Nationwide lung function monitoring from infancy in newborn-screened children with cystic fibrosis

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Shareable abstract (@ERSpublications)
This study shows that surveillance multiple breath washout testing in infants and toddlers with cystic fibrosis demonstrated lung function impairment, and revealed bacteriological risk factors and potential beneficial effects of therapeutic interventions https://bit.ly/3qiGBRf

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Abstract

Background Cystic fibrosis (CF) lung disease starts in infancy and can be assessed for structural lung abnormalities using computed tomography or magnetic resonance scans, or for lung function impairment using multiple breath washout (MBW). However, in infancy these two methods are not well correlated. Trajectories of CF lung disease assessed by MBW in infants and toddlers remain poorly described, which is why we aimed to 1) describe the trajectory of lung function, 2) explore risk factors for progression and 3) explore the real-life effect of lumacaftor/ivacaftor.

Methods This was a nationwide observational cohort study (2018–2021) using data collected as part of the routine clinical surveillance programme (including MBW and monthly endo-laryngeal suction sampling for bacterial pathogens) in children born after implementation of newborn screening for CF (May 2016). Lumacaftor/ivacaftor commenced from age 2 years in children homozygous for F508del. Ventilation distribution efficiency (VDE), recently described to have advantages over lung clearance index (LCI), was reported as the primary MBW outcome after z-score calculations based on published reference data. Mixed effect linear regression models were the main statistical analyses performed in this study.

Results 59 children, aged 2–45 months, contributed with 211 MBW occasions (median (interquartile range (IQR)) 3 (2–5) MBW occasions per child) with a median (IQR) follow-up time of 10.8 (5.2–22.3) months. An overall mean annual deterioration rate of −0.50 (95% CI −0.78– −0.22) z-VDE was observed, starting from an estimated mean z-VDE of −1.68 (95% CI −2.15– −1.22) at age 0.0 years (intercept). *Pseudomonas aeruginosa* "ever" (n=14, MBWs 50) had a significantly worse z-VDE trajectory *versus P. aeruginosa* "never" (mean difference 0.53 (95% CI 0.16–0.89) per year; p=0.0047) and lumacaftor/ivacaftor treatment (n=22, MBWs 46) significantly improved the trajectory of z-VDE (mean difference 1.72 (95% CI 0.79–2.66) per year; p=0.0004), leading to a stable mean z-VDE trajectory after start of treatment.

Conclusions Infants and toddlers with CF demonstrated progressive deterioration in z-VDE over the first years of life. *P. aeruginosa* isolation "ever" was associated with an accelerated deterioration in lung function, while lumacaftor/ivacaftor therapy significantly improved and stabilised the trajectory.

Introduction

Cystic fibrosis (CF) lung disease is progressive and starts early in life [1–4]. Diagnosis using newborn screening for CF (NBS-CF) [5] is routine in several countries and was implemented in Denmark in 2016 [6]. Early detection and monitoring of lung impairment are critical to enabling effective treatment for preventing or slowing progression [7]. Inert gas multiple breath washout (MBW), a well-recognised sensitive method for detecting and monitoring CF lung disease [7–11], is applicable from infancy [12–14], yet not widely used in clinical routine in CF care to date [15]. The availability of validated commercial sulfur hexafluoride (SF_6)-based MBW (SF_6MBW) equipment and methodology [16] led to its implementation as a routine surveillance tool starting in 2018 for all Danish infants and toddlers with CF (age range approximately 3 months to 4 years). Since 2019, CF transmembrane conductance regulator (CFTR) modulator therapy (lumacaftor/ivacaftor) has been routinely administered from age 2 years to those homozygous for F508del [17], creating a unique opportunity to investigate the real-life effect on the trajectory of CF lung disease within this age group.

We recently published modern SF_6MBW device-specific reference values covering the whole paediatric age range, including infancy [18]. In that publication, we also outlined the benefits of a new MBW index, ventilation distribution efficiency (VDE), in comparison to the lung clearance index (LCI) [18]. In contrast to LCI, VDE was linearly correlated to overall respiratory dead space ventilation, resulting in more consistent changes in VDE over the entire range of ventilation distribution inhomogeneity (disease severity) [18], which is advantageous when studying changes in ventilation inhomogeneity over time and any treatment intervention effects. VDE is calculated as 1/LCI and expressed as a percentage, where a lower value represents a worsening of ventilation inhomogeneity [18].

The use of MBW in the routine clinical surveillance programme, together with the published theoretical and physiological benefits of VDE, including an age-specific reference equation, created a unique opportunity to not only investigate 1) the benefit of using z-VDE as an outcome parameter in monitoring CF lung disease and treatment effects, 2) the trajectory of disease progression over the first years of life in children with CF, and 3) associations to risk factors (*e.g.* symptoms and pathogens), but also 4) the real-life effect of lumacaftor/ivacaftor on the trajectory of CF lung disease over the first year of CFTR modulator therapy.

Methods

Design and patients

This was a prospective, longitudinal cohort study. The two existing paediatric CF centres in Denmark (Aarhus and Copenhagen) follow all children with CF. As such, the entire national cohort of newborn-screened children with CF was eligible for inclusion in this study. Patients with CF-SPID (CF screen positive, inconclusive diagnosis) and patients with severe comorbidity or malformations preventing standard treatment and routine monitoring were not eligible. Patients with at least one technically acceptable SF_6MBW occasion performed sleeping in the supine position, available from MBW implementation (January 2018) until the end of data collection (October 2021), were included in the study.

A detailed description of the methodological and procedural aspects of the current study, including information on MBW recordings and quality control, sedation procedures, standard CF treatment, and monitoring of bacterial cultures in airway secretion, is provided in the supplementary material.

MBW recordings and quality control

SF_6MBW is part of the standard monitoring programme for patients with CF from diagnosis until the child does not have daytime naps anymore (around 3 years of age) in both Danish CF centres, aiming for quarterly testing during clinical stability. All patients underwent clinical assessment on the day of testing. MBW testing was postponed if the physician found evidence of respiratory exacerbation, defined per modified Fuchs criteria (any new or increased pulmonary symptoms, fever, or severe rhinitis) [19]. SF_6MBW was performed in the supine position during quiet sleep, natural or induced by sedation with either dexmedetomidine (Copenhagen) or chloral hydrate (Aarhus), using the EXHALYZER D MBW equipment (infant set-up, SPIROWARE 3.2.1; ECO MEDICS, Duernten, Switzerland) as described in previous publications [14, 16, 18, 20, 21].

Airway pathogens

As part of the monthly routine visits at the CF centres each patient underwent an endo-laryngeal suction sampling, cultured for bacteria and fungi (*Aspergillus*). Bacterial pathogens of interest were *Pseudomonas aeruginosa*, *Burkholderia* spp. and *Achromobacter* spp., as the most severe pathogens, along with *Staphylococcus aureus*, *Haemophilus influenzae* and *Moraxella catarrhalis*. Mixed oral flora (MOF) was considered a sample without bacterial growth.

