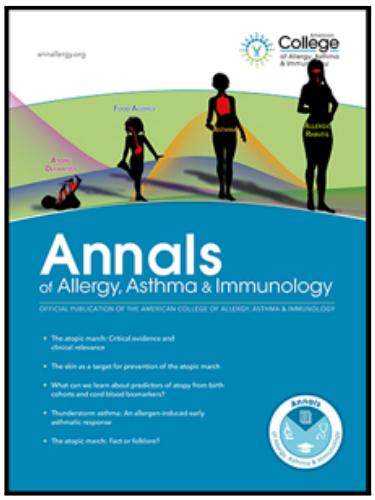
Accepted Manuscript



Differences in airway structural changes assessed by three-dimensional computed tomography in asthma and asthma-COPD overlap

Mitsuru Niwa , Tomoyuki Fujisawa , Masato Karayama , Kazuki Furuhashi , Kazutaka Mori , Dai Hashimoto , Hideki Yasui , Yuzo Suzuki , Hironao Hozumi , Noriyuki Enomoto , Yutaro Nakamura , Naoki Inui , Takafumi Suda

PII: S1081-1206(18)30666-5
DOI: https://doi.org/10.1016/j.anai.2018.08.006
Reference: ANAI 2664

To appear in: *Annals of Allergy, Asthma Immunology*

Received date: 17 May 2018
Revised date: 8 August 2018
Accepted date: 13 August 2018

Please cite this article as: Mitsuru Niwa , Tomoyuki Fujisawa , Masato Karayama , Kazuki Furuhashi , Kazutaka Mori , Dai Hashimoto , Hideki Yasui , Yuzo Suzuki , Hironao Hozumi , Noriyuki Enomoto , Yutaro Nakamura , Naoki Inui , Takafumi Suda , Differences in airway structural changes assessed by three-dimensional computed tomography in asthma and asthma-COPD overlap, *Annals of Allergy, Asthma Immunology* (2018), doi: https://doi.org/10.1016/j.anai.2018.08.006

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Differences in airway structural changes assessed by three-dimensional computed tomography in asthma and asthma-COPD overlap

Mitsuru Niwa, MD[1], Tomoyuki Fujisawa, MD, PhD[1], Masato Karayama, MD, PhD[1], Kazuki Furuhashi, MD, PhD[1], Kazutaka Mori, MD, PhD[2], Dai Hashimoto, MD, PhD[3], Hideki Yasui, MD, PhD[1], Yuzo Suzuki, MD, PhD[1], Hironao Hozumi, MD, PhD[1], Noriyuki Enomoto, MD, PhD[1], Yutaro Nakamura, MD, PhD[1], Naoki Inui, MD, PhD[4], Takafumi Suda, MD, PhD[1]

[1]Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan

[2]Department of Respiratory Medicine, Shizuoka City Shimizu Hospital, 1231 Miyakami, Shimizu-ku, Shizuoka, 424-8636, Japan

[3]Department of Respiratory Medicine, Seirei Hamamatsu General Hospital, 2-12-12 Sumiyoshi, Naka-ku, Hamamatsu, 430-8558, Japan

[4]Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu, 431-3192, Japan

Corresponding author: Tomoyuki Fujisawa, MD, PhD

1-20-1 Handayama Higashi-ku, Hamamatsu 431-3192, Japan

Tel: +81(53)435-2263

Fax: +81(53)435-2354

E-mail: fujisawa@hama-med.ac.jp

Financial supports : None

Clinical Trial Registration

UMIN Clinical Trials Registry (UMIN-CTR) system (http://www.umin.ac.jp/ctr/ UMIN000028913).

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Abbreviations: ACO=asthma-COPD overlap; ACT=the Asthma Control Test; Ai=airway inner luminal area; AL=airflow limitation; ALX=lowfrequency reactance area; BSA=body surface area; COPD=chronic obstructive pulmonary disease; FeNO= fractional exhaled nitric oxide; FEV1=forced expiratory volume in 1 second; Fres=resonant frequency; FVC=forced vital capacity; IQR=interquartile range; MDCT=multidetector row computed tomography; MMF=maximal mid-expiratory flow; R5=respiratory resistance at 5 Hz; R20=respiratory resistance at 20 Hz; WT=wall thickness; X5=respiratory reactance at 5 Hz; 95% CI=95% confidence interval; %pred=percentage of the predicted value

Keywords: asthma, asthma-chronic obstructive pulmonary disease overlap, multidetector row computed tomography, wall thickness, airway remodeling

Word count (introduction to discussions): 3,141 words

The number of tables and figures: 4 Tables, 2 figures, 2 eTables

Potential conflicts of interest

The authors declare that they have no conflicts of interests.

The authorship credit: (1) conception and design of the study: TF, MN, TS; (2) data generation: MN, MK, KF, DH, HY, HH, NE; (3) analysis and interpretation of the data: TF, MN, KM, YS, YN, NI, TS; (4) preparation of the manuscript: TF, MN.

