

Is Computed Tomography Airway Count Related to Asthma Severity and Airway Structure-function?

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ABSTRACT

Rationale: In patients with asthma, x-ray computed tomography (CT) has provided evidence of thickened airway walls and airway occlusions, but the total number of CT-visible airways and its relationship with disease severity is unknown.

Objectives: To measure CT total-airway-count (TAC) in asthma and evaluate relationships with asthma severity, airway morphology, pulmonary function and magnetic-resonance-imaging (MRI) ventilation.

Methods: Participants underwent post-bronchodilator inspiratory CT, and pre- and post-bronchodilator spirometry and hyperpolarized ^3He MRI. CT TAC was quantified as the sum of airways in the segmented airway-tree, and airway wall-area-percent (WA%) and lumen-area (LA) were measured. MRI ventilation abnormalities were quantified as the ventilation-defect-percent (VDP).

Measurements and Main Results: We evaluated 70 participants including 15 Global-Initiative-for-Asthma (GINA) steps 1-3, 19 GINA4 and 36 GINA5 asthmatics. As compared with GINA1-3, TAC was significantly diminished in GINA4 ($p=0.03$) and GINA5 ($p=0.045$). Terminal airway intraluminal occlusion was present in five (two GINA4, three GINA5) of 70 participants. Sub-subsegmental airways were CT-invisible or missing in 69/70 participants; the most common number of missing sub-subsegments was 10. Participants with ≥ 10 missing subsegments had worse WA% ($p<0.0001$), LA ($p<0.0001$) and VDP ($p=0.03$) than those with <10 missing subsegments. In a multivariable-model, TAC ($\beta=0.50$, $p=0.001$) independently predicted FEV_1 ($R^2=0.27$, $p=0.003$) and in a separate model, TAC ($\beta=-0.53$, $p<0.0001$) independently predicted airway WA% ($R^2=0.32$, $p=0.0001$).

Conclusions: TAC was significantly diminished in participants with greater asthma severity and was related to airway wall thickness and ventilation-defects. Fewer airways in severe as compared with mild asthma challenges our understanding of airways-disease in asthma.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject:

Computed tomography (CT) studies have provided evidence of thickened airway walls and abnormally narrowed and/or occluded airway lumens in patients with asthma compared with healthy subjects, but the relationship of asthma severity with the total number of CT-visible airways has not yet been investigated or determined.

What This Study Adds to the Field:

Similar to recent findings in patients with COPD, we observed that total airway count (TAC) measured using chest CT was significantly and negatively related to asthma disease severity. In 70 patients with asthma, a greater number of missing sub-subsegmental airways was associated with thicker airway walls (wall area %), narrower airway lumens and larger (worse) MRI ventilation defects; in multivariable models, TAC independently predicted FEV₁ and airway wall area. Terminal intraluminal airway occlusion, which was most likely mucus alone or in combination with cellular debris, was present in five (two GINA4, three GINA5) of 70 participants which suggests that apparently lost/missing or CT invisible airways were not strongly influenced by intraluminal occlusion. TAC was significantly diminished in severe asthma as compared to mild asthma, which challenges our understanding of the role of airways disease in asthma.

INTRODUCTION

In asthma, airways disease caused by smooth muscle abnormalities, inflammation and/or mucus hypersecretion leads to variable airflow obstruction that is reversible or improves post-bronchodilator (1). Airway abnormalities in asthma are believed to encompass the entire tracheobronchial tree from the large to the small airways (2) and small airways disease is recognized as a distinct phenotype of asthma (2-4). While small airway abnormalities remain difficult to directly measure, the large airways have been extensively investigated *in vivo*, using x-ray computed tomography (CT). Airway walls in asthmatics are thicker as compared to healthy controls (5-12) and this thickening tends to worsen with increasing asthma severity. Until now, CT studies of asthma have focused on morphological airway measurements and their relationships with clinical measurements, however the total number of CT-visible airways in patients with asthma has not been investigated.

Landmark studies of airways disease in COPD were performed using micro-CT (13-15) and more recently, *in vivo* CT total airway count (TAC) revealed missing distal airways that were associated with thinning airway walls in patients with mild COPD (16). We think this finding may have implications for airways disease in asthma and hypothesized that in severe asthma, thickened airways are concomitant with airway obstruction and/or occlusion, which could be quantified using TAC.

Accordingly, our objective was to measure and evaluate CT total airway count in patients with asthma across a range of severities and explore potential relationships of TAC with asthma severity, airway measurements, pulmonary function and pulmonary functional magnetic resonance imaging (MRI). A preliminary description of these results was previously reported in abstract form (17).

MATERIALS and METHODS

Study Participants and Design

Study participants with asthma according to the Global Initiative for Asthma (GINA) treatment step criteria (1) were recruited as a convenience sample between ages 18 to 70 years with <1 pack year smoking history from two tertiary care respirology clinics (Asthma Centre, St Joseph's Health Care London, Western University, London, Ontario, Canada; Firestone Institute for Respiratory Health, St Joseph's Health Care Hamilton, McMaster University, Hamilton, Ontario, Canada). Participants provided written informed consent to an ethics board and Health Canada-approved, registered (www.clinicaltrials.gov, NCT02351141) protocol for a single study visit (Robarts Research Institute, Western University, London Canada). Participants performed spirometry, plethysmography and MRI before and after bronchodilator and completed a single post-bronchodilator thoracic CT as well as the asthma control (ACQ-6) (18) and quality of life questionnaires (AQLQ) (19). Participants were stratified by asthma severity according to GINA treatment steps (1) as: GINA1-3, GINA4, and GINA5.

Pulmonary Function Tests

Spirometry and plethysmography were performed using a *MedGraphics Elite Series* plethysmograph (MGC Diagnostics Corporation, St. Paul, MN, USA). Spirometry was performed according to ATS guidelines (20) to measure FEV₁ and FVC, and plethysmography was performed to measure lung volumes and airways resistance. Post-bronchodilator measurements were acquired after four separate doses of 100 µg of Novo-Salbutamol HFA (Teva Novopharm Ltd., Toronto, ON, Canada) through a pressurized metered-dose inhaler using an *AeroChamber Plus* spacer (Trudell Medical International, London, ON, Canada). Participants withheld asthma

medications before the study visit according to ATS guidelines (20): short-acting β -agonists for ≥ 6 hours, long-acting β -agonists for ≥ 12 hours and long-acting muscarinic agents for ≥ 24 hours.

CT

Thoracic CT was acquired post-bronchodilator using a 64-slice LightSpeed VCT system (General Electric Healthcare, Milwaukee, WI, USA) as previously described (21) from apex to base under breath-hold conditions after inhalation of 1.0 L of N₂ gas from functional residual capacity. CT parameters were as follows: 64 x 0.625 collimation, 120 kVp, 100 mA, tube rotation time 500 ms, pitch 1.25, standard reconstruction kernel, 1.25 mm slice thickness and field of view (FOV) 36-40 cm². The total effective dose for each CT scan was 1.8 mSv, calculated using the manufacturer's settings and the ImPACT patient dosimetry calculator (based on the UK Health Protection Agency NRPB-SR250 software).

Thoracic CT images were analyzed by a single observer with four-years experience (RLE) using Pulmonary Workstation 2.0 (VIDA Diagnostics Inc., Coralville, IA, USA) to segment and label the airway tree and lung lobes. All airway segments in the segmented tree were summed to quantify total airway count (TAC) (16), and airway counts were also generated by tree generation from the trachea (generation 0) to generation 11.

