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Novel volumetric capnography indices measure ventilation inhomogeneity in cystic fibrosis

Sotirios Fouzas^{1*}, Anne-Christianne Kentgens^{2*}, Olga Lagiou¹, Bettina Sarah Frauchiger², Florian Wyler², Ilias Theodorakopoulos¹, Sophie Yammine², Philipp Latzin²

- ^{1.} Paediatric Respiratory Unit, University Hospital of Patras, Patras, Greece
- ^{2.} Division of Paediatric Respiratory Medicine and Allergology, Department of Paediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland

*Those authors contributed equally to this work

Corresponding author: Philipp Latzin

Hospital name: Inselspital, University of Bern,

Address: Freiburgstrasse 18, 3010 Bern, Switzerland

Email: Philipp.Latzin@insel.ch

Take home message: Novel volumetric capnography indices are promising markers of ventilation inhomogeneity in patients with cystic fibrosis.

1. Introduction

Volumetric capnography (VCap) is a simple and non-invasive 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*, that is characterized 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", that 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 (S_{III}) is considered an index of ventilation inhomogeneity (VI) 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 VI, 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 VI 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.

2. Methods

2.1 Study design and population

In this pilot study, novel VCap indices were validated using existing Nitrogen (N₂) MBW data from 4-18-year-old CF patients and healthy controls. All measurements were obtained at the University Children's Hospital of Bern, Switzerland, between 2013-2019 (see sample size estimation, below) [16-21]. All subjects were free from pulmonary exacerbations [16-21]. Only subjects with at least two good quality MBW trials were included. Local ethic committees approved all studies, and participants or caregivers provided informed written consent.

2.2 Lung function

MBW trials were performed with the children in sitting position while breathing normally, according to international guidelines [22]. N₂-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 CO₂ sensor (Capnostat, infrared single beam, dual-wavelength technology; rise time <60ms) with Spiroware version 3.1.6 or 3.2.1. All data were reloaded and reanalyzed using the updated software provided by the manufacturer Spiroware 3.3.1. 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.

2.3 Volumetric capnography

Volumetric capnograms were obtained from N₂-MBW trials after CO₂ signals were corrected for setup-dependent signal alignment and sensor-specific delays (Spiroware software). Breath-by-breath volumetric capnograms were obtained by plotting the CO₂ fraction against the corresponding V_E. The expired CO₂ volume (V_ECO₂) was calculated by integrating the CO₂ signal over V_E. The slope of phase II (S_{II}) and the S_{III} were obtained by fitting a linear regression line (least squares method) over the capnogram, between 10 and 60% of the end-tidal CO₂ value and 65-95% of V_E, respectively [6]. The capnographic index (KPI_{IV}) was calculated as the ratio S_{III}/S_{II}. The slopes were normalized by the corresponding mixed expired CO₂ fraction (F_ECO₂), which equals the V_ECO₂ 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 CO₂ (ETCO₂) was <3.5%; 2. their corresponding V_E deviated more than two SDs from trial average; 3. V_D was <10% or >30% of V_E; 4. intersection of S_{II} and S_{III} was not calculable, fell outside the capnogram range (i.e. intersection at V_E<0, V_E>V_E at the end of expiration, CO₂>CO₂max), or below the CO₂ curve; 5. S_{III} was steeper than S_{II}; 6. coefficient of determination (R²) for S_{III} fitting was <0.7. For all acceptable breaths of each MBW trial, we calculated average ETCO₂, S_{II}, S_{III}, normalized S_{II} (nS_{II}), normalized S_{III} (nS_{III}), KPI_{IV}, and V_D values. Calculation of VCap indices and quality control were performed using a custom Python script (Python Software Foundation, <https://www.python.org/>).

2.4 Capnographic Inhomogeneity Indices

2.4.1 Modeling 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 of 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 FACO_2 , which is the average CO_2 concentration of alveolar sub-compartments (i.e., FA1CO_2 , FA2CO_2 and FA3CO_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 (FECO_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 FDCO_2 (Figure 1).

