Parenchymal destruction in asthma: Fixed airflow obstruction and lung function trajectory

Kaoruko Shimizu, M.D. Ph.D.[a]*, Naoya Tanabe, M.D., Ph.D.[b]*, Akira Oguma, M.D..[a], Hirokazu Kimura, M.D., Ph.D.[a], Masaru Suzuki, M.D., Ph.D.[a], Isao Yokota, M.D., M.P.H.[c], Hironi Makita, M.D., Ph.D.[a,d], Susumu Sato, M.D., Ph.D.[b], Toyohiro Hirai, M.D., Ph. D.[b], Masaharu Nishimura, M.D., Ph. D.[a,d], Satoshi Konno, M.D., Ph. D.[a], The Hi-CARAT investigators

*These two authors contributed equally to the study

[a]Department of Respiratory Medicine, Faculty of Medicine, Hokkaido University, Sapporo, Japan

[b]Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[c]Department of Biostatistics, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

[d]Hokkaido Institute of Respiratory Diseases, Sapporo, Japan

Correspondence: Kaoruko Shimizu

Department of Respiratory Medicine, Faculty of Medicine Hokkaido University,

North 15 West 7, Kita-ku, Sapporo 060-8638, Japan

Fax: +81-11-706-7899 Tel: +81-11-706-5911

E-mail: okaoru@med.hokudai.ac.jp

A disclosure statements

The Hokkaido-based Investigative Cohort Analysis for Refractory Asthma (Hi-CARAT) Study is supported by the Ministry of Education, Culture, Sports, Science and Technology of Japan (24249049 to M.N., 26461151 to S.K.) and a research grant from the Japan Allergy Foundation, AstraZeneca, and Kyorin. M.S. reports grants from GlaxoSmithKline, Novartis, and AstraZeneca outside the current work. N.T., S.S., and T.H. report a grant from Fujifilm Medical outside the current work. I.Y. reports grants from Health, Labour and Welfare Policy Research Grants, and speaker fees from Chugai Pharmaceutical Co, AstraZeneca, Japan Tabacco Pharmaceutical Division, and Nippon Shinyaku Co, outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest. None of these companies had a role in the design or analysis of the study or in the writing of the manuscript.

Funding

None

Authorship

K.S.: study conception and design, CT analysis, statistical analysis, acquisition and interpretation of data, and drafting of the manuscript; N.T.: study conception and design of the study, CT analysis, interpretation of data, and editing of the manuscript; A.O.: CT analysis and interpretation of data; H.K., M.S., H.M.: acquisition and interpretation of data; Y.I. : Statistical advice, S.S., T.H.: interpretation of data; M.N., S.K.: acquisition and interpretation of data and finalizing of the manuscript.

Abstract (243 words / 250 words)

Background: Fixed airflow obstruction (FAO) in asthma, particularly in non-smoking subjects, is generally believed to be caused by airway remodeling. However, parenchymal destruction may also contribute to FAO and longitudinal decline in forced expiratory volume in 1 sec (FEV_1).

Objectives: To evaluate parenchymal destruction using emphysema indices, exponent D and low attenuation area percent (LAA%) on computed tomography (CT), and test whether the parenchymal destruction and airway disease are independently associated with FAO and FEV_1 decline in both smoking and non-smoking asthma.

Methods: D, LAA%, wall area percent (WA%) at segmental airways, and airway fractal dimension (AFD) in asthmatics were measured on inspiratory CT and compared to those in chronic obstructive pulmonary disease (COPD) patients.

Results: D was lower and LAA% was higher in COPD (N = 42) and asthma with FAO (N = 101) than in asthma without FAO (N = 88). The decreased D and increased LAA% were associated with FAO regardless of smoking status or asthma severity. In multivariable analysis, decreased D and increased LAA% were associated with an increased odds ratio of FAO and decreased FEV_1, irrespective of WA% and AFD. Moreover, decreased D affected the longitudinal decline in FEV_1 in severe asthmatics, independent of smoking status .

Conclusions: Asthmatics with FAO showed the parenchymal destruction regardless of smoking status and asthma severity. The parenchymal destruction was associated with an accelerated FEV_1 decline, suggesting the involvements of both airway and parenchyma in the pathophysiology of a subgroup of asthma.

