Novel volumetric capnography indices measure ventilation inhomogeneity in cystic fibrosis

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Abstract

Background Volumetric capnography (VCap) is a simpler alternative to multiple-breath washout (MBW) to detect ventilation inhomogeneity in patients with cystic fibrosis (CF). However, its diagnostic performance is influenced by breathing dynamics. We introduce two novel VCap indices, the capnographic inhomogeneity indices (CIIs), that may overcome this limitation and explore their diagnostic characteristics in a cohort of CF patients.

Methods We analysed 320 N_2-MBW trials from 50 CF patients and 65 controls (age 4–18 years) and calculated classical VCap indices, such as slope III (SIII) and the capnographic index (KPIv). We introduced novel CIIs based on a theoretical lung model and assessed their diagnostic performance compared to classical VCap indices and the lung clearance index (LCI).

Results Both CIIs were significantly higher in CF patients compared with controls (mean±SD CII_1 5.9±1.4% *versus* 5.1±1.0%, p=0.002; CII_2 7.7±1.8% *versus* 6.8±1.4%, p=0.002) and presented strong correlation with LCI (CII_1 r^2=0.47 and CII_2 r^2=0.44 in CF patients). Classical VCap indices showed inferior discriminative ability (SIII 2.3±1.0%/L *versus* 1.9±0.7%/L, p=0.013; KPIv 3.9±1.3% *versus* 3.5±1.2%, p=0.071), while the correlation with LCI was weak (SIII r^2=0.03; KPIv r^2=0.08 in CF patients). CIIs showed lower intra-subject inter-trial variability, calculated as coefficient of variation for three and relative difference for two trials, than classical VCap indices, but higher than LCI (CII_1 11.1±8.2% and CII_2 11.0±8.0% *versus* SIII 16.3±13.5%; KPIv 15.9±12.8%; LCI 5.9%±4.2%).

Conclusion CIIs detect ventilation inhomogeneity better than classical VCap indices and correlate well with LCI. However, further studies on their diagnostic performance and clinical utility are required.

Introduction

Volumetric capnography (VCap) is a simple and noninvasive lung function technique that describes the dynamics of carbon dioxide (CO_2) exhalation breath-by-breath [1]. The volume-based capnogram is the plot of CO_2 concentration against the exhaled air volume and consists of three phases: phase I represents the washout of the uppermost conductive airways that contain atmospheric – CO_2-free – air; phase II is characterised by a steep CO_2 rise and reflects the mixing between atmospheric air and CO_2-rich gas from the alveolar compartment; and phase III, the so-called "alveolar plateau", represents the expiration of alveolar gas. Volumetric capnography allows for the assessment of dead space ventilation and ventilation–perfusion abnormalities [2–7], while the slope of phase III (SIII) is considered an index of ventilation inhomogeneity in obstructive lung disorders, such as cystic fibrosis (CF) [7–11].

Since VCap does not require a complex measurement setup or extensive signal processing, it may be considered a simpler alternative to techniques aiming to detect ventilation inhomogeneity, such as multiple-breath washout (MBW) [2, 7, 10]. However, classical VCap indices are considerably dependent

on expiratory volume (V_E) and respiration dynamics [12–14]. This is particularly important in children, where variable breathing patterns may significantly affect the diagnostic performance of the method [6, 7, 9, 15].

In this paper, we introduce novel VCap indices called capnographic inhomogeneity indices (CIIs) that may overcome the above limitations. Moreover, they may serve as promising ventilation inhomogeneity indices in clinical settings where advanced MBW setups are not available, or as faster and more attainable markers in obstructive lung disorders that require continuous follow-up. We assess the feasibility and repeatability of these novel CIIs in clinical practice, and we explore their diagnostic performance in a cohort of CF patients.

Methods

Study design and population

In this pilot study, novel VCap indices were validated using existing nitrogen (N_2) MBW data from 4- to 18-year-old CF patients and healthy controls. All measurements were obtained at the University Children's Hospital of Bern, Switzerland, between 2013 and 2019 (see Sample size estimation) [16–21]. All subjects were free from pulmonary exacerbations [16–21]. Only subjects with at least two good-quality MBW trials were included. Local ethics committees approved all studies, and participants or caregivers provided informed written consent.

Lung function

MBW trials were performed with the children in sitting position while breathing normally, according to international guidelines [22]. N_2-MBW measurements were obtained as previously described [20], using the Exhalyzer D device (Ecomedics, Duernten, Switzerland) that incorporates an ultrasonic flowmeter and a main-stream CO_2 sensor (Capnostat, infrared single beam, dual-wavelength technology; rise time <60 ms) with Spiroware version 3.1.6 or 3.2.1. All data were reloaded and reanalysed using the updated software provided by the manufacturer of Spiroware 3.3.1 (Ecomedics, Duernten, Switzerland). MBW quality control was performed by experienced operators, based on established criteria [21, 22].

The following MBW parameters were investigated: functional residual capacity (FRC), lung clearance index (LCI), tidal volume, respiratory rate and minute ventilation [22]. Additional information on age, sex, weight and height were obtained from patient files.

Volumetric capnography

Volumetric capnograms were obtained from N_2-MBW trials after CO_2 signals were corrected for setup-dependent signal alignment and sensor-specific delays (Spiroware software). Breath-by-breath volumetric capnograms were obtained by plotting the CO_2 fraction against the corresponding V_E. The expired CO_2 volume (V_{ECO_2}) was calculated by integrating the CO_2 signal over V_E. The slope of phase II (SII) and the SIII were obtained by fitting a linear regression line (least squares method) over the capnogram, between 10 and 60% of the end-tidal CO_2 value and 65–95% of V_E, respectively [6]. The capnographic index (KPIv) was calculated as the ratio SIII/SII. The slopes were normalised by the corresponding mixed expired CO_2 fraction (F_{ECO_2}), which equals the V_{ECO_2} divided by V_E [12]. The airway dead space (V_D) was calculated using the equal-area method proposed by Fowler [23].