Statistical analyses

Parametric and non-parametric data are presented as mean with standard deviation and median (range and/or interquartile range (IQR)), respectively. We assumed that CF lung disease in patients from the two sites was similar in severity as they all followed the same surveillance programme. However, due to a considerable difference in the number of MBW occasions at the two sites, all analyses were performed for each site independently and for the total cohort to ensure no between-centre differences. A mixed effects linear regression model [22] with random intercept and random slope for each subject, together with an unstructured covariance structure, was used to estimate the annual change in z-VDE and the effect of pathogens and lumacaftor/ivacaftor on the trajectories for z-VDE. This method adjusts for different numbers of test occasions per child, improving the total useful sample size, as all data can be applied despite some children only having one test occasion. The resulting intercepts from the analyses are described as the estimated mean (95% confidence interval) value at age 0.0 years.

The within-subject variability between consecutive test occasions was calculated as the value of one test occasion minus the value from the previous test occasion in absolute values and percentage change. The median (range) values for changes, the 5th and 95th percentiles, and the 10th and 90th percentiles are given. Also, the coefficient of variation (CV%) is provided.

The covariance parameter estimate for the residuals in the different mixed model analyses was 1.22 for z-VDE and 0.96 for VDE (estimate of variability: $\sqrt{1.22}=1.10$ z-VDE and $\sqrt{0.96}=0.98$ VDE, respectively).

z-VDE was calculated using the reference equations described in a publication from our group [18]. The lower limit of normal (LLN) was set to −1.65 z-scores [23, 24], with an arbitrary threshold of −3.0 z-VDE to define severe abnormality.

All data are additionally presented using z-scores of the conventionally reported MBW outcome LCI (z-LCI; reference equation provided in the supplementary material) for comparisons to the z-VDE data. However, as changes in LCI (and the residual standard deviation) increased with the value of LCI, log transformation of LCI was necessary for optimal statistical use of data; the use of LCI and z-LCI was not optimal. We therefore also presented data as z-scores of the log-transformed LCI (z-$\log_{10}$(LCI); calculated using the published reference equation [18]).

p-values <0.05 were considered statistically significant. SAS Enterprise Guide 7.1 (SAS Institute, Cary, NC, USA) and Excel 2016 (Microsoft, Redmond, WA, USA) were used for statistical analysis and data display.

Ethics

The study was approved by the Danish Data Protection Agency (VD-2018-471, P-2020-1032) and the Capital Region of Denmark, Rigshospitalet. All caregivers provided written informed consent allowing clinical data from the MBW device and the medical reports to be collected and used in this study.

Results

MBW testing

Technically acceptable MBW data were obtained from 211 occasions in 59 children across the two centres from January 2018 to October 2021 (figure 1). The yearly number of Danish children diagnosed with CF (including those in the Faroe Islands and Greenland) is approximately 14 (in total, 77 children were diagnosed with CF from May 2016 to October 2021). 82% of the eligible cohort provided at least one MBW test occasion. Given the original aim of quarterly MBW testing, the surveillance programme captured 50% (263 out of 528) of all intended test occasions over the study period (for exclusion of tests and MBW quality, see figure 1 and supplementary material). The overall success rate of technically acceptable MBW occasions was 80% of performed test occasions: for CF Centre Aarhus it was 74% (improving from 35% to 98% after further MBW training in 2020) and for CF Centre Copenhagen it was 84% (improving from 0% to 97% after changing to the Rüsch silicone face masks in June 2018) (figure 1). The demographic data of the study patients and the MBW occasions are summarised in tables 1 and 2, respectively. 21 (84%) of the Copenhagen patients had at least three MBW occasions each, while the remaining four patients (all age <9 months when data collection was ended) had two MBW occasions. From CF Centre Aarhus, 11 (32%) patients had at least three MBW occasions each, 10 (29%) had two and 13 (38%) had only one technically acceptable MBW occasion (figure 2).

Longitudinal assessment

The mean VDE of children with CF at age 1.0 year was 12.7% (95% CI 12.3–13.2%), which was significantly lower (*i.e.* worse) (mean VDE −2.0% (95% CI −2.3– −1. 8%); p<0.0001) than the healthy

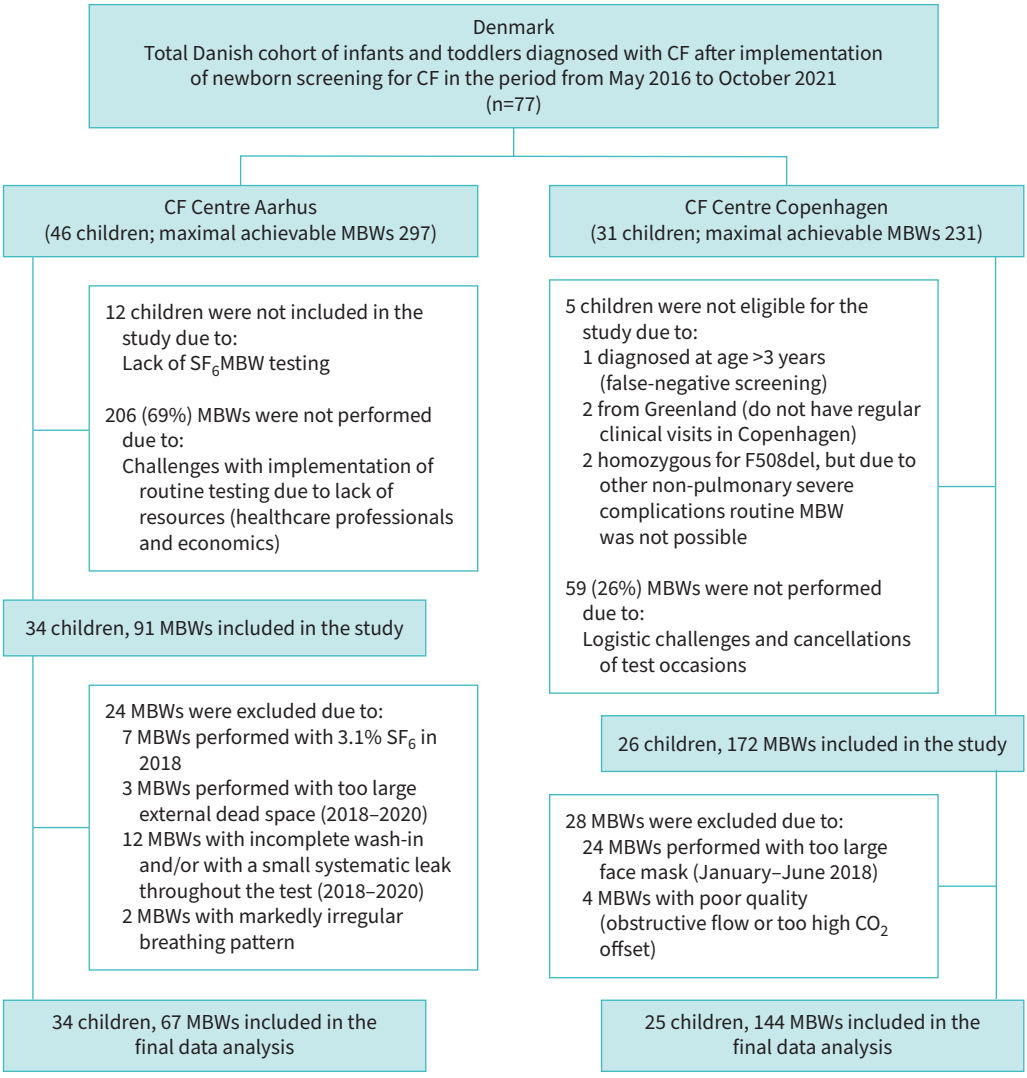

FIGURE 1 Flow diagram for children and multiple breath washouts (MBWs) in the study. Children were allocated to one of the two Danish cystic fibrosis (CF) centres depending on their home address. The number of maximal achievable MBW test occasions was calculated as four MBWs yearly from age 3 months until age 3 years or the end of the data collection period (October 2021), whichever came first. SF_6: sulfur hexafluoride; CO_2: carbon dioxide.

reference population from our previous publication (mean 14.8% (95% CI 14.6–14.9%)) [18]. In children with CF, the mean VDE trajectory was stable over time (0.6% (95% CI −0.0–1.3%) $\log_{10}(\text{age})^{-1}$; p=0.0653), in contrast to the trajectory found in healthy children, which increased (*i.e.* improved) significantly with time (mean 2.0% (95% CI 1.8–2.2%) $\log_{10}(\text{age})^{-1}$; p<0.0001) (figure 3a and b) [18].