We utilized a combination of automated airway segmentation and manual segmentation in the presence of complete airway lumen occlusions (possible mucus, cellular debris or its combination (22)). These were also recorded by manual inspection during airway segmentation as follows: 1) if there was completely visible lumen on both proximal and distal sides of an occlusion, the airway was manually segmented to its terminus and TAC was recorded at the distal terminus beyond the occlusion, and, 2) if there was lumen visible only on the proximal side of an occlusion which

terminated the airway, TAC was recorded at the proximal end of the occlusion and not beyond the occlusion. Anatomically equivalent segmental, subsegmental and sub-subsegmental airways for five airway paths (RB1, RB4, RB10, LB1, LB10; third to fifth generation) (23) were used to generate airway wall area percent (WA%) and lumen area (LA). We determined if subsegmental and sub-subsegmental daughter branches were missing for the five airway paths using the output file exported from Pulmonary Workstation 2.0. All airway segments were assigned a unique identifier that linked each parent airway to its corresponding daughter branches, and we defined participants with missing subsegments (generation 4) and sub-subsegments (generation 5) if one or more daughter branches were missing in the report. Airways were qualitatively assessed for segmental branch variants similar to previously published results (24).

MRI

Anatomical proton (^1H) and ^3He static ventilation MRI were acquired within five minutes of each other using a whole-body 3.0 T Discovery MR750 (General Electric Healthcare, Milwaukee, WI, USA) system with broadband imaging capability as previously described (25). Participants were instructed to inhale a gas mixture from a 1.0 L Tedlar[®] bag (Jensen Inert Products, Coral Springs, FL, USA) from functional residual capacity, and 15 coronal slices were acquired in 8-15 seconds under breath-hold conditions. ^1H MRI was performed before hyperpolarized ^3He during 1.0 L breath-hold of ultra-high purity, medical-grade nitrogen (N_2 ; Spectra Gases, Alpha, NJ, USA). ^3He gas was polarized to 30–40% (HeliSpin; Polarean, Durham, NC, USA) and static ventilation imaging was performed during 1.0 L breath-hold of hyperpolarized ^3He diluted to 25% by volume with N_2 .

Quantitative MRI analysis was performed by a single observer with four-years experience (RLE) using in-house segmentation software as previously described (26) and MRI ventilation

abnormalities were quantified as the ventilation defect percent (VDP; ventilation defect volume normalized to the MRI-measured thoracic cavity volume). VDP was also generated for each lung lobe by registering MRI to CT and normalizing the ventilation defect volume within each lobe to the corresponding lobe volume (27).

Statistical Analysis

All statistical analysis was performed using SPSS Statistics 25.0 (IBM Corporation, Armonk, NJ, USA). Data were tested for normality using Shapiro-Wilk tests and when not normally distributed, non-parametric tests were performed. Demographic, pulmonary function test and imaging measurements between treatment step groups were compared using one-way analysis of variance (ANOVA) or Kruskal-Wallis tests with post-hoc Holm-Bonferroni correction for multiple comparisons. An analysis of covariance (ANCOVA) was used to compare TAC by treatment step group adjusted by age, sex and body mass index (BMI) as potential covariates, with post-hoc Holm-Bonferroni correction for multiple comparisons. The number of participants with missing sub-subsegmental branches was plotted in a histogram for the number of missing sub-subsegments, and the mode from the histogram was used to dichotomize participants; groups were compared using unpaired t-tests. Receiver operator characteristic (ROC) curves were generated to determine thresholds for TAC and the number of missing sub-subsegments for differentiating mild (GINA1-3) from severe asthma (GINA4-5). Univariate relationships were evaluated using Pearson (r) or Spearman (ρ) correlation coefficients. Multivariable models were generated using the enter approach to determine the relative influence of significant univariate parameters on FEV₁ and WA%, with age, sex and BMI entered in the first step as potential covariates. All results were considered statistically significant when the probability of making a Type I error was less than 5% ($p < 0.05$).

RESULTS

Participant Demographics, Pulmonary Function and Imaging Measurements

We evaluated 70 participants with a clinical diagnosis of asthma as shown in Table 1. There were 15 participants in the GINA1-3 subgroup (6 females/9 males, 45 ± 12 years), 19 participants in the GINA4 subgroup (10 females/9 males, 51 ± 12 years) and 36 participants in the GINA5 subgroup (24 females/12 males, 48 ± 13 years). Participants in the GINA4 group had worse FEV_1 ($64 \pm 19\%_{\text{pred}}$ vs. $88 \pm 20\%_{\text{pred}}$, $p=0.003$) and FEV_1/FVC ($58 \pm 17\%$ vs. $74 \pm 11\%$, $p=0.006$) as compared to participants in the GINA1-3 subgroup. Participants in the GINA5 subgroup had worse FEV_1 ($65 \pm 22\%_{\text{pred}}$ vs. $88 \pm 20\%_{\text{pred}}$, $p=0.004$) and FVC ($80 \pm 18\%_{\text{pred}}$ vs. $94 \pm 14\%_{\text{pred}}$, $p=0.03$), WA% ($68.2 \pm 1.7\%$ vs. $66.7 \pm 1.5\%$, $p=0.006$) and VDP ($10 \pm 8\%$ vs. $3 \pm 2\%$, $p=0.02$) compared with participants in the GINA1-3 subgroup. There were no differences between the GINA4 and GINA5 subgroups. CT FOV differed to a small extent among study participants such that FOV=36x36 cm² for 47 participants, FOV=40x40 cm² for 20 participants, FOV=39x39 cm² for 2 participants and FOV=41x41 cm² for a single participant. Importantly, TAC was not significantly different between subgroups based on FOV ($p=0.7$) and FOV was not significantly different between GINA subgroups ($p=0.1$).

Is TAC Reduced with Increasing Asthma Severity?

In Figure 1, representative 3D reconstructed CT airway trees show that with greater asthma severity, the airway tree has fewer segmented airways. TAC was significantly lower in the GINA4 (145 ± 10 , $p=0.03$) and GINA5 (148 ± 7 , $p=0.045$) subgroups as compared to GINA1-3 subgroup (182 ± 11) when adjusting for covariates (age, sex, BMI). BMI was the only significant covariate ($p=0.003$), whereas age and sex were not significant ($p=0.05$ and $p=0.06$, respectively). We

performed an additional ANCOVA to include RV/TLC as a covariate, however RV/TLC was not significant ($p=0.07$) and TAC and GINA subgroup remained a significant interaction ($p=0.03$). TAC was not different between males and females (males TAC= 155 ± 42 vs. females TAC= 155 ± 50 , $p=1.0$), and there was no significant interaction between sex and GINA status for TAC ($p=0.2$). Figure 1 also shows that TAC was significantly reduced for generations 6 ($p=0.04$) and 7 ($p=0.01$) for the GINA4 subgroup and generations 6 ($p=0.04$), 7 ($p=0.01$), 10 ($p=0.01$) and 11 ($p=0.02$) for the GINA5 subgroup as compared to the GINA1-3 subgroup. The values for airway count by generation are shown in supplementary Table S1, as well as those previously published for healthy never-smokers (16) for comparison.

Is TAC Associated with Abnormal Airway Structure and Function?