In an ideal lung, FA1CO_2 , FA2CO_2 and FA3CO_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, FDCO_2 at end-expiration equals FACO_2 (Figure 1A). When time-dependent inhomogeneities exist, due to stratified $\dot{V}$ distal to the airway-alveolar interface and delayed emptying of respiratory units with low ventilation-perfusion ($\dot{V}/Q$) ratios [24, 25], it applies that $\text{FA1CO}_2 < \text{FA2CO}_2 < \text{FA3CO}_2$ (Figure 1B). In this case, the rate of exhaled CO_2 increases as expiration commences, and the S_{III} rises (Figure 1B). At end-expiration, FDCO_2 equals FA3CO_2 (Figure 1B). The magnitude of sequential inhomogeneity in this model is reflected by the difference among FA1CO_2 , FA2CO_2 and FDCO_2 .

(Figure 1B). However, since neither $FACO_2$ nor $FDCO_2$ can be measured *in vivo*, these differences cannot be computed.

2.4.2 Estimating $FDCO_2$

Let us consider the volumetric capnogram of Figure 2A and analyze 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 ($VDCO_2$) 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 $FDCO_2$ is the area *befc* divided by V_D . b) The total volume of CO_2 leaving the alveolar compartment ($VACO_2$) is the area of the trapezoid *aefd*; $FACO_2$ 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 S_{III}) by V_D . The $VECO_2$ was computed as the integral of the CO_2 signal over V_E , and the $VDCO_2$ as the integral of the extended part of the capnogram over V_D (Figure 1B); the $VACO_2$ was computed as the sum $VECO_2$ and $VDCO_2$. Then, by dividing $VECO_2$ by V_E , $VDCO_2$ by V_D , and $VACO_2$ by V_E , the $FECO_2$, $FDCO_2$ and $FACO_2$, respectively, were calculated. In addition, the concentration of CO_2 in the exhaled air coming exclusively from the alveolar compartment ($F_{ex}CO_2$) was calculated by dividing $VECO_2$ by the difference $V_E - V_D$.

2.4.3 Capnographic inhomogeneity indices

Two novel CIIs were calculated:

- A. CII1, which is the relative difference between $FDCO_2$ and $FACO_2$ or $(FDCO_2 - FACO_2) / FACO_2$ and represents a raw estimate of sequential inhomogeneity.

B. CII₂, which is the relative difference between FDCO₂ and FexCO₂ or $(FDCO_2 - FexCO_2) / FexCO_2$.

Since CII₂ reflects the difference of CO₂ concentration between the alveolar sub-compartments A1+A2 and A3 (Figure 1B), it should theoretically represent a more precise estimate of sequential VI.

2.5 Statistics

2.5.1 Sample size estimation

Since CII_s were introduced for the first time in clinical practice, no data were available for *a priori* sample size estimation. Based on the hypothesis that CII_s (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 $r \geq 0.3$ 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.

2.5.2 Statistical analyses

VCap indices were assessed both per subject (average 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 supplement (OLS). Between-group comparisons were performed using student's *t* test. The percentage of breaths acceptable for VCap analysis was calculated as percentage of all washout breaths. Linear and non-linear regression was applied to assess the relationship between SIII

and VE, and SIII and CII_s. 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 (between different trials of the same subject) variability was calculated using the coefficient of variation (CV). In presence of only two acceptable MBW, 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 cutoff values for each index were determined using Youden Index analysis. All analyses were performed in Stata (StataCorp, College Station, TX).

3. Results

All 320 MBW-trials from 50 patients with CF (137 trials) and 65 HCs (183 trials) were analyzed. 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 vs 6.2±0.4, $P<0.001$).

