SUPPLEMENTAL APPENDIX

LINKS BETWEEN MUCUS PLUGS, EOSINOPHILIA AND AIRFLOW OBSTRUCTION IN ASTHMA

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1. METHODS

1.1 Study Design

SARP is a 3-year longitudinal cohort study. Asthma patients and healthy controls were recruited as part of the Severe Asthma Research Program (SARP)-3 cohort across 7 centers. The clinical centers in the network were Brigham and Women's Hospital, The University of California at San Francisco, the University of Pittsburgh, The University of Virginia, the University of Wisconsin, Wake Forrest School of Medicine, and Washington University in St Louis (with co-investigators at the University of Iowa). All centers used the same characterization procedures and all assessments adhered to standardized protocols and techniques ensuring uniformity of data and adherence to safety precautions. The protocol includes three baseline visits in which asthma patients undergo detailed characterization, including sputum questionnaires, maximum bronchodilator reversibility tests, a systemic corticosteroid responsiveness test, and an optional multi-detector computed tomography (MDCT) scan of the lungs (Figure S5). Data reported here are from patients that had MDCT's as part of their characterization. Healthy subjects for MDCT scans were recruited at a single center (Washington University in St Louis) and for sputum cell analyses were recruited from all SARP-3 centers.

1.2 Asthma Patients

658 asthma patients were enrolled to the Severe Asthma Research Program (SARP) from November 1, 2012 to October 1, 2014 by eleven clinical research centers across the United States. 146 of the 658 subjects underwent multidetector computerized tomography (MDCT) of the lungs (Figure 1, Table S1). Among 146 asthma patients who had MDCT scans as part of the SARP-3 protocol, 25 patients also had MDCT lung scans available from their participation

in SARP-1 or SARP-2 protocols. These patients were enrolled at 3 sites (University of Pittsburgh, University of Wisconsin, and Washington University) and scans were performed 2-9 years prior to the SARP-3 MDCT scans (Table S2).

Inclusion criteria for SARP mandated that at least 60% of the asthmatic patients meet the American Thoracic Society/European Respiratory Society (ATS/ERS) definition for severe asthma[1]. All patients were non-smokers (<10 pack-years of tobacco use if >30y of age; <5 pack-years if <30y of age) and were required to have evidence of bronchial hyperresponsiveness (defined as a PC20 methacholine < 16mg/mL) or reversible airflow obstruction, as evidenced by an increase in FEV1 of ≥12% following albuterol inhalation (up to 720ug) with or without additional ipratropium bromide inhalation (136 mcg). Patients were excluded if they were pregnant or breastfeeding during the initial characterization period, had a history of premature birth (<35 weeks' gestation), or had a diagnosis of any other chronic pulmonary disorder, which, in the opinion of the investigator, contributed significantly to the patient's respiratory symptoms.

Patients completed comprehensive phenotypic characterization, including a physician-directed history, Asthma Control Test, spirometry, maximum bronchodilator reversibility, corticosteroid responsiveness, complete blood count with cell differential, induced sputum cell counts, serum IgE measurements, and FeNO measurement. In addition, subjects completed extensive questionnaires that characterized asthma symptoms, sputum symptoms, quality of life, medication use, and health care utilization (Figure S5). All subjects signed informed consents approved by their local institutional review boards.

1.3 Healthy Subjects

Adult healthy subjects were recruited at Washington University in St Louis (Table S1).

Inclusion criteria were as follows: non-smokers (<10 pack-years of tobacco use if >30y of age; <5 pack-years if <30y of age), and normal lung function (pre-bronchodilator FEV/FVC >0.70 and <12% increase in FEV1 following 4 puffs of albuterol). Subjects were excluded if they were pregnant or breastfeeding, or had a diagnosis of any lung disease.

1.4 Lung Function Testing

Spirometry, lung volume measurement, and maximum bronchodilation procedures were conducted according to a SARP manual of procedures, which conformed with ATS/ERS guidelines for spirometry [2] and lung volumes measurements [3]. Total Lung Capacity (TLC) and Residual Volume (RV) were measured by body plethysmography. A pant rate of <1 Hz was used during the mouthpiece occlusion, which was activated after the subject had attained a stable end-expiratory volume for at least 4 breaths; after the brief occlusion, subjects exhaled maximally to RV and then inhaled maximally to TLC. Subjects were asked to withhold bronchodilator medications prior to spirometry and plethysmography testing.

1.5 Procedures for withholding asthma and allergy medications

Subjects were asked to hold their bronchodilator medications prior to spirometry testing. The medication holds for SARP were as follows; short-acting beta agonists - 4 hours; short-acting anticholinergics - 6 hours; long-acting beta agonists - 12 hours; long-acting muscarinic antagonists - 24 hours; and leukotriene modifiers - 24 hours.

1.6 Multi Detector Computerized Tomography (MDCT) Protocol

MDCT was performed within 2 hours following maximal bronchodilation according to a standard protocol monitored by a SARP imaging center at the University of Iowa with institutional review board approval. The same scanning protocol was used in both asthma patients and healthy controls. Before beginning the MDCT scan, patients were carefully coached using standardized breathing instructions administered by the technologist and images of the lungs at Total Lung Capacity (TLC) were obtained from a single breath-hold at full inspiration. The MDCT parameters for each scanner model used are listed in Table S6. BMI (3 categories), lung volume (e.g. TLC) and scanner model were used to determine the CTDIvol and subsequently the effective mAs or mA settings appropriate for each subject (Table S7). Scanners at each center were regularly calibrated with a phantom (COPDgene® Phantom Model CCT162, The Phantom Laboratory - http://www.phantomlab.com/other-catphans/) and all scans were evaluated for protocol adherence by the SARP Imaging Center at the University of Iowa. De-identified image data (in standard digital format) were distributed to the radiologists for scoring. To blind the readers to the disease status of the subject, healthy subjects were given a SARP identification number and the scan date of the healthy scans were shifted forward 3 years to match the scanning period of the asthmatic scans. Evaluation for mucus was performed on scans taken at total lung capacity using a standard window width of 1200 HU and level of -600 HU[4].

1.7 Automated CT analysis

Quantitative airway morphology was measured from MDCT scans using automated, quantitative software that was designed to reliably label and segment the first five to six airway generations, and to allow the accurate measurement of airway walls and lumen diameters obtained perpendicular to the long axis of each airway (Apollo 1.2; VIDA Diagnostics; Iowa City, IA). Airway measurements of RB1, RB4, RB10, LB1, LB4, LB10 (4th generation) were made at each centerline voxel and were averaged over the middle third of the segment. The specific MDCT scan measurements used included airway wall thickness (WT), percentage of WT (WT%), wall area (WA), percentage of WA (WA%), luminal area (LA) and percentage of

LA (LA%) (Figure S6). The calculations are as follows: WT: average outer diameter - average inner diameter; WT%: (WT/average outer diameter) x 100; WA: total area (TA) - LA; WA%: (WA/TA) x 100; and LA%: (LA/TA) x 100. WA%, LA% and WT% were used in analysis, as these account for differences in airway size. Airway measurements of RB1, RB4, RB10, LB1, LB4, LB10 were averaged to give a summary estimate for each patient. WT% was reported in results but all 3 measurements gave similar results.