Introduction

Bronchial asthma is a chronic inflammatory disorder characterized by airway hyperresponsiveness and reversible airflow obstruction.[1, 2] Current practice guidelines emphasize the

ACCEPTED MANUSCRIPT

importance of inhaled corticosteroids as an anti-inflammatory therapy[3] that substantially contributes to improving disease control and quality of life in patients with asthma. Persistent airway inflammation that is not treated adequately results in structural changes in the large and small airways, such as edema of the airway wall, subepithelial fibrosis, thickening of the smooth muscle, and hyperplasia of the submucosal glands[4-6], and is known as remodeling of the airways. Airway remodeling leads to irreversible airflow obstruction in patients with asthma.[7-9]

Cigarette smoking is known to worsen the clinical outcome of asthma but is equally common in patients with asthma and the general population. Tobacco smoking is also the leading cause of chronic obstructive pulmonary disease (COPD), which is categorized as a distinct clinical entity among the chronic airway diseases.[10] Recently, an overlapping of asthma and COPD has been recognized and termed asthma-COPD overlap (ACO).[11, 12] ACO is characterized by persistent airway obstruction and is accompanied by several features of asthma and COPD as outlined in the guidelines developed by several international associations, such as the Global Initiative for Asthma (GINA) and Global Initiative for Chronic Obstructive Lung Disease (GOLD). ACO includes two main phenotypes, i.e., patients with asthma who continue to smoke and develop irreversible airflow obstruction and patients with COPD who develop asthma-like symptoms, e.g., high reversibility of airway obstruction and eosinophilic airway inflammation.[13-17] A history of tobacco smoking is considered to be a major cause of COPD, so smoking is usually regarded as a necessary factor when making a diagnosis of ACO in patients with asthma.[18, 19] Duration of disease could also affect airway remodeling in patients with asthma, considering that prolonged inflammation of the airways evolves into airway remodeling and incomplete reversibility of airflow in asthma.[20, 21]

The characteristics of ACO in patients with asthma have not been studied in depth. There are a few reports suggesting that patients with ACO have more frequent exacerbations, more frequent hospitalizations, poorer asthma control, and more impaired lung function than those with asthma alone[22-26] , which point to the importance of evaluating the difference between asthma and ACO. However, little is known about any differences in clinical features and structural changes in the airway between patients with asthma and those with ACO. Multidetector row computed tomography (MDCT) is a non-invasive technique for measurement of the dimensions of the airway structures in

ACCEPTED MANUSCRIPT

the lung. MDCT is useful for detection of airway remodeling in patients with asthma.[27, 28] Several studies using MDCT have demonstrated that the airway walls were thicker and the airway inner luminal area was smaller in patients with asthma than in healthy controls and that airway wall thickness (WT) was related to disease severity and degree of airflow obstruction in patients with asthma.[28-30]

Given that MDCT is non-invasive and can analyze gross pathologic changes in lung morphology in patients with asthma, we suspect that this technique may be able to differentiate features of airway remodeling between ACO and asthma. The aim of the present study was to compare the clinical features and structural changes in the airway between asthma and ACO in a clinical cohort of patients with asthma.

Methods

Subjects

We recruited patients aged >18 years who had bronchial asthma according to the GINA definition[3] and attended the outpatient clinic at Hamamatsu University Hospital for routine check-ups. The inclusion criteria were treatment with appropriate asthma therapy including an inhaled corticosteroid and fulfilment of the GINA criteria for "controlled" asthma. Patients were excluded if their treatment for asthma had changed in the 3 months before the start of the study or if they had another chronic pulmonary disease, e.g., lung cancer or pulmonary fibrosis. The patients were then allocated according to airflow limitation (AL) and smoking history to an asthma group (never-smokers and ex-smokers with a smoking history of <10 pack-years) or an ACO group (ever-smokers with a smoking history of ≥10 pack-years and a forced expiratory volume in 1 second [FEV_1]/forced vital capacity [FVC] <0.7). For further analysis, the asthma group was classified according to whether they did or did not have AL (FEV_1/FVC <0.7 or FEV_1/FVC ≥0.7). Reference subjects without asthma or COPD were enrolled as controls and were required to have normal pulmonary function on spirometry and to be never-smokers or ex-smokers with a smoking history of <10 pack-years.

ACCEPTED MANUSCRIPT

Study design

Subjects in this prospective observational study consecutively underwent fractional exhaled nitric oxide (FeNO) measurements, pulmonary function tests, forced oscillation technique (FOT) measurements, and MDCT of the chest. The patients' clinical data, including history and laboratory findings, were obtained from the medical records. Atopic asthma was defined by the presence of positive specific IgE antibodies for at least one common inhalant allergen. Clinical characteristics, pulmonary function test results, respiratory impedance measured with FOT, and findings on MDCT were compared between the asthma group, the ACO group, and the control group. We further compared the findings in the ACO group with those in the subgroup of patients with asthma with AL, both of which were matched for the degree of airway obstruction.

The study protocol complied with the Declaration of Helsinki and was approved by the institutional review board of Hamamatsu University School of Medicine. Each patient gave written informed consent to be included in the study. The study was registered in the UMIN Clinical Trials Registry (UMIN-CTR) system (http://www.umin.ac.jp/ctr/ UMIN000028913).