To avoid inclusion of irregular capnograms, MBW breaths were automatically excluded from analysis if: 1) end-tidal CO_2 ($ETCO_2$) was <3.5%; 2) their corresponding V_E deviated more than two $_{SD}$s from trial mean; 3) V_D was <10% or >30% of V_E; 4) intersection of SII and SIII was not calculable, fell outside the capnogram range (*i.e.*intersection at V_E<0, V_E>V_E at the end of expiration, CO_2>CO_2max) or below the CO_2 curve; 5) SIII was steeper than SII; and 6) coefficient of determination (r^2) for SIII fitting was <0.7. For all acceptable breaths of each MBW trial, we calculated mean $ETCO_2$, SII, SIII, normalised SII (nSII), normalised SIII (nSIII), KPIv and V_D values. Calculation of VCap indices and quality control were performed using a custom Python script (Python Software Foundation, Wilmington, DE, USA; www.python.org/).

Capnographic inhomogeneity indices

Modelling ventilation inhomogeneity

To understand the calculation of novel CIIs, we consider a lung model (figure 1) in which we define V_A, the volume of the alveolar compartment that contributes to expiration, V_D the volume of the dead space compartment and V_E the expired volume of air. Let us assume that the alveolar compartment comprises three equal sub-compartments connected in series, which empty sequentially without air mixing among them (figure 1). At end-inspiration, the dead space compartment is filled with atmospheric air (with a CO_2 concentration of zero). All CO_2 is contained in V_A, at a mixed alveolar concentration of F_{ACO_2}, which is

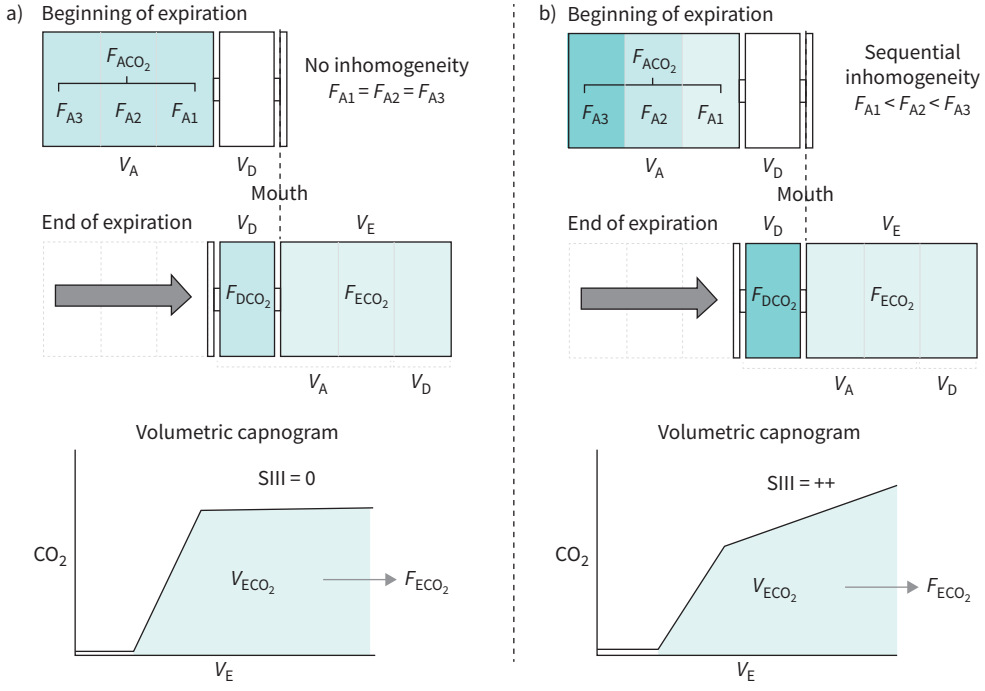

FIGURE 1 The three-compartment model of our study and the respective volumetric capnograms. a) Without inhomogeneity (ideal lung); b) with sequential inhomogeneity. See text for complete description. V_E: expiratory volume; V_A: alveolar compartment volume; V_D: dead space compartment volume; V_{ECO_2}: expired CO_2 volume; F_{ECO_2}: mixed expired CO_2 fraction; SIII: slope of phase III; F_{ACO_2}: CO_2 fraction in alveolar compartment; F_{DCO_2}: CO_2 fraction in dead space compartment; F_{A1-3}: alveolar sub-compartments.

the mean CO_2 concentration of alveolar sub-compartments (*i.e.* F_{A1CO_2}, F_{A2CO_2} and F_{A3CO_2}) (figure 1). As expiration commences, dead space air emerges first, followed by gas from the three alveolar sub-compartments. At end-expiration, the concentration of CO_2 in the expired air (F_{ECO_2}) results from the mixing between the CO_2-free dead space air and the CO_2-rich alveolar air; an amount of CO_2 remains in the dead space compartment at a concentration F_{DCO_2} (figure 1).

In an ideal lung, F_{A1CO_2}, F_{A2CO_2} and F_{A3CO_2} are equal (figure 1a). Thus, time-dependent inhomogeneities do not exist, the rate of CO_2 exhalation is constant and the phase III of the capnogram is horizontal (figure 1a). In this case, F_{DCO_2} at end-expiration equals F_{ACO_2} (figure 1a). When time-dependent inhomogeneities exist, due to stratified ventilation inhomogeneity distal to the airway–alveolar interface and delayed emptying of respiratory units with low ventilation–perfusion (V'/Q') ratios [24, 25], it applies that $F_{A1CO_2} < F_{A2CO_2} < F_{A3CO_2}$ (figure 1b). In this case, the rate of exhaled CO_2 increases as expiration commences, and the SIII rises (figure 1b). At end-expiration, F_{DCO_2} equals $F_{A3CO_2}2$ (figure 1b). The magnitude of sequential inhomogeneity in this model is reflected by the difference among F_{A1CO_2}, F_{A2CO_2} and F_{DCO_2} (figure 1b). However, since neither F_{ACO_2} nor F_{DCO_2} can be measured *in vivo*, these differences cannot be computed.

