**SUPPLEMENTAL APPENDIX**

# LINKS BETWEEN MUCUS PLUGS, EOSINOPHILIA AND AIRFLOW OBSTRUCTION IN ASTHMA

Eleanor M. Dunican, Brett M. Elicker, David S. Gierada, Scott K. Nagle, Mark L. Schiebler, John D. Newell Jr., Wilfred W. Raymond, Marrah E. Lachowicz-Scroggins, Selena Di Maio, Eric A. Hoffman, Mario Castro, Sean B. Fain, Nizar N. Jarjour, Elliot Israel, Bruce D. Levy, Serpil C. Erzurum, Sally E. Wenzel, Deborah A. Meyers, Eugene R. Bleecker, Brenda R. Phillips, David T. Mauger, Erin D. Gordon, Prescott G. Woodruff, Michael C. Peters, John V. Fahy for the National Heart Lung and Blood Institute Severe Asthma Research Program.

Corresponding author. Email: john.fahy@ucsf.edu

# 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 ($4^{th}$ 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.

Application and validation of the CT Mucus Score:

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 | All (n=144) | Mucus Score 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<br>M-110<br>L-165 | S-85<br>M-105<br>L-150 | S-80<br>M-100<br>L-145 | S-145<br>M-180<br>L-270 | S-145<br>M-180<br>L-270 | S-105<br>M-130<br>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 |