Measurement of FeNO, pulmonary function tests and FOT examination

FeNO was measured using the commercially available analyser NIOX MINO (Aerocrine AB, Solna, Sweden), according to the American Thoracic Society/European Respiratory Society recommendations.[31] Patients performed standardized spirometry using the Autospirometer System 7 (Minato Medical Science Co., Ltd., Osaka, Japan). All maneuvers met the standards of the American Thoracic Society[32] and Japanese Respiratory Society[33]. Respiratory impedance was measured using a commercial FOT device (Most-Graph 01; Chest MI, Tokyo, Japan) according to the standard recommendations reported previously.[34-36] Briefly, impulse oscillatory signals generated by a loudspeaker at intervals of 0.25 s were applied to the respiratory system through a mouthpiece during tidal breathing at rest. Mouth pressure and flow signals were measured and calculated to obtain the resistance and reactance properties against oscillatory frequencies from 4–36 Hz. We evaluated R5, R20, the difference between R5 and R20 (R5–R20), X5, Fres (resonant frequency; where the reactance crosses zero and the elastic and inertial forces are equal in magnitude and opposite), and

ACCEPTED MANUSCRIPT

low-frequency reactance area (the integral of reactance from 5 Hz to Fres). Each index was expressed as a mean value at whole-breath phase.

Multidetector row CT

All subjects were scanned using a 64-slice MDCT scanner (Aquilion-64; Toshiba Medical Systems, Tokyo, Japan) in the supine position at full-inspiration breath-hold. The scanner was calibrated every day following the manufacturer's recommendations. The scanning parameters were as follows: collimation, 64 × 0.5 mm; tube voltage, 120 kV; tube current, 200 mA; rotation time, 0.5 s; and pitch, 0.83. These parameters were varied by the scanner system to obtain the optimum radiation dose and image quality. Images were reconstructed using a standard (FC50) reconstruction algorithm for the lung using a slice thickness of 0.5 mm and a reconstruction interval of 0.5 mm.

Three-dimensional CT analysis

A three-dimensional reconstructed bronchial tree was created using image-analyzing software (Synapse Vincent; Fuji Film, Tokyo, Japan) as described previously[37-39] and airway measurements were recorded. The analysts were blinded to the patients' information. The bronchial pathway was identified automatically and reconstructed into multiplanar reconstruction images using a window width of 1600 HU and a window level of -600 HU. We selected six airway pathways (RB1, RB2, RB3, RB8, RB9, and RB10) in the right lung, and defined the segmental, subsegmental, and sub-subsegmental bronchi as the third, fourth, and fifth generations of bronchi, respectively. The airway inner luminal area (Ai) was automatically calculated as the area surrounded by the inner border pixels of the airway wall. WT was measured as the mean distance of the outer to inner edge of the airway (assuming that it is a "true" circle). Ai and WT were affected by body size, so were normalized by body surface area (BSA). RB1–3 were summarized as the upper lobe and RB8–10 were summarized as the lower lobe.

Statistical analysis

ACCEPTED MANUSCRIPT

Comparisons between the asthma group, ACO group, and healthy control group were performed using non-parametric tests. Categorical values, expressed as the number and proportion, were analyzed using the chi-square test and Fisher's exact test. Continuous or ordinal values were summarized as the median and interquartile range and analyzed using the Kruskal-Wallis test and Mann-Whitney *U* test. All statistical analyses were performed using R version 3.1.3 (The R Foundation for Statistical Computing, Vienna, Austria, 2015). A *p*-value <0.05 was considered statistically significant and all tests were two-sided.

Results

Patient characteristics

Forty-three of the 64 patients enrolled in this study were allocated to the asthma group and 16 to the ACO group. Five patients with a smoking history of ≥10 pack-years and an FEV_1/FVC >0.7 were excluded. In the asthma group, 20 patients had AL (FEV_1/FVC <0.7) and 23 did not have AL (FEV_1/FVC ≥0.7). Twenty-seven subjects were enrolled as controls (Figure 1). The patient characteristics are summarized in Table 1. The patients in the ACO group were older than those in the asthma and control groups; however, there was no statistically significant difference in age between the groups. All patients in the ACO group were male, as were 15 (34.9%) in the asthma group and 13 (44.9%) in the control group. There was no significant difference in height, weight, body mass index, or BSA between the study groups. All patients in the ACO group were former smokers and 33 (76.7%) in the asthma group and 25 (92.6%) in the control group were never-smokers. The duration of asthma was relatively longer in the ACO group than in the asthma groups but the difference was not statistically significant. All patients in the asthma and ACO groups were treated with an inhaled corticosteroid or an inhaled corticosteroid/long-acting beta-agonist. A long-acting muscarinic antagonist was used in 6 patients (37.5%) in the ACO group and in 5 patients (11.6%) in the asthma group.

Asthma control, FeNO, pulmonary function test results, and FOT test findings are shown in Table 2. Scores on the asthma control test tended to be lower in the ACO group than in the asthma group. There were no significant differences in the value of FeNO between the ACO group and the

ACCEPTED MANUSCRIPT

asthma group. No significant difference in FVC was observed between the three groups. FEV_1 % predicted, FEV_1/FVC, maximal mid-expiratory flow (MMF), and MMF % predicted were significantly lower in the ACO group than in the asthma and control groups. The values for the reactance elements (X5, Fres, and low-frequency reactance area) were significantly lower in the control group than in the asthma group or ACO group.