The healthy population was composed of 327 children, age range 0.14–18.39 years (median 1.38 years), 46% females, with a mean±SD z-weight of 0.25±0.85 and z-height of 0.12±1.12.

Disease progression

The overall estimated mean z-VDE at age 0.0 years (intercept of the analysis) was −1.68 (95% CI −2.15– −1.22) for the entire CF cohort, changing significantly by −0.50 (95% CI −0.78– −0.22) per year (p=0.0007) (figure 4a).

Figure 3 indicates that a greater proportion of patients had VDE below the LLN with increasing age and visually there was increased between-subject variation (*i.e.* some children develop more lung disease than

TABLE 1 Patient characteristics

	Copenhagen	Aarhus	Danish CF cohort
Study subjects (n) (MBW occasions (n))	25 (144)	34 (67)	59 (211)
Male	13 (52)	18 (53)	31 (52.5)
Age at first visit (years)	0.63 (0.25–2.06)	1.60 (0.25–2.48)	1.22 (0.24–2.48)
z-score for height (all MBW visits)	−0.15±0.96	−0.30±1.03	−0.20±0.98
z-score for weight (all MBW visits)	−0.34±1.05	−0.38±1.10	−0.35±1.07
z-score for BMI (all MBW visits)	−0.32±1.28	−0.27±1.16	−0.30±1.24
Diagnosed by other[#] than NBS-CF	6 (25)	4 (12)	10 (17)
Homozygote F508del	16 (64)	27 (79)	43 (73)
Heterozygote (F508del+other)	7 (28)	7 (21)	14 (24)
Heterozygote (other/other)	2[¶] (8)	0 (0)	2 (3.4)
Pancreas insufficiency	24 (96)	33 (97)	57 (97)
Patients on lumacaftor/ivacaftor[+]	12 (48)	10 (29)	22 (37)
Patients with *Pseudomonas aeruginosa* "ever"	6 (24)	7 (21)	13 (22)
Age at first *P. aeruginosa* infection (years)	1.97 (1.17–4.26)	1.37 (0.16–1.56)	1.47 (0.16–4.26)
Patients with *Burkholderia cenocepacia* "ever"	1 (4)	0	1 (1.7)

Data are presented as mean±SD, n (%) or median (minimum–maximum), unless otherwise stated. CF: cystic fibrosis; MBW: multiple breath washout; BMI: body mass index; NBS: newborn screening. *Aspergillus* spp., *Achromobacter xylosoxidans*, *Mycobacterium avium* or *Mycobacterium abscessus* were never isolated in any of the patients. [#]: diagnosed by meconium ileus (n=7), meconium plugs (n=2), clinical symptoms at 8 months old (false-negative NBS-CF) (n=1). [¶]: in Denmark (including patients from the Faroe Islands) the percentage of patients with CF homozygous for F508del is very high. In the Copenhagen area the percentage of homozygous is slightly less, due to a larger proportion of subjects with a mixed genetic background living in a large capital city. [+]: number of patients with MBW performed one or more times after start of lumacaftor/ivacaftor treatment (started from age 2 years).

others with age). Approximately 50% of measurements performed from the first 6 months of life had normal z-VDE, while this number dropped to 27% in the age group tested at age 18–24 months and further to 24% in measurements performed in children >24 months of age. The percentage of measurements with severe abnormality increased with age, from 12% in those <6 months of age to 39% among those tested at age 18–24 months, whereafter a small decrease to 37% was found in those >24 months of age (after initiation of lumacaftor/ivacaftor therapy in 22 of the children) (supplementary table E2 and supplementary figure E3).

TABLE 2 Multiple breath washout (MBW) characteristics

	Copenhagen	Aarhus	All CF
MBW occasions per child	6 (2–11, 4–8)	2 (1–4, 1–3)	3 (1–11, 2–5)
Triplicate runs	126 (88)	55 (82)	183 (87)
CV%[#] VDE	2.7±1.57	2.4±1.35	2.6±1.51
CV%[#] LCI	2.7±1.57	2.4±1.35	2.6±1.51
CV%[#] FRC	2.8±1.95	2.2±1.86	2.6±1.94
Sedation[¶]	122 (85)	67 (100)	189 (90)
Time between MBWs (months)	3.5 (2.5–10.5, 3.0–4.4)	3.3 (1.6–11.7, 3.1–7.2)	3.5 (1.6–11.7, 3.0–4.6)
Duration of monitoring each child (months)	19 (2–32, 11–25)	4 (0–14, 0–10)	10 (0–32, 2–16)
MBWs on lumacaftor/ivacaftor[+]	31 (21.5)	15 (22.3)	46 (21.8)
Duration of monitoring after start of lumacaftor/ivacaftor (months)	10 (4–16, 7–11)	6 (1–12, 1–7)	7 (1–16, 5–11)
Minimal symptoms at time of MBW[§]	45 (31)	20 (30)	65 (31)

Data are presented as median (minimum–maximum, interquartile range), n (%) or mean±SD. CF: cystic fibrosis; CV%: coefficient of variation; VDE: ventilation distribution efficiency; LCI: lung clearance index; FRC: functional residual capacity. [#]: CV% calculated for each MBW occasion (two to three runs). [¶]: two different procedures for sedation were used. In Copenhagen, dexmedetomidine (Dexdor) 2 mg·kg^{-1} body weight was administered as a nasal puff (MAD Nasal Intranasal Mucosal Atomization Device; Teleflex, Morrisville, NC, USA). CF Centre Aarhus used chloral hydrate 100 mg·kg^{-1} body weight as a rectal administration. [+]: lumacaftor/ivacaftor is available for children homozygous for F508del from age 2 years. [§]: MBW performed despite minimal rhinitis or few coughs starting the same day as MBW, without any other symptoms of respiratory tract infection. All other MBWs were performed at times of no symptoms of respiratory tract infection.

