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# Single-breath washout and association with structural lung disease in children with cystic fibrosis[1]

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## Conflict of interest

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**Single-breath washout and chest CT in children with CF**

**ABSTRACT 225/250**

**Background** In children with cystic fibrosis (CF) lung clearance index (LCI) from multiple-breath washout (MBW) correlates with structural lung disease. As a shorter test, single-breath washout (SBW) represents an attractive alternative to assess the ventilation distribution, however data for the correlation with lung imaging are lacking.

**Methods** We assessed correlations between phase III slope (SIII) of double-tracer gas SBW, nitrogen MBW indices (LCI and moment ratios for overall ventilation distribution, Scond and Sacin for conductive and mainly acinar ventilation, respectively) and structural lung disease assessed by chest computed tomography (CT) in children with CF.

**Results** In a prospective cross-sectional study data from MBW, SBW and chest CT were obtained in 32 children with CF with a median (range) age of 8.2 (5.2 – 16.3) years. Bronchiectasis was present in 24 (75%) children and air trapping was present in 29 (91%). Median (IQR) SIII of SBW was -138.4 (150.6) mg/mol. We found no association between SIII with either the MBW outcomes or CT scores (n = 23, association with bronchiectasis extent r = 0.10, p = 0.64). LCI and Scond were associated with bronchiectasis extent (n = 23, r = 0.57, p = 0.004; r = 0.60, p = 0.003, respectively).

**Conclusions** Acinar ventilation inhomogeneity measured by SBW was not associated with structural lung disease on CT. Double-tracer SBW added no benefit to indices measured by MBW.



## INTRODUCTION

Structural lung abnormalities begin early in life in children with cystic fibrosis (CF) and progress rapidly into irreversible lung damage [1,2]. Currently, chest computed tomography (CT) represents the gold standard for assessing the presence and extent of CF-related structural lung disease [1-4]. However, there is great interest to find radiation-free surrogates for the early assessment of CF lung disease.

Among lung function techniques, the multiple-breath washout (MBW) test has gained increasing interest as a sensitive, non-invasive test for assessment of ventilation distribution in children with CF [5-7]. The most commonly reported outcome is the lung clearance index (LCI), calculated as a ratio of the cumulative expired volume needed to wash out a tracer gas, divided by the functional residual capacity. LCI has shown good agreement with structural changes on chest CT in preschool [3] and school-aged children [3,6,8]. Even though MBW is becoming more widely used and better standardized [9], the long duration of the test is a potential drawback for daily clinical use.

One attractive alternative is to study the ventilation distribution based on one single breath, by performing the single-breath washout test (SBW). The most widely established SBW test is based on a vital capacity manoeuvre washing out nitrogen ($N_2$) [10]. A modification of SBW test is performed during tidal breathing and uses a double tracer gas (DTG) mixture of helium (He) and sulphur hexafluoride ($SF_6$) [11,12]. Molar mass of $SF_6$ is much higher than molar mass of He, which leads to differing speeds of diffusion. Thus, after equal distribution during bulk flow in larger convective airways, the DTG mixture separates into He and $SF_6$ in peripheral airways where diffusion dominates as main gas transport mechanism. The use of the DTG mixture aims to provide more specific information about ventilation inhomogeneity in these peripheral lung zones [12]. Main outcome parameter is the slope of the third phase of the expired tracer gas mixture (SIII) which is supposed to come from the peripheral, i.e. alveolar lung zones. The test was validated using commercial equipment [11], and promising results were reported with regards to discrimination between children with CF, mild asthma, primary ciliary dyskinesia, and healthy controls [12-14]. However, the exact underlying anatomical changes assessed by the test remain unclear since data on association with measures of lung structure are missing.

The aim of this study was to assess the correlation between DTG-SBW and CF-related structural lung disease measured by chest CT, the gold standard in children with CF. We hypothesised that SIII would be associated with structural abnormalities visualized by CT.

## METHODS

*Study design and population*

The study was performed as a prospective cross-sectional substudy of the AREST CF follow up. Children enrolled in the AREST CF research program undergoing annual surveillance or in regular outpatient visits at the Royal Children's Hospital in Melbourne, and the Princess Margaret Hospital in Perth, Australia, were eligible to take part in this study [1]. During a time period of 17 months children of the AREST CF additionally performed DTG-SBW after $N_2$MBW. For inclusion, successful performance of either two acceptable DTG-SBW or $N_2$MBW trials within ± 14 days of chest CT was necessary. Only successful trials of the first study visit per child were included. The ethics committee of each institution approved the study and the children's parents gave written informed consent to each aspect of the study separately.

*Chest computed tomography*

Spirometry-assisted volumetric chest CT was performed in school-age children (≥ 6.5 years) whereby inspiratory scans were obtained at total lung capacity and expiratory scans were collected at residual volume [3]. In younger preschool children (< 6.5 years), CT was performed under intravenous general anesthesia. Volumetric inspiratory scans were obtained at a positive airway opening pressure of 25 cm $H_2O$, and volumetric or limited slice expiratory scans were obtained at an airway opening pressure of 0 cm $H_2O$, *i.e.* relaxed end-expiratory volume [1,3].