Clinical implications (27/30 words)

Decreased D, together with increased LAA% on CT, reflecting parenchymal destruction, was associated with fixed airflow obstruction and accelerated FEV_1 decline in asthmatics irrespective of smoking status.

Capsule summary (33/35 words)

The contribution of parenchymal damages to pulmonary function impairments was independent of airway diseases, severity of asthma, smoking status, and blood eosinophil counts. This distinct feature broadens our insight into the pathophysiology of asthma.

Keywords

Asthma, computed tomography, fractal, low attenuation area, non-smokers, parenchyma

Abbreviations

AFD : airway fractal dimension, AQLQ : Asthma Quality of Life Questionnaire, ATS : American Thoracic Society, BSA : body surface area, CT : computed tomography, COPD : chronic obstructive pulmonary disease, D_{Lco} : carbon monoxide diffusing capacity, FAO : fixed airflow obstruction, FeNO : fractional exhaled nitric oxide, FEV_1 : forced expiratory volume in 1 sec, FVC : forced vital capacity, HU : Hounsfield Unit, ICS : inhaled corticosteroids, Kco : transfer coefficient, LABA : long acting β2 agonist, LAC : low attenuation cluster, LAA% : low attenuation area percent, LA : airway luminal area, OCS : oral corticosteroids, RB1 : right apical bronchus, RB8 : lateral basal bronchus, WA : airway wall area, V_A : alveolar volume, WA% : wall area percent

INTRODUCTION

Asthma has a complex pathophysiology with diverse disease history and therapeutic responses [1]. Despite advances in clinical management and treatment, such as inhaled corticosteroids (ICS), bronchodilators, and biologics, a subgroup of patients with asthma still develops fixed airflow obstruction (FAO) and shows an accelerated decline in lung function [2-4]. Therefore, uncovering its underlying mechanisms is urgently needed.

Airway disease is believed to be a main pathology of asthma that is characterized by wall remodeling and lumen narrowing [5, 6], and the involvement of small airway disease has been increasingly recognized, particularly in severe asthma [7]. Moreover, cigarette smoking evokes airway inflammation and potentiates structural airway changes [8, 9]. Meanwhile, autopsy studies have shown the destruction of alveolar walls attached to small airways, termed alveolar attachments [10], and centrilobular emphysema [11] in non-smoking asthmatics. A recent study by Tonga *et al.* [12] demonstrated the loss of elastic recoil in older longstanding non-smoking asthmatics with FAO, even after recommended treatments. However, whether parenchymal destruction has distinct functional roles, irrespective of airway remodeling, has not been elucidated.

Computed tomography (CT) enables comprehensive assessments of parenchyma and airways. The relative contribution of airways and emphysema to airflow limitation on CT has been studied in chronic obstructive pulmonary disease (COPD) [13], but less so in asthma, especially in non-smokers. CT studies have shown a decrease in lung density [14] and an increase in low attenuation area percentage (LAA%) in asthma [15], which is generally used as an emphysema index. Nonetheless, LAA% alone cannot fully address the question about whether emphysematous destruction was present, because simple local lung expansion without alveolar destruction would also increase LAA% on

CT.

Fractals can be used for morphological lung analysis. An object exhibiting self-similarity at various length scales possesses a fractal property, which is governed by a power law characterized by the exponent D. Mishima *et al.* discovered that the cumulative frequency of size distribution of low attenuation clusters on CT follows a power law characterized by the exponent D in COPD, and suggested in a spring network simulation that a decrease in D reflects alveolar wall destruction causing coalescence of neighboring airspaces [16]. Yuan *et al*. confirmed a close association between D on CT and emphysema on histology and suggested that D might enable sensitively detecting parenchyma destruction [17]. This concept was further confirmed by Tanabe *et al.* who showed in a computer simulation that when LAA% increases, a decrease in exponent D could reflect coalescence of low attenuation clusters representing emphysematous destruction rather than simple local lung expansion [18]. Meanwhile, Mitsunobu *et al.* showed a reduction in exponent D in severe asthma [15], whereas Gupta *et al.* showed no difference in the exponent D between severe asthmatics, mild to moderate asthmatics, and controls [19].