1.8 Development, application and validation of the MDCT Mucus Score

A scoring system to quantify mucus plugs in lung images generated using multi-detector computerized tomography was developed by a mucus score team (ED, JF, BE, DG, SN, MS, and JN). The scoring system was based on bronchopulmonary segmental anatomy. Each bronchopulmonary segment was given a score of 1 (mucus plug present) or 0 (mucus plug absent). The segment scores of each lobe were summed to generate a total mucus score for both lungs, yielding a mucus score ranging from 0-20. The score was tested and refined using 25 scans from patients with severe asthma recruited at UCSF for SARP. During development, the score was tested and modified twice to yield the final version as shown in Figure 1D and further explained below.

<u>Final scoring system applied in this study:</u>

1. Mucus plugs were defined as complete occlusion of a bronchus, irrespective of generation. When parallel to the scan plane, mucus plugs were recognized as tubular densities with or without branching. When oriented obliquely or perpendicularly to the scan plane, they were identified as oval or rounded opacities seen on sequential slices and differentiated from blood vessels by their continuity with non-impacted portions of the bronchial lumen and their position relative to adjacent blood vessels.

2. A 2 cm peripheral exclusion zone confined to the costal and diaphragmatic pleura was excluded from evaluation as the small caliber of these peripheral airways makes occlusion by mucus difficult to ascertain. The 2 cm peripheral zone adjacent to the mediastinal pleura was *not* excluded from evaluation owing to the larger airways adjacent to the mediastinum.
3. Use of a standard window width of 1200 HU and level -600 HU for bronchial wall evaluation.

<u>Application and validation of the CT Mucus Score:</u>

Before application of the scoring system to the SARP cohort, a teleconference was held which included a slide presentation with detailed description of the final scoring system followed by a 1-hour consensus reading session using a training-set of 3 CT scans. Five radiologists with sub-specialty training in thoracic radiology scored the MDCT's. To generate the mucus score, two radiologists were randomly assigned to independently score each scan. Each radiologist was provided with their individual set of scans in digital format. The radiologists entered the mucus score data in real-time into a secure online survey (Research Electronic Data Capture) (Figure S8). The average score of both raters was used to calculate the CT mucus score for each subject. This generated a continuous score ranging from 0 to 20 increasing in increments of 0.5. The validity of the mucus score was tested by analyzing for inter-rater bias followed by inter-rater and intra-rater agreement. Bias between raters, where one rater consistently over- or underscores relative to the other rater, was tested using paired analyses. No significant bias and was found between any of the pairs of raters ($p>0.05$). Once absence of bias was confirmed, inter-rater agreement of the CT mucus score could be assessed by intraclass correlation coefficient (ICC). An initial check of inter-rater agreement was made after half of the scans were scored, with a plan to recalibrate any rater(s) with outlying scores to the group mean. The ICC at interim analysis was 0.69 and retraining was provided in one instance. At the end of the study, the ICC for agreement between readers was 0.80 (95% CI 0.74 to 0.85) for all 171 scans and 0.79 (95% CI 0.72 to 0.85) for the 146 asthma scans alone. In addition, the intra-rater agreement for a random subset of 14 scans (3 healthy, 11 asthma) that was scored twice by each of the five radiologists was 0.99 (95% CI 0.99 to 1.00).

1.9 Sputum induction

Sputum induction was performed on visits 2 and 3 (Figure S6). For safety, induced sputum was only performed in patients with an FEV1 was > 50% predicted after albuterol pretreatment (360ug). Sputum was induced over 12 min with using hypertonic saline. Induced sputum was processed and analyzed at two SARP centers. The Wake Forest University center generated the sputum cell differential counts for SARP, and the University of California at San Francisco center extracted the RNA and measured gene expression for SARP. Total and differential cell counts were quantified in SARP subjects using methods previously described[5,6]. Gene expression of IL-4, IL-5, IL-13, and for airway gel-forming mucins (MUC5AC, MUC5B) and housekeeping genes were measured from RNA isolated from induced sputum cell pellets from 77 asthma subjects using previously described methods of real-time Taqman-based quantitative PCR (qPCR)[7]. The details of the specific design of the primers and probes are shown in Table S8.

<u>Sputum quality systems:</u>

1. Cell counts: Sputum samples were deemed of sufficient quality if squamous cell count was <80%.
2. qPCR: Only sputum samples with adequate cell counts were analyzed for qPCR. RNA quality was measured with the Agilent 2100 Bioanalyzer (Biogen, Weston, Mass), which performs electrophoretic separations according to molecular weight. The RNA integrity

number (RIN) was measured for each sample[8,9] and only samples whose RIN value was >5 were considered adequate for gene expression profiling[7].

1.10 Questionnaires

Sputum and Cough Questions

Questionnaires that were completed by asthma patients at study entry. Chronic mucus hypersecretion was defined using the ATS/WHO definition of chronic bronchitis, which assesses chronic cough and sputum production in the preceding 2 years[10]. The specific question used was: "*Have you had cough and sputum production on most days for at least 3 months a year for at least 2 consecutive years*". The answer options were: Yes, No, or Don't Know. The subjects that answered "Don't know" were recoded as "no".

Some patients did not have data for chronic mucus hypersecretion. Initially, the chronic bronchitis was a sub-question of the question "Have you ever had bronchitis?" Patients who answered "no" to this question were directed to skip the chronic bronchitis and this data was therefore not collected. This skip logic was removed in October 2013, and chronic bronchitis became independent question going forward. For this reason, data for "chronic bronchitis" are missing in 25 patients (17.1%).

Asthma Control Test (ACT)

This is a validated self-administered tool for identifying poorly controlled asthma [11,12]. ACT assesses the frequency of shortness of breath and general asthma symptoms, use of rescue medications, the effect of asthma on daily functioning, and overall self-assessment of asthma control in the previous 4 weeks rated using a 5-point scale. The score ranges from 5 (poor control) to 25 (complete control of asthma). An ACT <20 indicates poor control.