To assess CT-determined structural lung disease, two previously described scoring systems were used; a simplified CF CT scoring method based on a modified Brody score [1,15], and the

quantitative Perth-Rotterdam Annotated Grid Morphometric Analysis for CF (PRAGMA-CF) [3,4]. The CF-CT scoring represents a semi-quantitative extent score for four different disease parameters. For the scoring, each lung was considered in six lobes (with the lingula treated as a separate lobe) for volumetric scans, and zones (upper, mid, and lower; right and left) for limited slice scans. The presence (no = 0, yes = 1) and extent (≤50% of the zone = 1, >50% of the zone = 2) of bronchiectasis (outer-edge bronchus-artery cross-sectional area ratio >1), mucus plugging (high-density airway occlusion or tree-in-bud appearance), bronchial wall thickening (airway walls thicker than or with increased signal density relative to normal airways) on inspiratory scans, and trapped air (geographic low-density regions) on expiratory scans were assessed. Outcome parameters were scores of up to 12 for the total lung for each of the four disease parameters. For PRAGMA-CF, a grid overlaid on 10 equidistant axial slices was annotated hierarchically for the presence of the same four above-mentioned disease parameters. Outcome parameters were the extents of total airway disease (percent disease), bronchiectasis (percent bronchiectasis), and trapped air (percent air trapping) represented as volume proportions of the lung [3,4].

*Washout measurements*

Both DTG-SBW [11,12] and $N_2$MBW measurements [3,7] were performed using a validated and commercially available device (Exhalyzer D system, Eco Medics AG, Duernten, Switzerland) according to consensus guidelines [9]. For data recording, we used Spiroware 3.1.6.

*Double-tracer gas single-breath washout*

To assess acinar ventilation distribution within the timeframe of a single breath, DTG-SBW was applied as described previously [11,12,14]. The tracer gas mixture contained 26.3% helium (He), 5% sulphur hexafluoride (SF6), 21% oxygen (O2) and balance $N_2$. The total molar mass of DTG was equivalent to air. Hence, any detectable changes compared with normally expired

molar mass could be attributed to the tracer gas [11]. At least two acceptable DTG-SBW trials were obtained [9]. The primary outcome was the slope of the third phase (SIII) of the molar mass signal, calculated between 65% and 95% of the expired volume. Steep negative slopes represent abnormal results, *i.e.* of ventilation inhomogeneity arising at the entrance of the acinus. Phase III slopes were adjusted for breathing pattern by multiplication with tidal volume as recommended [9,16]. Signal processing and analyses were performed in Matlab (Lungsim, Matlab R2014a; The Mathworks Inc., Natick, MA, USA) (details attached in the online supplement).

*Multiple-breath nitrogen washout*

$N_2$MBW was performed to assess markers for global and peripheral ventilation distribution [3,7]. For this test the subject breaths 100% $O_2$ to wash out resident $N_2$ gas from the lungs. At least two successful $N_2$MBW trials were obtained with no evidence of leak or irregular breathing pattern [9]. Outcome parameters of global ventilation inhomogeneity included LCI, the first (M1/M0) and the second moment ratio (M2/M0). Specific diffusion-convection-dependent and convection-dependent ventilation inhomogeneity was estimated by Sacin and Scond, both derived from SIII analysis of multiple washout breaths. Sacin was calculated from the first SIII value and reflected ventilation distribution within diffusion-convection-dependent acinar airways. Scond was derived from the subsequent evolution of SIII values from lung turn over 1.5 to 6.0 reflecting ventilation distribution within convection-dependent conducting airways [9]. SIII values were normalised for mean gas concentration over their calculation interval and corrected for tidal volume [16,17]. Signal analysis was performed in Spiroware 3.1.6 (Eco Medics AG).

*Statistics*

The primary objective of this study was to determine the relationships between the outcome from the DTG-SBW, the SIII value, and the presence and extent of structural damage as assessed by chest CT. Secondary objectives were the relationships between the outcome from the $N_2$MBW as established markers of ventilation inhomogeneity and chest CT, and between the outcome from the DTG-SBW and $N_2$MBW. We estimated the sample size n = 26 based on a one-sample correlation test using the Fisher's z test assuming a hypothesized (h0) poor correlation coefficient (r) = 0.10, and an alternative strong r = 0.60, and set alpha = 0.050 and power = 0.80. Relationships between washout outcomes and the chest CT extent scores were explored with Spearman correlations. Subgroups of children were compared by Mann-Whitney test. All analyses were performed in Stata™ (Stata Statistical Software: Release 14. College Station, TX: StataCorp LP) and significance accepted at the level of $p < 0.05$.

**RESULTS**

A total of 32 children with chest CT were enrolled. Median (range) age was 8.2 (5.2 – 16.3) (Table 1). Twenty-six of these children performed at least two successful DTG-SBW trials. In three children the interval between DTG-SBW and CT exceeded 14 days, leaving 23 children for the analysis of relationships between DTG-SBW and chest CT (mean (SD) interval between DTG-SBW and CT 0.43 (1.04) days, range 0-3 days). Twenty-seven children performed at least two successful $N_2$MBW trials, four were excluded with chest CT more than 14 days apart, leaving 23 children for the analysis of relationships between $N_2$MBW and CT (mean (SD) interval 0.70 (1.26) days, range 0-3 days). Twenty-one children remained for assessment of relationships between $N_2$MBW and DTG-SBW. The three subgroups of children for analysis of the different relationships did not differ in age (Table 1).

All children exhibited either bronchiectasis or air trapping on chest CT (Table 1). Based on the Brody scoring, 66% (21/32) of the children had both bronchiectasis and air trapping present, 75% (24/32) had bronchiectasis, and 91% (29/32) air trapping. Mean (SD) disease

extent according to the PRAGMA-CF scoring was 4.3% (4.11) for total disease, 1.7% (2.61) for bronchiectasis, and 15.2% (16.68) for air trapping.