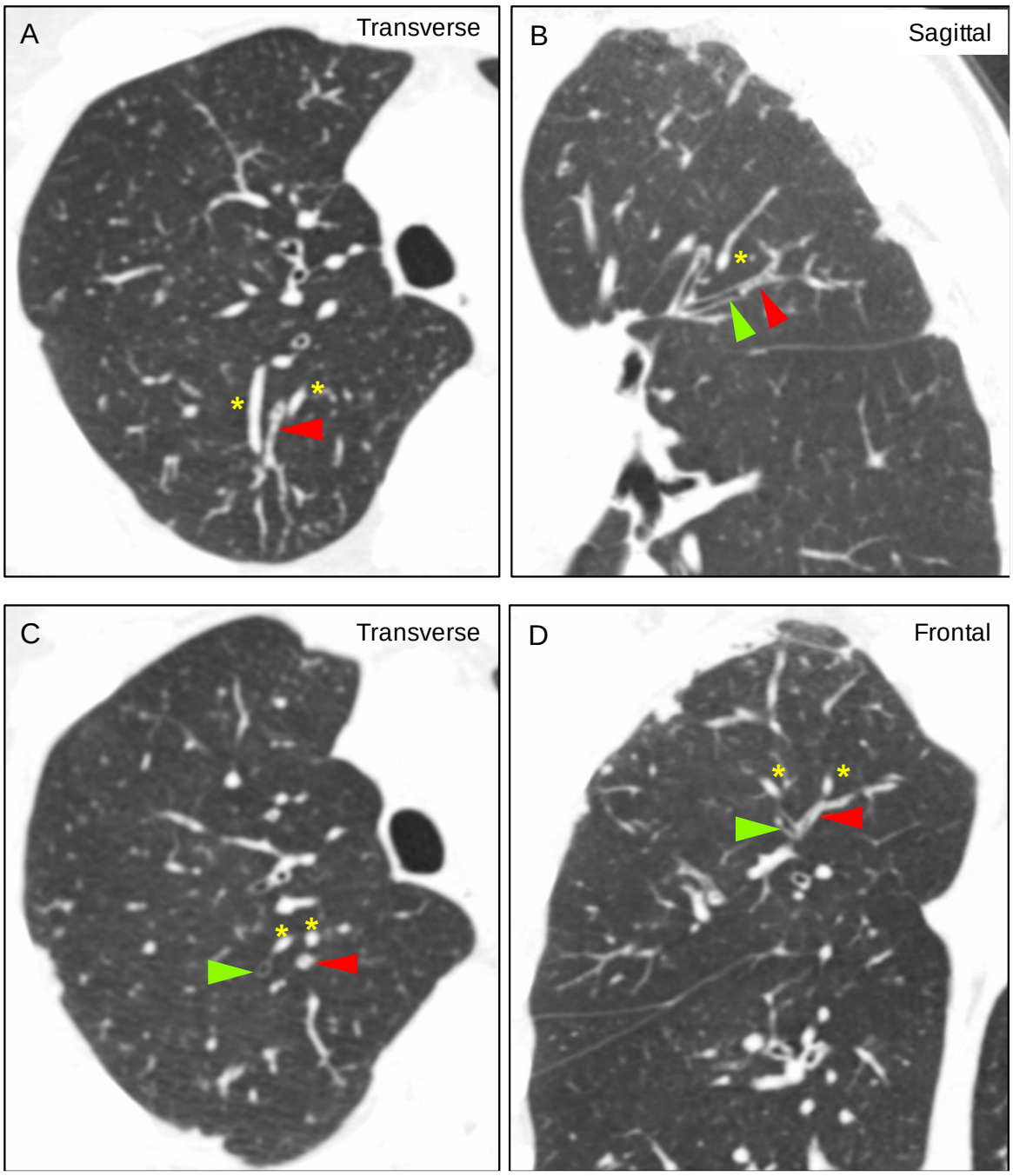


**Figure S1. Examples of mucus plugs shown in different planes on MDCT.**

**(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.

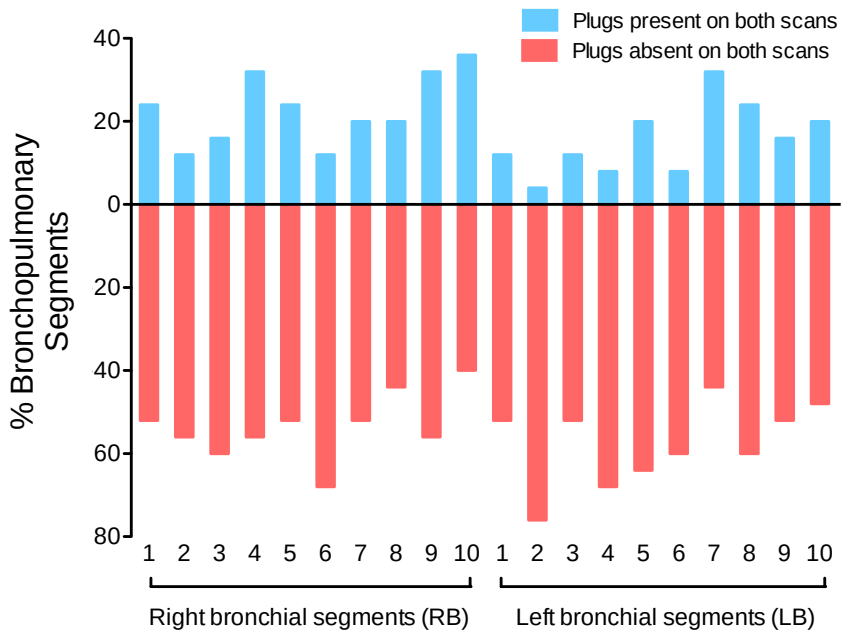


**Figure S2. Persistence of mucus phenotype by bronchopulmonary segment.**

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.

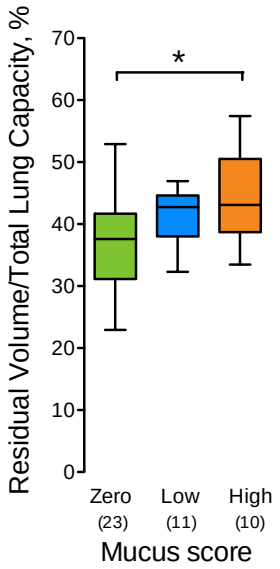


**Figure S3. Mucus plugging is associated with air-trapping.**

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 $p<0.05$.