Table 2 shows the number of participants with CT invisible or missing subsegmental (generation 4) and sub-subsegmental (generation 5) daughter branches. A total of 19 participants (27%) were missing subsegmental branches, which was most common in the right middle lobe (RB4; 13/19). A total of 69 participants (99%) were missing sub-subsegmental branches, and this was most common in the left lower lobe (LB10; 54/69) and right middle lobe (RB4; 48/69). The distribution of participants with missing sub-subsegments provided in Figure 2 shows that the most common number of missing sub-subsegments (mode) was 10; 34/70 participants or 49% were missing ≥ 10 (or $\geq 50\%$) of 20 potential total sub-subsegmental airways. Figure 2 also shows that asthma participants with ≥ 10 missing sub-subsegments ($n=34$) had increased WA% ($68.6\pm 1.4\%$ vs. $66.8\pm 1.3\%$, $p<0.0001$) and reduced LA ($9.1\pm 1.9\text{ mm}^2$ vs. $11.8\pm 2.2\text{ mm}^2$, $p<0.0001$) compared with participants with <10 missing sub-subsegments ($n=36$). Participants with ≥ 10 missing sub-subsegments also had greater pre-bronchodilator VDP compared with participants with <10 missing sub-subsegments ($7\pm 7\%$ vs. $11\pm 9\%$, $p=0.03$), but not post-bronchodilator VDP ($5\pm 7\%$ vs.

7±6%, $p=0.053$). VDP improved post-bronchodilator in both subgroups dichotomized by missing sub-subsegments (both $p<0.001$). ROC curves showed an area under the curve of 0.71 ($p=0.01$) for TAC. A TAC threshold of 165 discriminated between subgroups with a sensitivity of 60% and specificity of 67%.

The relationship between missing sub-segmental airways and VDP on a lobar level is shown in Figure 3. MRI ventilation defect percent (VDP) was significantly greater in the right middle lobe (RML, $p=0.04$), right lower lobe (RLL, $p<0.0001$) and left lower lobe (LLL, $p<0.0001$) as compared to the right upper lobe (RUL), and VDP was significantly greater in the RLL ($p<0.0001$) and LLL ($p<0.0001$) as compared to the left upper lobe (LUL). We also classified participants by the number of missing sub-subsegments on each of the five airway paths; as compared to participants with 0 missing sub-subsegments, participants with 3-4 missing on RB10 and LB1 had significantly greater VDP in the RLL and LUL, respectively (both $p=0.04$), whereas participants with 1-2 missing had significantly greater VDP in the LUL ($p=0.02$). Groups were not different for the RML ($p=0.7$), LLL ($p=0.3$), nor RUL ($p=0.5$; not shown).

As shown in Table 3, we also investigated airway branch variants at the segmental level. Airway branch variants were observed in 18 of 70 participants (26%). The most common branch variants were the accessory sub-superior segment ($n=10$, 14%) and the accessory left-medial basal segment ($n=4$, 6%). Other variants included an absent right-medial basal, accessory right anterior, accessory right-medial basal and accessory airway off the left main bronchus, before the left upper lobe bronchus. Participants with airway variants had significantly diminished pre-bronchodilator RV ($117\pm 28\%_{\text{pred}}$ vs. $137\pm 36\%_{\text{pred}}$, $p=0.03$), TLC ($99\pm 14\%_{\text{pred}}$ vs. $105\pm 13\%_{\text{pred}}$, $p=0.04$) and RV/TLC ($117\pm 24\%_{\text{pred}}$ vs. $130\pm 26\%_{\text{pred}}$, $p=0.050$), significantly diminished post-bronchodilator MRI VDP ($5\pm 6\%$ vs. $7\pm 7\%$, $p=0.048$), and significantly greater LA ($11.6\pm 2.4 \text{ mm}^2$ vs. 10.1 ± 2.4

mm²), compared with participants without airway variants. TAC was not significantly different between participants with and without airway variants (164±33 vs. 152±50, p=0.4).

Table 4 summarizes the number of participants with CT evidence of airway occlusions that either terminated or did not terminate airways and the total number of airway occlusions observed during the segmentation process. Intraluminal airway occlusions were identified in 20 of the 70 (29%) participants; five (7%) participants had occlusions that terminated the airway (11 total occlusions) and 15 participants (22%) had occlusions that did not terminate the airway segmentation distal to the plug (31 total occlusions). Of the five participants with occlusions terminating airways, two were GINA4 (2/19, 11%) and three were GINA5 (3/36, 8%). Of the 15 participants with occlusions that did not terminate the airways, one was GINA3 (1/15, 7%), four were GINA4 (4/19, 21%) and ten were GINA5 (10/36, 28%).

Is TAC Related to FEV₁ and Airway Wall Area?

Figure 4 shows that TAC was moderately related to post-bronchodilator FEV₁ (r=0.39, p=0.001; post-bronchodilator ΔFEV₁ r=-0.38, p=0.002 [not shown]), WA% (r=-0.55, p<0.0001) and LA (r=0.52, p<0.0001). In a subset of 60 participants who underwent hyperpolarized ³He MRI (two did not fit the MRI coil and eight were consented to ¹²⁹Xe MRI only), TAC was also related to post-bronchodilator VDP (ρ=-0.34, p=0.008). TAC was weakly related to RV/TLC (r=-0.28, p=0.02), but not FVC (r=0.24, p=0.051), RV (r=-0.21, p=0.09), TLC (r=-0.02, p=0.9) nor FOV (r=0.03, p=0.8; data not shown).

Multivariable models to determine the relative influence of TAC on FEV₁ and WA%, adjusted for covariates, are shown in Table 5. First, we determined the relative influence of CT imaging measurements on FEV₁ (Model 1: R²=0.27, p=0.003), for which TAC was the only predictor

($\beta=0.50$, $p=0.001$). Next, we determined the relative influence of CT and MRI on FEV₁ (Model 2: $R^2=0.49$, $p<0.0001$), and both CT TAC ($\beta=0.27$, $p=0.03$) and MRI VDP ($\beta=-0.53$, $p<0.0001$) significantly contributed. Finally, in a model for WA% (Model 3: $R^2=0.32$, $p=0.0001$), only TAC was a significant predictor ($\beta=-0.53$, $p<0.0001$). Some of these important relationships can be demonstrated in Figure 5 where 3D reconstructed airway trees are shown co-registered to ³He MRI static ventilation.

DISCUSSION

Recent work has shown that CT total airway count decreases with increasing severity in COPD (16) and based on these findings, we wondered whether airways also appear missing on CT in asthma and about the potential relationship of CT TAC with asthma severity. We evaluated 70 participants with asthma and made the following observations: 1) TAC was diminished in GINA4 and GINA5 participants compared with GINA1-3, 2) asthma participants with ≥ 10 missing sub-subsegmental airways had thicker airway walls, narrower airway lumens and worse MRI ventilation defects than asthma participants with <10 missing sub-subsegmental airways, and 3) TAC was moderately related to FEV₁, MRI VDP, airway wall thickness and lumen area, and in multivariate models, TAC independently predicted FEV₁ and WA%.