Median (IQR) SIII of DTG-SBW was -138.4 (150.6) mg/mol (Table 1). Published data using the same setup in a group of 48 healthy children, reported mean (SD) SIII values of -88.4 (129.1) mg/mol [12,] which applied to our data results in 38% (10/26) of children with SIII values above the upper limit of normal (mean + 1.96*SD). Median (IQR) LCI of $N_2$MBW was 8.2 (2.3) (Table1). Referring to published data of healthy children measured in the same $N_2$MBW setup [18], we found that 56% (15/27) of our children had abnormal LCI values, 48% (13/27) abnormal M1/M0, 56% (15/27) abnormal M2/M0, 74% (20/27) abnormal Scond and 19% (5/27) abnormal Sacin values. None of the multiple or single breath washout parameters were associated with age in our study population.

SIII was not significantly increased in children with the presence of bronchiectasis (p = 0.88, comparison by Mann-Whitney test) on chest CT (Table 1, Figure 1). There was no correlation between SIII and structural lung changes on chest CT, with either the Brody based scoring system or PRAGMA-CF scoring (Table 2, Figure 2a and 2b). Correlation between SIII and Sacin of $N_2$MBW did not reach statistical significance (r = -0.42, p = 0.055) (Table 3, Figure 3).



There was a significant correlation between the markers of global ventilation inhomogeneity LCI, M1/M0, M2/M0 and bronchiectasis Brody scores (r = 0.59, p = 0.003; r = 0.57, p = 0.005; r = 0.53, p = 0.009 respectively) and percent bronchiectasis in the PRAGMA-CF scoring (r = 0.57, p = 0.004; r = 0.57, p = 0.004; r = 0.55, p = 0.006 respectively) (Table 2). Additionally, LCI was significantly associated with mucus plugging Brody scores (r = 0.44, p = 0.035) and percent total disease in the PRAGMA-CF scoring (r = 0.43, p = 0.041). Scond

exhibited a significant correlation with bronchiectasis Brody scores (r = 0.47, p = 0.023), percent bronchiectasis (r = 0.60, p = 0.003) (Figure 4) and percent total disease in the PRAGMA-CF scoring (r = 0.51, p = 0.012). There were no associations between Sacin and any measures of structural lung damage.

## DISCUSSION

The objective of this study was to determine the association between DTG-SBW and structural lung disease in children with CF. We compared DTG-SBW against chest CT, the current gold standard method to detect structural lung disease in CF, and against MBW as a known monitoring tool for early CF lung disease, which has been shown to correlate with structural lung disease in preschool [3] and school-age children with CF [3,6,8]. In contrast to our hypothesis, we found no correlation between SIII from DTG-SBW, reflecting mainly diffusion-dependent acinar ventilation inhomogeneity and the presence and extent of structural abnormalities visualized by CT. In accordance, Sacin from MBW, which theoretically measures the same peripheral lung region, showed no correlation with structural lung changes. However, LCI and Scond, measuring global and convection-dependent ventilation inhomogeneity respectively, correlated with bronchiectasis and disease extent on chest CT.

SIII from DTG-SBW is theorised to measure the mainly diffusion-dependent ventilation distribution of the acinar lung region [11,12]. This is supported by the fact that in different disease entities with small airway disease such as children with CF, mild asthma, primary ciliary dyskinesia and adults with chronic obstructive pulmonary disease, SIII is elevated compared with healthy controls [12-14,19,20]. However, we found no correlation between SIII and structural lung changes in our children with CF. This seems surprising, particularly in a population of mainly school-age children with a high prevalence of bronchiectasis and abnormal LCI. A recent study by *Ramsey et al* [3], showed correlation of LCI and M2/M0 with

total disease extent and bronchiectasis in school-age children with CF, and a correlation with air trapping that we did not find most probably due to the smaller sample size we had. Furthermore, they showed increasing correlations between LCI and structural lung abnormalities with age, but a constantly low negative predictive value around 50% of LCI to detect bronchiectasis.

While our data support previous studies regarding the ability of LCI to reflect structural lung disease in school-age children with CF [3,6,8], they do not suggest the same for SIII of DTG-SBW. The validity of the SIII as a marker of peripheral ventilation inhomogeneity is supported by the fact that Sacin also showed no correlation with structural disease on chest CT, but did have weak correlation with SIII itself. On the other hand, Scond showed correlations with total disease extent and bronchiectasis, as seen for LCI. If we assume that SIII and Sacin are indeed measures of acinar ventilation distribution [21-23], there are several possible reasons for the lack of associations seen. Since chest CT scans depict approximately the first six airway generations [24] they might not capture all abnormalities in the peripheral airways. Trapped air is the CT outcome that best reflects peripheral airway disease [25], and thus would be the most likely measure to correlate with SIII and Sacin. However, a recent study from our group suggests that so-called trapped air on CT may actually also be the result of perfusion defects, rather than airway obstruction alone [26]. Effective gas exchange depends on well-matched ventilation and perfusion of the alveolar-capillary units. Thus, impaired perfusion might contribute to impaired ventilation, which might subsequently lead to trapped air. There is also evidence that defective cystic fibrosis transmembrane conductance regulator can alter the mechanical properties and cause remodelling of peribronchial vasculature [27]. Functional magnetic resonance imaging (MRI) represents a radiation-free alternative to chest CT and may shed more light on this conundrum [28]. However, lung MRI as a monitoring tool in CF, especially in children, is still relatively new and requires further validation before it can

be used routinely in the clinic. We learned from cross-sectional studies that elevated Scond appears to be an early event in CF, followed by elevated LCI, and only later in more severe disease course by elevated Sacin [17,29,30]. This may reflect a disease pathology of well-preserved alveolar structure until late in CF due to the predominant airway involvement, minimal loss of alveolar-capillary units, and improved ventilation-perfusion relationship due to the process of "claustration" [31,32]. While certain lung areas supplied by the same mucus-impacted bronchus become simultaneously hypoventilated and underperfused, the remaining well-preserved lung areas show enhanced ventilation – perfusion relationship.