2. TABLES AND FIGURES

Table S1. Characteristics of Healthy and Asthma Subjects

Characteristics	**Healthy**		**Asthma**	
	MDCT analysis (n=22)	**Sputum analysis** (n=35)	**MDCT analysis** (n=146)	**Plethysmography analysis** (n=43)
Mean age (years)	29.5 ± 11.5	39.2 ± 12.6	46.8 ± 16.0	51.1 ± 14.3
Female sex - no. (%)	15 (60.0)	21 (53.9)	91 (62.3)	26 (60.5)
Race, no. (%)				
American Indian or Alaska Native	0 (0)	0 (0)	0 (0)	0 (0)
Asian	1 (4)	3 (7.7)	10 (6.9)	1 (2.3)
Black or African American	3 (12)	6 (15.4)	34 (23.3)	5 (11.6)
Caucasian	17 (68)	25 (64.1)	90 (61.6)	36 (83.7)
Native Hawaiian or Pacific Islander	0 (0)	0 (0)	0 (0)	1 (2.3)
Mixed race	1 (4)	5 (12.8)	12 (8.2)	0 (0)
Unknown/refused to answer	3 (12)	0 (0)	0 (0)	0 (0)
Spirometry data				
FEV1 (% predicted)	98.2 ± 9.3	98.1 ± 11.3	72.2 ± 20.6	74.3 ± 22.3
FVC (% predicted)	100.1 ± 10.3	99.8 ± 13.3	85.5 ± 20.6	84.6 ± 17.5
FEV1/FVC	0.84 ± 0.03	0.98 ± 0.57	0.83 ± 0.13	0.86 ± 0.13
History of atopy	4 (16)	(0)	110 (75.3)	34 (79.0)
History of smoking[†]	0 (0)	(0)	0 (0)	0 (0)

Data reported as mean and standard deviation unless otherwise indicated. CT scans of healthy controls from SARP II and SARP III. Sputum measurements of healthy controls from SARP III. CT scans and plethysmography of asthma subjects from SARP III.

[†]Predicted values missing in one healthy male subject for sputum analysis (age 23 years; FEV1 4.65L, FVC 5.81, height measurement missing).

[‡] Smoking history refers to >5 pack years

Table S2. Characteristics of Asthma Subjects with repeat CT scans across SARP studies

Characteristics	Time points	
	SARP-1/ SARP-2	**SARP-3**
Mean age (years)*	44.3 ± 10.3	49.5 ± 11.7
Female sex - no. (%)	13 (52)	13 (52)
Spirometry data*		
FEV1 (% predicted)	67.7 ± 19.5	67.5 ± 20.8
FVC (% predicted)	80.4 ± 16.1	81.2 ± 17.2
FEV1/FVC	0.67 ± 0.11	0.81 ± 0.13
Max FEV1 (% predicted)	81.4 ± 21.1	77.9 ± 20.7
Max FVC (% predicted)	91.7 ± 15.5	89.2 ± 14.9
Sputum cell counts (%)		
Eosinophils	0.3 (0.001, 3.2)	0.6 (0.2, 2.4)
Neutrophils	62 (32.2, 76.3)	68.9 (42.9, 77.8)
FeNO (ppm)‡	22 (10.3, 39.6)	22 (14, 46)
Blood cell counts ($x10^6$/L) †		
Eosinophils	259 ± 232	313.5 ± 409.6
Neutrophils	4782 ± 2819	4599 ± 2106
Mucus Score, segments	2 (0,9)	6 (1,12)
Mucus Score, categories		
Zero	10 (40)	5 (20.0)
Low	4 (16)	4 (16.0)
High	11 (44)	16 (64.0)

Data reported as mean and standard deviation unless otherwise indicated.

* Age and spirometry data for SARP1-2 missing in 1 patient

† Spirometry data for SARP-1/-2 missing in 3 patients

‡ FeNO data for SARP-1/-2 missing in 8 patients

§ Sputum cell count data for SARP-1/-2 missing in 15 patients and for SARP-3 in 5 patents.

Table S3. Characteristics of Subjects with Asthma across Mucus Score Categories

Characteristic	All (n=146)	Mucus Score Zero (n=61)	Low (n=45)	High (n=40)
Mucus score	0.5 (0-4.5)	0 (0)	1.5 (0.5-2.5)	9.5 (6-12)
Spirometry - pre bronchodilator				
FEV1(% predicted)†‡	72.2 ± 20.6	81.0 ± 16.2	74.5 ± 20.8	56.1 ± 17.4
FVC (% predicted)†‡	85.5 ± 17.9	89.3 ± 14.0	88.3 ± 19.4	76.7 ± 19.0
FEV1/FVC (predicted)* †‡	0.83 ± 0.13	0.90 ± 0.10	0.83 ± 0.11	0.72 ± 0.11
Spirometry - post bronchodilator				
FEV1 (% predicted)†‡	82.7 ± 20.9	90.7 ± 15.9	85.3 ± 21.3	67.7 ± 19.3
FVC (% predicted)†‡	92.8 ± 17.0	95.1 ± 13.8	95.2 ± 17.9	86.6 ± 19.2
FEV1/FVC (predicted)†‡	0.89 ± 0.12	0.96 ± 0.09	0.89 ± 0.10	0.78 ± 0.11
Sputum cell counts (%)‖				
Neutrophils	58 (35,78)	62 (37,83)	60 (35,79)	47 (31,70)
Epithelial cells	4.7 (2,11.5)	4.3 (2.3,11.5)	4.3 (2.3,5.9)	6.9 (1.9,17)
Blood cell counts ($x10^6$/L) ¶				
Neutrophils	4286 ± 2350	4569 ± 2951	4030 ± 1934	4134 ± 1592
Total white blood cells	7279 ± 2548	7534 ± 3149	6953 ± 2138	7255 ± 1827
Total IgE (IU/mL) ¶	150 (52,363)	126 (32,482)	150 (74,335)	181 (79,363)
Exacerbations in last 12 months – no. (%)	74 (50.7)	29 (47.5)	23 (51.1)	22 (55.0)
Nasal polypectomy – no. (%)†	21 (14.4)	1 (1.6)	8 (17.8)	12 (30.0)
Sinus surgery – no. (%)†	19 (13.0)	3 (4.9)	8 (17.8)	8 (20.0)
ABPA - no. (%)**	3 (2.1)	0 (0)	2 (1.4)	0 (0)

Data reported as mean ± standard deviation or median (interquartile range). Zero represents the "mucus absent" group (mucus score=0). Low represents the group with mucus scores 0.5-3.5 and high represents the group with mucus scores ≥4, based on the median score of 3.5 in the "mucus present" group.

* $p<0.05$ for comparison of *zero* and *low* scores

† $p<0.05$ for comparison of *zero* and *high* scores

‡ $p<0.05$ for comparison of *low* and *high* scores

‖ Sputum cell counts were not available in 40 subjects due to ineligibility for sputum induction or because the induced sputum not meet quality metrics.

¶ Blood eosinophil measurements were not available for 2 subjects. Serum IgE was not available for 1 patient.

** Diagnosed using elevated total IgE, specific IgE to Aspergillus fumigatus, systemic eosinophilia, and radiographic changes consistent with ABPA.