TAC is Reduced with Increasing Asthma Severity

To our knowledge, this is the first time the relationship between CT airway count and asthma severity has been directly measured and we were surprised to observe that the total number of CT-visible airways was reduced in GINA4-5 participants. Moreover, mean TAC for all asthma participants in this study (154 ± 45) was less than mean TAC previously reported for never-smoker healthy participants in the CanCOLD cohort (221 ± 73) (16), suggesting the reduced number of

airways may be linked to asthma susceptibility or pathogenesis. In fact, TAC reported for GINA1-3 (183 ± 49) and GINA4-5 (148 ± 36 , 146 ± 47 , respectively) were qualitatively similar to TAC measurements previously reported in GOLD 1 (190 ± 66) and GOLD II COPD (152 ± 53), respectively (16). We note that BMI was the only significant covariate when comparing TAC between treatment steps; age, sex and RV/TLC were not significant covariates. To the best of our knowledge, there are no previously published TAC values for patients with asthma. Missing or CT-invisible airways started to be obvious at generation 6 in asthma which is similar to previous findings (generation 5) in COPD (16) and could be due to airway narrowing, obstruction and/or obliteration. We note that four participants with the lowest FEV₁/FVC were GINA4, which may dictate a need for treatment step-up (1). The GINA4 subgroup sample size was about half the GINA5 subgroup and the small sample size may have also influenced this result.

TAC is Associated with Abnormal Airway Structure and Function

For asthmatics with ≥ 10 missing sub-subsegmental airways, airway walls were thicker and airway lumens were narrower; this is in agreement with extensive CT studies demonstrating thickened airway walls in patients with asthma (5-12). COPD participants were previously dichotomized using the presence or absence of sub-subsegmental branches (16). However, we were alarmed that all but one participant had missing sub-subsegments and the dichotomization scheme previously described was not possible. In fact, approximately 50% of the 70 participants we evaluated were missing half or more (≥ 10) of the total 20 possible sub-subsegmental airways. We note that the mean number of missing sub-subsegments was 9, the median=8, and the mode=10 and that using any of these thresholds resulted in the same subgroup composition. Similar to previous work in COPD (16), our results suggest that in asthma, CT-invisible airways may be related to abnormal airway structure, which may be a combination of airway remodeling (28) and/or intraluminal

occlusion (22). Intraluminal airway occlusion (mucus, cellular debris or their combination) was identified in 20 participants, although such occlusions terminated the airways in a quarter of those with occlusions (5 of 20) which is 7% (5 of 70) of all participants evaluated. All but one participant with occlusions reported severe asthma, which is similar to previous reports (22), although we observed fewer occlusions or plugs in fewer participants overall than previously reported. Together, this suggests that intraluminal occlusions did not have a large impact on TAC in this study. The presence of thicker airway walls and narrowed lumens in subsegmental airways that were missing daughter branches suggest that it is obstruction (via airway wall remodeling or collapse), rather than airway destruction that is responsible for our findings in participants with asthma. Quantitative CT phenotypes have been identified in patients with asthma (29) largely based on proximal airway morphology including airway luminal narrowing and wall thickness. TAC provides a complementary quantitative CT measurement that reflects the architecture of the airway tree and may help to further enrich imaging-based phenotypes of asthma. We also investigated the functional consequences of reduced TAC and observed worse MRI ventilation defects in participants with more missing sub-subsegments on the whole-lung level and worse MRI VDP in lung lobes with greater prevalence of missing sub-subsegments. Previous work (30) demonstrated a relationship between thickened airway walls and MRI ventilation defects in asthma, which is congruent with our finding that TAC and abnormal airway structure contribute to abnormal MRI ventilation on whole-lung and regional lobar levels.

Similar to recently published findings in COPD (24), we observed airway variants in 18 of 70 or 26% of participants. The most common variant was an accessory sub-superior segment which was observed 14% of participants. These results are in good agreement with published results in 3,000 COPD participants (24), with very similar prevalence of airway variants and the presence of the

accessory sub-superior segment. Interestingly, MRI VDP was worse in participants with conventional segmental airway anatomy compared with participants with airway variants, which may be explained by the smaller airway lumens also observed in participants with conventional airway tree architecture. We were not powered to investigate relationships for individual airway variants which should be investigated in larger-scale studies.

TAC is Related to FEV_1 and Airway Wall Area

TAC was significantly, albeit weakly to moderately, related to FEV_1 , VDP, WA% and LA. In addition, TAC was inversely and positively correlated with WA% and LA, respectively. This suggests that thickened airway walls and narrow airway lumens help explain diminished TAC in asthma, in contrast to COPD where missing airways were related to thinning airway walls (16). The relationship between TAC and VDP was especially weak, possibly because a large proportion of participants reported VDP<5%. We note that VDP values here were similar to previously reported values in mild-moderate (3-4% (30, 31)) and severe asthma (6-10% (31, 32)). Univariate relationships were used to drive multivariable models to investigate these relationships and showed that among the CT measurements investigated, TAC was the only independent predictor of FEV_1 . In a separate model, CT TAC and MRI VDP together explained a greater proportion of FEV_1 variability. Hence, CT airway count provided unique information related to FEV_1 , independent of airway morphology. Previous work in COPD (16) also showed that TAC and lumen area both helped to explain FEV_1 . To more deeply explore this finding and our own results, we generated an additional multivariable model using only CT wall area percent ($\beta=-0.24$, $p=0.2$) and CT lumen area ($\beta=0.04$, $p=0.8$), neither of which was significant (model $R^2=0.13$, $p=0.1$). It is important to note that airway wall and lumen measurements were generated for anatomically equivalent segmental, subsegmental and sub-subsegmental airways in five airway paths (23). BMI was a

significant covariate in the multivariable models (participant age and sex were not) which may help explain the weak to moderate univariate relationships. CT in combination with MRI offers highly complementary information about lung structure and function in patients with asthma and generates an understanding of the functional consequences of structural abnormalities. TAC also uniquely explained airway wall morphology (WA%) which suggests there is some form of interaction between airway thickening and apparently lost/missing or CT invisible airways.

Limitations and Unanswered Questions

Limitations of our study include the fact that the study was based on a convenience sample dominated by more severe disease, such that we were underpowered to individually evaluate GINA1-3 (GINA1 n=4, GINA2 n=2 and GINA3 n=9). A population-based sample would provide more participants with milder disease which would allow for the detection of differences between all GINA subgroups. It is important to note that CT images were acquired at functional residual capacity plus 1.0 L to volume-match CT and MRI datasets. We pondered the relationship between CT/MRI lung volume and airway count and the fact that most studies acquire CT close to TLC (16). CT lung volume acquisition differences could impact participants with larger lung volumes because at a lower fraction of vital capacity, the elastic forces tethering the airways open would also be lowered which would impact lumen diameters and potentially contribute to reduced TAC. We note however that TAC was not significantly related to the fraction of lung volume for imaging normalized to vital capacity ($r=0.04$, $p=0.7$) or total lung capacity ($r=0.045$, $p=0.7$). We did not acquire expiratory CT and therefore we have no CT measurements of air trapping (33, 34) so it is not possible to compare parametric response map or expiratory CT lucency measures with TAC in this study. Although CT FOV differed to a small extent among study participants, we confirmed that CT FOV was not different between treatment step groups and was not correlated with TAC,

nor was TAC different between participant groups with different FOV. We also acknowledge the lack of repeat and longitudinal follow-up CT imaging at this time. Future work will be needed to evaluate the reproducibility of TAC in participants with asthma and potential changes in TAC over time. Whereas repeat and sometimes longitudinal CT in patients with asthma is not common due to radiation burden, MRI allows for repeat evaluations without added risk to patients. Missing airways on CT were related to worse MRI ventilation defects on whole-lung and lobar levels, and previous work has demonstrated spatial relationships between focal ventilation defects and abnormally remodeled airways (30, 35). Although MRI and CT measure different but complimentary information, this supports the notion that ventilation defects are indicative of abnormal airways in severe asthma and can be used to guide treatment decisions or localized therapies with the goal of resolving ventilation defects and thus, airway abnormalities and asthma control.

