of Medicine, University Medical Centre Mannheim, Mannheim, Baden-Württemberg, Germany
2 Department of Pulmonology and Critical Care Medicine, Thoraxklinik at University Hospital Heidelberg, Translational Lung Research Centre Heidelberg (TLRC), Member of German Centre for Lung Research (DZL), Heidelberg, Baden-Württemberg, Germany
3 Department of Biomedical Informatics, Centre for Preventive Medicine & Digital Health Baden-Württemberg, University Medical Centre Mannheim, Mannheim, Germany
4 Department of Neuroradiology, University Medical Centre Mannheim, Mannheim, Germany
5 Institute for Clinical Radiology and Nuclear Medicine, University Medical Centre Mannheim, Mannheim, Germany
6 DZHK (German Centre for Cardiovascular Research), Mannheim, Germany

Acknowledgements We thank all subjects who agreed to take part in the study, as well as our technical assistants Maria Moritz and Sabrina Kraemer (1st Department of Medicine, University Medical Centre Mannheim) for their help in acquiring lung function data.

Contributors All authors read and approved the final version of the manuscript. FT: writing the first draft of the manuscript, study design, data management, data analysis. MM: data analysis, statistical advice. KR: data collection, data management. AH: data management, data collection, JUC: data management, data analysis, JC: data analysis, study design, data management. JoS: study design, data analysis. IA: study design, revision of the first draft of the manuscript. MB: study design, revision of the manuscript. FJFH: study design, data analysis, revision of the manuscript. TG: data analysis, data management, revision of the first draft of the manuscript.

Funding Parts of our work received external funding from Ms Ester Knorr (Germany, private donation) and Markedsmodningsfonden (Denmark, public grant). Internal financial support was given by Heidelberg University (MEAMEDMA, internal grant).

Competing interests The following financial activities outside the submitted work exist: FT received travel support from Actelion, Berlin Chemie, Boehringer Ingelheim, Chiesi, Novartis, Mundipharma and TEVA, as well as speaker or consultation fees from AstraZeneca, Berlin Chemie, Boehringer Ingelheim, Bristol-Myers Squibb, Chiesi, GlaxoSmithKline, Roche, Novartis and Sanofi-Aventis. JoS received travel support and speaker fees from Boehringer Ingelheim, GlaxoSmithKline, Novartis and Roche. MB received speaker or consultation fees from Bayer, Boehringer Ingelheim, Daiichi Sankyo, Impulse Dynamics and Zoll Medical. IA received travel support as well as speaker or consultation fees from Abiomed, Bayer, Boehringer Ingelheim and St Jude Medical.

Patient consent for publication Not required.

Ethics approval The study protocol was approved by the Medical Ethics Committee II of the Medical Faculty Mannheim (Heidelberg University), compliant with the Declaration of Helsinki.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement All data relevant to the study are included in the article or uploaded as supplementary information. The models generated and analysed during the current study are available for download from an online repository (https://github.com/ftrinkmann).

Supplemental material This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

Open access This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/.

ORCID iDs
Frederik Trinkmann http://orcid.org/0000-0001-5877-877X
Máté Maros http://orcid.org/0000-0002-1589-8699
REFERENCES

1 Woodruff PG, Barr RG, Bleeker E, et al. Clinical significance of symptoms in smokers with preserved pulmonary function. *N Engl J Med* 2016;374:1811–21.

2 Trinkmann F, Henzler T, Saur J. Symptoms in smokers with preserved pulmonary function. *N Engl J Med* 2016;375:895–6.

3 Kjellberg S, Houlzé B, Zetterström G, et al. Clinical characteristics of adult asthma associated with small airway dysfunction. *Respir Med* 2016;117:92–102.

4 Trinkmann F, Saur D, Roth K. Multiple breath washout (MBW) using sulfur hexafluoride – proof of concept in COPD. *Eur Respir J* 2016;48:9A3440.

5 Crisafulli E, Pisi R, Aiello M, et al. Prevalence of small-airway dysfunction among COPD patients with different gold stages and its role in the impact of disease. *Respiration* 2017;93:32–41.

6 O’Donnell DE, Revill SM, Webb KA. Dynamic hyperinflation and exercise intolerance in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;164:770–7.