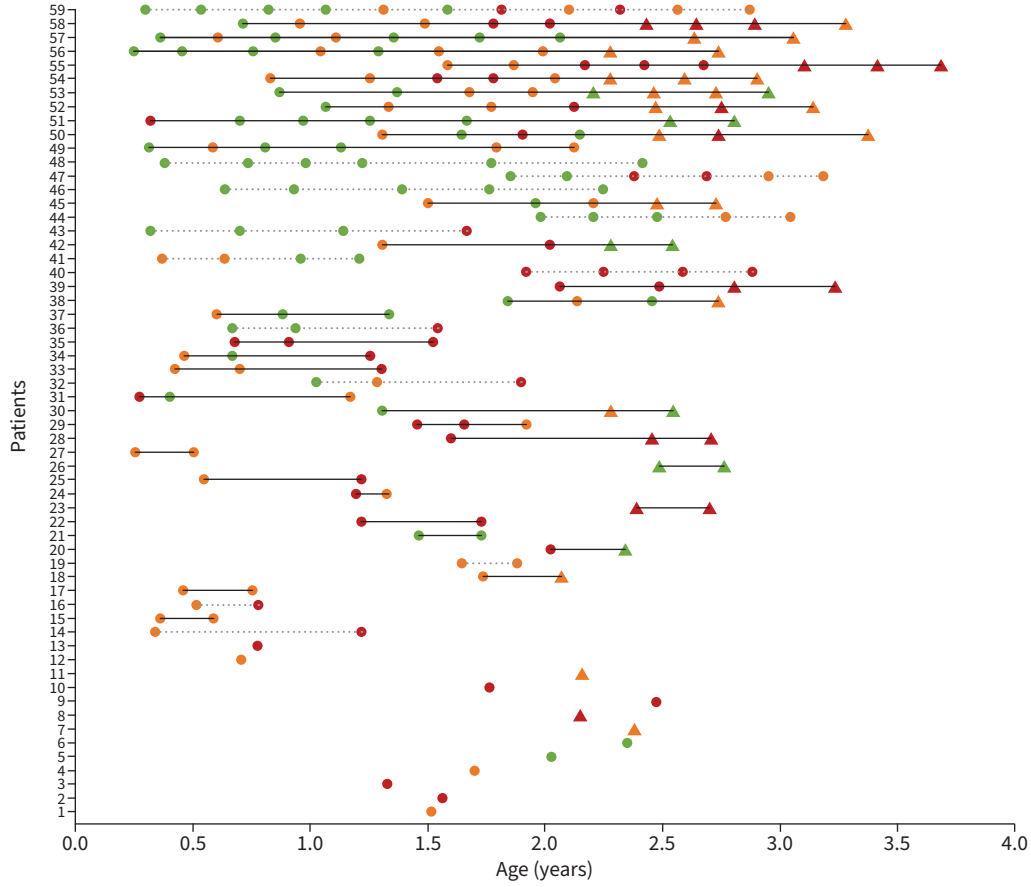

FIGURE 2 Each of the 59 patient's multiple breath washout (MBW) occasions are presented separately within the horizontal lines, according to the patient's age when they were performed. Patients are arranged with increasing numbers of test occasions from bottom to top. Patients 1–13, 18–27, 29–37 and 42 are from Arhus; patients 14–17, 28, 38–41 and 43–59 are from Copenhagen. VDE: ventilation distribution efficiency; SF_6: sulfur hexafluoride. Green: z-VDE > −1.65 (normal); orange: z-VDE −1.65– −2.9 (mildly abnormal); red: z-VDE < −3 (severely abnormal). Circles: SF_6MBW performed without the patient receiving treatment with lumacaftor/ivacaftor; triangles: SF_6MBW performed after start of treatment with lumacaftor/ivacaftor. Black lines between measurements: patients homozygous for F508del; grey dotted lines between measurements: patients heterozygous.

Variability of ventilation inhomogeneity

Out of the 211 MBW occasions in this dataset, a within-subject change between two consecutive MBW outcomes could be calculated 147 times. After excluding all MBW occasions performed at times of minimal clinical symptoms, only 90 within-subject between-test differences could be calculated. Data of variability are presented in table 3.

In 90% of consecutive MBW occasions, the difference in VDE between the two MBW occasions (5–95th percentile) varied between −16% (or −2.2 percentage points of VDE) and 25% (or 2.8 percentage points of VDE), and in 80% of the consecutive test occasions (10–90th percentile) resulted in a difference between −11% (or −1.5 percentage points of VDE) and 17% (or 1.8 percentage points of VDE).

The change in VDE was correlated to the value of VDE, such that those with the best VDE values (high value) had the largest worsening in VDE and those with low VDE (more ventilation inhomogeneity) had the largest improvements between test occasions, a phenomenon of regression towards the mean.

In total, 18 (12%) consecutive MBW test occasions resulted in a worsening of more than −10.0% (approximately 1.4 percentage points of VDE); however, this number was reduced to seven (8%) tests, after excluding tests performed at times with minimal respiratory tract symptoms. The CV% for VDE in this dataset was 5.21%, and 4.26% when excluding tests without any symptoms. For comparison, the same

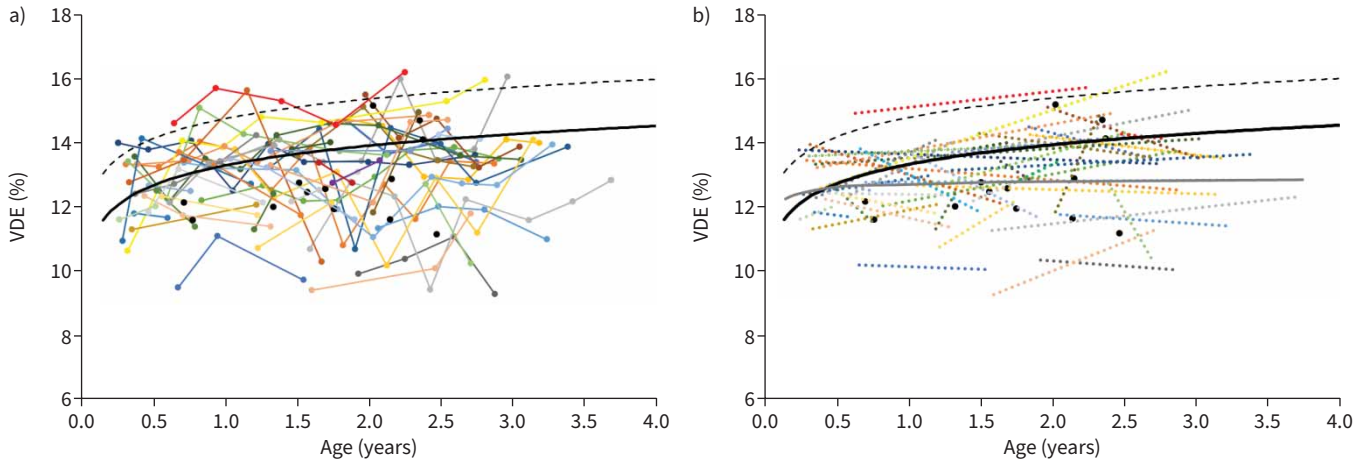

FIGURE 3 a) Line plot of ventilation distribution efficiency (VDE) *versus* age from 211 sulfur hexafluoride-based multiple breath washout (SF_6MBW) test occasions performed in 59 Danish children with cystic fibrosis (CF) (each colour represents a separate child, black dots represent the children with only one MBW occasion each). The bold black line represents the lower limit of normal (LLN; predicted mean −1.65SD), while the dashed black line above represents the predicted mean of VDE. b) Trendline plot of VDE *versus* age from 211 SF_6MBW test occasions performed in 59 Danish children with CF (each colour represents a separate child, black dots represent the children with only one MBW occasion each). The bold black line represents the LLN (predicted mean −1.65SD), while the dashed black line above represents the predicted mean of VDE. The grey solid line represents the mean VDE for the study group in total.

data for LCI is also provided in table 3. When examining the variability between test occasions using VDE and LCI, the numbers were comparable. Only the upper and lower percentiles for LCI and VDE showed slightly smaller variability for VDE.