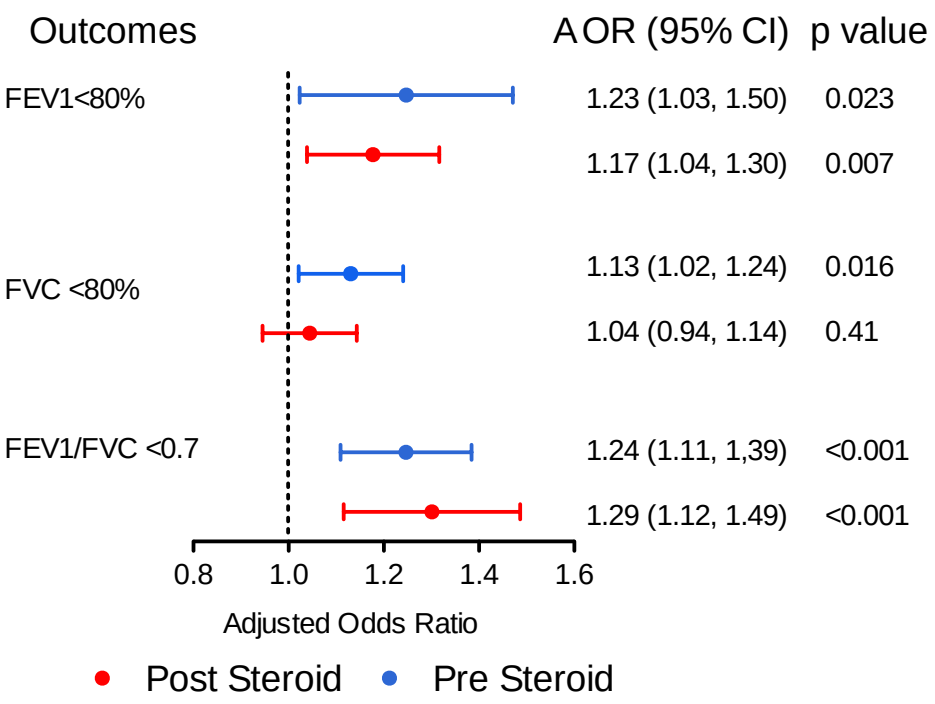


**Figure S4. Logistic regression of the effects of mucus score on lung function**

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.

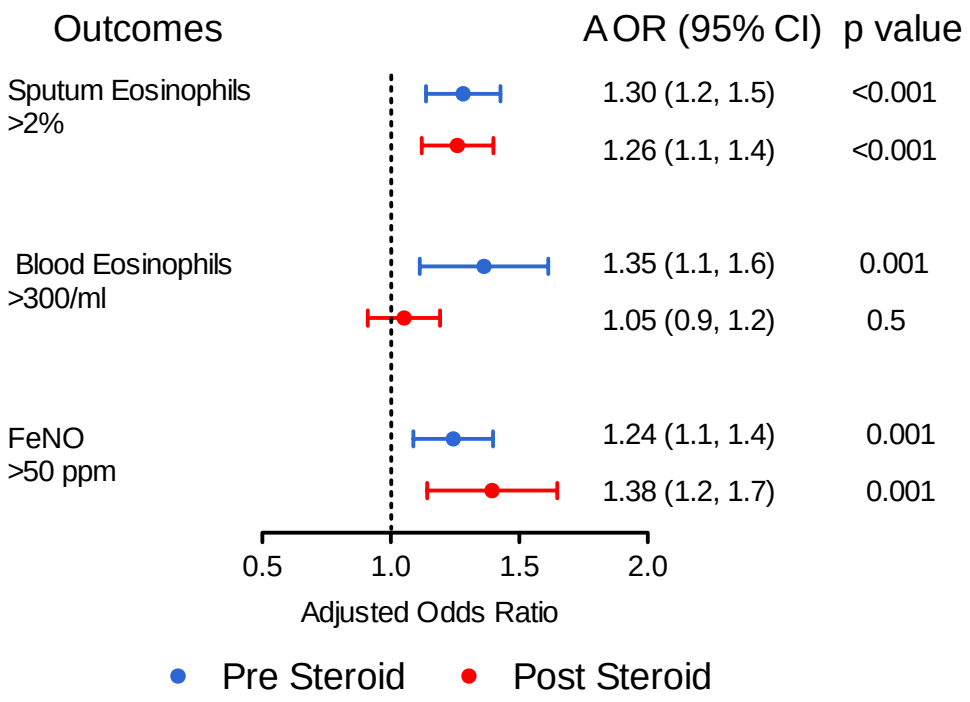


**Figure S5. Logistic regression of mucus score on markers of type 2 inflammation.** Forrest plot of the association between mucus plugging and markers of type 2 inflammation before and after steroid treatment in subjects with asthma. Associations were derived from multivariable logistic regression models. 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.