This study raises more questions than it answers. What are the underlying pathophysiological processes that drive airway drop-out in severe asthma? Do mucus or other types of occlusions play a role and were these more dominant at airway termini at a timepoint prior to our evaluation? In other words, is the relative lack of mucus at most airway termini definitive for the process underlying missing airways? Are some patients preprogrammed for low TAC which may be coincident to, or predictive of the development of asthma? Is TAC a missing link between asthma-COPD overlap or the asthma transition to loss of post-bronchodilator reversibility and COPD? How does TAC change over time, with and without treatment? Future studies ought to investigate histologic airway wall remodeling (28), inflammatory markers including sputum cell counts or exhaled nitric oxide (32, 36) and/or CT scores for intraluminal obstructions (22, 37) to elucidate the underlying mechanisms for missing airways. Integration of TAC with ‘omic investigations or

genome-wide association studies (GWAS) (38, 39) may help determine possible predispositions for reduced TAC and development of obstructive lung disease. Finally, longitudinal studies are also required to understand the stability of TAC over time and how it may help monitor disease progression or changes in response to therapy.

Conclusions

Whilst chronic airflow obstruction and airway obliteration have been described in COPD (13-15), airways disease in asthma is regarded as temporally variable and reversible within an entire tracheobronchial tree. Here we show that the airway tree is truncated in patients with severe asthma and the reduced number of terminal airways is similar in magnitude to what was previously reported in moderate COPD (16). The reduced number of airways detected using CT in asthma may be related to airway obstruction (luminal plugging, airway collapse or wall thickening) rather than destruction or obliteration. How TAC may change with treatment or over time in patients with asthma remains to be determined. In severe asthma, MRI ventilation heterogeneity was recently likened to a canary in the coal mine (40) because MRI ventilation abnormalities uniquely explained asthma control (40) and also predicted the transition of asthma to fixed obstruction (41). In a similar manner, the airway tree may represent the tunneling shafts that support the coal mining operation; once blocked or destroyed, the entire enterprise is threatened and sometimes doomed.

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FIGURE CAPTIONS

Figure 1. CT airway count by airway tree generation.

Three-dimensional reconstruction of the segmented airway tree for representative participants with asthma by Global Initiative for Asthma (GINA) treatment steps for asthma severity shows reduced airways as asthma severity increases (top). Total airway count was significantly less for GINA4 ($p=0.03$) and GINA5 ($p=0.045$) participants compared with GINA1-3 participants. GINA4 participants had significantly less airways at airway tree generations 6 and 7 compared with GINA1-3, whereas GINA5 participants had significantly less airways at generations 6, 7, 10 and 11 compared with GINA1-3. Stars (*) indicate significantly different from GINA1-3.

Figure 2. Airway morphology and VDP by number of missing sub-subsegmental airways.

Participants were dichotomized by the number of missing sub-subsegmental airways less than and greater than the mode of the number of missing sub-subsegmental airways (mode=10); 36 participants had <10 missing sub-subsegmental airways and 34 had ≥ 10 missing sub-subsegmental airways. For participants with ≥ 10 missing sub-subsegmental airways, wall area percent (WA%) was significantly greater and lumen area (LA) was significantly less (both $p<0.0001$), compared with participants with <10 missing sub-subsegmental airways. Pre-bronchodilator (BD) ventilation defect percent (VDP) was significantly worse in participants with ≥ 10 missing sub-subsegments ($p=0.03$) but post-BD VDP was not different ($p=0.053$).

Figure 3. MRI VDP by lung lobe.

MRI ventilation defect percent (VDP) was significantly greater in the right middle lobe (RML), right lower lobe (RLL) and left lower lobe (LLL) as compared to the right upper lobe (RUL; $*p<0.05$); VDP was significantly greater in the RLL and LLL as compared to the left upper lobe

(LUL; $\dagger p < 0.05$). As compared to participants with 0 missing sub-subsegments, participants with 3-4 missing sub-subsegments on RB10 and LB1 had significantly greater VDP in the RLL and LUL, respectively (both $p = 0.04$), whereas participants with 1-2 missing sub-subsegments had significantly greater VDP in the LUL ($p = 0.02$). Groups were not different for the RML nor LLL.

Figure 4. CT total airway count relationships.

Total airway count was significantly related to post-bronchodilator FEV₁ ($r = 0.39$, $p = 0.001$) and MRI ventilation defect percent (VDP; $r = -0.34$, $p = 0.008$), as well as CT wall area percent (WA%; $r = -0.55$, $p < 0.0001$) and lumen area (LA; $r = 0.52$, $p < 0.0001$).

Figure 5. Airway count and wall area relationship with MRI ventilation defects and FEV₁.

Three-dimensional reconstruction of the segmented airway tree co-registered to two-dimensional coronal centre slice ³He MRI static ventilation (cyan) and anatomical ¹H (grey-scale) for participants with <10 (left) and ≥10 (right) missing sub-subsegments. The participant with <10 missing sub-subsegments (45-year-old female) has greater TAC, less MRI ventilation defects and greater FEV₁ than the participant with ≥10 missing sub-subsegments (31-year-old female). The two-dimensional airway paths to RB1 (below) show the CT-visible sub-subsegmental daughter branch (RB1ai) with less abnormal airway wall thickening at the subsegmental level (RB1a) for the participant with <10 missing sub-subsegments, versus the missing sub-segmental daughter branch with marked airway wall thickening at the segmental level for a participant with ≥10 missing sub-subsegments.

Table 1. Participant demographics, pulmonary function and imaging measurements.

Parameter (±SD)	ALL (n=70)	GINA1-3 (n=15)	GINA4 (n=19)	GINA5 (n=36)
Age years	48 (12)	45 (12)	51 (12)	48 (13)
Female n (%)	40 (57)	6 (40)	10 (53)	24 (67)
BMI kg/m ²	29 (5)	28 (5)	28 (6)	30 (5)
ACQ-6 Score	1.6 (1.2)	1.6 (0.9)	1.3 (1.3)	1.7 (1.3)
AQLQ Score	5.0 (1.4)	5.1 (1.1)	5.5 (1.3)	4.7 (1.4)
FEV ₁ % _{pred}	70 (22)	88 (20)	64 (19)*	65 (22)*
BD ΔFEV ₁ %	17 (20)	8 (11)	16 (21)	20 (22)
FVC % _{pred}	85 (17)	94 (14)	86 (14)	80 (18)*
FEV ₁ /FVC %	65 (14)	74 (11)	58 (17)*	64 (12)
RV L	2.41 (0.67)	2.29 (0.55)	2.67 (0.53)	2.32 (0.77)
RV % _{pred}	131 (35)	125 (35)	144 (24)	128 (39)
TLC L	5.98 (1.18)	6.47 (0.89)	6.19 (1.10)	5.63 (1.27)
TLC % _{pred}	104 (14)	105 (11)	110 (10)*	100 (15)
RV/TLC % _{pred}	127 (26)	118 (25)	131 (19)	128 (30)
R _{aw} % _{pred}	195 (118)	167 (93)	190 (80)	210 (244)
CT FOV cm ²	37 (2)	37 (2)	38 (2)	37 (2)
TAC n	154 (45)	183 (49)	148 (36)*	146 (47)*
WA%	67.7 (1.6)	66.7 (1.5)	67.5 (1.1)	68.2 (1.7)*
LA mm ²	10.5 (2.4)	11.5 (2.0)	10.4 (2.4)	9.7 (2.7)
VDP % [†]	9 (8)	3 (2)	10 (10)	10 (8)*