Comparison of MDCT parameters

The WT/$\sqrt{}$BSA at the level of the third-generation to fifth-generation bronchi was significantly greater in the asthma and ACO groups than in the control group (Table 3). The WT/√BSA at the level of the third-generation bronchi was greater in the ACO group than in the asthma group (upper lobe, 1.36 mm/m vs 1.27 mm/m, p=0.086; lower lobe, 1.29 mm/m vs 1.18 mm/m, p=0.042; Table 3 and Figure 2). There was no significant difference in Ai/BSA between the three groups, except for the fifth-generation bronchi in the lower lobe (Table 4). Previous studies showed that airway WT was related to the degree of airflow obstruction in asthma[28, 29], so there was a possibility that a difference in the degree of airway obstruction between the ACO and asthma groups might affect the airway WT in this study. Therefore, we compared the MDCT parameters between the ACO group and the subgroup of asthma with AL. Age, disease duration and the degree of airway obstruction on pulmonary function tests, including FEV_1 % predicted, FEV_1/FVC, and MMF % predicted, were comparable between the two groups (eTable 1). As shown in eTable 2, the WT/√BSA at the level of the third-generation bronchi in the lower lobe was significantly greater in the ACO group than in the subgroup of asthma with AL.

Discussion

In the present study, we classified patients with clinical asthma into an asthma group and an ACO group according to their AL and smoking history and assessed the differences in clinical features and structural changes in the airway between asthma and ACO. We found that patients with ACO were older and more likely to be male and to have worse AL than those with asthma. The asthma and ACO groups had greater airway WT on assessment of airway dimensions by MDCT than the control group. Notably, the airway WT at the level of the third-generation bronchi was

ACCEPTED MANUSCRIPT

significantly greater in the patients with ACO than in those with asthma, indicating that structural changes in the airway are more prominent in ACO than in asthma alone. Even when comparing ACO and asthma with AL (FEV_1/FVC <70%), patients with ACO had thicker airway walls at the level of the third-generation bronchi in the lower lobe than those with asthma with AL. To our knowledge, this is the first study to investigate the difference in airway remodeling between asthma and ACO using MDCT in a cohort of patients with clinical asthma.

The ACO group, which included patients with asthma, an extensive history of cigarette smoking, and airway obstruction, had a lower $\%FEV_1$ and a thicker airway WT on assessment of airway dimensions by MDCT than the asthma group. Smoking is known to have detrimental effects on asthma outcomes, including increased cough, wheezing, sputum production, and frequency of asthma attacks, resulting in an accelerated decline of lung function.[40-42] Consistent with previous reports, in the present study, patients with asthma and an extensive smoking history (the ACO group and 5 patients without obstruction and a smoking history ≥10 pack-years) had a lower $\%FEV_1$ than those without an extensive smoking history (the asthma group) (data not shown). Smoking also induced pathologic changes in the epithelium of the airways in patients with asthma. Martine et al. showed that patients with asthma who were current smokers had more goblet cells and a thicker epithelium than those with no smoking history.[43] In the present study, patients with ACO had thicker airway walls than those with asthma when measured on MDCT, so chronic exposure to cigarette smoke could affect the difference in morphologic changes in the airway between asthma and ACO. In our study, the duration of asthma tended to be longer in the ACO group than in the asthma group but the difference was not statistically significant. When the ACO group was compared with the subgroup of asthma with AL, the duration of asthma and degree of AL (e.g., FEV_1/FVC, $\%FEV_1$) were almost identical between the two groups (eTable 1), and WT at the level of the third-generation bronchi in the lower lobe was significantly greater in the ACO group than in the subgroup of asthma with AL (eTable 2). These findings suggest that there is a difference in the process of airway remodeling between asthma and ACO and that continuous exposure to cigarette smoke may affect morphologic changes in the airway in patients with ACO.

ACCEPTED MANUSCRIPT

ACO is defined as "the coexistence of asthma and COPD in patients with chronic airway obstruction".[44] However, it is also recognized that ACO has various phenotypes, as does asthma and COPD.[45, 46] Patients with ACO may have different characteristics along the spectrum of features in the definitions of asthma and COPD. For example, patients with asthma who develop fixed airflow obstruction after a significant smoking habit are considered to have ACO. Patients with a long-standing diagnosis of COPD who present with the characteristics of asthma (e.g., reversibility of AL, eosinophilic airway inflammation, history of atopy or allergic rhinitis) are also categorized as having ACO. A number of investigators have proposed diagnostic criteria for ACO that have varied depending on the aims of their research. In the present study, we identified patients with ACO on the basis of a significant smoking habit and clinical asthma with obstructive changes in the airways and found that airway remodeling was more severe in patients with ACO than in those with asthma alone. Most of the previous studies have focused on patients with ACO in a COPD population. Hadrin et al. demonstrated the difference of clinical features, including CT analyses, between COPD and ACO.[18] They found that patients with ACO had more frequent respiratory exacerbations, greater airway WT of a 10-mm luminal perimeter, and less emphysema than those with COPD alone in a COPD patient cohort. The phenotypes of ACO (e.g., asthma with a smoking history and eosinophilic COPD) could affect the type of changes in airway morphology and clinical features. These changes will be the subject of future research.

There are some limitations to this study. First, it was performed at a single institution and the sample size was relatively small, and therefore not generalizable. Second, any pathologic differences in the structure of the airways between the ACO group and the asthma group could not be determined, although the airway WT was significantly greater on MDCT in patients with ACO than in those with asthma. Remodeling of the airway in a patient with asthma includes subepithelial fibrosis, increased smooth muscle mass, and goblet cell hyperplasia in the airway, which contributes to thickening of the airway walls.[47] Chronic exposure to cigarette smoke prevents adequate regeneration of the epithelium, leading to excessive proliferation[43], which may lead to thickening of the epithelial layer in patients with ACO. Finally, the subjects in this study included patients with asthma or ACO, but did not include those with COPD. Clinically, patients with ACO have been reported to have an

ACCEPTED MANUSCRIPT

increased frequency of exacerbation and more impairment of lung function than those with asthma or COPD alone.[23] However, no studies have compared the morphologic changes in the airway between ACO, asthma, and COPD using MDCT. Prospective observational studies are required to clarify the difference in the clinical course and structural changes in the airway in larger cohorts of patients with asthma, COPD, and ACO.