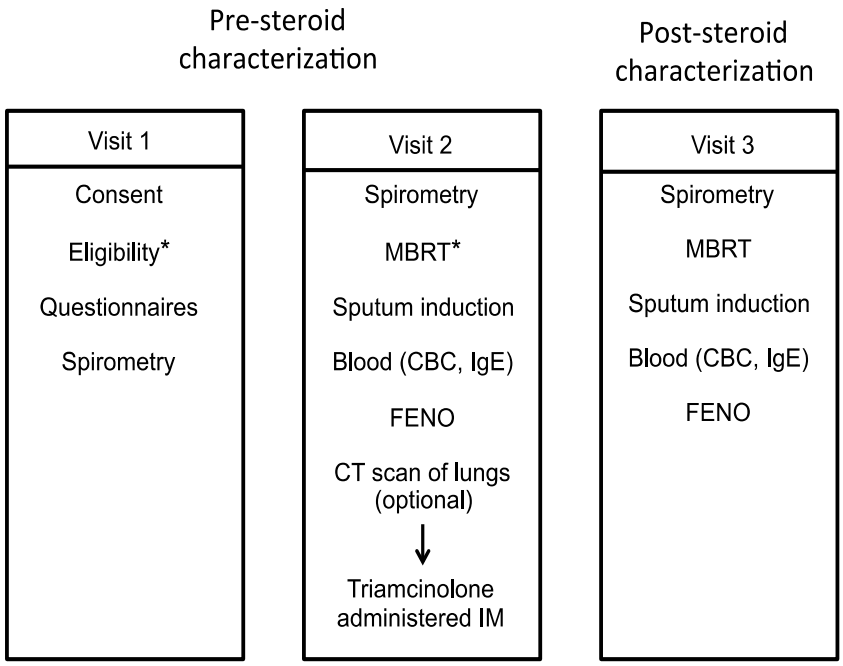


**Figure S6. Visit procedures for patient characterization at baseline in SARP.** Eligibility was determined by maximum bronchodilator reversibility test (MBRT) or methacholine challenge on visit 1. If MBRT was performed more than 6 weeks before visit 2 it was repeated at visit 2. Visit 3 was 18 ± 3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

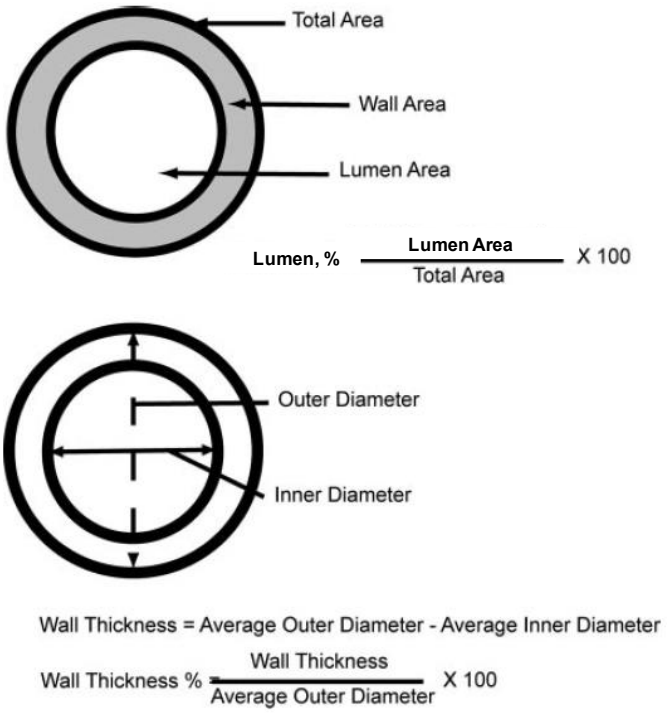


**Figure S7. Airway measures by MDCT scan.** The specific MDCT scan measurements used included airway wall thickness (WT), percentage of WT (WT%), luminal area (LA) and percentage of LA (LA%).