It was hypothesized that in addition to airway disease, parenchymal destruction occurs in a subgroup of asthmatics regardless of smoking status and that both the airway disease and parenchymal destruction could be involved in FAO and accelerated lung function decline in a subgroup of asthmatics. To test this hypothesis, we evaluated parenchymal destruction using a combination of exponent D and LAA% in asthmatics and COPD patients. Then we explored the relative contributions of the exponent D, LAA%, and CT airway disease indices to an increased risk of FAO and lower forced expiratory volume in 1 sec (FEV_1) at the baseline, and greater longitudinal decline in

138 FEV_1 in the prospective asthma cohort including smokers.

METHODS

This study was approved by the Ethics Committee of the Hokkaido University School of Medicine (approval number, 02-001) and registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) system (https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno = R000003917; ID no. 000003254). All subjects provided written informed consent, and 213 subjects (127 with severe and 86 with non-severe asthma) were eligible for the initial study [20]. All participants stayed at the Hokkaido University Hospital for 2 days of initial screening (baseline visit), and patients with severe asthma were followed up yearly on an outpatient basis for 5 years. Detailed information is described in the online supplement.

Asthma patients

Subjects were participants of the Hokkaido-based Investigative Cohort Analysis for Refractory Asthma (Hi-CARAT). Those with CT data suitable for further assessment at baseline entered this study. We classified the subjects into two groups based on the number of cigarette packs they smoked (non-smokers (<10 pack-years) and smokers (≥10 pack-years). Subjects with severe asthma were categorized based on the American Thoracic Society (ATS) criteria of refractory asthma in 2000, with slight modifications of the inhaled corticosteroid doses due to the availability in Japan [20]. Hi-CARAT study participants were scheduled to undergo four times pre-bronchodilator and post-bronchodilator spirometry at inhalation of 400 μg of salbutamol and 400 μg of oxytropium on the first day, followed by 400 μg of salbutamol on the second day at the baseline examinations. We adopted FEV_1/forced vital capacity (FEV_1/FVC) when the best FEV_1 was obtained among the four spirometry (two pairs of pre- and post-bronchodilator spirometry) during the baseline examinations. Then we defined patients

with $FEV_1/FVC < 0.7$ as asthmatics with FAO.

COPD Patients

For morphological and physiological comparisons with asthma patients, we included mild to moderate COPD patients whose post-bronchodilator FEV_1 (% of predicted) was 50% or more to match spirometric impairment to that of asthmatics. We selected patients who completed the exams of the fifth-year visit of the Hokkaido COPD cohort [21, 22], which was the nearest visit to the baseline exams of this asthma cohort. COPD patients underwent CT examinations and pulmonary function tests under the same conditions as asthmatic patients did.

Pulmonary function tests

Pulmonary function tests met the requirements of the Japanese Respiratory Society Guidelines [23]. Carbon monoxide diffusing capacity (D_{Lco}) and transfer coefficient (D_{Lco}/V_A, Kco), based on the single breath method, were measured in all patients according to these guidelines.

Quantitative chest CT

Asthma and COPD patients underwent chest full-inspiration CT in the supine position using a multidetector row spiral CT scan with a 64-detector array (Aquilion Multi, TSX-101A/6A; Toshiba Medical Systems, Tochigi, Japan) and pulmonary function tests on the same day at the Hokkaido University Hospital. The acquisition parameters were 120 kVp, 300 mA, 64 detectors, 0.5 mm collimation, slice thickness of 0.5 mm, 0.5 s/rotation, helical pitch of 41, and smooth and sharp reconstruction kernels (FC03 and FC52). Parenchymal analysis was conducted using FC03, while airway analysis was done using FC52.

Assessment of D, LAA%, WA%, and AFD

LAA% was calculated as the volume percentages of low attenuation voxels < −950 and < -910 Hounsfield Unit (HU) (LAA%950 and LAA%910, respectively) [24]. Additionally, neighbouring voxels < −910 HU were three-dimensionally identified as a low attenuation cluster (LAC), and the volume of each LAC was obtained. The log-transformed volume of the LACs and the log-transformed cumulative count of LACs larger than the given volume were plotted on the x and y-axis, respectively. The absolute slope of the linear regression line was measured as the exponent D [25]. A lower D indicates greater extent of parenchymal destruction.

To quantify airway structure, the airway tree was three-dimensionally segmented, and airway fractal dimension (AFD) was calculated based on the box-counting method as reported [15, 26, 27], Moreover, airway luminal area (LA), airway wall area (WA), wall area percent (a ratio of wall area to summed area from wall and lumen (WA%)) at the right apical (RB1) and lateral basal (RB8) segmental airways were measured, and averaged LA and WA were normalized by body surface area (BSA).