Estimating F_{DCO_2}

Let us consider the volumetric capnogram of figure 2a and analyse it according to the concept of Aitken and Clark-Kennedy [26], as follows: a) The volume of CO_2 that remains in the dead space compartment at end-expiration (V_{DCO_2}) can be obtained by extending the line of phase III to the right (line *be*) using linear regression until the distance *cf* becomes equal to V_D, and calculating the area of the trapezoid *befc*; the F_{DCO_2} is the area *befc* divided by V_D. b) The total volume of CO_2 leaving the alveolar compartment (V_{ACO_2}) is the area of the trapezoid *aefd*; F_{ACO_2} can be obtained by dividing this area by V_E (figure 2a).

The above concept was applied to volumetric capnograms of our study (figure 2b). After the V_D was calculated by Fowler's method [23], each capnogram was extended to the "right" (linear extension according to SIII) by V_D. The V_{ECO_2} was computed as the integral of the CO_2 signal over V_E, and the

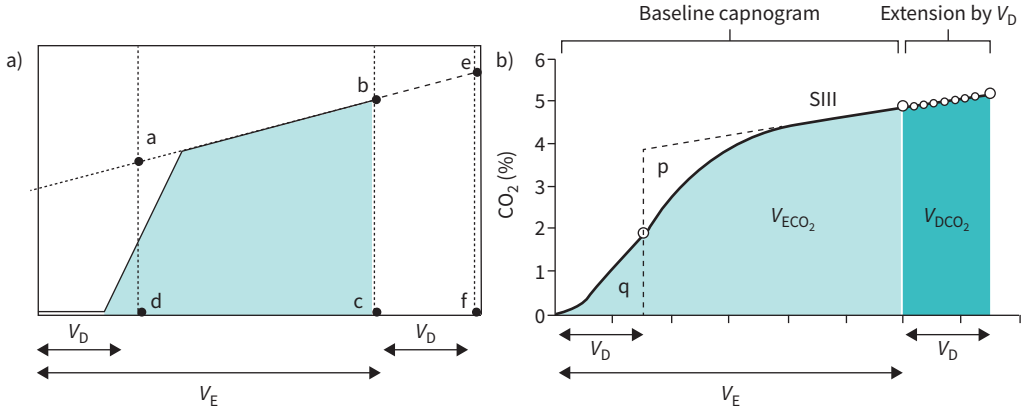

FIGURE 2 VCap analysis with extension by V_D. a) As originally proposed by Aitken and Clark-Kennedy [26]; b) as applied in our study. See text for detailed explanations. V_E: expiratory volume; V_D: dead space compartment volume; SIII: slope of phase III; V_{ECO_2}: expired CO_2 volume; V_{DCO_2}: CO_2 volume in dead space compartment.

V_{DCO_2} as the integral of the extended part of the capnogram over V_D (figure 1b); the V_{ACO_2} was computed as the sum of V_{ECO_2} and V_{DCO_2}. Then, by dividing V_{ECO_2} by V_E, V_{DCO_2} by V_D, and V_{ACO_2} by V_E, the F_{ECO_2}, F_{DCO_2} and F_{ACO_2}, respectively, were calculated. In addition, the concentration of CO_2 in the exhaled air coming exclusively from the alveolar compartment (F_{exCO_2}) was calculated by dividing V_{ECO_2} by the difference $V_E - V_D$.

Capnographic inhomogeneity indices

Two novel CIIs were calculated:

A. CII1, which is the relative difference between F_{DCO_2} and F_{ACO_2} or $(F_{DCO_2} - F_{ACO_2})/F_{ACO_2}$ and represents a raw estimate of sequential inhomogeneity.

B. CII2, which is the relative difference between F_{DCO_2} and F_{exCO_2} or $(F_{DCO_2} - F_{exCO_2})/F_{exCO_2}$. Since CII2 reflects the difference of CO_2 concentration between the alveolar sub-compartments A1+A2 and A3 (figure 1b), it should theoretically represent a more precise estimate of sequential ventilation inhomogeneity.

Statistics

Sample size estimation

Since CIIs were introduced for the first time in clinical practice, no data were available for *a priori* sample size estimation. Based on the hypothesis that CIIs (as markers of VI) would be correlated with the LCI, we estimated that MBW measurements from at least 112 subjects would be required to reveal a significant correlation ($p<0.05$), with a Pearson's $r \geqslant 0.3$ and at least 90% power. By reviewing the database of our laboratory (different independent studies [16–21]), we found that such a sample with at least two acceptable MBW trials could be obtained by including the children measured between January 2013 and December 2019 (n=115; 50 CF and 65 healthy controls). We preferred to include all 115 children (instead of 112) for reasons of consistency. *Post hoc* effect size calculation was also performed.

Statistical analyses

VCap indices were assessed both per subject (mean of at least two acceptable trials) and per MBW trial (analysis per trial) to allow better physiological appraisal. Per subject data are presented throughout the manuscript, while per trial analyses are presented in the online supplementary material. Between-group comparisons were performed using t-test. The percentage of breaths acceptable for VCap analysis was calculated as percentage of all washout breaths. Linear and nonlinear regression was applied to assess the relationship between SIII and V_E, and SIII and CIIs. Pearson correlation analysis was used to assess the relationship between LCI and VCap outcomes. The intra-trial (*i.e.* between breaths of the same trial) and inter-trial (*i.e.* between different trials of the same subject) variability was calculated using the coefficient of variation (CV). In the presence of only two acceptable MBW trials, the inter-trial variability was calculated as relative difference between the two trials. Receiver operation characteristics analysis was used to estimate the overall diagnostic ability of LCI and VCap indices by means of area under the

curve (AUC). Optimal cut-off values for each index were determined using Youden Index analysis. All analyses were performed in Stata (StataCorp, College Station, TX, USA).