Table S4. Aeroallergen Sensitivity				
Allergen		**Mucus Score**		
	All (n=144)	**Zero** (n=61)	**Low** (n=44)	**High** (n=39)
Fungal				
Aspergillus fumigatus, no. (%)	30 (20.8)	11 (18.0)	11 (25.0)	8 (20.5)
Cladosporium herbarum, no. (%)	21 (13.9)	8 (13.1)	9 (20.5)	4 (10.3)
Alternaria alternata, no. (%)	37 (25.7)	15 (24.6)	15 (34.1)	7 (18.0)
Furred animal				
Cat dander, no. (%)	82 (56.6)	32 (52.5)	28 (62.2)	32 (56.4)
Dog dander, no. (%)	78 (53.8)	33 (54.1)	26 (57.8)	19 (48.7)
Mouse urine proteins, no. (%)	16 (11.0)	6 (9.84)	7 (15.6)	3 (7.7)
Rat urine proteins, no. (%)	21 (14.5)	10 (16.4)	7 (15.6)	4 (10.3)
Mites and insects				
Dermatoph pteronyssinus, no. (%)	70 (48.3)	31 (50.8)	23 (51.1)	16 (41.0)
Dermatoph fariane, no. (%)	71 (49)	32 (52.5)	24 (53.3)	15 (38.5)
Cockroach, no. (%)	29 (20.1)	16 (26.2)	7 (15.9)	6 (15.4)
Plant				
Ragweed, no. (%) *	44 (30.6)	25 (41.0)	13 (29.6)	6 (15.4)
Weed mix, no. (%)	41 (28.5)	23 (37.7)	12 (27.3)	6 (15.4)
Grass mix, no. (%)	42 (29.0)	18 (29.5)	13 (29.0)	11 (28.2)
Tree mix, no. (%)	45 (31.3)	20 (32.8)	14 (31.8)	11 (28.2)

Aeroallergen sanitization defined as specific IgE >0.35 IU on Immunocap test (Phadia, Uppsala Sweden)
Blood measurements were not available for 2 subjects.
* P<0.05

Table S5. Characteristics stratified by chronic mucus hypersecretion and mucus plugging				
Characteristic	**Chronic mucus hypersecretion***		**Mucus plugging**	
	Absent (n=80)	**Present** (n=41)	**Zero** (n=61)	**High** (n=40)
Anthropometrics				
Mean age (years)	44.3 ± 16.5	52.4 ± 15.3†	43.3 ± 15.4	52.2 ± 16.5†
Female sex - no. (%)	53 (66.3)	27 (65.9)	43 (70.5)	22 (55.0)
Body Mass Index (kg/m^2)	31.2 ± 8.7	34.5 ± 9.4	34.3 ± 9.9	30.7 ± 6.3
Asthma control and Exacerbations				
Asthma Control Test score	20 (16, 21)	15 (10, 19)‡	19 (15,21)	16.5 (13,19)‡
High dose inhaled steroids use - no. (%)	53 (66.3)	31 (75.6)	36 (59.0)	36 (90.0)‡
Chronic systemic steroids use - no. (%)	6 (7.5)	5 (12.2)	3 (4.9)	9 (22.5)†
Exacerbations in last 12 months - no. (%)¶	28 (35.0)	29 (70.7)§	29 (47.5)	22 (55.0)
Spirometry‖				
FEV1 (% predicted)	77.6 ± 18.8	67.2 ± 21.9‡	81.0 ± 16.2	56.1 ± 17.4§
FVC (% predicted)	90.1 ± 16.3	81.6 ± 18.2‡	89.3 ± 14.0	76.7 ± 19.0‡
FEV1/FVC (predicted)	0.85 ± 0.12	0.81 ± 0.14	0.90 ± 0.10	0.72 ± 0.11§
Inflammation				
Airway measures				
FeNO (ppm)**	20 (12,35)	20 (11,29)	18 (10,27)	28 (19,40)‡
Sputum eosinophil count (%)††	0.7 (0.2,3.5)	0.6 (0,4.5)	0.2 (0,0.9)	7.3 (1.5,21.4)§
Sputum neutrophil count (%)††	59 (33,77)	66 (42,83)	62 (37,83)	47 (31,70)
Blood measures‡‡				
Blood eosinophil count (x10^6/L)	284 ± 202	338 ± 347	209 ± 153	459 ± 349§
Blood neutrophil count (x10^6/L)	4278 ± 2541	4450 ± 2258	4569 ± 2951	4134 ± 1592
Total IgE (IU/mL)	138 (46,306)	129 (35,406)	125 (32,482)	181 (79,363)
Sputum cell gene expression				
IL-4	15 (13, 17)	15 (12, 17)	15 (14, 17)	17 (15, 18)
IL-5	18 (16, 21)	18 (17, 20)	17 (15, 19)	20 (18, 22) ‡
IL-13	20 (17, 21)	20 (18, 21)	19 (17, 21)	22 (20, 22) †
IL-17	18 (18,20)	19 (17,20)	18 (17,20)	18 (17,19)
MUC5AC/MUC5B	0.99 (0.9,1.1)	0.99 (0.9,1.1)	0.95 (0.86,1)	1.1 (1.0,1.2) ‡
CT Findings				
Bronchiectasis on CT - no. (%)	15 (18.8)	9 (22.0)	7 (11.5)	11 (27.5)

Data reported as mean ± standard deviation or median (interquartile range).

* Questionnaire data for chronic bronchitis are available for 121 patients (see supplementary appendix)

† p<0.05 for comparison between absent and present or zero and high groups

‡ p<0.01 for comparison between absent and present or zero and high groups

§ p<0.001 for comparison between absent and present or zero and high groups

|| Pre bronchodilator

¶ Exacerbations defined as taking a short course of oral corticosteroids for asthma (min. 3 days) in the last year

** Fraction of nitric oxide in exhaled breath (FeNO) was not measured in 4 subjects.

†† Sputum cell counts were not available in 26 subjects due to ineligibility for sputum induction or because the induced sputum not meet quality metrics.

‡‡ Blood measurements were not available for 1 subject

Table S6. CT parameters: Total Lung Capacity (TLC) protocol

Scanner Model	SIEMENS Definition (AS Plus) 128 slice	SIEMENS Definition (DS) 64 slice	SIEMENS Sensation 64 slice	GE VCT 64 slice/ Discovery STE	GE Discovery CT 750HD 64 slice	PHILIPS Brilliance 64 slice
Scan Type	Spiral	Spiral Single Source	Spiral	Helical	Helical - Standard	Spiral Helix
Scan FOV	No selection	No Selection	No selection	Large	Large	No selection
Rotation Time (s)	0.5	0.5	0.5	0.5	0.5	0.5
Det. Configuration	128x0.6	64x0.6	64x0.6	64x0.625	64x0.625	64 x 0.625
Pitch	1.0	1.0	1.0	0.984	0.984	0.923
kVp	120	120	120	120	120	120
Effective mAs	S-90 M-110 L-165	S-85 M-105 L-150	S-80 M-100 L-145	S-145 M-180 L-270	S-145 M-180 L-270	S-105 M-130 L-190
Dose modulation	Care Dose OFF	Care Dose OFF	Care Dose OFF	Auto mA OFF	Auto mA OFF	Dose Right (ACS) OFF
Std. Algorithm	B35	B35	B35	Standard	Standard	B
Lung Algorithm	B30	B31	None	Detail	Detail	YB
Additional Image filters	No Selection	No Selection	No Selection	No Selection	IQ Enhance OFF	Adaptive Filtering OFF
Thickness (mm)	0.75	0.75	0.75	0.625	0.625	0.67
Interval (mm)	0.5	0.5	0.5	0.5	0.5	0.5
Iterative reconstruction	IRIS OFF	IRIS OFF	No Selection	ASIR OFF	ASIR OFF	iDOSE OFF
Scan Time (Sec) 30cm length	<10	<10	<10	<10	<10	<10
Recon Mode	N/A	N/A	N/A	Plus	Plus	N/A
Smart mA	N/A	N/A	N/A	OFF	OFF	N/A

* Effective mAs: Siemens = Eff. mAs, GE = mA setting, Philips = mAs. S= small, M= medium, and L= large. BMI categories as defined in Table S7.