Statistical analyses

For group comparisons of asthma with and without FAO in non-smokers and smokers, and in non-severe and severe asthmatics, Student-T test or Wilcoxon signed rank test were used. For comparisons among asthma with and without FAO, and COPD, ANOVA followed by Tukey's multiple comparison test, Dunn test for continuous variables, and chi-square test for categorical variables were used. Spearman's correlation analysis was performed to examine the relationships between D, LAA%910, and %FEV_1. Longitudinal FEV_1 changes were calculated using values from the first-year visit (one year after the screening examination including CT) to the sixth-year visit. Patients with more than 3 FEV_1 values were eligible (N = 102). We excluded pulmonary function test values at the

screening examination, as they were disproportionately higher compared to those obtained in following years. (Online supplementary Table E1).

Multivariable logistic regression analysis was used to test the association between D or LAA%910 and FAO, and multivariable linear regression analyses were used to examine the association between D or LAA%910 and $\%FEV_1$ after adjustment for sex, asthma severity and atopic status, as a categorical variable and age, body mass index (BMI), pack-years, blood eosinophil counts, AFD and WA% for as a continuous variable. Further multivariable linear regression models were used to examine associations between D or LAA%910 and the longitudinal FEV_1 changes, after adjustment for the abovementioned factors excluding asthma severity.

RESULTS

Of 189 eligible asthma patients, 101 were categorized as having FAO and were compared with COPD patients (Online supplementary Figure E1).

Comparisons between asthma with and without FAO and COPD

Clinical, physiological, and CT imaging characteristics are shown in Table 1.

Asthmatics without FAO were more predominantly female compared to asthmatics with FAO and COPD patients. Duration of asthma was longer in asthmatics with FAO than those without FAO. Although the severity of asthma and CT measured lung volume (adjusted by predicted TLC value) did not differ between asthmatics with and without FAO, $\%FEV_1$ and FEV_1/FVC were lower and %RV and RV/TLC were higher in asthmatics with FAO. Moreover, LA/BSA and AFD were lower, WA% and LAA%950 were higher in asthmatics with FAO. To increase the sensitivity to detect mild

parenchymal destruction, LAA%910 was also measured. In Figure 1, larger clusters of low attenuation area < −910 HU were found in asthmatics with FAO (the exponent D = 0.84) compared to those without FAO (the exponent D = 1.35), as visualized by different regression line slopes. Figure 2 further shows that D decreased and LAA%910 increased in asthmatics with FAO and COPD patients compared to asthmatics without FAO. In contrast, D and LAA%910 did not differ between severe and non-severe asthmatics.

Associations of D and LAA%910 with FAO in asthmatics depending on severity and smoking status

LAA%910 was higher in smokers with asthma than in non-smokers with asthma (p=0.01), while D showed no significant difference between -smokers with asthma and non-smokers with asthma (p=0.09). Since smoking could affect airway and parenchyma structure, comparisons of physiological and CT indices between asthmatics with and without FAO were performed in non-smokers and smokers, separately. Table 2 and Figure 3 show that D decreased, LAA%950 and LAA%910 increased in asthmatics with FAO compared with in asthmatics without FAO both in non-smokers and smokers while no difference in CT derived lung volume adjusted by predicted TLC value was found. Furthermore, in subgroup analysis of severe or non-severe asthmatics (Supplementary Table E2), decreased D and increased LAA%910 were found in non-severe and severe asthmatics with FAO (Figure 4). Meanwhile, D and LAA%910 showed no differences between severe asthma and non-severe asthma both in non-smokers and smokers. (Online supplementary Figure E2)

Associations of D and LAA%910 with FAO, %FEV_1 at baseline, and longitudinal

FEV_1 decline

Multivariable analyses were performed to explore whether the parenchymal destruction estimated using LAA%910 and exponent D and airway disease estimated using WA% and AFD on CT were associated with FAO (Table E3) and FEV_1 independent of demographics, and pack-years (Table 3). Due to a close association between D and LAA%910, these were separately included in models. Decreased D and AFD as well as increased LAA%910 and WA% were independently associated with FAO and $\%FEV_1$ after adjustment for age, sex, BMI, pack-years, asthma severity, atopy, and blood eosinophil count.