Results

All 320 MBW trials from 50 patients with CF (137 trials) and 65 HCs (183 trials) were analysed. The clinical characteristics and lung function parameters of the two study groups are presented in table 1. The LCI was significantly higher in CF patients compared to healthy children (mean±SD LCI 7.8±1.7 *versus* 6.2±0.4, p<0.001).

VCap indices

Calculation of VCap indices was feasible in all trials of all study participants, independent of disease status. The percentage of breaths within each trial that were acceptable for VCap analysis was higher in CF patients compared with healthy children (72.4±17.8 (42.7–98.3)% *versus* 63.5±19.3 (19.7–96.7)%). Further information regarding non-accepted breaths (percentages, stratification per exclusion criteria) is presented in supplementary table S2. Slope III and KPIv were higher in CF patients compared with healthy children (SIII 2.3±1.0%/L *versus* 1.9±0.7%/L, p=0.013; KPIv 3.9±1.3% *versus* 3.5±1.2%, p=0.07), but only SIII was significantly higher. The CIIs were also significantly higher in CF patients compared with healthy controls (CII1 5.9±1.4% *versus* 5.1±1.0%, p=0.002; CII2 7.7±1.8% *versus* 6.8±1.4%, p=0.002). *Post hoc* effect size analysis revealed that these differences yield a study power of 71% and 68%, respectively, for the given level of statistical significance, or 96% and 89.5%, respectively, for a p-value <0.05. VCap parameters and indices are presented in tables 2 and 3, and figure 3. AUC values and the corresponding optimal cut-off values of LCI and VCap indices are presented in supplementary table S5). Above the cut-off value of LCI were classified 76% (n=38) of patients with CF and 10.8% (n=7) of controls, above the cut-off value of SIII 34% (n=17) of patients with CF and 10.8% (n=7) of controls, above the cut-off value of KPIv 76% (n=38) of patients with CF and 47.7% (n=31) of controls, above the cut-off value of CII1 44% (n=22) of patients with CF and 20% (n=13) of controls, and above the cut-off value of CII2 48% (n=24) of patients with CF and 18.5% (n=12) of controls.

Correlations of VCap indices

There was a strong inverse curvilinear relationship between SIII and V_E and a weak linear relationship between CII1 and SIII and CII2 and SIII (figure 4, supplementary figure S3). The correlations between classical VCap indices and CIIs are shown in supplementary table S6. The correlation SIII–LCI and

TABLE 1 Characteristics and lung function of the study groups

	Cystic fibrosis	Healthy controls
Subjects, n	50	65
General characteristics		
Male sex, n (%)	54.0 (50.3)	53.8 (50.2)
Age years	9.8±4.1	10.1±3.9
Weight kg	32.3±14.3	37.8±17.6
Weight z-score#	−0.1±0.8	0.4±0.8
Height cm	134.4±19.7	140.7±23.0
Height z-score#	−0.1±0.8	0.4±1.0
Body mass index kg·m^{-2}	17.0±2.6	17.9±3.1
Body mass index z-score#	0.0±0.8	0.2±0.8
N_2-MBW		
Number of trials, n	137	183
Tidal volume mL	402.9±175.8	437.4±199.0
Tidal volume per kg (mL·kg^{-1})	12.7±2.8	12.0±2.7
Respiratory rate (per minute)	18.7±4.1	18.2±4.8
Minute ventilation mL·min^{-1}·kg^{-1}	231.2±46.0	215.3±67.6
FRC mL·kg^{-1}	40.2±7.8	41.4±8.9
LCI 2.5%	7.8±1.7	6.2±0.4*

Data are presented as mean±SD, unless stated otherwise. N_2-MBW: nitrogen multiple-breath washout; FRC: functional residual capacity; LCI: lung clearance index; CDC: Centers for Disease Control and Prevention; WHO: World Health Organization. #: weight, height and body mass index z-scores were calculated according to WHO growth charts. Weight z-scores for children older than 10 years of age were calculated according to CDC growth charts; *: statistically significant difference (p<0.05 using the t-test).

TABLE 2 VCap parameters and indices

	Cystic fibrosis	Healthy controls	Mean difference (95% CI)
V_E mL	402.9±174.6	438.7±201.6	35.7 (−35.2–106.7)
V_E per kg (mL·kg^{-1})	12.7±3.1	12.1±2.7	−0.7 (−1.8–0.4)
V_D mL	88.6±27.5	98.3±34.3	9.8 (−2.0–21.5)
V_D per kg (mL·kg^{-1})	2.9±0.5	2.8±0.5	−0.1 (−0.3–0.1)
V_D % of V_E (%)	23.7±4.9	23.9±4.3	0.2 (−1.5–1.9)
P_{ETCO_2} %	5.3±0.5	5.3±0.5	0.0 (−0.1–0.2)
V_{CO_2} mL	16.3±5.9	18.2±7.9	1.9 (−0.7–4.6)
$ETCO_2$ %	3.9±0.4	4.0±0.4	0.0 (−0.1–0.2)
SII %/L	59.3±19.2	54.5±16.8	−4.8 (−11.5–1.9)
Normalised SII 1/L	15.0±4.2	13.7±3.9	−1.2 (−2.7–0.3)
SIII %/L	2.3±1.0	1.9±0.7	−0.4 (−0.7– −0.1)*
Normalised SIII 1/L	0.6±0.3	0.5±0.2	−0.1 (−0.2–0.0)*
KPIv %	3.9±1.3	3.5±1.2	−0.4 (−0.9–0.0)

Data are presented as mean±SD, unless stated otherwise. VCap: volumetric capnography; V_E: expiratory volume; V_D: dead space volume; $ETCO_2$: end-tidal CO_2 fraction; V_{CO_2}: expired CO_2 volume; F_{ECO_2}: mixed expired CO_2 fraction; SII: slope of phase II; SIII: slope of phase III; KPIv: capnographic index. *: statistically significant difference (p<0.05 using the t-test).