Table S7. CTDIvol as a function of BMI

Body Size	**BMI Range**	**CTDIvol (mGy)**
Small	15 to 19	11.4
Medium	20 to 30	7.6
Large	>30	6.1

Table S8. Gene Primers and Probes	
Gene Primers	**Sequence**
PPIA-outer forward	ATGAGAACTTCATCCTAAAGCATACG
PPIA-outer reverse	TTGGCAGTGCAGATGAAAAACT
PPIA-inner forward	ACGGGTCCTGGCATCTTGT
PPIA-probe	ATGGCAAATGCTGGACCCAACACA
PPIA-inner reverse	GCAGATGAAAAACTGGGAACCA
GAPDH-outer forward	CAATGACCCCTTCATTGACCTC
GAPDH-outer reverse	CTCGCTCCTGGAAGATGGTGAT
GAPDH-inner forward	GATTCCACCCATGGCAAATTC
GAPDH-probe	CGTTCTCAGCCTTGACGGTGCCA
GAPDH-inner reverse	GGGATTTCCATTGATGACAAGC
YWHAZ-outer forward	CTTCTGTCTTGTCACCAACCATTC
YWHAZ-outer reverse	CAACTAAGGAGAGATTTGCTGCAG
YWHAZ-inner forward	TGGAAAAAGGCCGCATGAT
YWHAZ-probe	TGGCTCCACTCAGTGTCTAAGGCACCCT
YWHAZ-inner reverse	TCTGTGGGATGCAAGCAAAG
PSMB2-outer forward	CCATATCATGTGAACCTCCTCCT
PSMB2-outer reverse	GTCGAGGATACTGAGAGTCAGGAA
PSMB2-inner forward	TCCTCCTGGCTGGCTATGAT
PSMB2-probe	ACAGCGCTGGCCCTTCATGCTC
PSMB2-inner reverse	GGCTGCCAGGTAGTCCATGT
IL4-outer forward	GGGTCTCACCTCCCAACTGC
IL4-outer reverse	TGTCTGTTACGGTCAACTCGGT
IL4-inner forward	GCTTCCCCCTCTGTTCTTCCT
IL4-probe	TCCACGGACACAAGTGCGATATCACC
IL4-inner reverse	GCTCTGTGAGGCTGTTCAAAGTT
IL5-outer forward	GCCATGAGGATGCTTCTGCA
IL5-outer reverse	GAATCCTCAGAGTCTCATTGGCTATC
IL5-inner forward	AGCTGCCTACGTGTATGCCA
IL5-probe	CCCCACAGAAATTCCCACAAGTGCA
IL5-inner reverse	GTGCCAAGGTCTCTTTCACCA
IL13-outer forward	CAACCTGACAGCTGGCATGT
IL13-outer reverse	CCTTGTGCGGGCAGAATC
IL13-inner forward	GCCCTGGAATCCCTGATCA
IL13-probe	TCGATGGCACTGCAGCCTGACA
IL13-inner reverse	GCTCAGCATCCTCTGGGTCTT
IL17-outer forward	ACTGCTACTGCTGCTGAGCCT
IL17-outer reverse	GGTGAGGTGGATCGGTTGTAGT
IL17-inner forward	CAATCCCACGAAATCCAGGA
IL17-probe	CCCAAATTCTGAGGACAAGAACTTCCCC
IL17-inner reverse	TTCAGGTTGACCATCACAGTCC
MUC5B-outer forward	TACATCTTGGCCCAGGACTACTGT
MUC5B-outer reverse	AGGATCAGCTCGTAGCTCTCCAC
MUC5B-inner forward	CATCGTCACCGAGAACATCC
MUC5B-probe	CTGTGGGACCACCGGCACCAC
MUC5B- inner reverse	AAGAGCTTGATGGCCTTGGA
MUC5AC-outer forward	TGTGGCGGGAAAGACAGC
MUC5AC-outer reverse	CCTTCCCATGGCTTAGCTTCAGC
MUC5AC-inner forward	CGTGTTGTCACCGAGAACGT
MUC5AC-probe	CTGCGGCACCACAGGGACCA
MUC5AC- inner reverse	ATCTTGATGGCCTTGGAGCA