ACQ=asthma control questionnaire; AQLQ=asthma quality of life questionnaire; BD=bronchodilator; BMI=body mass index; FEV₁=forced expiratory volume in one second; FOV=field of view; FVC=forced vital capacity; GINA=Global Initiative for Asthma; LA=lumen area; R_{aw}=airways resistance; RV=residual volume; SD=standard deviation; TAC=total airway count; TLC=total lung capacity; VDP=ventilation defect percent; WA%=wall area percent; %_{pred}=percent predicted.

*Significantly different from GINA Steps 1-3 (p<0.05) using one-way ANOVA for parametric variables or Kruskal Wallis tests for non-parametric variables, both with post-hoc Holm Bonferroni corrections.

[†]n=60

Table 2. Participants with missing subsegmental and sub-subsegmental daughter branches by whole-lung and lung lobe.

Parameter n (%)	Number of Participants n=70	
	Subsegments	Sub-subsegments
Whole-lung	19 (27)	69 (99)
RUL (RB1)	2 (3)	21 (30)
RML (RB4)	13 (19)	48 (69)
RLL (RB10)	2 (3)	45 (64)
LUL (LB1)	5 (7)	42 (60)
LLL (LB10)	4 (6)	54 (77)

LLL=left lower lobe; LUL=left upper lobe; RLL=right lower lobe;
 RML=right middle lobe; RUL=right upper lobe.

All percentages shown as fraction of total n=70.

Table 3. Segmental airway branch variants.

Parameter n (%)	Number of Participants n=70
None	52 (74)
Any	18 (26)
Accessory sub-superior (RB6)*	10 (14)
Absent right-medial basal (RB7)	1 (1)
Accessory left-medial basal (LB7)	4 (6)
Accessory right anterior (RB3)	1 (1)
Accessory right-medial basal (RB7)	1 (1)
Accessory left main bronchus†	1 (1)

*Accessory right sub-superior (RB6) observed in 10 of 70 participants, 2 of which also had accessory left sub-superior (LB6), and 1 of which also had absent right-medial basal (RB7).

†Accessory branch off left main bronchus, before left upper lobe bronchus.

Table 4. CT airway count and airway occlusion

Parameter	ALL (n=70)	GINA1-3 (n=15)	GINA4 (n=19)	GINA5 (n=36)
<i>Occlusions terminating airways</i>				
Participants n (%)	5 (7)	0	2 (11)	3 (8)
Occlusions n	11	0	3	8
<i>Occlusions not terminating airway</i>				
Participants n (%)	15 (22)	1 (7)	4 (21)	10 (28)
Occlusions n	31	1	10	20
<i>No occlusions</i>				
Participants n (%)	50 (71)	14 (93)	13 (68)	23 (64)

GINA=Global Initiative for Asthma.

Table 5. Multivariable models.

Parameter	Unstandardized		Standardized	p
	B	Standard Error	β	
MODEL 1: FEV₁ %_{pred} (<i>R</i>²=0.27, <i>p</i>=0.003)				
TAC	0.22	0.07	0.50	0.001
WA%	-0.58	2.44	-0.05	0.8
LA	-0.62	1.62	-0.07	0.7
MODEL 2: FEV₁ %_{pred} (<i>R</i>²=0.49, <i>p</i><0.0001)				
TAC	0.13	0.06	0.27	0.03
WA%	-0.25	2.25	-0.02	0.9
LA	-0.18	1.45	-0.02	0.9
VDP	-1.34	0.28	-0.53	<0.0001
MODEL 3: WA% (<i>R</i>²=0.32, <i>p</i>=0.0001)				
TAC	-0.02	0.01	-0.53	<0.0001
FEV ₁	-0.01	0.01	-0.07	0.7
VDP	-0.02	0.04	-0.09	0.5

B=regression coefficient; β=standardized regression coefficient; FEV₁=forced expiratory volume in one second; LA=lumen area; TAC=total airway count; VDP=ventilation defect percent; WA%=wall area percent; %_{pred}=percent predicted.

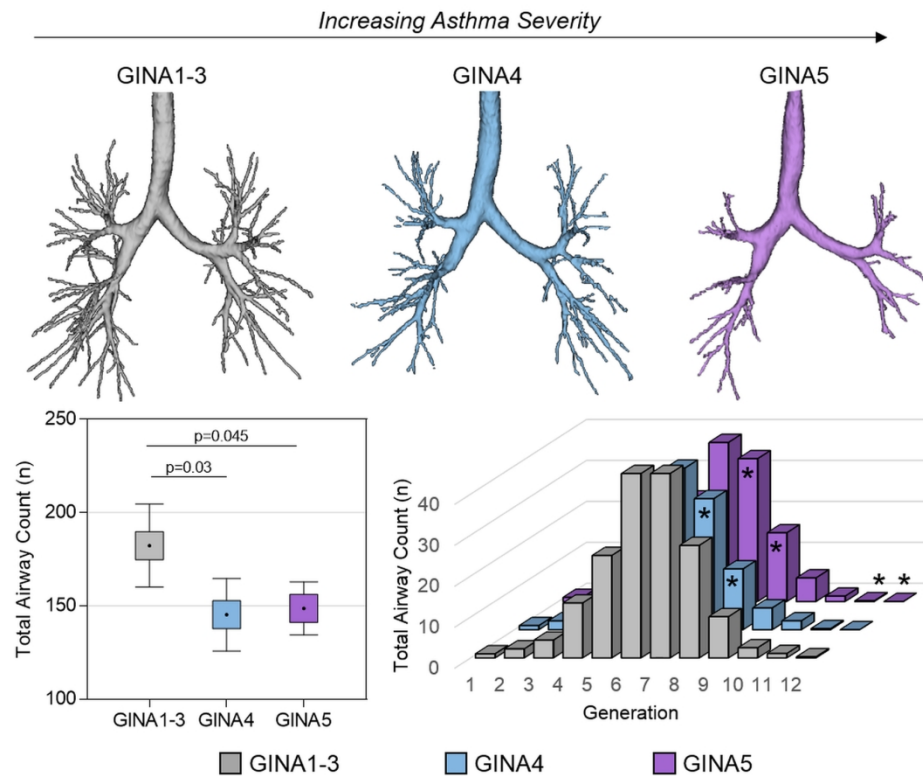


Figure 1. CT airway count by airway tree generation.

Three-dimensional reconstruction of the segmented airway tree for representative participants with asthma by Global Initiative for Asthma (GINA) treatment steps for asthma severity shows reduced airways as asthma severity increases (top). Total airway count was significantly less for GINA4 ($p=0.03$) and GINA5 ($p=0.045$) participants compared with GINA1-3 participants. GINA4 participants had significantly less airways at airway tree generations 6 and 7 compared with GINA1-3, whereas GINA5 participants had significantly less airways at generations 6, 7, 10 and 11 compared with GINA1-3. Stars (*) indicate significantly different from GINA1-3.