KPIv–LCI was weak (SIII–LCI r^2=0.03; KPIv–LCI r^2=0.08 in CF patients), while the correlation between CIIs and LCI was stronger (CII1–LCI r^2=0.47 and CII2–LCI r^2=0.44 in CF patients). Overall (all participants), the Pearson's correlation coefficient between CII1 and LCI was 0.572 and between CII2 and LCI 0.557; both values yield a study power of 100% (p<0.001). More correlations between VCap parameters and LCI are presented in table 4 and supplementary table S7. Both CII1 and CII2 were significantly correlated with age (r^2=0.162 and r^2=0.118, respectively), while age was also significantly correlated with the LCI (r^2=0.09). There was no relationship between CIIs and sex in our cohort (data not shown).

Intra- and inter-trial variability

The intra-trial variability CVs of SIII and KPIv were higher compared with CII1 and CII2 (CV of SIII 37.5±19.2%, KPIv 35.3±15.5% *versus* CII1 31.1±8.9% and CII2 31.7±9.4%, in all trials). For all these VCap indices, the intra-trial variability was lower in CF patients compared with controls (supplementary table S8). Similarly, the inter-trial (intra-subject) variability of SIII and KPIv was higher compared with CII1 and CII2 in all trials (SIII 16.3±13.5%, KPIv 15.9±12.8% vs CII1 11.1±8.2% and CII2 11.0±8.0%). However, the inter-trial variability was comparable between CF patients and controls (supplementary table S9). The LCI showed lowest inter-trial variability (LCI 5.9±4.2%) (supplementary table S9, figure S2).

Discussion

In this pilot study, we introduced novel capnographic indices of ventilation inhomogeneity and we assessed their diagnostic performance in comparison with classical VCap parameters (*i.e.* SIII and KPIv) and the LCI. We found that SIII and the novel CIIs were higher in CF patients than in controls. However, the novel capnographic inhomogeneity indices showed better correlation with the LCI and lower intra-trial and inter-trial variability than SIII and KPIv, although their overall diagnostic performance was inferior to the LCI.

TABLE 3 Novel volumetric capnography (VCap) parameters and capnographic inhomogeneity indices

	Cystic fibrosis	Healthy	Mean difference (95% CI)
Subjects n	50	65	
F_{ACO_2} %	5.1±0.5	5.2±0.5	0.1 (−0.1–0.2)
F_{DCO_2} %	5.4±0.5	5.5±0.5	0.0 (−0.1–0.2)
F_{exCO_2} %	5.1±0.5	5.1±0.5	0.1 (−0.1–0.2)
CII1 %	5.9±1.4	5.1±1.0	−0.7 (−1.2– −0.3)*
CII2 %	7.7±1.8	6.8±1.4	−1.0 (−1.5– −0.3)*

Data are presented as mean±SD, unless stated otherwise. F_{ACO_2}: CO_2 fraction in alveolar compartment; F_{DCO_2}: CO_2 fraction in dead space compartment; F_{exCO_2}: CO_2 fraction in the air expired from the alveolar compartment; CII: capnographic inhomogeneity index (see text for details). *: statistically significant difference (p<0.05 using the t-test).