3.1 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)% vs 63.5±19.3 (19.7-96.7)%). Further information regarding non-accepted breaths (percentages, stratification per exclusion criteria) is presented in Table S2. Slope III and KPI_v were higher in CF patients compared with healthy children (SIII 2.3±1.0%/L vs 1.9±0.7%/L, $P=0.013$; KPI_v 3.9±1.3% vs 3.5±1.2%, $P=0.07$), but only SIII was significantly higher. The CII_s were also significantly higher in CF patients compared with healthy controls (CII₁ 5.9±1.4% vs 5.1±1.0%,

$P=0.002$; CII2 $7.7 \pm 1.8\%$ vs $6.8 \pm 1.4\%$, $P=0.002$). 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 Table 2, Table 3, and Figure 3. AUC values and the corresponding optimal cutoff values of LCI and VCap indices are presented in the OLS (Table S5). Above the cutoff value of LCI were classified 76% ($n=38$) of patients with CF and 10.8% ($n=7$) of controls, above the cutoff value of SIII 34% ($n=17$) of patients with CF and 10.8% ($n=7$) of controls, above the cutoff value of KPIV 76% ($n=38$) of patients with CF and 47.7% ($n=31$) controls, above the cutoff value of CII1 44% ($n=22$) of patients with CF and 20% ($n=13$) of controls, and above the cutoff value of CII2 48% ($n=24$) of patients with CF and 18.5% ($n=12$) of controls.

3.2 Correlations of VCap indices

There was a strong inverse curvilinear relationship between SIII and VE and a weak linear relationship between CII1 and SIII and CII2 and SIII (Figure 4, Figure S3). The correlations between classical VCap indices and CIIs are shown in the OLS (Table S6). The correlation SIII-LCI and 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 -value < 0.001). More correlations between VCap parameters and LCI are presented in Table 4 and 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).

3.3 Intra- and inter-trial variability

The intra-trial variability CVs of S_{III} and $KPIv$ were higher compared with CII_1 and CII_2 (CV of S_{III} $37.5 \pm 19.2\%$, $KPIv$ $35.3 \pm 15.5\%$ vs CII_1 $31.1 \pm 8.9\%$ and CII_2 $31.7 \pm 9.4\%$, in all trials). For all these VCap indices, the intra-trial variability was lower in CF patients compared with controls (Table S8). Similarly, the inter-trial (intra-subject) variability of S_{III} and $KPIv$ was higher compared with CII_1 and CII_2 in all trials (S_{III} $16.3 \pm 13.5\%$, $KPIv$ $15.9 \pm 12.8\%$ vs CII_1 $11.1 \pm 8.2\%$ and CII_2 $11.0 \pm 8.0\%$). However, the inter-trial variability was comparable between CF patients and controls (Table S9). The LCI showed lowest inter-trial variability (LCI $5.9 \pm 4.2\%$) (Table S9, Figure S2).

4. Discussion

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

4.1 Performance of VCap indices

As expected, S_{III} and $KPIv$ were increased in CF patients. These findings are in line with previous studies, suggesting that classical VCap indices may be useful VI markers in adults and children with CF [8-11, 27]. In CF, VI 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 S_{III} to S_{II} ratio) increases respectively [8]. Thus, increased S_{III} 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 S_{III} and LCI or KPI_v 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 S_{II} and S_{III} on an “averaged”, user-defined capnogram in their study [10], as opposed to breath-by-breath calculation of VCap indices using well-defined criteria in ours, might explain these differences.

Conversely, CII_1 and CII_2 presented better discriminative characteristics than the classical VCap indices. In addition, both CII s correlated significantly with the LCI - a robust index of VI in CF patients [28, 29], and did so better than the classical VCap parameters (Table 4). Thus, CII s may be considered valid measures of VI, which may also relate to the severity of the disease.

4.2 Theoretical advantages of CII s over classical VCap indices

The S_{III} reflects the rate of CO_2 exhalation beyond mid-expiration and is an unstandardized index that depends on the dynamics of expiration, especially on VE [12-14]. However, normalization of S_{III} by VE [11] is not justified, because as shown in previous studies [12-14] and confirmed in ours (Figure 4A, Figure S3A), the S_{III} - VE relationship is not linear. S_{III} normalization by the $FECO_2$ (i.e. the normalized S_{III}) may allow for intra- or inter-subject adjustment for different CO_2 concentrations, but does not eliminate the dependency from VE . Therefore, in subjects with variable respiratory patterns and/or changing lung volumes, as it typically is in children, the utility of S_{III} is limited [14]. In our study, this disadvantage is also reflected by the unexpected negative correlation between S_{III} 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 S_{III} is lower due to the strong S_{III} - VE relationship (Figure 4A, Figure S3A).