165x130mm (300 x 300 DPI)

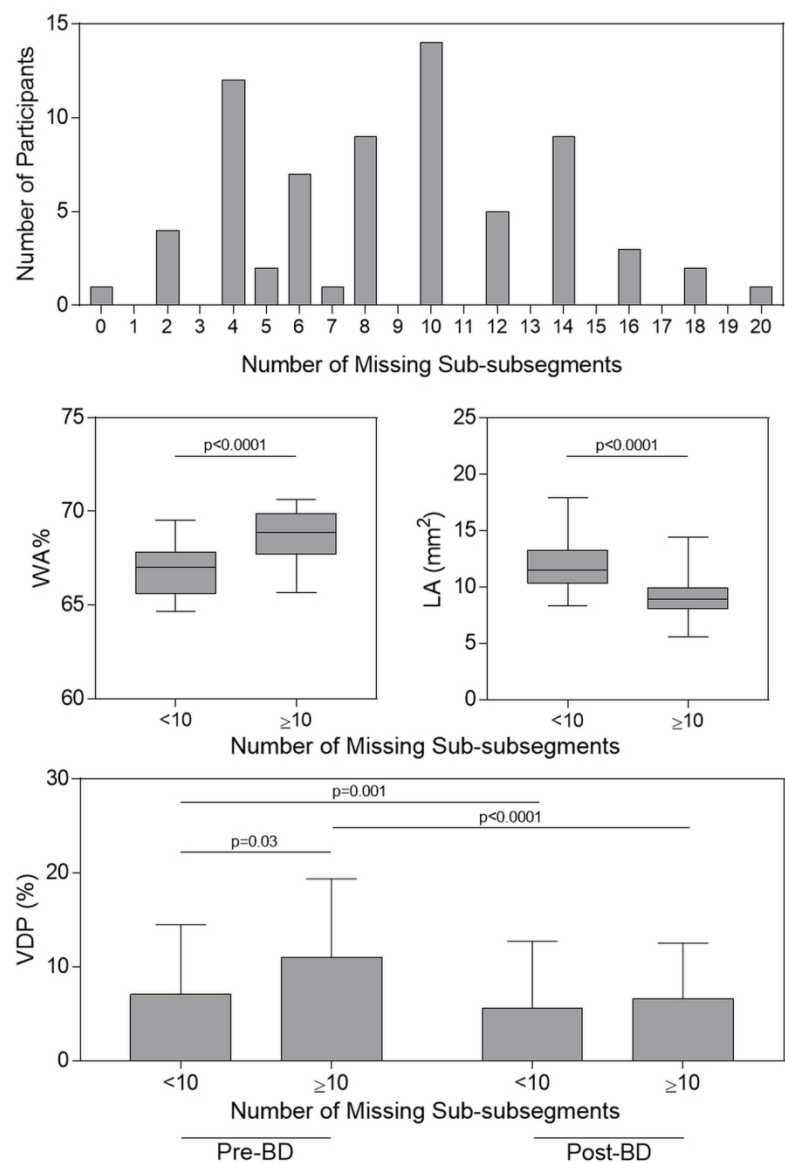


Figure 2. Airway morphology and VDP by number of missing sub-subsegmental airways. Participants were dichotomized by the number of missing sub-subsegmental airways less than and greater than the mode of the number of missing sub-subsegmental airways (mode=10); 36 participants had <10 missing sub-subsegmental airways and 34 had ≥10 missing sub-subsegmental airways. For participants with ≥10 missing sub-subsegmental airways, wall area percent (WA%) was significantly greater and lumen area (LA) was significantly less (both $p<0.0001$), compared with participants with <10 missing sub-subsegmental airways. Pre-bronchodilator (BD) ventilation defect percent (VDP) was significantly worse in participants with ≥10 missing sub-subsegments ($p=0.03$) but post-BD VDP was not different ($p=0.053$).

127x177mm (300 x 300 DPI)

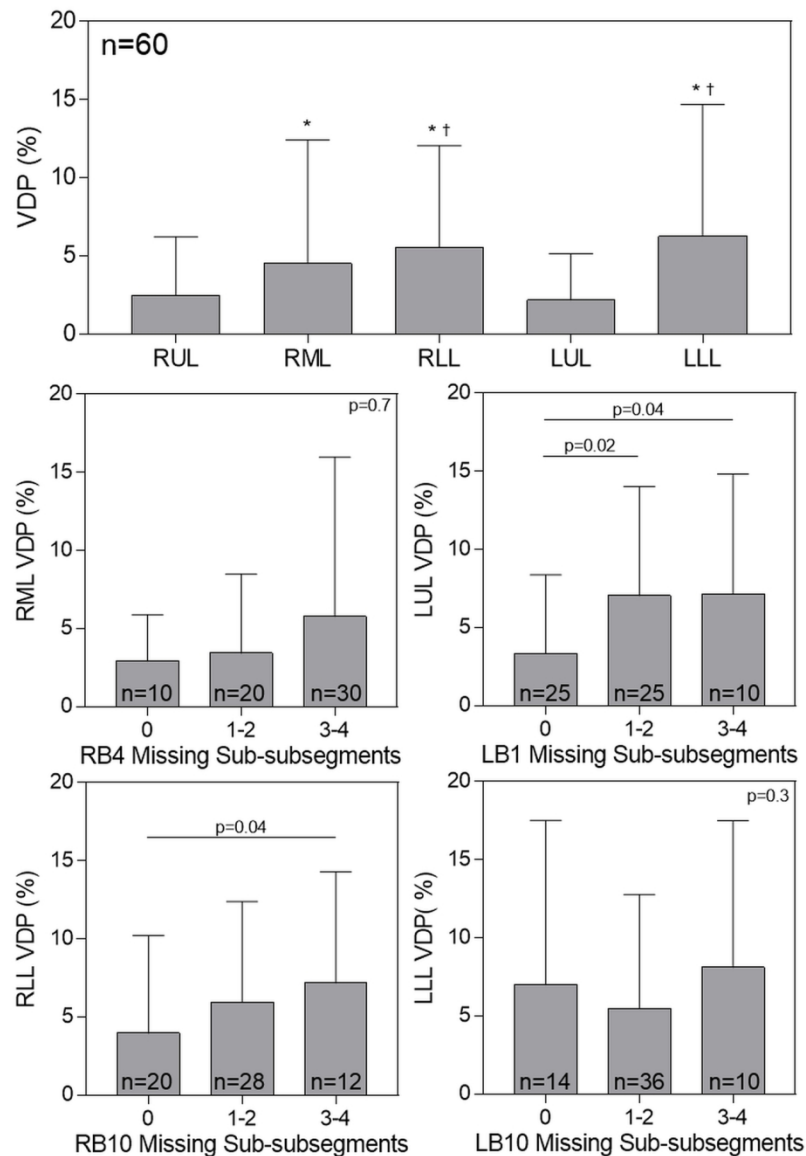


Figure 3. MRI VDP by lung lobe.