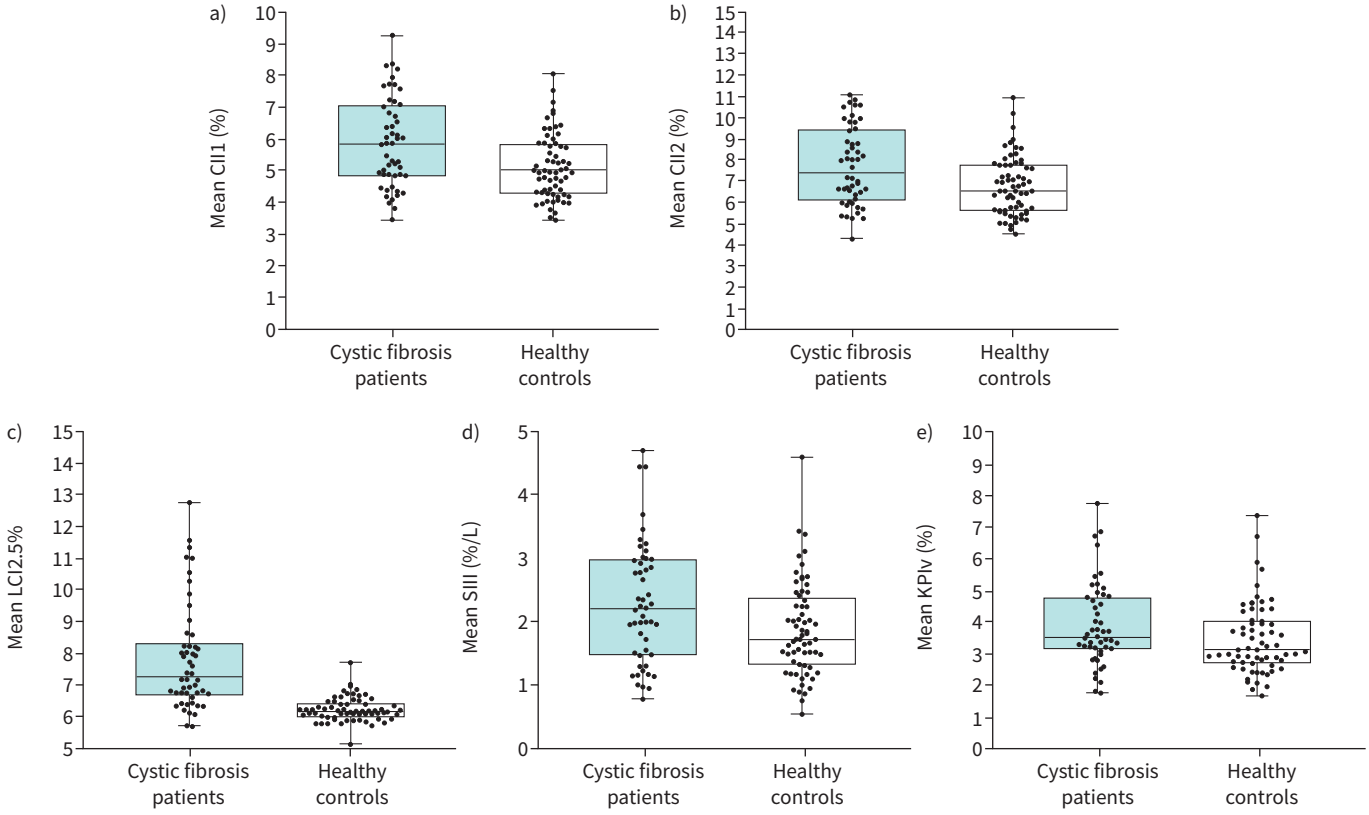

FIGURE 3 Boxplots showing the difference between patients with cystic fibrosis and healthy controls in mean values of a) CII1 and b) CII2, c) LCI, d) SIII and e) KPIv. The individual black dots represent mean values per subject. CII: capnographic inhomogeneity index; LCI: lung clearance index; SIII: slope of phase III; KPIv: capnographic index.

Performance of VCap indices

As expected, SIII and KPIv were increased in CF patients. These findings are in line with previous studies, suggesting that classical VCap indices may be useful ventilation inhomogeneity markers in adults and children with CF [8–11, 27]. In CF, ventilation inhomogeneity results in delayed CO_2 mixing within the conductive airways and, eventually, to non-homogeneous CO_2 exhalation (*i.e.* steeper phase III) [1, 3, 8] (figure 1); the KPIv (*i.e.* the SIII to SII ratio) increases respectively [8]. Thus, increased SIII and KPIv are consistent findings in CF [9–11, 27], albeit their discriminative ability is moderate [11] and, in any case, inferior to that of the LCI [10]. Of note, the correlation between SIII and LCI or KPIv and LCI was rather weak. The latter contrasts the findings of Fuchs *et al.* [10] who showed a stronger correlation between those indices and LCI. The calculation of SII and SIII on an "averaged", user-defined capnogram in their

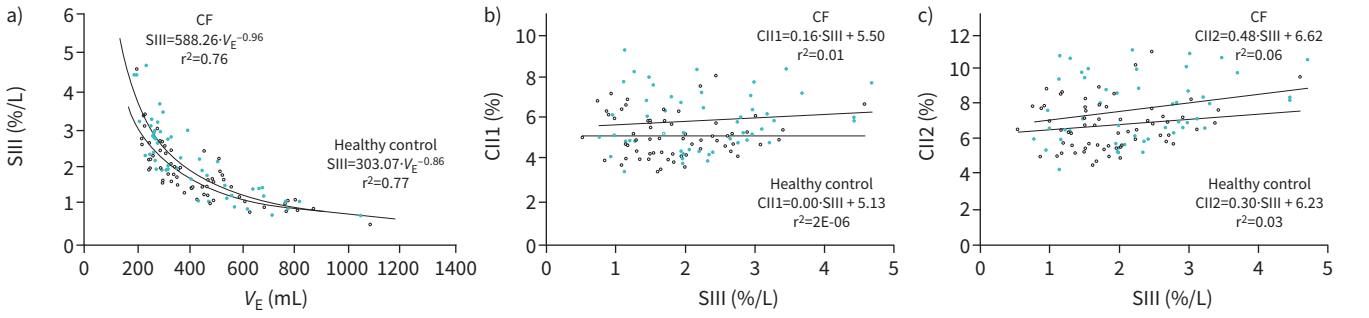

FIGURE 4 Relationship between a) SIII and V_E, b) CII1 and SIII and c) CII2 and SIII in CF (green dots) and healthy control trials (black open dots). CF: cystic fibrosis; SIII: slope of phase III; V_E: expiratory volume; CII: capnographic inhomogeneity index.

TABLE 4 Correlations between VCap parameters and the lung clearance index

	Cystic fibrosis			Healthy controls		
	r	r^2	p-value[#]	r	r^2	p-value[#]
SII	−0.52	0.27	<0.001	−0.04	0.00	0.764
Normalised SII	−0.44	0.20	0.002	−0.01	0.00	0.912
SIII	−0.18	0.03	0.209	0.04	0.00	0.771
Normalised SIII	−0.07	0.01	0.623	0.06	0.00	0.629
KPIv	0.29	0.08	0.04	0.124	0.02	0.325
CII1	0.68	0.47	<0.001	0.06	0.00	0.620
CII2	0.66	0.44	<0.001	0.11	0.01	0.389

Data are presented as Pearson correlation coefficients (r) and coefficients of determination (r^2). VCap: volumetric capnography; SII: slope of phase II; SIII: slope of phase III; KPIv: capnographic index; CII: capnographic inhomogeneity index. [#]: t-test.

study [10], as opposed to breath-by-breath calculation of VCap indices using well-defined criteria in ours, might explain these differences.

Conversely, CII1 and CII2 presented better discriminative characteristics than the classical VCap indices. In addition, both CIIs correlated significantly with the LCI – a robust index of ventilation inhomogeneity in CF patients [28, 29], and did so better than the classical VCap parameters (table 4). Thus, CIIs may be considered valid measures of ventilation inhomogeneity, which may also relate to the severity of the disease.