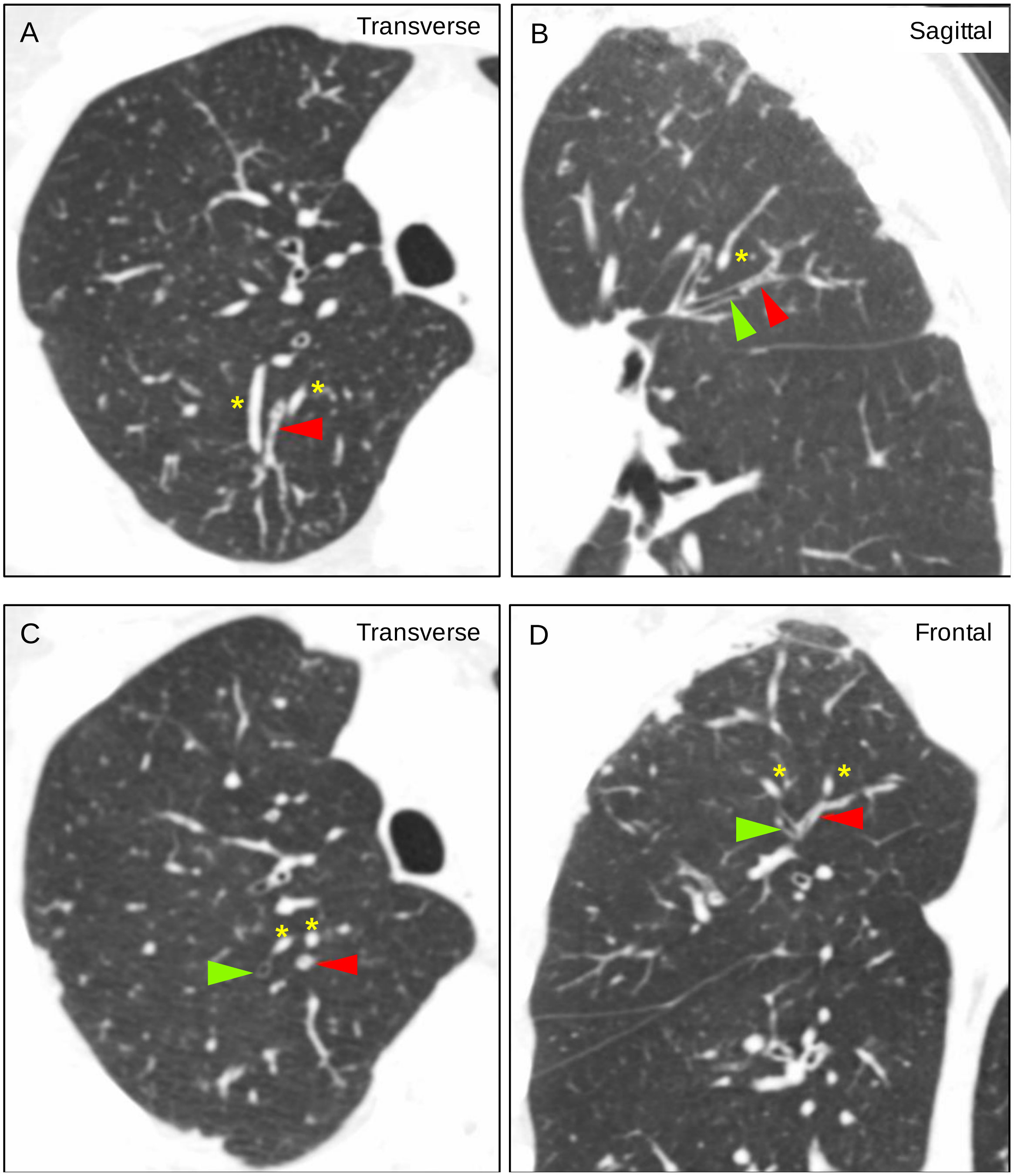


Figure S1. Examples of mucus plugs shown in different planes on MDCT.

(A) Transverse plane: Intraluminal mucus plug (red arrow) in longitudinal section on transverse plane. The accompanying bronchopulmonary vessels are indicated with yellow asterisks. **(B) Sagittal plane:** The mucus plug in (A) is now seen on the sagittal plane (red arrow) with patent airway lumen (green arrow) visible proximally. **(C) Transverse plane:** Intraluminal mucus plug in cross section appears as a rounded opacification (red arrow) on transverse plane. Adjacent patent airway (green arrow) and bronchopulmonary vessels (yellow asterisks) are also shown. **(D) Frontal plane:** The plugged airway in (C) is now seen in longitudinal section as a tubular opacification (red arrow), and a patent airway (green arrow) is seen branching off proximally.

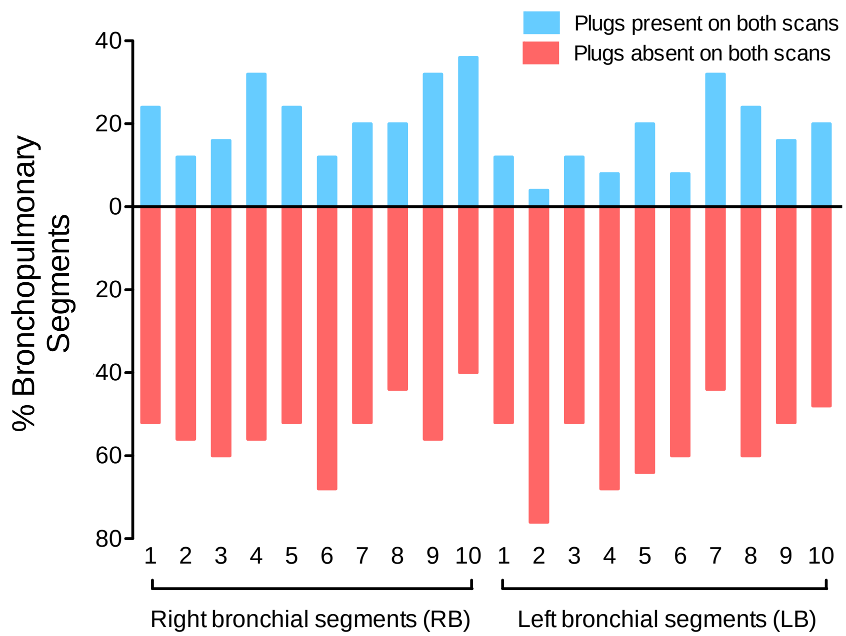


Figure S2. Persistence of mucus phenotype by bronchopulmonary segment.

Persistent presence or absence of mucus plugs from first to second scan, while very variable, were seen with similar frequency across all bronchopulmonary segments. There was no apical or basal pattern of involvement.

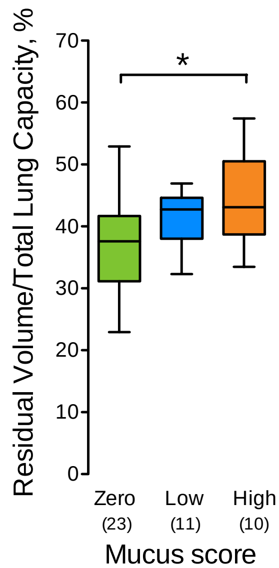


Figure S3. Mucus plugging is associated with air-trapping.

The RV/TLC % was higher in patients with a high-mucus score than patients with a zero-mucus score. Data was performed by body plethysmography and represents post-bronchodilator values. * indicates $p<0.05$.

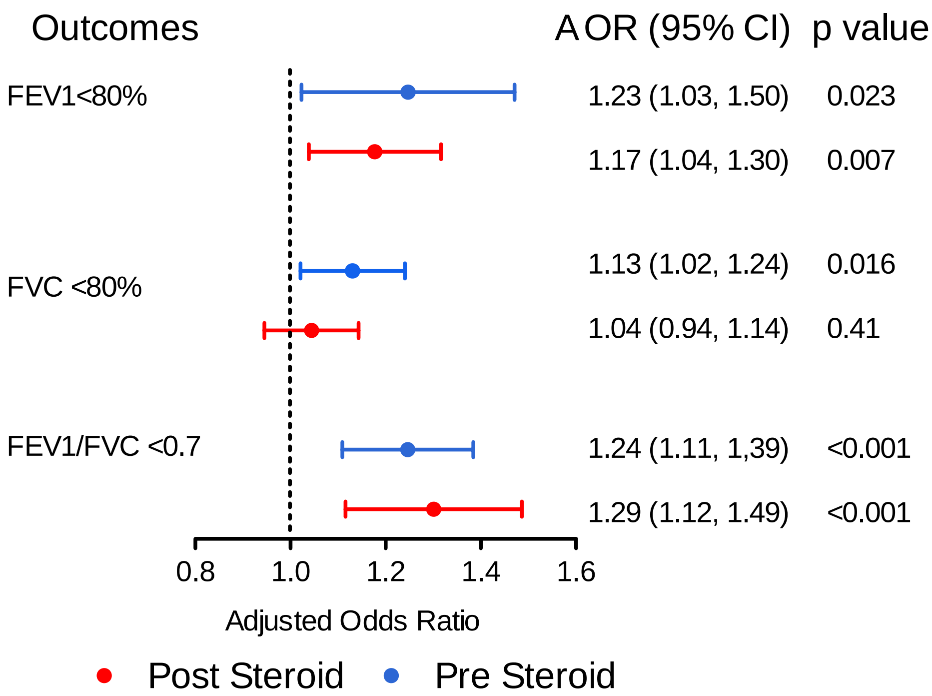


Figure S4. Logistic regression of the effects of mucus score on lung function
Forrest plot of the association between mucus plugging and lung function outcomes in asthma. Associations were derived from multivariable logistic regression models. Shown in the figure are the adjusted odds ratios (aOR) for subjects having FEV1 <80%, FVC <80% and FEV1/FVC <0.07, predicted by the mucus score (ranging 0-20). Age, gender, and wall thickness (surrogate for airway remodeling) were included in the model as covariates.