MRI ventilation defect percent (VDP) was significantly greater in the right middle lobe (RML), right lower lobe (RLL) and left lower lobe (LLL) as compared to the right upper lobe (RUL; * $p < 0.05$); VDP was significantly greater in the RLL and LLL as compared to the left upper lobe (LUL; † $p < 0.05$). As compared to participants with 0 missing sub-segments, participants with 3-4 missing sub-segments on RB10 and LB1 had significantly greater VDP in the RLL and LUL, respectively (both $p = 0.04$), whereas participants with 1-2 missing sub-segments had significantly greater VDP in the LUL ($p = 0.02$). Groups were not different for the RML nor LLL.

127x173mm (300 x 300 DPI)

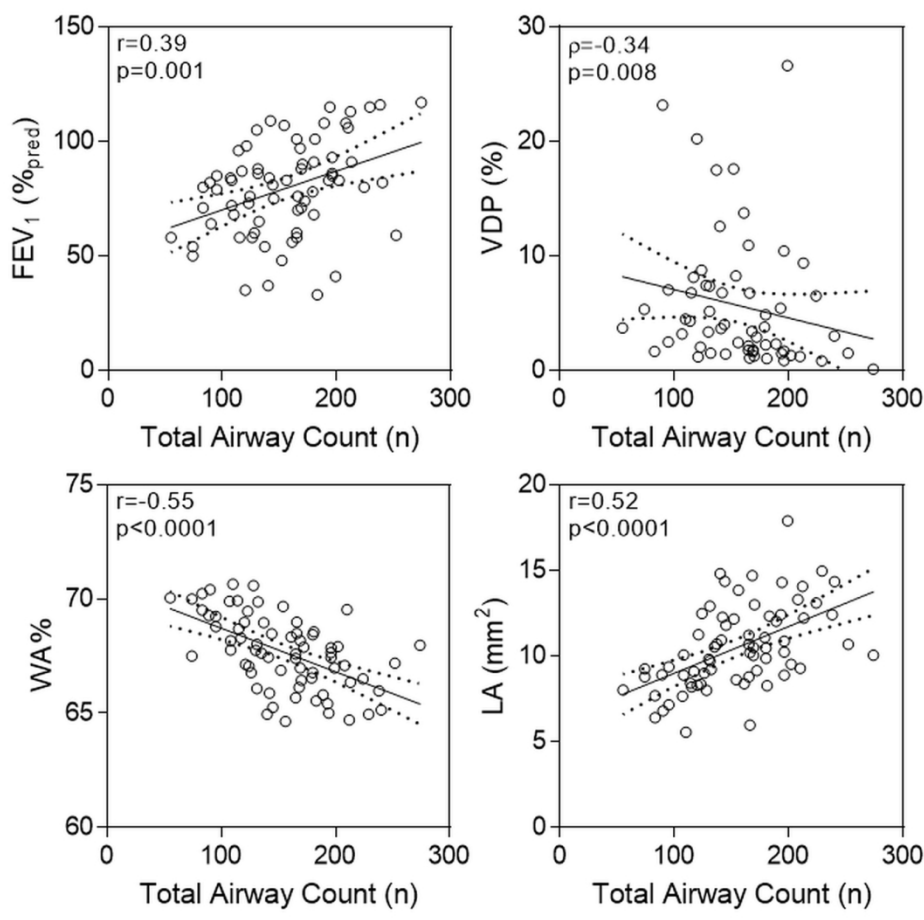
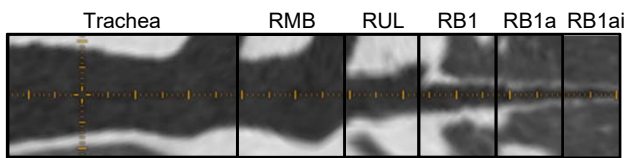


Figure 4. CT total airway count relationships.
Total airway count was significantly related to post-bronchodilator FEV₁ ($r=0.39$, $p=0.001$) and MRI ventilation defect percent (VDP; $r=-0.34$, $p=0.008$), as well as CT wall area percent (WA%; $r=-0.55$, $p<0.0001$) and lumen area (LA; $r=0.52$, $p<0.0001$).

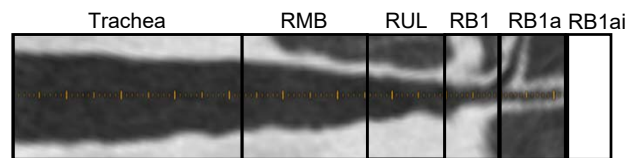
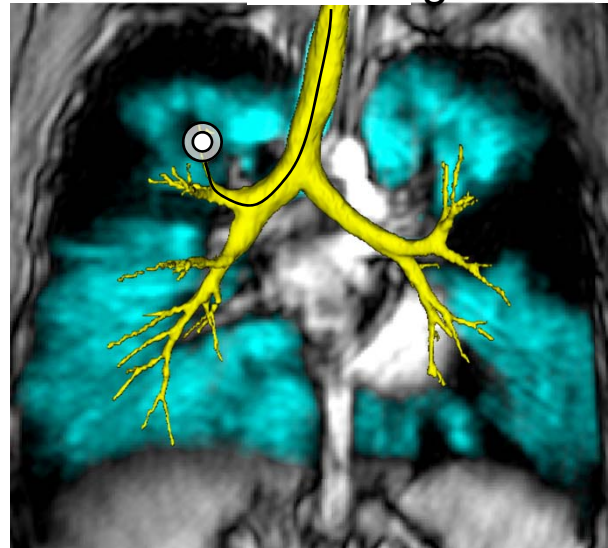
127x121mm (300 x 300 DPI)

<10 Missing

*Missing sub-subsegments n=4* $FEV_1 = 103\%_{pred}$

TAC=196

VDP=3%

 $WA_{RB1a} = 65\%$ ≥ 10 Missing*Missing sub-subsegments n=16* $FEV_1 = 33\%_{pred}$

TAC=74

VDP=16%

 $WA_{RB1a} = 73\%$

ONLINE DATA SUPPLEMENT**Is Computed Tomography Airway Count Related to Asthma Severity and Airway Structure-function?**

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Supplementary Table S1. Total airway count by airway generation.

Generation Count ($\pm$ SD)	Never-smokers¹	GINA1-3 (n=15)	GINA4 (n=19)	GINA5 (n=36)
0	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)
1	2.0 (0.1)	2.0 (0.0)	2.0 (0.0)	2.0 (0.0)
2	4.0 (0.1)	4.3 (0.8)	4.5 (1.4)	4.2 (0.5)
3	13.0 (0.8)	13.4 (0.6)	13.9 (1.1)	13.5 (1.0)
4	24.2 (1.3)	25.0 (2.2)	24.9 (1.7)	25.0 (2.5)
5	43.5 (6.2)	45.3 (6.2)	39.4 (7.3)	38.8 (10.1)
6	52.3 (17.4)	46.9 (16.3)	32.0 (15.4)*	34.9 (17.3)
7	42.1 (22.9)	27.5 (16.3)	14.8 (8.8)*	13.8 (12.1)*
8	23.3 (16.4)	10.1 (10.5)	5.3 (4.7)	5.8 (5.5)
9	10.8 (9.8)	2.5 (3.3)	2.2 (2.7)	1.3 (2.0)
10	3.8 (4.6)	1.1 (1.8)	0.3 (0.6)	0.2 (0.6)*
11	-	0.3 (0.7)	0.0 (0.0)	0.0 (0.0)*

GINA=Global Initiative for Asthma. ¹From Kirby et al., 2018. *Significantly different from treatment steps 1-3, $p<0.05$.