Furthermore, Table 4 shows that D, but not LAA%910, was significantly associated with FEV_1 decline (-33.8±23.4 ml/year, (mean±SD)) after adjustment for age, sex, BMI, pack-years, atopy, and blood eosinophil count in severe asthma patients (N = 102). Online supplementary Figure E3 shows no significant correlations between D and blood eosinophil count or the percentage of eosinophils or neutrophils in sputum. (rho = -0.04, p = 0.54, rho =- 0.08, p = 0.31, rho = -0.02, p = 0.75, respectively)

DISCUSSION

This study showed that D was lower and LAA%910 was higher in asthmatics with FAO than in those without FAO regardless of smoking status. It further revealed that the parenchymal destruction estimated from decreased D and increased LAA%910 as well as the airway diseases estimated from decreased AFD and increased WA% were independently associated with a higher odds ratio of FAO and a lower $\%FEV_1$ after adjusting for severity of asthma, smoking history, and other potentially confounding

283 factors. Moreover, a decrease in D at the baseline was associated with a greater
284 longitudinal FEV_1 decline in a five-year observation of severe asthma. These data suggest
285 that the parenchymal destruction occurs in asthmatics with FAO, and the parenchymal
286 destruction and airway disease may independently affect trajectory of lung function in
287 both smokers and non-smokers with asthma.

288 Airway remodeling is a well-established asthma feature [28], and parenchyma in
289 asthma was believed to remain intact. However, this concept is inconsistent with several
290 reports on parenchymal disorders in never smokers with moderate to severe asthma,
291 including the destruction of alveolar attachments [10], presence of centrilobular
292 emphysema [11], and loss of elastic recoil [12]. In this context, the found associations of
293 decreased D with FAO, lower FEV_1, and an accelerated FEV_1 decline (regardless of
294 smoking status and airway diseases) substantially increase the understanding of the
295 functional role of parenchyma in patients with asthma, which could be extended to that
296 in patients with Asthma – COPD overlap in the future.

297 One could argue that LAA% increases due to local lung expansion without the
298 parenchymal destruction. However, we deem this unlikely, since an increase in
299 LAA%910 and LAA%950 was accompanied by a decrease in D in non-smoking and
300 smoking asthmatics with FAO, and because the combinational change in LAA% and D
301 in asthmatics was comparable to that in COPD. Furthermore, the finding that CT derived
302 lung volume adjusted by predicted TLC in asthmatics with and without FAO did not differ
303 ($92.4 \pm 17.0\%$ and $88.1 \pm 17.4\%$) suggests that a decrease in D cannot be explained solely
304 by lung expansion. Alternatively, a reduction in D can be explained by a previous work
305 by Mishima *et al.* who showed that a rupture of alveolar walls and coalescence of
306 damaged areas would be required for a decrease in D [16]. Further, Tanabe *et al.* showed

that when LAA% increased, a decrease in D was induced by coalescence of neighboring pre-existing low-density CT regions, and not by simple enlargement of pre-existing low-density regions presumably reflecting local lung expansion [18]. Therefore, the observed combinational change in LAA% and D in asthmatics with FAO could be at least partially reflective of alveolar airspace enlargement due to alveolar wall destruction besides local lung expansion.

Notably, the parenchymal destruction assessed as the combination of increased LAA% and decreased D was found in both smoking and non-smoking asthmatics with FAO. Moreover, the CT finding of the parenchymal destruction was accompanied by a decrease in Kco in smoking asthmatics with FAO, suggesting that the parenchymal destruction in smoking asthmatics with FAO could be consistent with emphysematous destruction observed in smoking-related COPD as previously reported [29]. In contrast, a decrease in K_{CO} was not found in non-smoking asthmatics with FAO. We postulate that morphological changes and functional impairments induced by the parenchymal destruction in non-smoking asthmatics with FAO may not be exactly the same as those in smoking asthmatics with FAO.