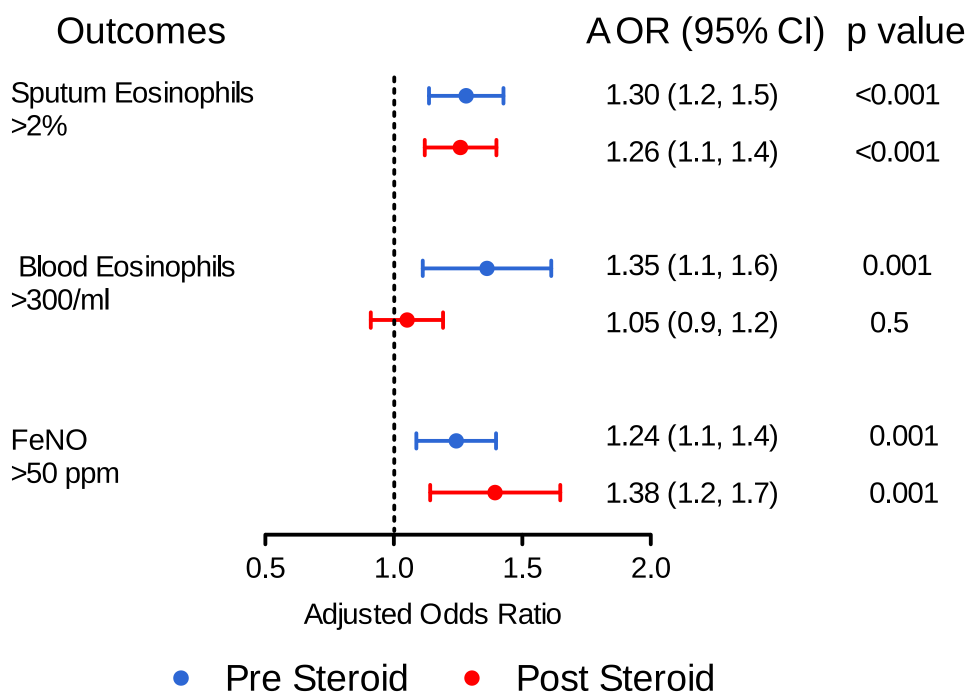


Figure S5. Logistic regression of mucus score on markers of type 2 inflammation. Forrest plot of the association between mucus plugging and markers of type 2 inflammation before and after steroid treatment in subjects with asthma. Associations were derived from multivariable logistic regression models. Shown in the figure are the adjusted odds ratios (aOR) for subjects having sputum eosinophilia (>2%), blood eosinophilia (>300/mL) or high FeNO (>50ppm), predicted by the mucus score (ranging 0-20). Age and gender were included in the model as covariates. Analyses were confined to subjects that had paired pre and post steroid data. Sputum eosinophil% = 90 subjects, Blood eosinophils = 73 subjects, FeNO=136 subjects.

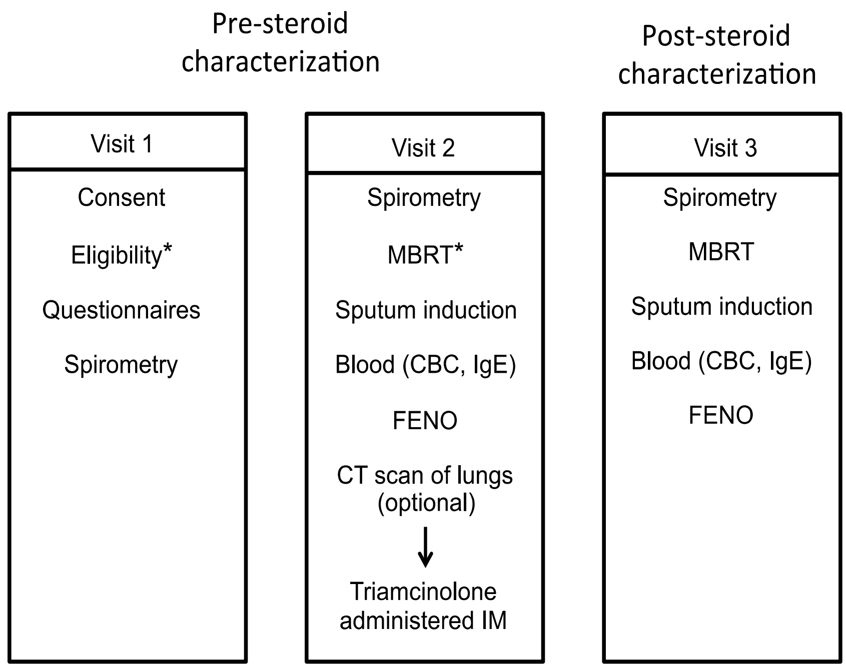


Figure S6. Visit procedures for patient characterization at baseline in SARP. Eligibility was determined by maximum bronchodilator reversibility test (MBRT) or methacholine challenge on visit 1. If MBRT was performed more than 6 weeks before visit 2 it was repeated at visit 2. Visit 3 was 18 ± 3 days after visit 2.