The theoretical background for calculation of CII is different: in lung disease, variable gas mixing within the respiratory units (serial inhomogeneities) and/or regional $\dot{V}/Q$ 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 $\dot{V}/Q$ [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 CII, 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. $\text{VCO}_2 - \text{VE}$ fractions) also include an inherent normalization for VE and their use may thus overcome the limitations of classical VCap indices. The significant positive correlation between CII and LCI and the weak correlation between CII and S_{III} further support this hypothesis.

4.3 Feasibility and repeatability of CII

CII calculation was feasible in all MBW trials, independent of disease status. The percentage of acceptable breaths for VCap analysis was approximately 75% in CF and 65% in control trials. We found lower variability in CII compared with S_{III} and KPI_{IV} , 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.

4.4 Strengths and limitations

This study presents an innovative method for VI 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 CIIIs showed a good association with the more complicated LCI, thus confirming their clinical potential. The use of N₂-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 analyzed 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, Posteriori effect size calculations yielded study power in the range of 68-100% for the differences between CIIIs in CF and controls and the Pearson's correlation coefficients between CIIIs and LCI.

Inevitably, our study also has some limitations. First, VCap analysis was performed retrospectively using previously collected N₂-MBW data from one center. 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 CIIIs (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 CIIIs and increase their diagnostic performance. Second, the effect of 100% O₂ concentration (N₂-MBW technique) on VCap parameters is unknown. However, the effect, if any, would not be different between CF and controls.

Finally, CIIIs 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 VD [23], where the capnogram is extended "to the left".

4.5 Clinical implications

Volumetric capnography is a simple non-invasive technique that does not require complex respiratory maneuvers, exogenous gases, extensive signal processing or operator's expertise [2,5,7]. Current evidence suggests that classical VCap indices, such as S_{III} 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 CIIIs 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 CIIIs 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.

5. Conclusions

In conclusion, the herein introduced CIIIs performed better than the classical VCap parameters in detecting VI in CF patients. The CIIIs 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 VI. 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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Conflicts of interest:

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Table 1. Characteristics and lung function of the study groups

	Cystic Fibrosis (n=50)	Healthy Controls (n=65)
<i>General characteristics</i>		
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)
<i>N₂-MBW</i>		
Number of trials	137	183
Tidal volume (mL)	402.9 (175.8)	437.4 (199.0)
Tidal volume per kg (ml/kg)	12.7 (2.8)	12.0 (2.7)
Respiratory rate (per minute)	18.7 (4.1)	18.2 (4.8)
Minute ventilation (ml/kg x min)	231.2 (46.0)	215.3 (67.6)
FRC (ml/kg)	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

* Statistically significant difference (P<0.05 using the Student's T test)

Weight, height and BMI z-scores were calculating according WHO growth charts. Weight z-scores for children older than 10 years of age were calculated according to CDC growth charts.

N₂-MBW: nitrogen multiple breath washout, FRC: functional residual capacity, LCI: lung clearance index, CDC: Centers for Disease Control and Prevention (CDC)