In summary, we have compared the clinical features and structural changes in the airway seen on MDCT between ACO and asthma alone in a cohort of patients with clinical asthma and found that patients with ACO had thicker airway walls at the level of the third-generation bronchi than patients with asthma. When the ACO group was compared with the subgroup of asthma with AL, WT was still greater in the patients with ACO than in those with asthma with AL. This study highlights a distinct difference in the structural changes in the airway between patients with asthma and those with ACO. When assessed by MDCT, airway remodeling is more prominent in patients with ACO than in those with asthma.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

REFERENCES

1. Brusasco V, Crimi E, Pellegrino R. Airway hyperresponsiveness in asthma: not just a matter of airway inflammation. Thorax. 1998;53:992-998.
2. McCracken JL, Veeranki SP, Ameredes BT, Calhoun WJ. Diagnosis and Management of Asthma in Adults: A Review. Jama. 2017;318:279-290.
3. Global Initiative for Asthma. Global strategy for asthma management and prevention; 2018 [Internet]. Available from: http://ginasthma.org/ [cited 2018, April 30].
4. Lambrecht BN, Hammad H. The airway epithelium in asthma. Nat Med. 2012;18:684-692.
5. Saglani S, Lloyd CM. Novel concepts in airway inflammation and remodelling in asthma. Eur Respir J. 2015;46:1796-1804.
6. Djukanovic R. Airway inflammation in asthma and its consequences: implications for treatment in children and adults. J Allergy Clin Immunol. 2002;109:S539-548.
7. Kato M, Fujisawa T, Hashimoto D, et al. Plasma connective tissue growth factor levels as potential biomarkers of airway obstruction in patients with asthma. Ann Allergy Asthma Immunol. 2014;113:295-300.
8. James AL, Bai TR, Mauad T, et al. Airway smooth muscle thickness in asthma is related to severity but not duration of asthma. Eur Respir J. 2009;34:1040-1045.
9. Bai TR. Evidence for airway remodeling in chronic asthma. Current opinion in allergy and clinical immunology. 2010;10:82-86.
10. Cosio MG, Saetta M, Agusti A. Immunologic aspects of chronic obstructive pulmonary disease. N Engl J Med. 2009;360:2445-2454.
11. Gibson PG, Simpson JL. The overlap syndrome of asthma and COPD: what are its features and how important is it? Thorax. 2009;64:728-735.
12. Diagnosis of Disease of Chronic Airflow Limitation. Asthma COPD and Asthma-COPD Overlap Syndrome (ACOS); 2014 [Internet]. Available from: http://ginasthma.org/ [cited 2018, April 30].

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

13. Gelb AF, Yamamoto A, Verbeken EK, et al. Further Studies of Unsuspected Emphysema in Nonsmoking Patients With Asthma With Persistent Expiratory Airflow Obstruction. Chest. 2018;153:618-629.

14. Hynes G, Pavord ID. Asthma-like Features and Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2016;194:1308-1309.

15. Chanez P, Vignola AM, O'Shaugnessy T, et al. Corticosteroid reversibility in COPD is related to features of asthma. Am J Respir Crit Care Med. 1997;155:1529-1534.

16. Lee JH, Lee YK, Kim EK, et al. Responses to inhaled long-acting beta-agonist and corticosteroid according to COPD subtype. Respir Med. 2010;104:542-549.

17. Saha S, Brightling CE. Eosinophilic airway inflammation in COPD. Int J Chron Obstruct Pulmon Dis. 2006;1:39-47.

18. Hardin M, Cho M, McDonald ML, et al. The clinical and genetic features of COPD-asthma overlap syndrome. Eur Respir J. 2014;44:341-350.

19. Nielsen M, Barnes CB, Ulrik CS. Clinical characteristics of the asthma-COPD overlap syndrome--a systematic review. Int J Chron Obstruct Pulmon Dis. 2015;10:1443-1454.

20. Kaminska M, Foley S, Maghni K, et al. Airway remodeling in subjects with severe asthma with or without chronic persistent airflow obstruction. J Allergy Clin Immunol. 2009;124:45-51.e41-44.

21. Al-Muhsen S, Johnson JR, Hamid Q. Remodeling in asthma. J Allergy Clin Immunol. 2011;128:451-462; quiz 463-454.

22. Milanese M, Di Marco F, Corsico AG, et al. Asthma control in elderly asthmatics. An Italian observational study. Respir Med. 2014;108:1091-1099.

23. Postma DS, Rabe KF. The Asthma-COPD Overlap Syndrome. N Engl J Med. 2015;373:1241-1249.

24. Sin DD, Miravitlles M, Mannino DM, et al. What is asthma-COPD overlap syndrome? Towards a consensus definition from a round table discussion. Eur Respir J. 2016;48:664-673.

25. Tommola M, Ilmarinen P, Tuomisto LE, et al. Differences between asthma-COPD overlap syndrome and adult-onset asthma. Eur Respir J. 2017;49.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

26. Menezes AMB, Montes de Oca M, Perez-Padilla R, et al. Increased risk of exacerbation and hospitalization in subjects with an overlap phenotype: COPD-asthma. Chest. 2014;145:297-304.