SIII derived markers have some intrinsic challenges. Since they are based on one single breath (SIII, Sacin), or a sequence of successive breaths (Scond) they are more variable than other lung function parameters such as LCI and moment ratios [12,14,19]. Changes in breathing pattern such as tidal volume and flow might influence the measure [16]. Adult adopted standardized breathing protocols of 1 L tidal volume targets were found to be invalid for use in children due to non-systematic and heterogeneous changes of washout parameters [33]. Whether an adapted protocol for children with incentive breathing within a certain tidal volume per weight range might be helpful has to be examined. In adults, *Husemann et al.* showed only a minor influence of tidal volume, flow, and lung volume on the total SIII variance [19]. Systematic analysis of this in children is missing. Generally, SIII showed quite heterogeneous results within different disease groups [13,14,20]. Through collective interpretation of different lung function tests it is possible to assign heterogeneous SIII results to different physiological [30] and disease [20] phenotypes.

Recently, methodological concerns about the use of a dual gas mixture in the DTG-SBW have been expressed [34]. One important pitfall raised by *Verbanck et al.* is that an absence of change in SIII cannot exclude an intra-acinar effect, for instance when some structural acinar changes

affect both He and $SF_6$ phase III slope to the same extent. Thus, there may be a further interpretation for our lack of correlation between SIII and structural changes; changes may have affected proximal and peripheral acinar structures to the same extent and thus were not captured by the $SF_6$-He SIII. However, we would still expect a correlation between chest CT outcomes and Sacin, which was also not evident.

Strengths of the present study include the use of both DTG-SBW and the established $N_2$MBW in a group of well-defined children with CF. We used two different CT scoring methods, the novel PRAGMA-CF which aims specifically at quantifying the extent of early structural abnormalities in CF [4] and the more classical semi-quantitative Brody based CF-CT scoring.

The most important limitation of the study is the rather small sample size, which might make the analysis prone to type II errors. However, in view of the strong associations found between LCI, moment ratios and Scond and bronchiectasis we are still confident about the validity of the results. The small sample size represents a clear limitation. Ideally, we need future studies with bigger sample size that demonstrate the reproducibility of our results. One of the reasons for the limited number of acceptable DTG-SBW tests was the fact that DTG-SBW measurement was performed within a spectrum of other tests of the AREST CF follow-up. Thus, it was not possible to prioritize DTG-SBW. Further limitation of the study include the lack of a healthy control group and therefore upper limits of normal for washout parameters, particularly for SIII. As a result, analysis about the validity of the DTG-SBW test were not possible. The cross-sectional study design did not allow a longitudinal follow-up with repeated measures, to determine if these lung function measures track the development and the progression of structural changes over time. The potent greenhouse effect of $SF_6$ and therefore limited availability represents a drawback for potential future, widespread application.

Since we found no correlation between SIII and structural lung changes in children with CF, DTG-SBW added no benefit to indices measured by MBW in this study. Thus, although fast and simple to perform, DTG-SBW cannot replace MBW and does not meet the requirements to serve as a monitoring tool for structural lung disease as seen for LCI in preschool and school-aged children [3]. At the moment, SIII rather serves as a single piece among other lung function parameters to reflect pathophysiological conditions [20,30]. *Nyilas et al* were able to identify different physiological phenotypes independently of the underlying disease entity of the children [30]. Thereby SIII of DTG-SBW was one of the lung function parameters that differed significantly between the phenotypes. Characterization of such physiological phenotypes may help to personalize future diagnostic and therapeutic procedures. Whether or not DTG-SBW will maintain a role in the assessment of pathophysiological aspects is too early to say.

In conclusion, our study is the first to show that structural lung disease on CT scans in children with CF were not reflected by impaired acinar ventilation measured by SBW and Sacin, but were associated with changes in LCI and Scond. Structural disease in acinar regions may not be detected on CT or they may be too mild to be detected using inert gas washout tests at this age. In this study, SBW added no benefit to indices measured by MBW.



**FIGURE LEGENDS**

**Figure 1. Slope III (SIII) of double-tracer gas single-breath washout in children with cystic fibrosis with absence and presence of bronchiectasis.** Data are presented as box and whiskers plots representing the 25$^{th}$ and 75$^{th}$ centiles and 10$^{th}$ and 90$^{th}$ centiles respectively. There were no differences in SIII between those children without (n = 5; white box) and with (n = 18; grey box) bronchiectasis on chest CT.

**Figure 2a. Changes in Slope III of the double-tracer gas single-breath washout with increasing bronchiectasis Brody scores.** Increasing bronchiectasis Brody scores on chest CT

showed no association with negatively increasing slope III (SIII) of the double-tracer gas single-breath washout (r = 0.05, p = 0.82).

**Figure 2b. Changes in Slope III of the double-tracer gas single-breath washout with increasing percent bronchiectasis in the PRAGMA-CF scoring.** Increasing bronchiectasis percent in the PRAGMA-CF scoring showed no association with negatively increasing slope III (SIII) of the double-tracer gas single-breath washout (r = 0.12, p = 0.62).

**Figure 3. Changes in Slope III of the double-tracer gas single-breath washout with increasing acinar ventilation inhomogeneity measured by multiple-breath washout.** Increasing Sacin of the nitrogen multiple-breath washout reflecting acinar ventilation inhomogeneity showed a borderline association with negatively increasing slope III (SIII) of the double-tracer gas single-breath washout (r = -0.42, p = 0.055).