Decreased D was associated with a longitudinal FEV_1 decline, irrespective of airway disease and established factors leading to it, including smoking habits [30] and blood eosinophil count [31, 32]. This finding has augmented the significance of the parenchymal destruction in asthma. There are few reports that CT metrics of airways and parenchyma possibly serve as predictive markers of lung function decline or exacerbations [33]. The present data showed no significant correlations between D and blood eosinophil count or eosinophil% and neutrophil% in sputum, despite treatment with anti-inflammatory agents such as inhaled (ICS) and oral corticosteroid (OCS) under

adequate adherence. This finding is concordant with a previous study by Tonga *et al.* [12] showing that no changes were observed in eosinophil or neutrophil counts and inflammatory cytokines in bronchoalveolar lavage of non-smoking older asthmatics with FAO after 2 months of ICS/long acting β2 agonist (LABA) treatment. Collectively, these findings suggest that a one-fits-all anti-inflammatory drug strategy does not improve the trajectory of pulmonary function in asthmatics who are characterized by both the airway disease and parenchymal destruction.

Moreover, in autopsy studies, Maud. *et al.* [10] showed an increase in abnormal alveolar attachments and a decrease in elastic fiber content in small airways and peri-bronchial alveoli in fetal asthma with no clinical evidence of emphysema, whereas Gelb *et al.* showed diffuse mild centrilobular emphysema in non-smokers with asthma [11]. In COPD, inflammation of small airway disease, imbalance of proteases and anti-proteases, oxidative stress [34], and exaggerated mechanical force [35, 36] could drive emphysema development. We speculate that protease activity and mechanical force on alveolar walls might be enhanced in a subgroup of asthmatics who eventually develop disrupted normal tissue integrity and FAO. This phenomenon may be partly concordant with the evidence that a bronchoconstriction without inflammation causes airway remodeling [37].

No significant difference in D between severe and non-severe asthmatics was found in this study. This is consistent with a study by Gupta *et al.* [19], but not with a study by Mitsunobu *et al.*, who showed a significant reduction in exponent D in non-smokers with severe asthma compared to those with mild to moderate asthma (please see further discussion in the online supplement) [15].

This study defined low attenuation regions using CT values of −910HU and −950 HU as cut-offs to calculate LAA% and of −910HU as cut-off to calculate the exponent D.

Moreover, this study also used fractals to evaluate airway structure using AFD [25, 26]. Few papers have performed combinational analysis using the two power law indicators [27], D and AFD. These topics are further discussed in the online supplemental discussion.

The current study has several limitations. First, only CT data at baseline are available. Consecutive CT data would confirm the results and broaden the perspectives for the clinical significance and proper utilization of exponent D as a CT-based biomarker. Second, annual FEV_1 decline was calculated from one year after baseline examination, while the CT scan was performed at baseline. Participants with severe asthma had been prescheduled to undergo baseline examinations during a two-day hospital stay, but to undergo follow-up examinations by visiting the hospital as out-patient . Consequently, the results of spirometry at the baseline examination were disproportionally better than those at the follow-up examinations in many patients, possibly due to adequate rest, less allergen burden, or less stimuli causing the worsening of asthma control. Therefore, we did not include the baseline data to calculate the longitudinal change in FEV_1 in the present analyses. Nonetheless, we believe that the FEV1 decline data should be accurate because it was calculated using serial data obtained annually from year 1 to year 5 visits. Third, the longitudinal analysis on FEV_1 was performed in severe asthma patients, so future studies should determine the effect of parenchymal destruction on FEV_1 in patients across different severities.

In conclusion, parenchymal evaluation with a combination of LAA% and D on CT showed that the parenchymal destruction occurs in asthmatics with persistent airflow limitation regardless of smoking status and asthma severity. Moreover, decreased D and increased LAA% were associated with airflow limitation, and decreased D affected the longitudinal FEV_1 decline independent of WA% and AFD in asthma. Of note, no

379 association between D and inflammatory markers was found. Therefore, more attention
380 should be paid to the possibility that both airway disease and parenchymal destruction
381 underlie physiological impairments and may affect clinical outcomes in a subgroup of
382 asthma, who requires personalized managements and novel interventions in the future.
383

ACKNOWLEDGMENTS

The authors thank the Hokkaido-based Investigative Cohort Analysis for Refractory Asthma investigators for recruitment of patients as well as Hideka Ashikaga, Ayako Kondo, and Yuko Takagi of the Central Office of the Hokkaido-based Investigative Cohort Analysis for Refractory Asthma (Sapporo, Japan) and the staff of Exam Co., Ltd. (Sapporo, Japan) for data management, Tsukasa Sasaki in the Division of Radiology, Department of Diagnosis and Treatment Support, the staff in the Division of Pulmonary Function, Department of Laboratory Medicine, Hokkaido University Hospital for technical support