27. Hoshino M, Matsuoka S, Handa H, Miyazawa T, Yagihashi K. Correlation between airflow limitation and airway dimensions assessed by multidetector CT in asthma. Respir Med. 2010;104:794-800.

28. Aysola RS, Hoffman EA, Gierada D, et al. Airway remodeling measured by multidetector CT is increased in severe asthma and correlates with pathology. Chest. 2008;134:1183-1191.

29. Niimi A, Matsumoto H, Amitani R, et al. Airway wall thickness in asthma assessed by computed tomography. Relation to clinical indices. Am J Respir Crit Care Med. 2000;162:1518-1523.

30. Shimizu K, Hasegawa M, Makita H, Nasuhara Y, Konno S, Nishimura M. Comparison of airway remodelling assessed by computed tomography in asthma and COPD. Respir Med. 2011;105:1275-1283.

31. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med. 2005;171:912-930.

32. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152:1107-1136.

33. Guideline of respiratory function tests--spirometry, flow-volume curve, diffusion capacity of the lung. Nihon Kokyuki Gakkai zasshi. 2004;Suppl:1-56.

34. Mikamo M, Fujisawa T, Oyama Y, et al. Clinical Significance of Forced Oscillation Technique for Evaluation of Small Airway Disease in Interstitial Lung Diseases. Lung. 2016;194:975-983.

35. Mori K, Shirai T, Mikamo M, et al. Colored 3-dimensional analyses of respiratory resistance and reactance in COPD and asthma. COPD. 2011;8:456-463.

36. Oostveen E, MacLeod D, Lorino H, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. Eur Respir J. 2003;22:1026-1041.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

37. Karayama M, Inui N, Mori K, et al. Respiratory impedance is correlated with morphological changes in the lungs on three-dimensional CT in patients with COPD. Sci Rep. 2017;7:41709.

38. Yasui H, Inui N, Furuhashi K, et al. Multidetector-row computed tomography assessment of adding budesonide/formoterol to tiotropium in patients with chronic obstructive pulmonary disease. Pulm Pharmacol Ther. 2013;26:336-341.

39. Nishimoto K, Karayama M, Inui N, et al. Relationship between fraction of exhaled nitric oxide and airway morphology assessed by three-dimensional CT analysis in asthma. Sci Rep. 2017;7:10187.

40. Althuis MD, Sexton M, Prybylski D. Cigarette smoking and asthma symptom severity among adult asthmatics. The Journal of asthma : official journal of the Association for the Care of Asthma. 1999;36:257-264.

41. Chaudhuri R, McSharry C, McCoard A, et al. Role of symptoms and lung function in determining asthma control in smokers with asthma. Allergy. 2008;63:132-135.

42. Thomson NC, Chaudhuri R, Livingston E. Asthma and cigarette smoking. Eur Respir J. 2004;24:822-833.

43. Broekema M, ten Hacken NH, Volbeda F, et al. Airway epithelial changes in smokers but not in ex-smokers with asthma. Am J Respir Crit Care Med. 2009;180:1170-1178.

44. Yanagisawa S, Ichinose M. Definition and diagnosis of asthma-COPD overlap (ACO). Allergol Int. 2018;67(2):172-178.

45. Kostikas K, Clemens A, Patalano F. The asthma-COPD overlap syndrome: do we really need another syndrome in the already complex matrix of airway disease? Int J Chron Obstruct Pulmon Dis. 2016;11:1297-1306.

46. Joo H, Han D, Lee JH, Rhee CK. Heterogeneity of asthma-COPD overlap syndrome. Int J Chron Obstruct Pulmon Dis. 2017;12:697-703.

47. Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. Am J Respir Crit Care Med. 2003;167:1360-1368

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Figure legends

Figure 1. Study profile.

FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Figure 2. Comparison of WT / $\sqrt{}$BSA among healthy control, the asthma group and the ACO group. WT, airway wall thickness; BSA, body surface area.

*p<0.05 and **p<0.01 vs controls; #p<0.05 vs the asthma group.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 1. Patient characteristics

	Control group (n=27)	Asthma group (n=43)	ACO group (n=16)	*p*-value
Age (years)	65 [54, 75]	66 [55, 72]	71 [66, 76] [#]	0.071[a]
Sex				<0.001[b]
Male	11 (40.7%)	15 (34.9%)	16 (100.0%)* [##]	
Female	16 (59.3%)	28 (65.1%)	0 (0%)	
Height (m)	1.57 [1.52, 1.63]	1.57 [1.52, 1.62]	1.60 [1.58, 1.65]	0.241[a]
Weight (kg)	55.5 [45.8, 60.6]	56.4 [51.2, 59.3]	57.2 [54.6, 66.1]	0.170[a]
BMI	22.1 [19.3, 24.6]	22.3 [21.2, 25.1]	23.3 [21.6, 24.3]	0.443[a]
BSA (m^2)	1.49 [1.44, 1.65]	1.56 [1.47, 1.64]	1.60 [1.54, 1.70]	0.086[a]
Smoking status				<0.001[b]
Current	0 (0%)	0 (0%)	0 (0%)	
Former	2 (7.4%)	10 (23.3%)	16 (100.0%)* [##]	
Never	25 (92.6%)	33 (76.7%)	0 (0%)	
Smoking history (pack-years)	0 [0, 0]	0 [0, 0]	37 [19, 54]* [##]	<0.001[a]
Duration of asthma (years)		18 [10, 30]	30 [12, 61]	0.133[c]
Atopic asthma		29 (67.4%)	6 (37.5%)	0.072[d]
Comorbid allergic rhinitis		23 (53.5%)	5 (31.2%)	0.153[d]
Treatment				
ICS		5 (11.6%)	2 (12.5%)	1.000[d]
ICS/LABA		38 (88.4%)	14 (87.5%)	1.000[d]
LAMA		5 (11.6%)	6 (37.5%)	0.054[d]
LTRA		23 (53.5%)	7 (43.8%)	0.567[d]
Theophylline		4 (9.3%)	1 (6.3%)	1.000[d]