**Figure 4. Changes in Scond of the nitrogen multiple-breath washout with the increasing percent bronchiectasis in the PRAGMA-CF scoring.** Increasing bronchiectasis percent in the PRAGMA-CF scoring was associated with increasing Scond of nitrogen multiple-breath washout reflecting convection-dependent ventilation inhomogeneity (r = 0.60, p = 0.003).



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Figure caption

TABLES

Table 1. Demographics of Study population

| Characteristics | All children | DTG-CT | $N_2$MBW-CT | DTG-$N_2$MBW |
|---|---|---|---|---|
| Number of children | 32 | 23 | 23 | 21 |
| Age, yr | 9.46 (3.21) | 10.16 (3.39) | 9.66 (3.54) | 10.17 (3.43) |
| Sex, n (males/females) | 15/17 | 11/12 | 11/12 | 10/11 |
| Height, z-score (95% CI) | 0.003 (1.07) | 0.14 (1.07) | 0.21 (1.04) | 0.18 (1.00) |
| Weight, z-score (95% CI) | 0.08 (0.93) | 0.38 (0.91) | 0.29 (0.96) | 0.28 (1.08) |
| **Double-tracer gas SBW (n)** | 26 | 23 | - | 21 |
| SIII (mg/mol) | -138.4 (150.6) | -152.9 (170.2) | - | -134.0 (106.8) |
| **Nitrogen MBW (n)** | 27 | - | 23 | 21 |
| Lung clearance index | 8.19 (2.32) | - | 8.19 (2.40) | 8.34 (1.89) |
| First moment ratio | 1.75 (0.37) | - | 1.75 (0.37) | 1.78 (0.32) |
| Second moment ratio | 6.44 (3.46) | - | 6.44 (3.57) | 6.52 (3.06) |

| | | | | |
|---|---|---|---|---|
| Scond | 0.048 (0.037) | - | 0.048 (0.039) | 0.048 (0.037) |
| Sacin | 0.055 (0.076) | - | 0.064 (0.081) | 0.059 (0.069) |
| **Chest CT** | | | | |
| **Adapted Brody scores (n)** | 32 | 23 | 23 | 21 |
| Bronchiectasis present | 75% (24/32) | 78% (18/23) | 70% (16/23) | 71% (15/21) |
| Bronchiectasis score | 2 (6) | 2 (6) | 1 (7) | 2 (6) |
| Air trapping present | 91% (29/32) | 91% (21/23) | 96% (22/23) | 95% (18/21) |
| Air trapping score | 6.5 (5.5) | 6 (5) | 6 (5) | 6 (4) |
| Airway wall thickening present | 91% (29/32) | 87% (20/23) | 87% (20/23) | 86% (18/21) |
| Airway wall thickening score | 8.5 (5.5) | 9 (7) | 8 (6) | 8 (6) |
| Mucus plugging present | 44% (14/32) | 48% (11/23) | 35% (8/23) | 38% (8/21) |
| Mucus plugging score | 0 (2) | 0 (2) | 0 (2) | 0 (2) |
| **PRAGMA-CF scores (n)** | 32 | 23 | 23 | 21 |
| Bronchiectasis extent, % | 0.59 (2.14) | 0.66 (2.09) | 0.66 (2.10) | 0.86 (1.84) |
| Air trapping extent, % | 9.54 (17.37) | 9.72 (18.19) | 7.71 (17.13) | 10.14 (16.07) |
| Disease extent, % | 3.21 (5.04) | 2.63 (6.29) | 2.19 (5.30) | 2.63 (5.30) |

*Definition of abbreviations*: CI = confidence interval; SBW = single-breath washout; MBW = multiple-breath washout; CT = computed tomography; PRAGMA-CF = Perth-Rotterdam Annotated Grid Morphometric Analysis for Cystic Fibrosis.

Data are presented as mean (SD) for age, weight and height, and as median (interquartile ranges) for non-normally distributed washout and CT results, or as percentage (proportion). Height and weight z-scores were calculated using World Health Organization growth standards [35].

**Table 2. Association between double-tracer gas SBW outcomes, and between MBW outcomes and extent of structural lung disease in chest CT.**

| | SIII | LCI | M1/M0 | M2/M0 | Scond | Sacin |
|---|---|---|---|---|---|---|
| Adapted Brody scores | | | | | | |
| Bronchiectasis | r = 0.05, p = 0.82 | **r = 0.59, p = 0.003** | **r = 0.57, p = 0.005** | **r = 0.53, p = 0.009** | **r = 0.47, p = 0.023** | r = 0.26, p = 0.23 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Air trapping | r = -0.04, p = 0.87 | r = 0.41, p = 0.053 | r = 0.38, p = 0.08 | r = 0.31, p = 0.15 | r = 0.31, p = 0.15 | r = 0.12, p = 0.58 |
| Airway wall thickening | r = -0.15, p = 0.50 | r = 0.40, p = 0.06 | r = 0.38, p = 0.08 | r = 0.33, p = 0.12 | r = 0.40, p = 0.056 | r = 0.18, p = 0.41 |
| Mucus plugging | r = 0.02, p = 0.94 | **r = 0.44, p = 0.035** | r = 0.41, p = 0.05 | r = 0.37, p = 0.08 | r = 0.33, p = 0.12 | r = 0.26, p = 0.23 |
| **PRAGMA-CF scores** | | | | | | |
| Bronchiectasis extent, % | r = 0.10, p = 0.64 | **r = 0.57, p = 0.004** | **r = 0.57, p = 0.004** | **r = 0.55, p = 0.006** | **r = 0.60, p = 0.003** | r = 0.24, p = 0.27 |
| Air trapping extent, % | r = -0.03, p = 0.89 | r = 0.28, p = 0.19 | r = 0.25, p = 0.25 | r = 0.20, p = 0.35 | r = 0.36, p = 0.09 | r = -0.02, p = 0.92 |
| Disease extent, % | r = 0.07, p = 0.75 | **r = 0.43, p = 0.041** | r = 0.41, p = 0.05 | r = 0.37, p = 0.08 | **r = 0.51, p = 0.012** | r = -0.03, p = 0.88 |