REFERENCES

1. 2020 GINA MAIN REPORT2020 GINA Report, Global Strategy for Asthma Management and Prevention https://ginasthma.org/gina-reports/March.20.2021
2. Newby C, Agbetile J, Hargadon B, Monteiro W, Green R, Pavord I, ,et.al. . Lung function decline and variable airway inflammatory pattern: longitudinal analysis of severe asthma. J Allergy Clin Immunol. 2014 Aug;134:287-94.
3. Postma DS, Bush A, van den Berge M. Risk factors and early origins of chronic obstructive pulmonary disease. Lancet 2015 Mar;385:899-909.
4. Hastie AT, Mauger DT, Denlinger LC, Coverstone A, Castro M, Erzurum S, , et.al. Mixed sputum granulocyte longitudinal impact on lung function in the Severe Asthma Research Program. Am J Respir Crit Care Med. 2021 Feb. doi: 10.1164/rccm.202009-3713OC.
5. Shimizu K, Hasegawa M, Makita H, Nasuhara Y, Konno S, Nishimura M. . Comparison of airway remodeling assessed by computed tomography in asthma and

COPD. Respir Med. 2011 Sep;105:1275-83.

6. Oguma T, Hirai T, Fukui M, Tanabe N, Marumo S, Nakamura H ,et.al. Longitudinal shape irregularity of airway lumen assessed by CT in patients with bronchial asthma and COPD. Thorax. 2015 Aug;70:719-24.

7. Postma DS, Brightling C, Baldi S, Van den Berge M, Fabbri LM, Gagnatelli A, , et.al. Exploring the relevance and extent of small airways dysfunction in asthma (ATLANTIS): baseline data from a prospective cohort study. Lancet Respir Med. 2019 May;7:402-416.

8. Diaz AA, Hardin ME, Come CE, San José Estépar R, Ross JC, Kurugol S , et.al. Childhood-onset asthma in smokers. association between CT measures of airway size, lung function, and chronic airflow obstruction. Ann Am Thorac Soc. 2014 Nov;11:1371-8.

9. Tanabe N, Sato S, Oguma T, Shima H, Kubo T, Kozawa S et.al.. Influence of Asthma Onset on Airway Dimensions on Ultra-high-resolution Computed Tomography in Chronic Obstructive Pulmonary Disease. J Thorac Imaging. 2020 Nov. doi: 10.1097/RTI.0000000000000568.

10. Mauad T, Silva LF, Santos MA, Grinberg L, Bernardi FD, Martins MA , et.al.. Abnormal alveolar attachments with decreased elastic fiber content in distal lung in fatal asthma. Am J Respir Crit Care Med. 2004 Oct;170:857-62.

11. Gelb AF, Yamamoto A, Verbeken EK, Nadel JA. . Unraveling the pathophysiology of the asthma-COPD overlap syndrome: Unsuspected mild centrilobular emphysema is responsible for loss of lung elastic recoil in never smokers with asthma with persistent expiratory airflow limitation. Chest. 2015 Aug;148:313-320.

12. Tonga KO, Chapman DG, Farah CS, Oliver BG, Zimmermann SC , et.al. Reduced

lung elastic recoil and fixed airflow obstruction in asthma. Respirology. 2020 Jun;25:613-619.

13. Nakano Y, Muro S, Sakai H, Hirai T, Chin K, Tsukino M et.al. Computed tomographic measurements of airway dimensions and emphysema in smokers. Correlation with lung function. Am J Respir Crit Care Med. 2000 Sep;162:1102-1108.

14. Biernacki W, Redpath AT, Best JJ, MacNee W. Measurement of CT lung density in patients with chronic asthma. Eur Respir J. 1997 Nov;10(11):2455-9.

15. Mitsunobu F, Ashida K, Hosaki Y, et.al. Complexity of terminal airspace geometry assessed by computed tomography in asthma Am J Respir Crit Care Med. 2003 Feb;167:411-417.

16. Mishima M, Hirai T, Itoh H, Nakano Y, Sakai H, Muro S , et.al. Complexity of terminal airspace geometry assessed by lung computed tomography in normal subjects and patients with chronic obstructive pulmonary disease. Proc Natl Acad Sci U S A. 1999 Aug;96:8829-8834.