Values are shown as the number (percentage) or median [interquartile range]. ACO, asthma-chronic obstructive pulmonary disease overlap; BMI, body mass index; BSA, body surface area; ICS, inhaled corticosteroids; LABA, long-acting beta-agonists; LAMA, long-acting muscarinic antagonists; LTRA, leukotriene receptor antagonists. *p<0.001 vs controls; [#]p<0.05 vs asthma group; [##]p<0.001 vs asthma group. [a]Kruskal-Wallis test. [b]Chi-square test. [c]Mann-Whitney *U* test. [d]Fisher's exact test

ACCEPTED MANUSCRIPT

Table 2. Baseline measurements

	Control group (n=27)	Asthma group (n=43)	ACO group (n=16)	*p*-value
ACT		24 [22, 25]	20 [19, 24]	0.052[a]
FeNO (ppb)		33 [22, 57]	18 [15, 34]	0.122[a]
FVC (L)	2.61 [2.33, 2.84]	2.56 [2.24, 3.37]	3.09 [2.38, 3.56]	0.344[b]
% predicted	97.4 [84.8, 106.7]	94.9 [86.6, 109.0]	89.7 [82.1, 108.4]	0.784[b]
FEV_1 (L)	1.99 [1.84, 2.40]	1.82 [1.43, 2.22]	1.79 [1.23, 2.34]	0.081[b]
% predicted	89.2 [82.7, 106.8]	87.6 [73.4, 92.9]*	71.8 [50.7, 82.6]*[#]	<0.001[b]
FEV_1/FVC (%)	78.8 [74.6, 84.4]	71.0 [62.2, 78.5]*	58.1 [50.1, 64.2]*[##]	<0.001[b]
MMF (L)	1.94 [1.53, 2.52]	1.16 [0.70, 1.65]*	0.75 [0.44, 0.98]*[##]	<0.001[b]
% predicted	76.1 [56.2, 88.0]	44.0 [29.0, 61.2]*	24.2 [18.0, 34.8]*[##]	<0.001[b]
R5	2.78 [2.20, 3.51]	3.63 [2.18, 4.61]	2.98 [2.08, 4.83]	0.284[b]
R20	2.25 [1.92, 3.02]	3.10 [2.30, 3.89]	2.43 [1.93, 3.55]	0.057[b]
R5-R20	0.44 [0.25, 0.54]	0.37 [0.10, 0.91]	0.56 [0.11, 1.26]	0.583[b]
X5	-0.29 [-0.47, -0.10]	-0.61 [-1.11, -0.29]*	-0.76 [-2.29, -0.48]*	<0.001[b]
Fres	7.09 [5.87, 8.49]	10.4 [7.57, 13.1]*	11.3 [8.72, 21.3]*	<0.001[b]
ALX	1.03 [0.55, 1.93]	2.83 [1.01, 6.08]*	3.29 [1.89, 23.5]*	<0.001[b]

Measurements are shown as the median [interquartile range]. ACO, asthma-chronic obstructive pulmonary disease overlap; ACT, asthma control test; ALX, low frequency reactance area (cmH_2O/L); FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 second; Fres, resonant frequency (Hz); FVC, forced vital capacity; MMF, maximal mid-expiratory flow; R5, respiratory resistance at 5 Hz ($cmH_2O/L/s$); R 20, respiratory resistance at 20 Hz ($cmH_2O/L/s$): X5, respiratory reactance at 5 Hz ($cmH_2O/L/s$). *p<0.01 vs controls; [#]p<0.05 and [##]p<0.01 vs the asthma group. [a]Mann-Whitney *U* test. [b]Kruskal-Wallis test.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 3. WT/√BSA on three-dimensional computed tomography imaging of the lungs

WT/√BSA (mm / m)		Control group	Asthma group	ACO group	*p*-value[a]
3rd	Upper lobe	1.17 (1.12 - 1.28)	1.27 (1.19 - 1.38)**	1.36 (1.30 - 1.44)**	<0.001
	Lower lobe	1.10 (1.04 - 1.17)	1.18 (1.07 - 1.33)*	1.29 (1.21 - 1.37)**[#]	<0.001
4th	Upper lobe	1.05 (0.98 - 1.09)	1.12 (1.03 - 1.26)*	1.15 (1.09 - 1.19)*	0.015
	Lower lobe	1.00 (0.96 - 1.07)	1.08 (1.02 - 1.19)**	1.11 (1.04 - 1.18)*	0.007
5th	Upper lobe	0.95 (0.90 - 0.99)	1.02 (0.92 - 1.08)	0.98 (0.85 - 1.00)	0.033
	Lower lobe	0.91 (0.88 - 0.95)	0.97 (0.93 - 1.06)**	1.03 (0.94 - 1.07)**	0.002