Table 2. VCap parameters and indices

	Cystic Fibrosis (n= 50)	Healthy Controls (n=65)	Mean difference (95% confidence interval)
VE (mL)	402.9 (174.6)	438.7 (201.6)	35.7 (-35.2 to 106.7)
VE per kg (mL/kg)	12.7 (3.1)	12.1 (2.7)	-0.7 (-1.8 to 0.4)
VD (mL)	88.6 (27.5)	98.3 (34.3)	9.8 (-2.0 to 21.5)
VD per kg (mL/kg)	2.9 (0.5)	2.8 (0.5)	-0.1 (-0.3 to 0.1)
VD % of VE (%)	23.7 (4.9)	23.9 (4.3)	0.2 (-1.5 to 1.9)
ETCO ₂ (%)	5.3 (0.5)	5.3 (0.5)	0.0 (-0.1 to 0.2)
VCO ₂ (mL)	16.3 (5.9)	18.2 (7.9)	1.9 (-0.7 to 4.6)
FECO ₂ (%)	3.9 (0.4)	4.0 (0.4)	0.0 (-0.1 to 0.2)
SII (%/L)	59.3 (19.2)	54.5 (16.8)	-4.8 (-11.5 to 1.9)
Normalized SII (1/L)	15.0 (4.2)	13.7 (3.9)	-1.2 (-2.7 to 0.3)
SIII (%/L)	2.3 (1.0)	1.9 (0.7)	-0.4 (-0.7 to -0.1)*
Normalized SIII (1/L)	0.6 (0.3)	0.5 (0.2)	-0.1 (-0.2 to 0.0)*
KPIv (%)	3.9 (1.3)	3.5 (1.2)	-0.4 (-0.9 to 0.0)

Data are presented as mean (SD), unless stated otherwise

* Statistically significant difference (P<0.05 using the Student's T test)

VCap: volumetric capnography, VE: expiratory volume, VD: dead space volume, ETCO₂: end-tidal CO₂ fraction, VCO₂: expired CO₂ volume, FECO₂: mixed expired CO₂ fraction, SII: slope of phase II, SIII: slope of phase III, KPIv: capnographic index

Table 3. Novel VCap parameters and CIIs

	Cystic Fibrosis (n=50)	Healthy Controls (n=65)	Mean difference (95% confidence interval)
FACO ₂ (%)	5.1 (0.5)	5.2 (0.5)	0.1 (-0.1 to 0.2)
FDCO ₂ (%)	5.4 (0.5)	5.5 (0.5)	0.0 (-0.1 to 0.2)
FexCO ₂ (%)	5.1 (0.5)	5.1 (0.5)	0.1 (-0.1 to 0.2)
CII1 (%)	5.9 (1.4)	5.1 (1.0)	-0.7 (-1.2 to -0.3)*
CII2 (%)	7.7 (1.8)	6.8 (1.4)	-1.0 (-1.5 to -0.3)*

Data are presented as mean (SD), unless stated otherwise

* Statistically significant difference (P<0.05 using the Student's T test)

VCap: volumetric capnography, FACO₂: CO₂ fraction in alveolar compartment, FDCO₂: CO₂ fraction in dead space compartment, FexCO₂: CO₂ fraction in the air expired from the alveolar compartment, CII: capnographic inhomogeneity index (see text for details)

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
Normalized 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
Normalized 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

Dara are presented as Pearson correlation coefficients (r) and coefficients of determination (R²)

* Student's T test

VCap: volumetric capnography, LCI: lung clearance index, SII: slope of phase II, SIII: slope of phase III,

KPIv: capnographic index, CII: capnographic inhomogeneity index

Novel volumetric capnography indices measure ventilation inhomogeneity in cystic fibrosis

ONLINE SUPPLEMENT

Sotirios Fouzas^{1*}, Anne-Christianne Kentgens^{2*}, Olga Lagiou¹, Bettina Sarah Frauchiger², Florian Wyler², Ilias Theodorakopoulos¹, Sophie Yammine², Philipp Latzin²

- ^{1.} Paediatric Respiratory Unit, University Hospital of Patras, Patras, Greece
- ^{2.} Division of Paediatric Respiratory Medicine and Allergology, Department of Paediatrics, Inselspital, Bern University Hospital, University of Bern, Switzerland