Theoretical advantages of CIIs over classical VCap indices

The SIII reflects the rate of CO_2 exhalation beyond mid-expiration and is an unstandardised index that depends on the dynamics of expiration, especially on V_E [12–14]. However, normalisation of SIII by V_E [11] is not justified, because as shown in previous studies [12–14] and confirmed in ours (figure 4a, supplementary figure S3A), the SIII–V_E relationship is not linear. SIII normalisation by the F_{ECO_2} (*i.e.* the normalised SIII) may allow for intra- or inter-subject adjustment for different CO_2 concentrations but does not eliminate the dependency from V_E. Therefore, in subjects with variable respiratory patterns and/or changing lung volumes, as it typically is in children, the utility of SIII is limited [14]. In our study, this disadvantage is also reflected by the unexpected negative correlation between SIII and LCI (table 4). Higher LCI values are typically seen in older CF patients due to the progression of the disease [21]; but since older children also have larger lung volumes, their SIII is lower due to the strong SIII–V_E relationship (figure 4a, supplementary figure S3A).

The theoretical background for calculation of CIIs is different: in lung disease, variable gas mixing within the respiratory units (serial inhomogeneities) and/or regional V'/Q' variations (parallel inhomogeneities) result in sequential variations of CO_2 concentration distal to the airway–alveolar interface, which are exacerbated further by the delayed emptying of respiratory units with altered mechanical properties and low V'/Q' [23, 24]. Overall, these phenomena result in time-dependent inhomogeneities of CO_2 concentration that can be detected at the airway opening [1, 12]. The CIIs, which according to the proposed model (figure 1) are calculated as differences of CO_2 concentrations, reflect these time-dependent inhomogeneities. The CO_2 concentrations (*i.e.* $V_{CO_2} - V_E$ fractions) also include an inherent normalisation for V_E, and their use may thus overcome the limitations of classical VCap indices. The significant positive correlation between CIIs and LCI and the weak correlation between CIIs and SIII further support this hypothesis.

Feasibility and repeatability of CIIs

CIIs calculation was feasible in all MBW trials, independent of disease status. The percentage of acceptable breaths for VCap analysis was ∼75% in CF and 65% in control trials. We found lower variability in CIIs compared with SIII and KPIv, but higher compared with LCI. The higher percentage of acceptable breaths together with a lower intra-trial variability found in CF patients may be because they were familiar with the MBW procedure and, thus, able to maintain more stable breath patterns during MBW measurement.

Strengths and limitations

This study presents an innovative method for ventilation inhomogeneity assessment that in principle requires only flow and CO_2 signals, thus being potentially more attainable in clinical settings. In our large

cohort of both healthy children and children with CF, with a wide age range and diversity in disease severity, these novel CIIs showed a good association with the more complicated LCI, thus confirming their clinical potential. The use of N_2-MBW files facilitated direct comparison of MBW and VCap outcomes derived from the same files, limiting the effect of possible influencing factors (*e.g.* related to breathing pattern or testing circumstances). All MBW trials were analysed with the newest Spiroware version (*i.e.* 3.3.1), so that our results are not affected by the recently revealed sensor crosstalk error in the Exhalyzer D device [30]. Overall, *a posteriori* effect size calculations yielded study power in the range of 68–100% for the differences between CIIs in CF and controls and the Pearson's correlation coefficients between CIIs and LCI.

Inevitably, our study also has some limitations. First, VCap analysis was performed retrospectively using previously collected N_2-MBW data from one centre. This implies that the baseline quality-control criteria (*e.g.* those related to breathing pattern and variability) were specific to MBW [21, 22] and not to capnography. This may have affected the intra- and inter-trial variability of CIIs (which was larger than the inter-trial variability of the LCI) and influenced their discriminative ability. Prospective data acquisition for capnographic analysis, including instructions and/or incentives to reduce breathing variability and VCap-specific quality control, may improve the quality of capnograms, decrease the variability of CIIs and increase their diagnostic performance. Second, the effect of 100% O_2 concentration (N_2-MBW technique) on VCap parameters is unknown. However, the effect, if any, would not be different between CF and controls.

Finally, CIIs calculation was based on the extension of capnograms "to the right". Arguably, the evolution of events that determine phase III cannot be predicted [25]. Yet, if we assume that these events remain stable during the end of expiration, a forward extension of capnograms may be justified [26]. In fact, this assumption is the basis of the universally accepted Fowler's method to calculate V_D [23], where the capnogram is extended "to the left".

Clinical implications

Volumetric capnography is a simple noninvasive technique that does not require complex respiratory manoeuvres, exogenous gases, extensive signal processing or operator's expertise [2, 5, 7]. Current evidence suggests that classical VCap indices, such as SIII and KPIv, may be sensitive markers of early lung changes in patients with CF [11]; however, their diagnostic performance is limited due to the strong dependence on breathing dynamics [12–14]. Our results indicate that novel CIIs have lower variability compared to classical VCap indices, thus suggesting that they may be less influenced by the breathing pattern; however, further studies are needed to clarify this important issue. Future research should also focus on defining the methodological requirements and the proper quality-control criteria to obtain high-quality capnograms that might reduce variability and increase the performance of these novel indices. The novel CIIs should be assessed using simpler (*i.e.* non-MBW) setups, ideally on multiple occasions to allow for repeatability assessment, and in large cohorts with a range of obstructive lung disorders (*e.g.* asthma). Finally, further multicenter research is required to assess the external validity and the potential clinical value of this method.

Conclusions

In conclusion, the herein introduced CIIs performed better than the classical VCap parameters in detecting ventilation inhomogeneity in CF patients. The CIIs also correlated well with the LCI and had lower variability compared to classical VCap indices. Their calculation was feasible in all study participants, independently of disease status. Thus, although their overall diagnostic performance was inferior to the LCI, they may be considered as promising and simpler markers of ventilation inhomogeneity. Further research is required to define the exact methodological requirements, improve the diagnostic performance and assess the true clinical value of CIIs, especially at the bedside.