Airway pathogens

The mean±SD number of endo-laryngeal suctions for each patient was 9.6±1.9 per year, resulting in 1512 airway secretion samples, with zero to three different bacteria isolated in each. The median (IQR) number of bacteria isolated per airway secretion sample was 0.92 (0.11–1.75). The percentage of suctions without any bacterial isolation (including MOF) for each patient ranged from 0% to 78%, with a median (IQR) of 33% (20–48%). All analyses assessing the effect of positive bacterial cultures other than *P. aeruginosa* on lung function trajectories are shown in supplementary table E3 (none of them significant). *P. aeruginosa* was isolated at least once (*P. aeruginosa* "ever") in 13 patients (22%); none of them was considered chronically infected. *B. cenocepacia* was isolated in one patient (1.7%). Children in whom *P. aeruginosa* was ever isolated had a mean z-VDE of −3.43 (95% CI −4.12– −2.73) and children in whom *P. aeruginosa* was never isolated (*P. aeruginosa* "never") had a mean z-VDE of −2.23 (95% CI −3.70– −0.76), a significant mean difference in z-VDE of 1.20 (95% CI 0.42–1.97; p=0.0027). Children in whom *P. aeruginosa* was ever isolated had a significant decline in z-VDE (mean −0.80 (95% CI −1.15– −0.46) per year; p<0.0001), whereas children in whom *P. aeruginosa* was never isolated showed stable z-VDE (mean −0.27 (95% CI −0.55–0.01) per year; p=0.0571). There was a significant difference in the trajectory of z-VDE between the two groups (mean 0.53 (95% CI 0.17–0.89) per year; p=0.0047) (figure 4b).

Treatment effect of CFTR modulator therapy

22 out of the 32 patients (69%) reaching age 2 years during the data collection period were homozygous for F508del and started lumacaftor/ivacaftor therapy at a mean±SD age of 2.18±0.21 years. In total, 46 MBW occasions (median (range) 2 (1–4) per child) were recorded following treatment start (supplementary table E4). Dividing the dataset into those without lumacaftor/ivacaftor therapy (59 children, 166 MBW occasions) and with lumacaftor/ivacaftor (22 children, 46 MBW occasions), we found a significant mean annual difference in z-VDE trajectories between the two groups (1.72 (95% CI 0.79–2.66) per year; p=0.0004). The mean trajectories of z-VDE for the two groups were −0.87 (95% CI −1.19– −0.55) per year and 0.85 (95% CI −0.40–2.10) per year, respectively (figure 4c).

When only including MBWs performed in the 32 patients (71 MBW occasions) after 2 years of age, a significant mean difference (1.29 (95% CI 0.005–2.58) per year; p=0.0492) was also found between the