*Those authors contributed equally to this work

Corresponding author: Philipp Latzin

Hospital name: Inselspital, University of Bern,

Address: Freiburgstrasse 18, 3010 Bern, Switzerland

Email: Philipp.Latzin@insel.ch

	Cystic Fibrosis (n=137)	Healthy Control (n=183)	Mean differences (95% confidence interval)
Tidal volume, mL	390.8 (173.9)	424.8 (203.6)	33.9 (-8.6 to 76.5)
Tidal volume per kg, mL/kg	12.5 (2.8)	12.0 (2.9)	-0.5 (-1.1 to 0.1)
Respiratory rate, per minute	18.9 (4.2)	18.6 (5.1)	-0.4 (-1.4 to 0.7)
Minute ventilation, mL/kg x min	232.0 (46.1)	219.8 (68.7)	-12.1 (-25.5 to 1.2)
FRC, mL/kg	39.9 (8.0)	40.9 (8.8)	1.0 (-0.9 to 2.9)
Mean LCI2.5%	7.8 (1.7)	6.2 (0.5)	-1.6 (-1.8 to -1.3)*

Table S1. Basic Lung function characteristics and the Lung Clearance Index 2.5% in Cystic Fibrosis and healthy control (analysis per trial)

Data are presented as mean (SD), unless stated otherwise

* Statistically significant difference ($P < 0.05$ using the Student's T test)

FRC: functional residual capacity, LCI: lung clearance index

	Low ETCO ₂	VE outlier	VD outlier	Intersection was not calculable	SII-SIII Intersection outside the capnogram range	SII-SIII intersection below CO ₂ curve	SIII steeper than SII	R ² fit SIII <0.7	All error
Cystic Fibrosis	1.0 (1.7)	4.3 (2.0)	13.5 (14.2)	0.6 (2.0)	0.0 (0.3)	0.3 (1.1)	0.1 (0.5)	4.5 (7.5)	24.4 (16.5)
Healthy Control	1.2 (2.6)	4.4 (2.1)	15.3 (16.3)	0.5 (1.4)	0.1 (0.5)	0.3 (1.2)	0.1 (0.3)	13.0 (17.6)	34.8 (19.2)
All trials	1.1 (2.3)	4.4 (2.0)	14.5 (15.4)	0.5 (1.7)	0.1 (0.4)	0.3 (1.1)	0.1 (0.4)	9.3 (14.8)	30.4 (18.8)

Table S2. N₂-MBW breaths that met one of the exclusion criteria as a proportion of the total breaths within a trial (%) (analysis per trial).

Meeting one exclusion criteria lead to direct exclusion and no further evaluation of other exclusion criteria.

Data are presented as mean (SD), unless stated otherwise

ETCO₂: end-tidal CO₂ fraction, VE: expiratory volume, VD: dead space volume, SII: slope of phase II, SIII: slope of phase III

	Cystic Fibrosis (n=137)	Healthy Control (n=183)	Mean differences (95% confidence interval)
VE (mL)	390.6 (173.1)	426.0 (206.5)	35.4 (-7.5 to 78.3)
VE per kg (mL/kg)	12.6 (3.0)	12.1 (3.0)	-0.50 (-1.2 to 0.2)
VD (mL)	86.9 (26.0)	95.9 (33.5)	9.0 (2.2 to 15.8)*
VD per kg (mL/kg)	2.9 (0.5)	2.8 (0.5)	-0.1 (-0.2 to 0.0)
VD % of VE (%)	23.9 (5.2)	24.0 (4.9)	0.10 (-1.0 to 1.2)
ETCO ₂ (%)	5.3 (0.5)	5.3 (0.5)	0.0 (-0.1 to 0.1)
VCO ₂ (mL)	15.9 (5.8)	17.7 (8.1)	1.8 (0.2 to 3.4)*
FECO ₂ (%)	3.9 (0.4)	3.9 (0.4)	0.0 (-0.1 to 0.1)
SII (%/L)	60.3 (19.7)	55.4 (16.9)	-4.9 (-8.9 to -0.8)*
Normalized SII (1/L)	15.2 (4.2)	14.1 (3.9)	-1.1 (-2.0 to -0.2)*
SIII (%/L)	2.3 (1.0)	1.9 (0.8)	-0.4 (-0.6 to -0.2)*
Normalized SIII (1/L)	0.6 (0.3)	0.5 (0.2)	-0.1 (-0.2 to 0.0)*
KPIv (%)	3.9 (1.4)	3.5 (1.3)	-0.4 (-0.7 to -0.1)