7 Tzani P, Crisafulli E, Nicolini G, et al. Effects of beclomethasone/formoterol fixed combination on lung hyperinflation and dyspnea in COPD patients. *Int J Chron Obstruct Pulmon Dis* 2011;6:503–9.

8 Postma DS, Brightling C, Baldi S, et al. Lung volumes and forced ventilatory flows. *Am J Respir Crit Care Med* 2018.

9 Robinson PD, Latzin P, Verbanck S, et al. Consensus statement for inert gas washout measurement using multiple- and single-breath tests. *Eur Respir J* 2013;41:507–22.

10 Sullivan L, Forno E, Pedersen K, et al. Measurement using multiple- and single-breath tests. *Eur Respir J* 2013;41:507–22.

11 Kane M, Rayment JH, Jensen R, et al. Ventilation heterogeneity in the acinar and conductive zones of the normal ageing lung. *Thorax* 2012;67:789–95.

12 Houltz B, Green K, Lindblad A. Tidal N2 washout ventilation inhomogeneity indices in a reference population aged 7–70 years. European Respiratory Journal 2012;40:P3797.

13 Verbanck S, Paiva M. Gas mixing in the airways and airspaces. *Compr Physiol* 2011;1:809–34.

14 Dutnieue B, Vanholsbeeck F, Verbanck S, et al. A human acinar structure for simulation of realistic alveolar plateau slopes. *J Appl Physiol* 2000;89:1859–67.

15 Kim J, Heise RL, Reynolds AM, et al. Aging effects on airflow dynamics and lung function in human bronchioles. *PLoS One* 2017;12:e0183654.

16 Parraga G, Mathew L, Etemad-Rezaei R, et al. Hyperpolarized 3He magnetic resonance imaging of ventilation defects in healthy elderly volunteers: initial findings at 3.0 Tesla. *Acaad Radiol* 2008;15:776–85.

17 Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. *Eur Respir J* 2005;26:948–68.

18 Cochrane GM, Prieto F, Clark TJ. Intra-subject variability of maximal expiratory flow volume curve. *Thorax* 1977;32:171–6.

19 Trinkmann F, Gawlitza J, Künster M, et al. Small airway disease in pulmonary Hypertension-Additional diagnostic value of multiple breath washout and impulse oscillometry. *J Clin Med* 2018;7:30901017. [Epub ahead of print: 25 Feb 2018].

20 Clemensen P, Christensen P, Norsø P, et al. A modified photo- and magnetooptic multisig anaalyzer applied in gas exchange measurements. *J Appl Physiol* 1994;76:2832–9.

21 Lenherr N, Ramsey KA, Jost E, et al. Leaks during multiple-breath washout: characterisation and influence on outcomes. *ERI Open Res* 2018;4. doi:10.1183/23120541.00011-2017. [Epub ahead of print: 23 Feb 2018].

22 Nielsen JC. Lung clearance index: should we really go back to nitrogen washout? *Eur Respir J* 2014;43:655–6.

23 Lenherr N, Ramsey KA, Jost K, et al. Inert gas washout ventilation inhomogeneity on indices derived from multiple breath nitrogen washout. *PLoS One* 2013;8:e56868.

24 Nielsen N, Nielsen JG, Horsley AR, et al. Evaluation of the impact of alveolar nitrogen concentration on indices derived from multiple breath nitrogen washout. *PLoS One* 2013;8:e73335.

25 Gugliani L, Kasi A, Starks M. Difference between SF6 and N2 multiple breath washout kinetics is due to N2 back diffusion and error in N2 offset. *J Appl Physiol* 2018. doi:10.1152/japplphysiol.00326.2018

26 Quanjer PH, Tammeling GJ, Cotes JE, et al. Lung volumes and forced ventilatory flows. *Report Working Party standardization of lung function tests, European community for steel and coal. official statement of the European respiratory society. Eur Respir J Suppl* 1999;165–60.

27 Vogel J, Smidt U, Oscillometry I. Analysis of lung mechanics in general practice and clinic, epidemiological and experimental research. Frankfurt: PMI-Verlagsguppe, 1994.

28 Trinkmann F, Götzmann J, Saur D, et al. Multiple breath washout testing in adults with pulmonary disease and healthy controls - can fewer measurements eventually be more? *BMC Pulm Med* 2017;17:185.

29 R Core Team. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. 2017. https://www.R-project.org/