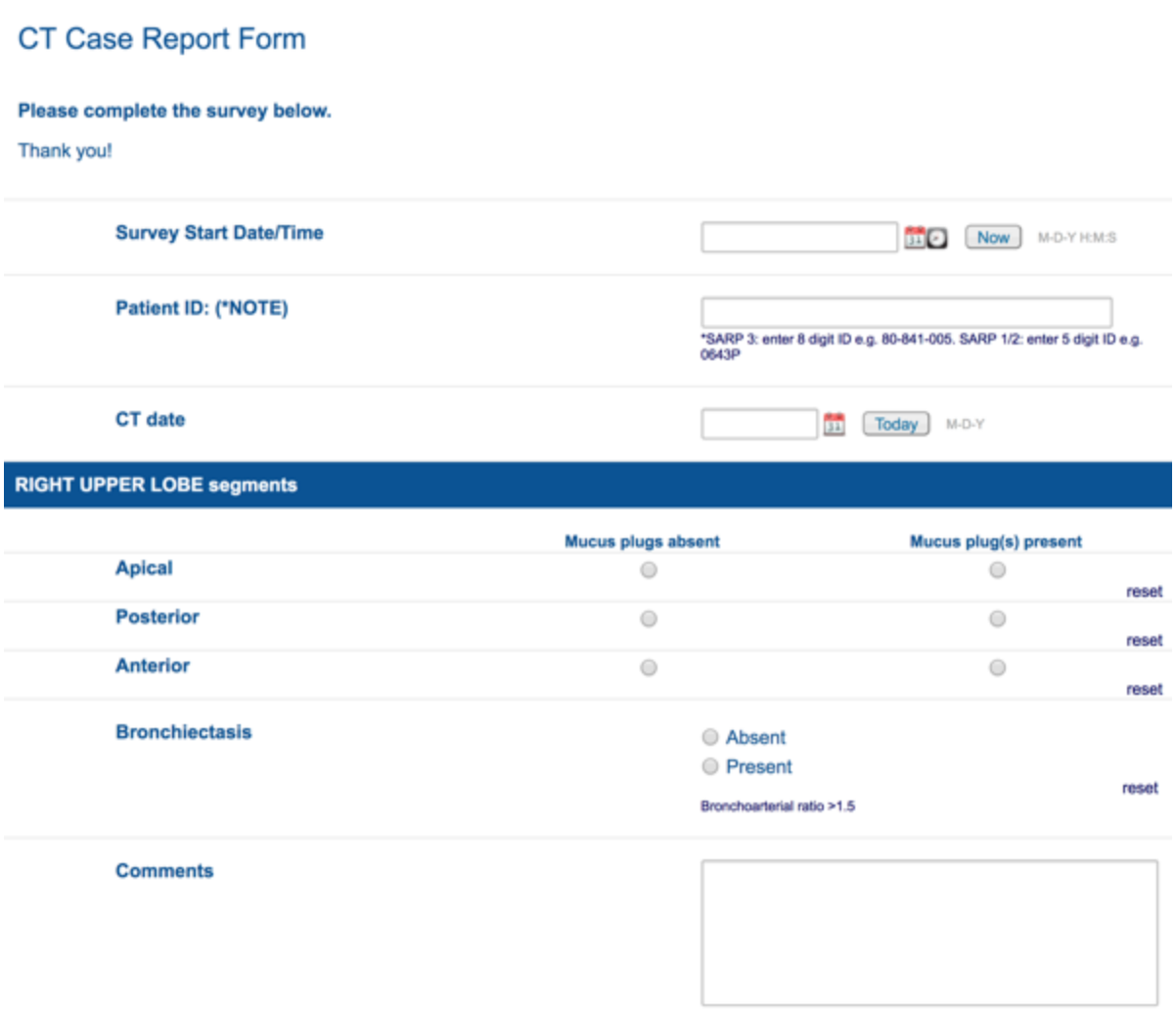


**Figure S8. Modified web-based data capture tool used for longitudinal measurements in a subset of the SARP cohort with repeat MDCT scans.** The figure shows a screen capture of the web based survey form that was modified from the original data capture tool to measure mucus plugging at a segmental level 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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**A. Severe Asthma Research Program (SARP)**: We thank the investigators and coordinators in the SARP (Table) who contributed to this manuscript by recruiting and characterizing participants, collecting biospecimens, developing systems for lung imaging and analysis, and coordinating data collection and analysis.

| **Table: NHLBI Severe Asthma Research Program** | |
|---|---|
| **(i). SARP Data Coordinating Center** | |
| Pennsylvania State University, Hershey, PA. | Brenda Phillips and David Mauger. |
| **(ii). SARP Clinical Centers** | |
| University of California San Francisco, San Francisco, CA. | John Fahy, Eleanor Dunican, Michael Peters, Erin Gordon, Prescott Woodruff, Kelly Wong McGrath, Jennifer Soh, Alexandra Perri, Nathalie Y, Gina Evans Young, Andrew Manies and Sheena Kerr. |
| Kaiser Permanente Division of Research, Oakland, CA. | Carlos Iribarren and Gabriella Sanchez. |
| Brigham and Women's Hospital and Harvard Medical School, Boston, MA. | Elliott Israel, Bruce Levy, Juan Carlos Cardet, Nawal Ali, Carrie Nettles, and Gabriela Sauza. |
| University of Pittsburgh, Pittsburgh, PA. | Sally Wenzel, Merritt Fajt, Anne Marie Irani, Joseph Leader, Louise Martin, and Jenelle Mock. |