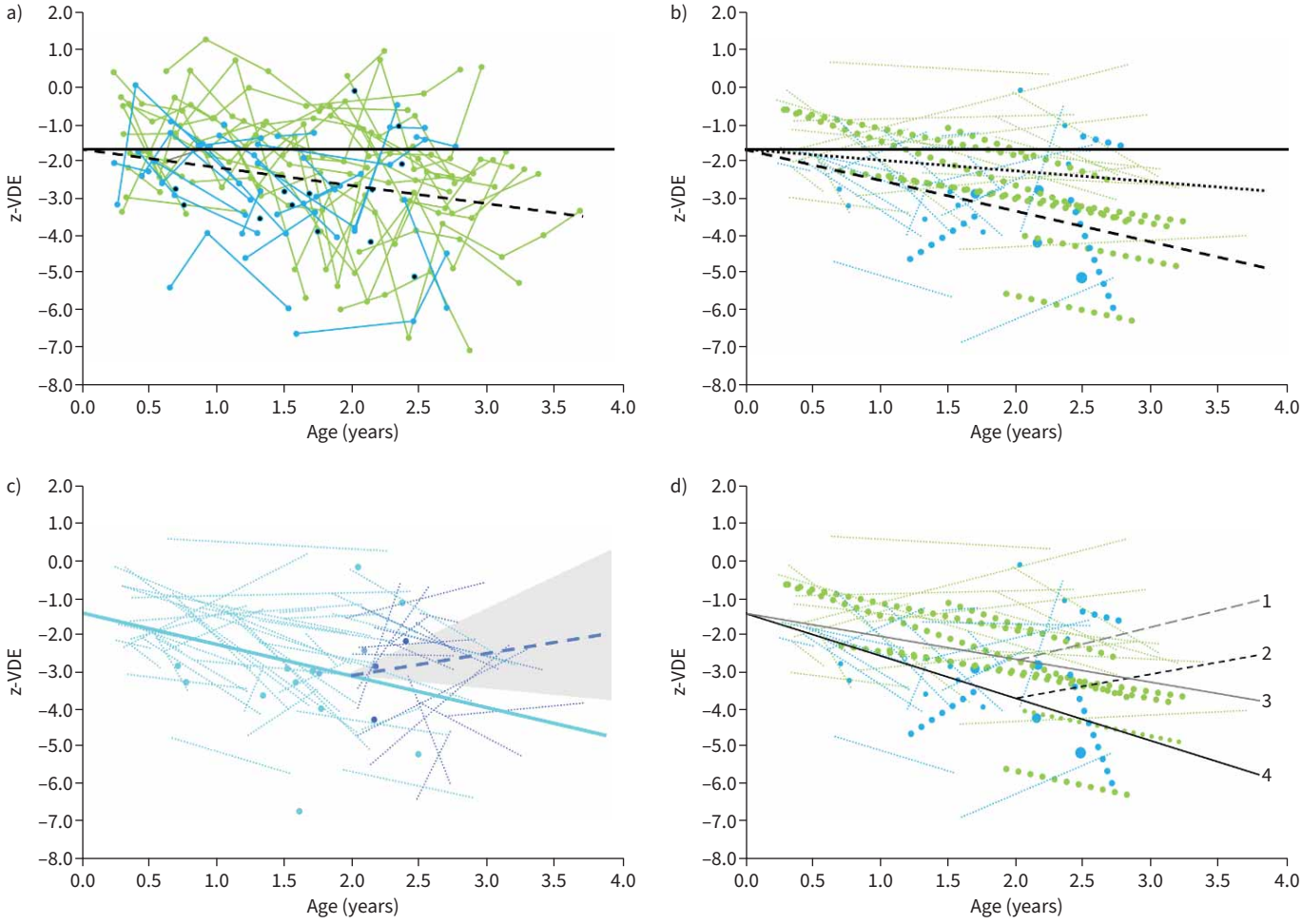

FIGURE 4 a) Line plot of individual and mean trends of z-scores of ventilation distribution efficiency (z-VDE) *versus* age from 211 sulfur hexafluoride (SF_6)-based multiple breath washout (SF_6MBW) test occasions performed in 59 Danish children with cystic fibrosis (CF) (green: data from Copenhagen; blue: data from Aarhus). The dashed black line represents the average z-VDE of the entire CF study cohort, while the solid black line represents the lower limit of normal (LLN; −1.65 z-VDE). b) Trendline plot of individual and mean trends of z-VDE *versus* age from 211 SF_6MBW test occasions performed in 59 Danish children with CF (green: data from Copenhagen; blue: data from Aarhus). Bold trendlines and dots represent data from children ever colonised with *Pseudomonas aeruginosa.* The dashed black line represents the average z-VDE trend of children ever colonised with *P. aeruginosa* and the dotted black line represent children never colonised with *P. aeruginosa*. The solid black line represents the LLN (−1.65 z-VDE). c) Trendline plot of individual and mean trends of z-VDE *versus* age from 211 SF_6MBW test occasions performed in 59 Danish children with CF. Bright blue trendlines represent children before or without lumacaftor/ivacaftor treatment and the bold bright blue line represents their mean annual decrease in z-VDE. Dark blue trendlines represent MBW data from 22 (73%) children above 2 years old, after start of lumacaftor/ivacaftor treatment. The dashed dark blue line represents the mean change in z-VDE after start of lumacaftor/ivacaftor treatment with the light grey area showing the 95% confidence interval. d) Trendline plot of individual and mean trends of z-VDE *versus* age from 211 SF_6MBW test occasions performed in 59 Danish children with CF (green: data from Copenhagen; blue: data from Aarhus). Thin trendlines represent MBW occasions from children never colonised with *P. aeruginosa* and bold trendlines represent those ever colonised with *P. aeruginosa*. 1) Mean z-VDE for *P. aeruginosa* "never" plus lumacaftor/ivacaftor treatment. 2) Mean z-VDE for *P. aeruginosa* "ever" plus lumacaftor/ivacaftor treatment. 3) Mean z-VDE for *P. aeruginosa* "never" without lumacaftor/ivacaftor treatment. 4) Mean z-VDE for *P. aeruginosa* "ever" without lumacaftor/ivacaftor treatment.

annual trajectories of z-VDE for those with lumacaftor/ivacaftor (22 children, 46 MBW occasions) and those without lumacaftor/ivacaftor (10 children, 25 MBW occasions) treatment. Both groups had a statistically stable mean z-VDE (0.60 (95% CI −1.68–2.87) per year; p=0.6177 and −0.69 (95% CI −1.68–0.30) per year; p=0.1612, respectively), despite the statistically significant difference between the groups. The same interpretation of results was found if only including children homozygous for F508del before and after the start of lumacaftor/ivacaftor treatment (table 4).

TABLE 3 Variability of ventilation distribution efficiency (VDE) and lung clearance index (LCI)

	All CF (n=46)	All CF with no respiratory symptoms (n=35)
VDE		
Change between occasions (VDE)	−0.05 (−5.4–3.8, −0.6–0.8)	0.06 (−3.1–3.2, −0.5–0.9)
% change between occasions (VDE)	−0.4 (−34–35, −5–7)	0.4 (−22–30, −4–7)
Change between occasions (z-VDE)	−0.22 (−6.4–4.1, −1.0–0.7)	−0.22 (−3.6–3.4, −0.8–0.8)
CV% (VDE)	5.2 (0.5–14.7, 3.4–7.1)	4.3 (0.4–11.7, 2.9–5.5)
10–90th percentile (%diff VDE)	−11–17	−9–16
5–95th percentile (%diff VDE)	−16–25	−11–20
LCI		
Change between occasions (LCI)	0.03 (−2.5–3.3, −0.5–0.4)	−0.03 (−2.4–2.0, −0.6–0.3)
% change between occasions (LCI)	0.4 (−26–52, −6–5)	−0.4 (−23–28, −7–4)
Change between occasions (z-LCI)	0.22 (−6.4–9.2, −1.1–1.2)	0.16 (−6.4–5.6, −1.1–1.0)
CV% (LCI)	5.3 (0.5–15.3, 3.4–7.0)	4.2 (0.4–11.7, 2.9–5.5)
10–90th percentile (%diff LCI)	−14–12	−14–10
5–95th percentile (%diff LCI)	−20–19	−17–12

Data are presented as median (minimum–maximum, interquartile range) or percentiles. CV%: coefficient of variation within-patient between-MBW occasions; %diff: percentage change between consecutive occasions. Outcome presented for all CF (46 children with CF who performed two or more MBW occasions during the study period; in total 147 differences between 193 MBW occasions) and all CF with no respiratory symptoms (35 children with CF who performed two or more MBW occasions without any respiratory symptoms; in total 90 differences between 125 MBW occasions). For reference equations, see [18] and supplementary material.

CFTR modulator therapy and P. aeruginosa *isolation status*

When including lumacaftor/ivacaftor therapy and *P. aeruginosa* "ever"/"never" status in the multivariate mixed model analysis, significant effects of lumacaftor/ivacaftor treatment were demonstrated on z-VDE trajectories both among children in whom *P. aeruginosa* was ever or never isolated (mean difference 1.94 (95% CI 0.47–3.42) per year; p=0.0102 and 1.50 (0.43–2.57) per year; p=0.0067, respectively) (figure 4d: 2+1). Among patients not receiving lumacaftor/ivacaftor treatment (n=54), decreasing z-VDE (mean −1.19 (95% CI −1.59– −0.79) per year; p<0.0001) was found in the subset in whom *P. aeruginosa* was ever isolated (n=9) (figure 4d: 4), whereas a stable z-VDE (mean −0.62 (95% CI −1.43–0.20) per year; p=0.1362) was found in the subset in whom *P. aeruginosa* was never isolated (figure 4d: 3). After start of lumacaftor/ivacaftor treatment, both the *P. aeruginosa* "ever" group (n=8) and the *P. aeruginosa* "never" group (n=14) had stable mean z-VDE trajectories (0.68 (95% CI −1.17–2.53) per year; p=0.4807 and 0.89 (95% CI −1.00–2.78) per year; p=0.3620, respectively).

Table 4 summarises the results of the mixed model analyses for z-VDE, z-$\log_{10}$(LCI) and z-LCI, performed for the two sites separately and for the total CF cohort and only those with no respiratory symptoms. The results revealed similar patterns for z-LCI and z-$\log_{10}$(LCI) as observed for z-VDE. The effects caused by age, *P. aeruginosa* and CFTR modulator treatment, however, were larger in absolute values, and with wider 95% confidence intervals when using z-$\log_{10}$(LCI) and especially z-LCI, compared with z-VDE.

Discussion

Summary of results

Several important results emerged in this study of longitudinal MBW surveillance testing in infants and toddlers with CF diagnosed after the implementation of NBS-CF. First, lung function deteriorated with age in the CF cohort, as demonstrated by decreasing VDE z-scores despite the normal clinical and nutritional status. Second, a significant detrimental influence on z-VDE was observed from positive culture(s) of *P. aeruginosa*. Finally, CFTR modulator therapy (lumacaftor/ivacaftor) was associated with a significant beneficial effect on lung function trajectory, both in children with or without evidence of *P. aeruginosa* colonisation. The SF_6MBW findings appeared clinically relevant and supported the consideration of infant MBW testing as part of routine clinical monitoring at CF centres.