Table S3. Basic classical capnographic lung function characteristics in Cystic Fibrosis and healthy control (analysis per trial)

Data are presented as mean (SD), unless stated otherwise

* Statistically significant difference (P<0.05 using the Student's T test)

VE: expiratory volume, VD: dead space volume, ETCO₂: end-tidal CO₂ fraction, VCO₂: expired CO₂ volume, FECO₂: mixed expired CO₂ fraction, SII: slope of phase II, SIII: slope of phase III, KPIv: capnographic index

	Cystic Fibrosis (n=137)	Healthy Control (n=183)	Mean difference (95% confidence interval)
FACO ₂ (%)	5.2 (0.5)	5.2 (0.5)	0.0 (−0.1 to 0.1)
FDCO ₂ (%)	5.4 (0.5)	5.4 (0.5)	0.0 (−0.1 to 0.1)
FexCO ₂ (%)	5.1 (0.5)	5.1 (0.5)	0.0 (−0.1 to 0.1)
CII1 (%)	5.8 (1.5)	5.1 (1.1)	−0.7 (−1.0 to −0.5)*
CII2 (%)	7.7 (1.9)	6.7 (1.5)	−1.0 (−1.3 to −0.6)*

Table S4. Novel capnographic inhomogeneity indices in Cystic Fibrosis and healthy control

(analysis per trial)

Data are presented as mean (SD), unless stated otherwise

* Statistically significant difference ($P < 0.05$ using the Student's T test)

FACO₂: CO₂ fraction in alveolar compartment, FDCO₂: CO₂ fraction in dead space compartment,

FexCO₂: CO₂ fraction in the alveolar expirate, CII: capnographic inhomogeneity index (see text for details)

	AUC (95% confidence Interval)	Optimal Cutoff	Sensitivity at optimal cutoff	Specificity at optimal cutoff
LCI	0.87 (0.80 to 0.94)	6.68	0.76	0.89
SIII	0.62 (0.51 to 0.73)	2.75	0.36	0.89
KPIv	0.61 (0.50 to 0.71)	3.16	0.76	0.52
CII1	0.65 (0.55 to 0.75)	6.02	0.46	0.80
CII2	0.65 (0.55 to 0.75)	7.98	0.48	0.82

Table S5. ROC analysis (analysis per subject)

ROC: Receiver operator characteristic, AUC: area under the curve, LCI: lung clearance index, SIII: slope of phase III, KPIv: capnographic index, CII: capnographic inhomogeneity index. Optimal Cutoffs were determined using the Youden Index analysis.

Table S6. Correlations between classical VCap indices and CII (analysis per trial)

	Cystic Fibrosis				Healthy Controls		
	SII	SIII	KPIv		SII	SIII	KPIv
CII1	-0.429 (-0.184)	0.142 (0.020)	0.595 (0.354)		-0.515 (-0.265)	0.120 (0.014)	0.555 (0.308)
CII2	-0.385 (0.148)	0.284 (0.081)	0.747 (0.558)		-0.465 (-0.217)	0.282 (0.079)	0.718 (0.516)

Data are Pearson correlation coefficients with R² (in parentheses)

SII: slope of phase II, SIII: slope of phase III, KPIv: capnographic index, CII: capnographic inhomogeneity index

	Cystic Fibrosis			Healthy Control trials		
	r	R ²	P-value*	r	R ²	P-value*
SII	-0.48	0.23	<0.001	-0.07	0.01	0.318
Normalized SII	-0.42	0.17	<0.001	-0.04	0.00	0.590
SIII	-0.13	0.02	0.129	0.03	0.00	0.660
Normalized SIII	-0.02	0.00	0.774	0.05	0.00	0.520
KPIv	0.32	0.10	0.000	0.12	0.01	0.100
CII1	0.65	0.42	<0.001	0.06	0.00	0.425
CII2	0.64	0.41	<0.001	0.11	0.01	0.146