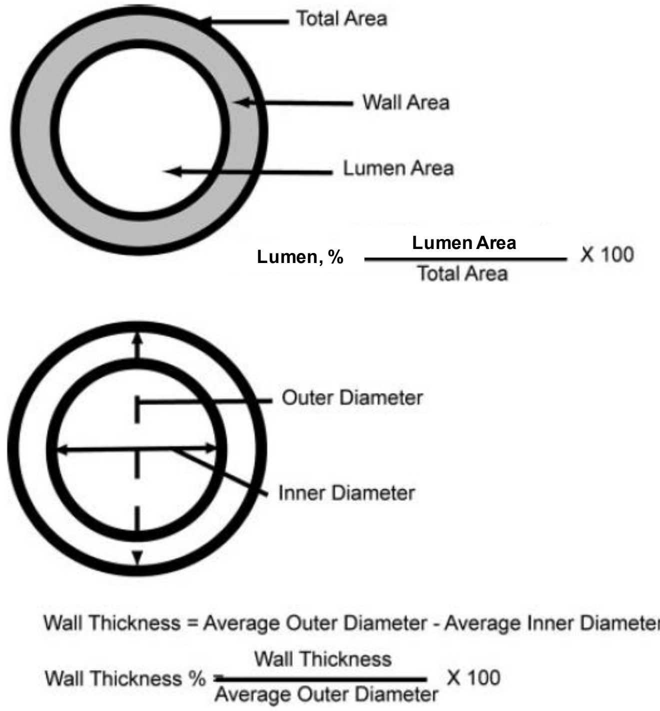
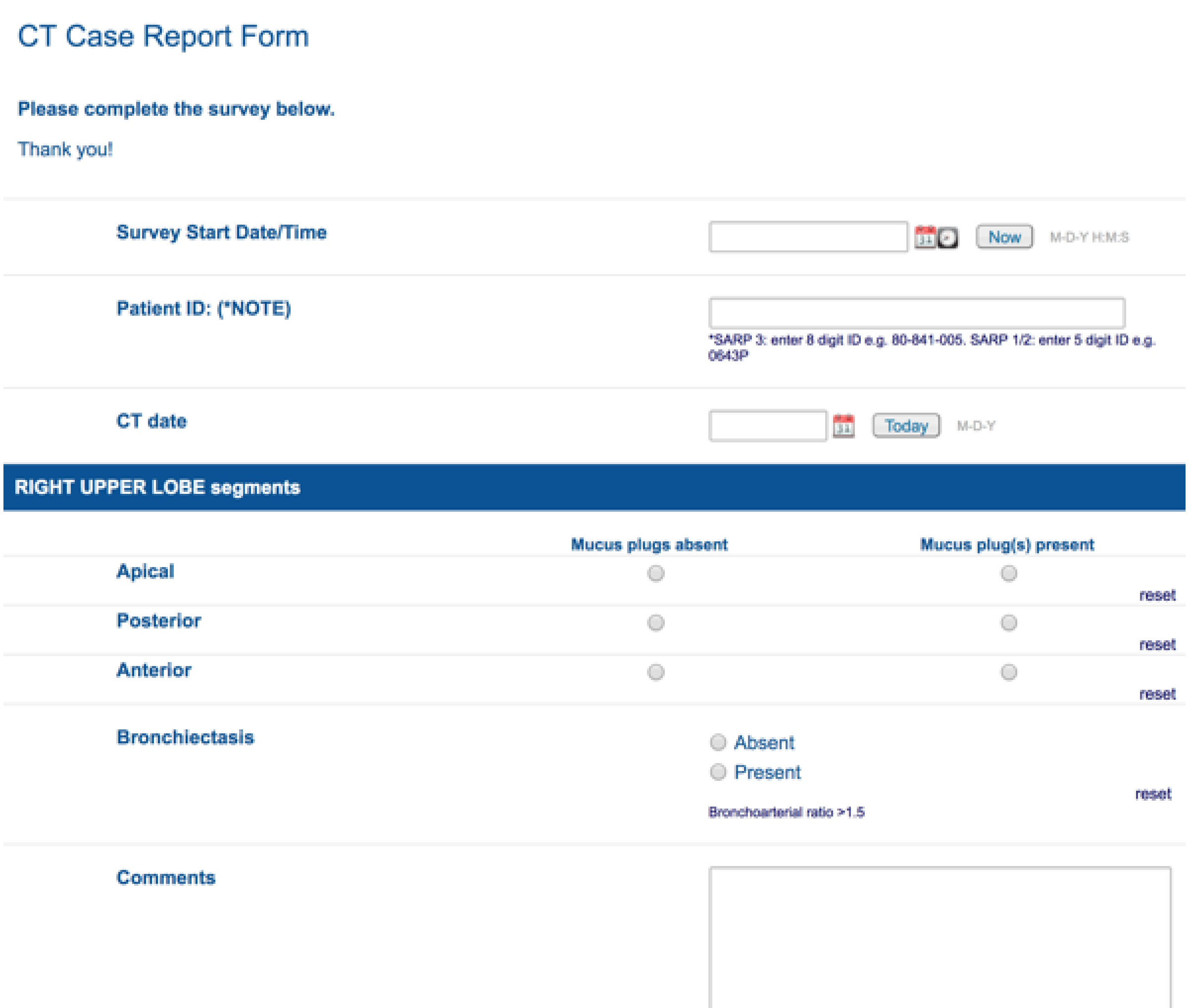


Figure S7. Airway measures by MDCT scan. The specific MDCT scan measurements used included airway wall thickness (WT), percentage of WT (WT%), luminal area (LA) and percentage of LA (LA%).


CT Case Report Form

Please complete the survey below.

Thank you!

Survey Start Date/Time	Now	M-D-Y H:M:S
Patient ID: (*NOTE)	*SARP 3: enter 8 digit ID e.g. 80-841-005. SARP 1/2: enter 5 digit ID e.g. 0643P	
CT date	Today	M-D-Y

RIGHT UPPER LOBE segments

	Mucus plugs absent	Mucus plug(s) present	
Apical			reset
Posterior			reset
Anterior			reset

Bronchiectasis: Absent / Present — reset

Bronchoarterial ratio >1.5

Comments

Figure S8. Modified web-based data capture tool used for longitudinal measurements in a subset of the SARP cohort with repeat MDCT scans. The figure shows a screen capture of the web based survey form that was modified from the original data capture tool to measure mucus plugging at a segmental level for comparison within the same patient over time. The same scoring criteria were displayed at the top of the form and the radiologists entered the data into the data fields as shown here. The data capture shown here is for each segment of right upper lobe – additional fields were available in the tool for the segments in other lung lobes.

3. SUPPLEMENTAL VIDEO

Video S1: CT scan demonstrating mucus plugs in relation to anatomical features in the right upper lobe. A patent sub segmental airway and 2 adjacent segmental bronchopulmonary vessels are labelled. Over sequential HRCT slices, airways that have patent lumens proximally (indicated by green arrow heads) are seen to transition into opacified airway lumens (red arrow). These opacified lumens meet the criteria for mucus plugs in the scoring system.

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Table: NHLBI Severe Asthma Research Program	
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(v). SARP CT Mucus Score Development Group	
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- We thank all of the volunteers who participated in the severe asthma research program.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	✔
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	✔
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	✔
Objectives	3	State specific objectives, including any prespecified hypotheses	✔
Methods			
Study design	4	Present key elements of study design early in the paper	✔
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	✔
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	✔
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	✔
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	✔
Bias	9	Describe any efforts to address potential sources of bias	✔
Study size	10	Explain how the study size was arrived at	✔
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	✔
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	✔
		(*b*) Describe any methods used to examine subgroups and interactions	✔
		(*c*) Explain how missing data were addressed	✔
		(*d*) If applicable, explain how loss to follow-up was addressed	
		(*e*) Describe any sensitivity analyses	
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	✔
		(b) Give reasons for non-participation at each stage	✔
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	✔
		(b) Indicate number of participants with missing data for each variable of interest	✔
		(c) Summarise follow-up time (eg, average and total amount)	✔
Outcome data	15*	Report numbers of outcome events or summary measures over time	✔
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	✔
		(*b*) Report category boundaries when continuous variables were categorized	✔
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	✔
Discussion			
Key results	18	Summarise key results with reference to study objectives	✔
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	✔
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	✔
Generalisability	21	Discuss the generalisability (external validity) of the study results	✔
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	✔

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.