VDE compared with LCI

By comparing the z-score results of VDE with those of LCI and $\log_{10}$(LCI) (table 4), we have shown that the magnitudes of changes in z-VDE were smaller than those found using z-LCI or z-$\log_{10}$(LCI), as a result of LCI overestimating disease severity at higher LCI values, and thereby also overestimating the changes in disease severity over time or after treatment [18]. We also showed that z-VDE resulted in narrower 95% confidence intervals and more significant p-values (table 4). The improvements reported in

TABLE 4 Results of the mixed model analyses

	Copenhagen	Aarhus	All CF	All CF (no respiratory symptoms)
z-VDE				
Annual trajectory	−0.49 (−0.84– −0.15) p=0.0071	−0.36 (−0.91–0.20) p=0.19	−0.50 (−0.78– −0.22) p=0.0007#	−0.42 (−0.73– −0.11) p=0.0087
P. aeruginosa "ever" *versus* "never"¶	−0.70 (−1.14– −0.25) p=0.0026#	−0.33 (−1.00–0.34) p=0.2964	−0.53 (−0.89– −0.17) p=0.0047	−0.53 (−0.89– −0.17) p=0.0046
Lumacaftor/ivacaftor (all)¶	1.68 (0.64–2.72) p=0.0018#	2.59 (0.06–5.13) p=0.0460	1.72 (0.79–2.66) p=0.0004#	1.71 (0.52–2.90) p=0.0056
Lumacaftor/ivacaftor (F/F)¶	1.54 (0.39–2.69) p=0.0094	2.53 (−0.17–5.23) p=0.0632	1.49 (0.47–2.52) p=0.0049	1.54 (0.27–2.82) p=0.0193
z-$\log_{10}$(LCI)				
Annual trajectory	0.48 (0.07–0.89) p=0.0250	0.25 (−0.44–0.94) p=0.4549	0.47 (0.13–0.80) p=0.0070	0.36 (−0.00–0.72) p=0.0525
P. aeruginosa "ever" *versus* "never"¶	0.85 (0.31–1.38) p=0.0022#	0.42 (−0.41–1.25) p=0.2872	0.66 (0.23–1.09) p=0.0030#	0.64 (0.22–1.05) p=0.0035
Lumacaftor/ivacaftor (all)¶	−1.98 (−3.23– −0.72) p=0.0024#	−2.94 (−6.10–0.21) p=0.0640	−2.00 (−3.13– −0.86) p=0.0007#	−1.95 (−3.35– −0.56) p=0.0069
Lumacaftor/ivacaftor (F/F)¶	−1.77 (−3.16– −0.37) p=0.0137	−2.87 (−6.31–0.56) p=0.0913	−1.67 (−2.93– −0.41) p=0.0100	−1.73 (−3.22– −0.24) p=0.0243
z-LCI				
Annual trajectory	0.47 (−0.04–0.97) p=0.0705	0.07 (−0.82–0.97) p=0.8729	0.41 (0.00–0.81) p=0.0497	0.28 (−0.15–0.71) p=0.1959
P. aeruginosa "ever" *versus* "never"¶	1.06 (0.40–1.71) p=0.0018#	0.62 (0.42–1.65) p=0.2158	0.86 (0.34–1.38) p=0.0015#	0.80 (0.31–1.29) p=0.0018#
Lumacaftor/ivacaftor (all)¶	−2.34 (−3.90– −0.77) p=0.0039	−3.40 (−7.45–0.64) p=0.0905	−2.35 (−3.76– −0.93) p=0.0013#	−2.30 (−3.96– −0.65) p=0.0074
Lumacaftor/ivacaftor (F/F)¶	−2.05 (−3.80– −0.31) p=0.0218	−3.32 (−7.79–1.15) p=0.1273	−1.89 (−3.48– −0.31) p=0.0198	−2.01 (−3.78– −0.24) p=0.0269

Data are presented as annual change with 95% CI, unless otherwise stated. CF: cystic fibrosis; VDE: ventilation distribution efficiency; LCI: lung clearance index; *P. aeruginosa*: *Pseudomonas aeruginosa*; *P. aeruginosa* "ever": *P. aeruginosa* isolated from a respiratory secretion sample once or more in the period from birth until October 2021; *P. aeruginosa* "never": *P. aeruginosa* never isolated from a respiratory secretion sample in the period from birth until October 2021; F/F: homozygous for F508del. Analyses were performed for effects of age, infection with *P. aeruginosa* and treatment with lumacaftor/ivacaftor on z-VDE, z-$\log_{10}$(LCI) and z-LCI for CF Centre Copenhagen (n=25, multiple breath washouts (MBWs) 144), CF Centre Aarhus (n=34, MBWs 67), and for all 59 Danish children with CF aged 0.25–3.69 years including all measurement (n=59, MBWs 211) and only those with no respiratory symptoms (n=56, MBWs 146). For reference equations, see [18] and supplementary material. #: analyses that are also statistically significant using Bonferroni-corrected p-values of <0.0031 (corrected for 16 analyses for each outcome parameter); ¶: results are given as the difference between the z-score trajectory.

this study regarding the use of VDE are important for longitudinal and interventional research projects, as a smaller number of patients/MBWs will be required to demonstrate true differences or changes in outcomes. Furthermore, these findings, coupled with the previously documented benefits of VDE's linear correlation with tidal volume-adjusted ventilatory dead space (V_D/V_T) [18], strengthen the argument for using VDE instead of LCI in the clinical assessment of lung function for monitoring children with CF.

In terms of clinimetric properties [25], VDE shows similar, and even slightly better, levels of reliability, validity and responsiveness compared with LCI. The reliability of both outcomes, as assessed by observer variability, is equal as both outcome parameters derive from the same MBW occasion. Therefore, the observer assessment of the MBW quality is the same. The consistency of each MBW run, measured by CV% across two to three runs in one MBW occasion, is also identical for VDE and LCI (table 2). Also, consistency over time, measured by the mean CV% for the cohort (variability between test occasions for each child), was comparable between VDE and LCI (table 3). The validity of VDE is improved compared with LCI if the outcomes are believed to reflect V_D/V_T. However, using VDE or LCI as a surrogate to reflect structural lung disease, further studies are needed to provide data on the correlations between VDE and structural lung disease (*e.g.* PRAGMA-CF (Perth–Rotterdam annotated grid morphometric analysis for CF), airway and artery ratio scores, and magnetic resonance imaging score) compared with LCI *versus* these scores. Similarly, new studies are required to determine whether VDE during early childhood (*e.g.* infancy or preschool age) has the same prognostic ability as LCI to predict lung function during school-age years. These data can be easily obtained by recalculating previously reported data, as calculation of VDE is a straightforward process (VDE=1/LCI).