*Definition of abbreviations*: CT = computed tomography; SBW = single-breath washout; MBW = multiple-breath washout; SIII = slope III of the double-tracer gas SBW; LCI = lung clearance index; M1/M0 = first moment ratio; M2/M0 = second moment ratio; PRAGMA-CF = Perth-Rotterdam Annotated Grid Morphometric Analysis for Cystic Fibrosis.

The r value represents the correlation coefficient of the calculated Spearman's rank correlation. Bold values are statistically significant, $p < 0.05$.

**Table 3. Association between phase III slope of double-tracer gas SBW and $N_2$MBW outcomes.**

| $N_2$MBW | Correlation coefficient | p-value |
|---|---|---|
| LCI | -0.22 | 0.33 |
| M1/M0 | -0.24 | 0.28 |
| M2/M0 | -0.27 | 0.23 |
| Scond | 0.01 | 0.96 |
| Sacin | -0.42 | 0.055 |

*Definition of abbreviations*: SBW = single-breath washout; $N_2$MBW = nitrogen multiple-breath washout; LCI = lung clearance index; M1/M0 = first moment ratio; M2/M0 = second moment ratio. The correlation coefficient represents the r value of the calculated Spearman's rank correlation.

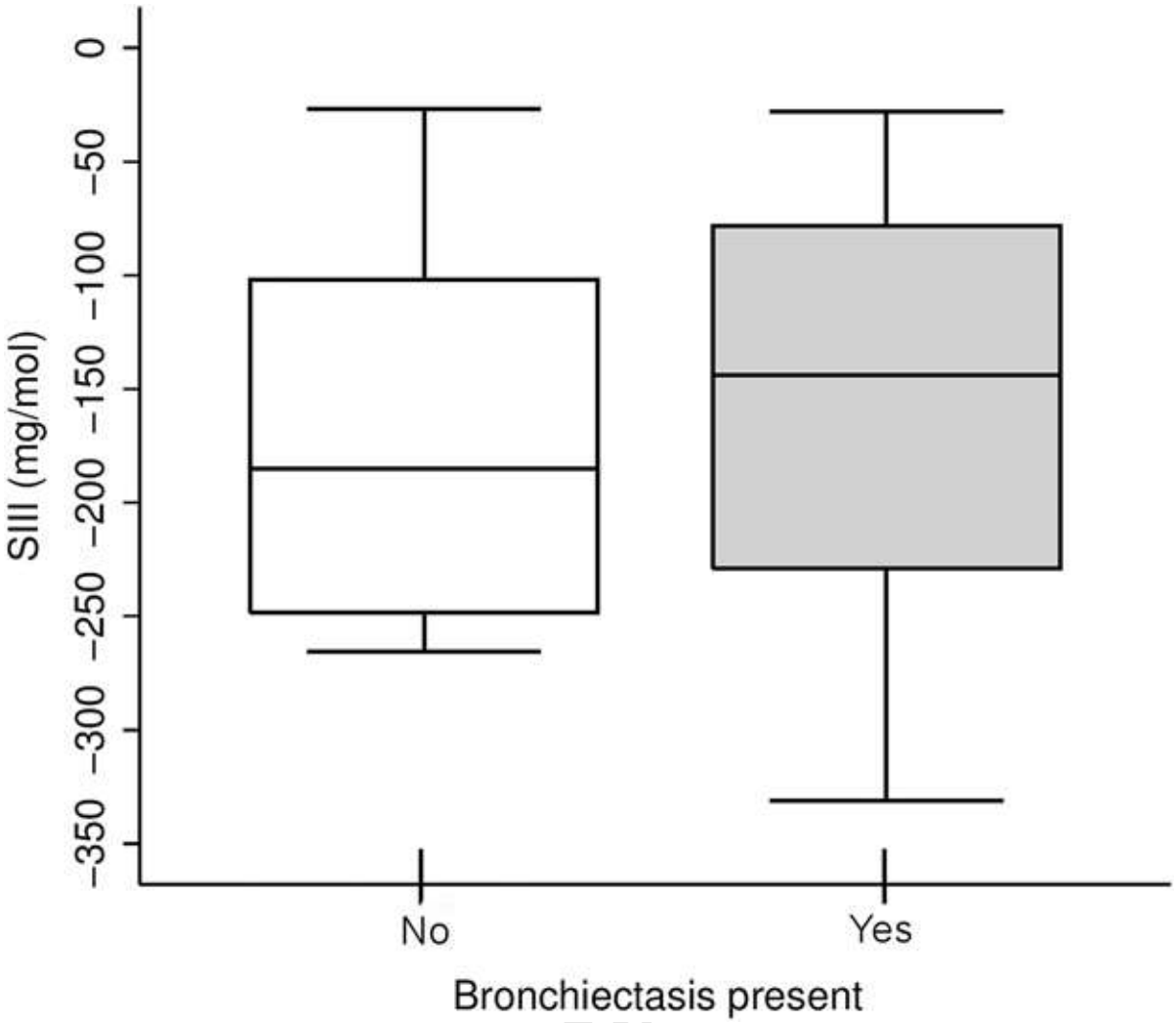


**Figure 1**

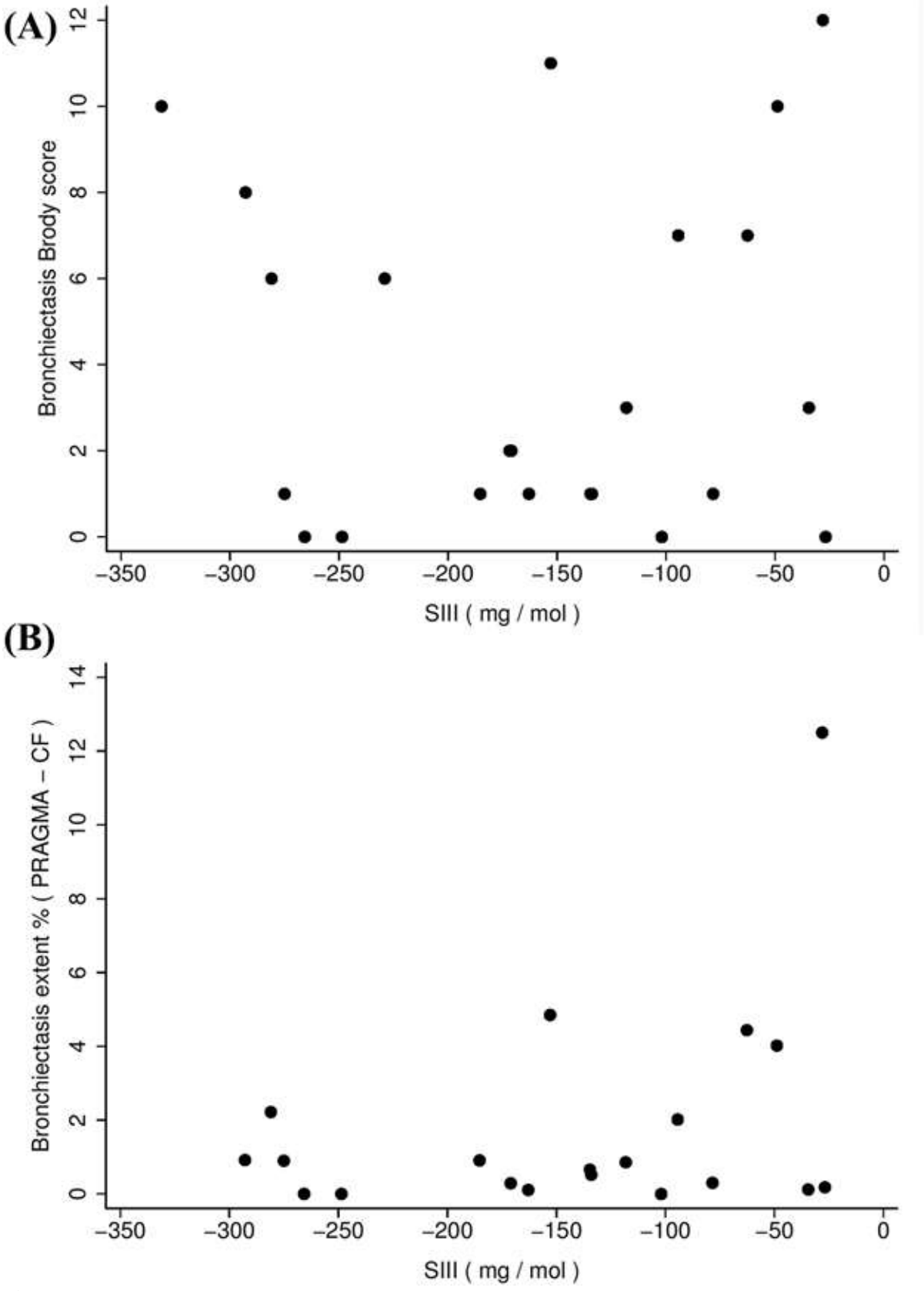
(A)
Bronchiectasis Brody score
0
2
4
6
8
10
12
−350
−300
−250
−200
−150
−100
−50
0
SIII ( mg / mol )
(B)
Bronchiectasis extent % ( PRAGMA – CF )
0
2
4
6
8
10
12
14
−350
−300
−250
−200
−150
−100
−50
0
SIII ( mg / mol )