| Washington University, St Louis, MI. | Mario Castro, Rebecca Schutz, Rachel Weaver, Alicia Cross, Michael Harrod, Jim Kozlowski, and Bori Oginni. |
| Cleveland Clinic, Cleveland, Ohio. | Serpil Erzurum, Benjamin Gaston, Elise Baldarelli, Marybeth Boyle, and Michelle Koo. |
| Wake Forest University, Winston-Salem, NC. | Eugene Bleecker, Deborah Meyers, Regina Smith, Annette Hastie, and Wendy Moore. |
| University of Wisconsin, Madison, WI. | Nizar Jarjour, Ron Sorkness, Loren Denlinger, Mark Schiebler, Sean Fain, Holly Eversoll, Jan Yakey, Evelyne Falbene, Maranda Hyde and Michele Wolf. |
| **(iii). SARP Imaging Core** | |
| University of Iowa, Iowa, IA. | Eric Hoffmann, Mark J. Escher, Jered P. Sieren and Ching-Long Lin. |
| **(iv). SARP Imaging Working Group** | |
| University of Iowa, Iowa, IA. | Eric Hoffman and Ching-Long Lin. |
| Washington University, St Louis, MI. | Mario Castro and David Gierada. |
| University of Wisconsin, Madison, WI | Nizar Jarjour, Mark Schiebler and Sean Fain. |
| University of Pittsburgh, Pittsburgh, PA. | Merritt Fajt. |
| Brigham and Women's Hospital and Harvard Medical School, Boston, MA. | George Washko. |
| Cleveland Clinic, Cleveland, Ohio. | Jason Lempel. |
| University of California San Francisco, San Francisco, CA. | Eleanor Dunican, John Fahy. |
| **(v). SARP CT Mucus Score Development Group** | |
| University of California, San Francisco, San Francisco, CA. | Eleanor Dunican, Brett Elicker, and John Fahy. |
| University of Wisconsin, Madison, Madison, WI. | Mark Schiebler, Scott Nagle. |
| Washington University, St Louis, MO. | David Gierada. |
| University of Iowa, Iowa, IA. | John Newell. |

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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.