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References

1 Fletcher R, Jonson B, Cumming G, *et al.* The concept of deadspace with special reference to the single breath test for carbon dioxide. *Br J Anaesth* 1981; 53: 77–88.
2 Suarez-Sipmann F, Bohm SH, Tusman G. Volumetric capnography: the time has come. *Curr Opin Crit Care* 2014; 20: 333–339.
3 Fletcher R. Airway deadspace, end-tidal CO_2, and Christian Bohr. *Acta Anaesthesiol Scand* 1984; 28: 408–411.
4 Tusman G, Suarez-Sipmann F, Bohm SH, *et al.* Capnography reflects ventilation/perfusion distribution in a model of acute lung injury. *Acta Anaesthesiol Scand* 2011; 55: 597–606.
5 Kremeier P, Böhm SH, Tusman G. Clinical use of volumetric capnography in mechanically ventilated patients. *J Clin Monit Comput* 2020; 34: 7–16.
6 Fouzas S, Hacki C, Latzin P, *et al.* Volumetric capnography in infants with bronchopulmonary dysplasia. *J Pediatr* 2014; 164: 283–8.e1-3.
7 Verscheure S, Massion PB, Verschuren F, *et al.* Volumetric capnography: lessons from the past and current clinical applications. *Crit Care* 2016; 20: 184.
8 Krauss B, Deykin A, Lam A, *et al.* Capnogram shape in obstructive lung disease. *Anesth Analg* 2005; 100: 884–888.
9 Strömberg NO, Gustafsson PM. Ventilation inhomogeneity assessed by nitrogen washout and ventilation-perfusion mismatch by capnography in stable and induced airway obstruction. *Pediatr Pulmonol* 2000; 29: 94–102.
10 Fuchs SI, Junge S, Ellemunter H, *et al.* Calculation of the capnographic index based on expiratory molar mass-volume-curves: a suitable tool to screen for cystic fibrosis lung disease. *J Cyst Fibros* 2013; 12: 277–283.
11 Almeida-Junior A, Marson FAL, Almeida CCB, *et al.* Volumetric capnography versus spirometry for the evaluation of pulmonary function in cystic fibrosis and allergic asthma. *J Pediatr (Rio J)* 2020; 96: 255–264.
12 Neufeld GR, Schwardt JD, Gobran SR, *et al.* Modelling steady state pulmonary elimination of He, SF_6 and CO_2: effect of morphometry. *Respir Physiol* 1992; 88: 257–275.
13 Schwardt JD, Gobran SR, Neufeld GR, *et al.* Sensitivity of CO_2 washout to changes in acinar structure in a single-path model of lung airways. *Ann Biomed Eng* 1991; 19: 679–697.
14 Ream RS, Schreiner MS, Neff JD, *et al.* Volumetric capnography in children. Influence of growth on the alveolar plateau slope. *Anesthesiology* 1995; 82: 64–73.
15 Schmalisch G. Current methodological and technical limitations of time and volumetric capnography in newborns. *Biomed Eng Online* 2016; 15: 104.
16 Korten I, Kieninger E, Yammine S, *et al.* The Swiss Cystic Fibrosis Infant Lung Development (SCILD) cohort. *Swiss Med Wkly* 2018; 148: w14618.
17 Fuchs O, Latzin P, Kuehni CE, *et al.* Cohort profile: the Bern infant lung development cohort. *Int J Epidemiol* 2012; 41: 366–376.
18 Yammine S, Nyilas S, Casaulta C, *et al.* Function and ventilation of large and small airways in children and adolescents with inflammatory bowel disease. *Inflamm Bowel Dis* 2016; 22: 1915–1922.
19 Nyilas S, Bauman G, Pusterla O, *et al.* Ventilation and perfusion assessed by functional MRI in CF patients: reproducibility in comparison to lung function. *J Cyst Fibros* 2019; 18: 543–550.
20 Frauchiger BS, Binggeli S, Yammine S, *et al.* Longitudinal course of clinical lung clearance index in children with cystic fibrosis. *Eur Respir J* 2020; 24: 2002686.
21 Frauchiger BS, Carlens J, Herger A, *et al.* Multiple breath washout quality control in the clinical setting. *Pediatr Pulmonol* 2021; 56: 105–112.
22 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: 507–522.
23 Fowler WS. Lung function studies. II: the respiratory deadspace. *Am J Physiol* 1948; 154: 405–416.
24 Anthonisen NR, Fleetham JA. Ventilation: total, alveolar and dead space. *In:* Fishman AP, Farhi LE, Tenney SM, Geiger SR, eds. Handbook of Physiology: Section 3: The Respiratory System. Volume IV: Gas Exchange. Bethesda, American Physiological Society, 1987; pp. 113–129.
25 Piiper J, Scheid P. Diffusion and convection in intrapulmonary gas mixing. *In:* Fishman AP, Farhi LE, Tenney SM, Geiger SR, eds. Handbook of Physiology: Section 3: The Respiratory System. Volume IV: Gas Exchange. Bethesda, American Physiological Society, 1987; pp. 51–69.
26 Aitken RS, Clark-Kennedy AE. On the fluctuation in the composition of the alveolar air during the respiratory cycle in muscular exercise. *J Physiol* 1928; 65: 389–411.
27 Veronez L, Moreira MM, Soares ST, *et al.* Volumetric capnography for the evaluation of pulmonary disease in adult patients with cystic fibrosis and noncystic fibrosis bronchiectasis. *Lung* 2010; 188: 263–268.

28 Gustafsson PM, Aurora P, Lindblad A. Evaluation of ventilation maldistribution as an early indicator of lung disease in children with cystic fibrosis. *Eur Respir J* 2003; 22: 972–979.
29 Aurora P, Gustafsson P, Bush A, *et al.* Multiple breath inert gas washout as a measure of ventilation distribution in children with cystic fibrosis. *Thorax* 2004; 59: 1068–1073.
30 Wyler F, Oestreich MH, Frauchiger BS, *et al.* Correction of sensor crosstalk error in Exhalyzer D multiple-breath washout device significantly impacts outcomes in children with cystic fibrosis. *J Appl Physiol (1985)* 2021; 131: 1148–1156.