**Figure 2**

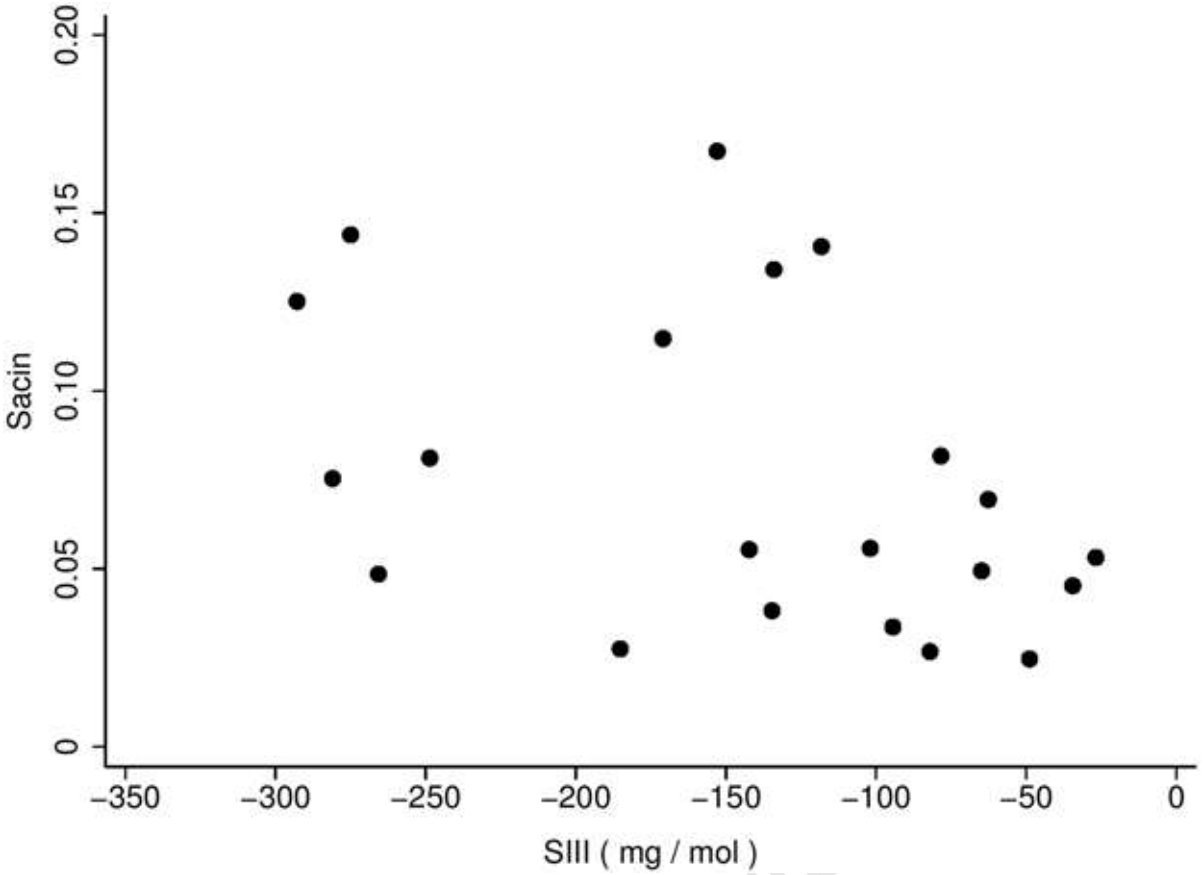


**Figure 3**

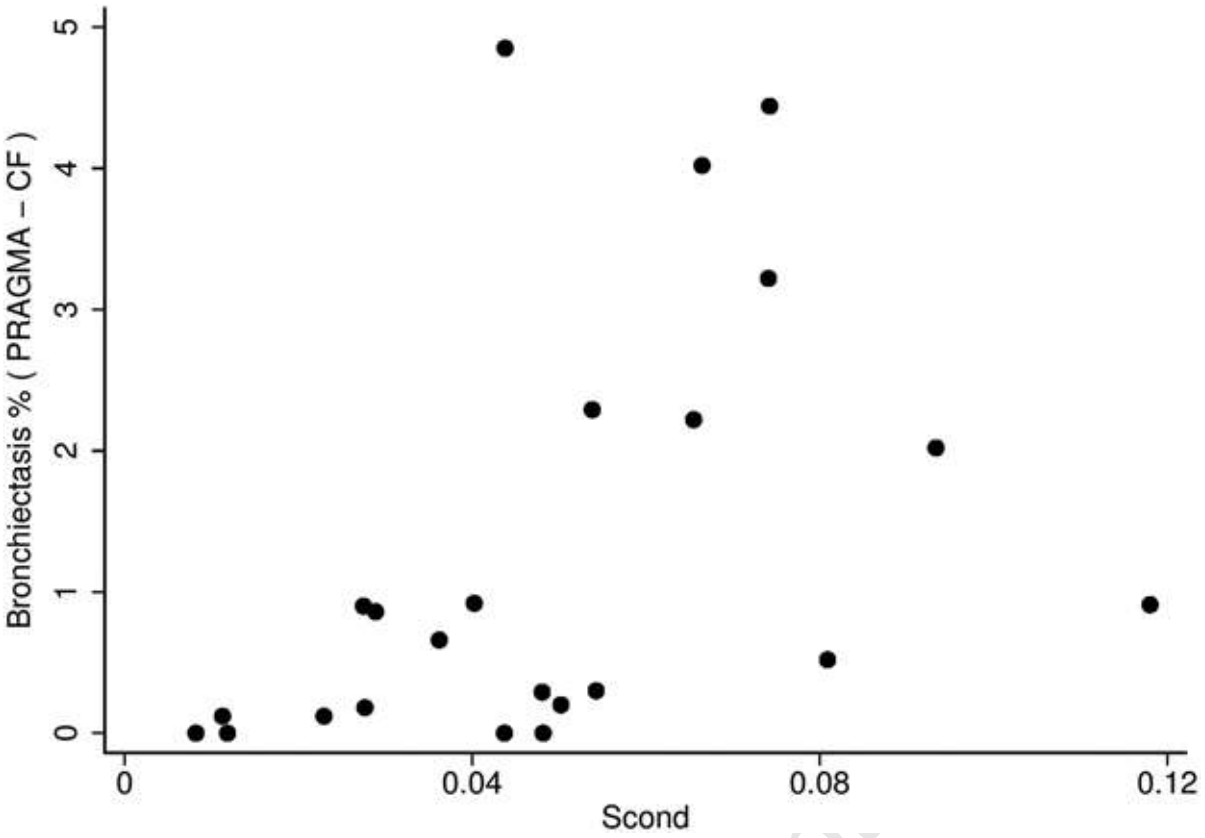


**Figure 4**