Early signs of lung deterioration

In the present study, only a few children demonstrated normal ventilation distribution inhomogeneity on all test occasions (two children with longitudinal data; six children in the total cohort) (supplementary material). The proportion of MBW occasions with abnormal ventilation distribution inhomogeneity increased with age, starting at 53% in infants <6 months of age at the time of testing and increasing to 76% in children >24 months of age. These data stress the importance of early disease detection and optimisation of the availability of early, effective treatment to prevent lung function deterioration during these first years of life. The ratio of MBW abnormality in this study is similar (although at the higher end) to published abnormality ratios of 19–67% in previous cross-sectional infant (age 0–2 years) MBW studies [1, 2, 11, 13, 26]. Detailed longitudinal MBW data have been lacking in this age group, where the four main studies to date are from the London CF Collaborative (LCFC) [4, 27], the AREST CF surveillance programme (Australia) [28] and a randomised clinical trial from Germany [29]. Direct comparisons between studies are challenging due to differences in MBW devices, software versions, numbers of longitudinal measurements, use of reference data and the method for bacterial detection. The LCFC questioned the value of infant MBW as impairments were mild and transient [4, 27]. The AREST CF group stated that LCI might be useful for longitudinal monitoring as pro-inflammatory bacteria (including *P. aeruginosa*) were significantly associated with both cross-sectionally and longitudinal LCI data [28]. Stahl *et al*. [29] used LCI in a double-blinded randomised trial and found that LCI was able to detect treatment effects in agreement with our data.

Effect of CFTR modulator therapy as measured by VDE

This is the first study to demonstrate the ability of z-VDE to detect the real-life effect of lumacaftor/ivacaftor treatment on lung function trajectories within this young age group. In a recent state-of-the-art review [30] by international experts in the field, the value of studies of this nature was strongly advocated for, discouraging medication approval in young children based on safety data alone. The fact that two phase 3, open-label studies in children aged 2–5 years did not find evidence of significant improvement in nitrogen (N_2)-based MBW (N_2MBW) (SPIROWARE 3.1.6)-derived LCI with lumacaftor/ivacaftor after 24 weeks (mean±SD change −0.58±1.16; p=0.06) and 96 weeks (mean −0.20 (95% CI −0.99–0.60); p=0.64) of treatment [17, 31] may reflect two important aspects: 1) a type 2 error due to the small portion of subjects who performed the MBW at both/all visits (17 (30%) and 17 (55%), respectively) and 2) those studies used no reference data from healthy children or children with CF on placebo as comparison groups. Both could have helped reveal a positive treatment effect. A randomised, placebo-controlled study using both MRI and N_2MBW (SPIROWARE 3.2.1) as the primary end-point for the treatment effect of lumacaftor/ivacaftor in children aged 2–5 years showed a numerically beneficial effect of treatment, although not to a statistically significant level. All three studies lacked the use of z-scores and they all used old software for N_2MBW in awake children with none, or only very few, children in the age range of 2–3 years. The relevance of the aforementioned aspects is highlighted by the findings of our study, where we demonstrated a significant difference in z-VDE trajectories "with" *versus* "without" treatment, resulting in a stable z-VDE after the start of treatment. The same results were found using LCI (supplementary material), z-LCI and z-$\log_{10}$(LCI) (table 4). The fact that some patients' lung function improved markedly with modulator therapy, while others did not, agrees with the effects seen in older children in previous CFTR studies. This variation in effect of treatment highlights the value of monitoring an individual response. Whether the use of N_2MBW with the old software version has influenced the results in previous studies cannot be rejected. However, a review indicates that conclusions from studies performed with old versions of EXHALYZER D N_2MBW are valid after re-analyses using the new software [32]. Using the old software, the values of LCI are significantly increased, leading to an overestimation of disease severity and treatment effectiveness compared with the use of VDE [18].

Strengths and limitations

The nationwide recruitment of patients, including 82% of the Danish cohort of eligible patients, is a major study strength. The EXHALYZER D SF_6MBW approach used in this study has been validated against the gold standard test, *i.e.* the respiratory mass spectrometer [16]. It has a reference dataset available, allowing for z-score calculations [18], strengthening our study results. The SF_6MBW reference data were mostly (90%) collected during natural sleep [18]. Whether the different sedation agents used in this study have biased our results and z-score calculations cannot be rejected. However, a previous study did not show any effect of sedation on ventilation inhomogeneity results [14].

The estimated z-scores at age 0.0 years (the intercept from the mixed model analyses) are subject to some uncertainties, as the calculation of z-scores relies on a dataset [18] in which no child under 1 month of age was investigated. However, no children with CF performed MBW before the age of 2.99 months (0.25 years), so the age range of the reference equation matched our cohort.

The reference equation [18] was built on a dataset with only very few children aged 2–4 years, which was an important age range in this study as we assessed the effect of CFTR modulator treatment starting at age 2 years. This could have led us to biased conclusions on the treatment effect of lumacaftor/ivacaftor. However, our conclusions on this real-life effect study are similar to those from randomised clinical trials. Also, as VDE (and LCI) improves with age in health, and no other reference dataset is available for SF_6MBW using EXHALYZER D, the use of this reference data was the best possible solution.

Implementing routine SF_6MBW in children with CF aged 0–3 years was particularly challenging due to a lack of resources (economically and MBW-trained operators) in CF Centre Aarhus. Both centres struggled with the logistics of finding new test dates when measurements were postponed due to respiratory symptoms and cancellations during the coronavirus disease 2019 lockdown, which prolonged intervals between some test occasions. The low rate of good quality MBWs in the Aarhus CF cohort during the first 1.5 years after implementing routine infant SF_6MBW once noticed was addressed by re-training [12, 33], with a marked improvement in test acceptability (from 35% to 98%). Despite these challenges, the overall success of the implementation of quarterly MBW in a routine clinical setting was 52% of all possible MBW measurements. The few test occasions for each child in Aarhus resulted in much wider 95% confidence intervals and higher p-values compared with those from Copenhagen (table 4). Merging data from the two sites strengthened the study by including a larger number of patients, providing greater statistical power (table 4). Whether or not the missing data of some patients could have biased the results is unknown. However, there was no systematic selection of which children performed the test occasions or not, as all children were supposed to have MBW testing as per the testing protocol. Physicians in the clinics were not blinded to the LCI values and may have considered these for clinical decisions. However, there were no clear guidelines available on when or how to use the MBW outcomes and the direct effect of LCI on management decisions is likely minimal.

Finally, the sample size for our study was opportunistic, including all eligible patients (80% of the Danish cohort). We found a significant effect of lumacaftor/ivacaftor therapy on the trajectory of z-VDE, leading to a stable trajectory after treatment initiation (statistically non-significant improvement in z-VDE, as the 95% confidence intervals overlapped zero). It is unclear from the available data whether the absence of significant regression of CF lung disease, as measured by ventilation inhomogeneity, is due to limited sample size and insufficient statistical power or if it is simply because the effectiveness of lumacaftor/ivacaftor is limited to stabilising the disease and preventing further deterioration.

Conclusions

In this nationwide study on infants and toddlers with CF diagnosed after NBS-CF, z-VDE as the primary MBW outcome detected progression in lung function abnormality over time and was able to detect associations to known risk factors and a subsequent beneficial treatment effect with CFTR modulator therapy. This, together with the demonstrated feasibility of prospective MBW testing from early infancy, suggests that a window of opportunity exists to prevent or reverse lung function impairment in children with CF using MBW surveillance testing as part of CF clinical care following NBS-CF diagnosis. Future addition of hypertonic saline inhalations or CFTR modulator treatment from very early infancy aims to prevent lung impairment in children with CF.

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Data sharing statement: For original data, please contact Rikke M. Sandvik (rikke.mulvad.sandvik.01@regionh.dk).

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