Table S7. Correlation between VCap indices and LCI (analysis per trial)

Dara are presented as Pearson correlation coefficients (r) and coefficients of determination (R²)

*Student's T-test

VCap: volumetric capnography, LCI: lung clearance index, SII: slope of phase II, SIII: slope of phase III,

KPIv: capnographic index, CII: capnographic inhomogeneity index

	Cystic Fibrosis Trials (n=137)	Healthy Control Trials (n=183)	All Trials (n=320)
SII	12.6 (16.4)	10.5 (5.5)	11.4 (11.5)
Normalized SII	12.0 (14.0)	10.9 (4.9)	11.4 (9.9)
SIII	34.4 (19.0)*	39.9 (19.0)*	37.5 (19.2)
Normalized SIII	37.0 (21.7)*	43.2 (22.0)*	40.6 (22.0)
KPIv	31.6 (13.0)*	38.1 (16.6)*	35.3 (15.5)
CII1	29.4 (8.4)*	32.4 (9.1)*	31.1 (8.9)
CII2	29.7 (8.7)*	33.2 (9.7)*	31.7 (9.4)

Table S8. Intra-trial variability of VCap indices (analysis per trial)

Data are presented as mean coefficient of variation (SD)%

* Statistically significant difference between Cystic Fibrosis and Healthy Control Trials

(P<0.05 using the Student's T test)

VCap: volumetric capnography, SII: slope of phase II, SIII: slope of phase III, KPIv: capnographic index,
CII: capnographic inhomogeneity index

	Cystic Fibrosis Trials (n=137)	Healthy Control Trials (n=183)	All Trials (n=320)
SII	8.4 (7.6)	8.6 (6.7)	8.5 (7.1)
Normalized SII	7.0 (7.4)	6.4 (5.3)	6.6 (6.2)
SIII	14.0 (12.3)	18.1 (14.2)	16.3 (13.5)
Normalized SIII	14.7 (12.7)	19.0 (14.8)	17.2 (14.0)
KPIv	14.8 (11.7)	16.8 (13.7)	15.9 (12.8)
CII1	10.5 (7.9)	11.6 (8.5)	11.1 (8.2)
CII2	10.7 (7.4)	11.3 (8.4)	11.0 (8.0)

Table S9. Inter-trial (intra-subject) variability of VCap indices

Data are presented as mean coefficient of variation (SD)%

No statistically significant difference between Cystic Fibrosis and Healthy Control Trials

($P < 0.05$ using the Student's T test)

VCap: volumetric capnography, SII: slope of phase II, SIII: slope of phase III, KPIv: capnographic index,

CII: capnographic inhomogeneity index

Figure S1. Boxplots showing the difference between Cystic Fibrosis and healthy control in mean values of CII I and II, LCI, SIII and KPIV (analysis per trial).

The individual black dots represent mean values per trial.

CII: capnographic inhomogeneity index, LCI: lung clearance index, SIII: slope of phase III, KPIV: capnographic index.

Figure S2. Inter-trial (intra-subject) variability) of LCI, CII I and CII II in patients with CF (black dots) and healthy controls (white dots).

The inter-trial (intra-subject) variability was calculated as the coefficient of variation in presence of three MBW trials, or as the relative difference in presence of two MBW trials. The red and green bars symbolize the mean inter-trial (intra-subject) variability for patients with CF and healthy controls respectively.

LCI: lung clearance index, CII: capnographic inhomogeneity index, CF: cystic fibrosis, MBW: Multiple Breath Washout.

Figure S3. Relationship between SIII and VE (A), CII1 and SIII (B) and CII2 and SIII (C) in CF (black dots) and healthy control trials (open dots) (analysis per trial). CF: cystic fibrosis, SIII: slope of phase III, VE: expiratory volume, CII: capnographic inhomogeneity index.