Values are shown as the median [interquartile range]. ACO, asthma-chronic obstructive pulmonary disease overlap; BSA, body surface area; WT, airway wall thickness. *p<0.05 and **p<0.01 vs controls; [#]p<0.05 vs the asthma group. [a]Kruskal-Wallis test.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Table 4. Ai/BSA on three-dimensional computed tomography imaging of the lungs

Ai/BSA (mm^2/m^2)		Control group	Asthma group	ACO group	*p*-value[a]
3rd	Upper lobe	12.3 (10.3 - 14.5)	11.1 (8.3 - 14.1)	13.5 (9.4 - 17.2)	0.219
	Lower lobe	10.7 (8.4 - 13.0)	9.3 (7.3 - 11.5)	11.3 (8.6 - 13.5)	0.183
4th	Upper lobe	6.8 (5.1 - 8.7)	5.6 (4.1 - 7.8)	6.7 (4.4 - 8.9)	0.285
	Lower lobe	6.2 (4.5 - 7.2)	5.0 (3.9 - 6.7)	6.7 (5.2 - 7.3)	0.162
5th	Upper lobe	4.1 (2.9 - 5.0)	3.2 (2.2 - 4.3)	3.4 (1.6 - 4.6)	0.116
	Lower lobe	4.4 (3.7 - 5.8)	3.1 (2.2 - 4.2)**	3.3 (2.7 - 3.6)**	0.003

Values are shown as the median [interquartile range]. ACO, asthma-chronic obstructive pulmonary disease overlap; Ai, airway inner luminal area; BSA, body surface area. *p<0.05 and **p<0.01 vs controls. [a]Kruskal-Wallis test.

ACCEPTED MANUSCRIPT

eTable 1. Characteristics of patients with asthma and airway limitation and patients with asthma-chronic obstructive pulmonary disease overlap

	Asthma with AL group (n=20)	ACO group (n=16)	*p*-value
Age (years)	69 [60, 73]	71 [66, 76]	0.271
Sex			<0.001
Male	7 (35.0%)	16 (100.0%)	
Female	13 (65.0%)	0 (0%)	
BMI	22.2 [21.3, 23.1]	23.3 [21.6, 24.3]	0.200
BSA (m^2)	1.52 [1.47, 1.59]	1.60 [1.54, 1.70]	0.046
Smoking status			<0.001
Former	3 (15.0%)	16 (100.0%)	
Never	17 (85.0%)	0 (0%)	
Smoking history (pack-years)	0 [0, 0]	37 [19, 54]	<0.001
Duration of asthma (years)	24 [15, 30]	30 [12, 61]	0.417
FVC % predicted	98.4 [83.6, 109.0]	89.7 [82.1, 108.4]	0.787
FEV_1 % predicted	72.9 [65.5, 83.8]	71.8 [50.7, 82.6]	0.463
FEV_1/FVC (%)	60.9 [56.7, 65.0]	58.1 [50.1, 64.2]	0.290
MMF % predicted	28.4 [20.5, 31.6]	24.2 [18.0, 34.8]	0.775
R5 $cmH_2O/L/s$	3.86 [2.41, 4.88]	2.98 [2.08, 4.83]	0.551
R20 $cmH_2O/L/s$	3.26 [2.31, 4.00]	2.43 [1.93, 3.55]	0.142
R5-R20 $cmH_2O/L/s$	0.36 [0.11, 0.93]	0.56 [0.11, 1.26]	0.766
X5 $cmH_2O/L/s$	-0.60 [-1.23, -0.26]	-0.76 [-2.29, -0.48]	0.417
Fres Hz	10.3 [8.3, 14.8]	11.3 [8.7, 21.3]	0.596
ALX cmH_2O/L	2.7 [1.2, 7.6]	3.3 [1.9, 23.5]	0.567

Values are shown as the number (percentage) or median [interquartile range]. ACO, asthma-chronic obstructive pulmonary disease overlap; ACT, asthma control test; ALX, low frequency reactance area (cmH_2O/L); BMI, body mass index; FEV_1, forced expiratory volume in 1 second; Fres, resonant frequency (Hz); FVC, forced vital capacity; MMF, maximal mid-expiratory flow; R5, respiratory resistance at 5 Hz ($cmH_2O/L/s$); R20, respiratory resistance at 20 Hz ($cmH_2O/L/s$): X5, respiratory reactance at 5 Hz ($cmH_2O/L/s$);

eTable 2. Three-dimensional computed tomography imaging of the lungs between patients with asthma-chronic obstructive pulmonary disease overlap (n=16) and patients with asthma who were non-smokers and had airway obstruction (n=20)

Third-generation		Asthma with AL group	ACO group	*p*-value
WT/$\sqrt{}$BSA (mm/m)	Upper lobe	1.33 (1.18 - 1.43)	1.36 (1.30 - 1.44)	0.180
	Lower lobe	1.22 (1.09 - 1.34)	1.29 (1.21 - 1.37)	0.019
Ai/BSA (mm^2/m^2)	Upper lobe	11.3 (8.2 – 13.1)	13.5 (9.4 - 17.2)	0.156
	Lower lobe	9.6 (8.1 - 11.7)	11.3 (8.6 - 13.5)	0.215

Values are shown as the median [interquartile range]. ACO, asthma-chronic obstructive pulmonary disease overlap; Ai, airway inner luminal area; AL, airflow limitation; BSA, body surface area; WT, airway wall thickness