17. Yuan R, Nagao T, Paré PD, Hogg JC, Sin DD, Elliott MW , et.al. Quantification of lung surface area using computed tomography. Respir Res. 2010 Oct 31;11(1):153

18. Tanabe N, Muro S, Hirai T, et.al. Impact of exacerbations on emphysema progression in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2011 Jun;183:1653-1659.

19. Gupta S, Hartley R, Khan UT, Singapuri A, Hargadon B, Monteiro W , et.al. .Quantitative computed tomography-derived clusters: redefining airway remodelling in asthmatic patients. J Allergy Clin Immunol. 2014 Mar;133:729-738.e18.

20. Kimura H, Konno S, Nakamaru Y, Makita H, Taniguchi N, Shimizu K , et.al. Sinus computed tomographic findings in adult smokers and nonsmokers with asthma.

456 Analysis of Clinical Indices and Biomarkers. Ann Am Thorac Soc. 2017 Mar;14:332-
457 341.
458 21. Nishimura M, Makita H, Nagai K, Konno S, Nasuhara Y, Hasegawa M , et.al. Annual
459 change in pulmonary function and clinical phenotype in chronic obstructive
460 pulmonary disease. Am J Respir Crit Care Med. 2012 Jan;185:44-52.
461 22. Suzuki M, Makita H, Konno S, Shimizu K, Kimura H, Kimura H , et al. Asthma-like
462 features and clinical course of chronic obstructive pulmonary disease. An Analysis
463 from the Hokkaido COPD Cohort Study. Am J Respir Crit Care Med. 2016
464 Dec;194:1358-1355.
465 23. Guidelines for pulmonary function tests: spirometry, flow-volume curve, diffusion
466 capacity of the lung. Tokyo, Japan: The Japanese Respiratory Society; 2004. Available
467 at https://www.ncbi.nlm.nih.gov/pubmed/15565748 (In Japanese) March.20.2021
468 24. Bodduluri S, Reinhardt JM, Hoffman EA, Newell JD Jr, Nath H , et.al. Signs of gas
469 trapping in normal lung density regions in smokers. Am J Respir Crit Care Med. 2017
470 Dec;196:1404-1410.
471 25. Shimizu K, Tanabe N, Tho NV, Suzuki M, Makita H, Sato S , et.al. .Per cent low
472 attenuation volume and fractal dimension of low attenuation clusters on CT predict
473 different long-term outcomes in COPD. Thorax. 2020 Feb;75:116-122.
474 26. Bodduluri S, Puliyakote ASK, Gerard SE, Reinhardt JM, Hoffman EA, Newell JD
475 Jr, , et.al. Airway fractal dimension predicts respiratory morbidity and mortality in
476 COPD. J Clin Invest. 2018 Dec;128:5374-5382.
477 27. Tanabe N, Sato S, Suki B, Hirai T. , et.al. Fractal analysis of lung structure in chronic
478 obstructive pulmonary disease. Front Physiol. 2020 Dec;11:603197
479 28. Mauad T, Bel EH, Sterk PJ Asthma therapy and airway remodeling. J Allergy Clin

480 Immunol. 2007 Nov;120:997-1009
481 29. Shimizu K, Konno S, Makita H, Kimura H, Kimura H, Suzuki M, ,et.al. Transfer
482 coefficients better reflect emphysematous changes than carbon monoxide diffusing
483 capacity in obstructive lung diseases. J Appl Physiol. 2018 Jul;125(1):183-189.
484 30 Apostol GG, Jacobs DR Jr, Tsai AW, Crow RS, Williams OD, Townsend MC,et al.
485 Early life factors contribute to the decrease in lung function between ages 18 and 40:
486 The coronary artery risk development in young adults study. Am J Respir Crit Care
487 Med 2002;166-172
488 31. Hancox RJ, Pavord ID, Sears MR. Associations between blood eosinophils and
489 decline in lung function among adults with and without asthma. Eur Respir J. 2018
490 Apr 19;51(4):1702533713OC.
491 32. Semprini R, Williams M, Semprini A, McDouall A, Fingleton J, Holweg C , et.al.
492 Type 2 biomarkers and prediction of future exacerbations and lung function decline
493 in adult asthma. J Allergy Clin Immunol Pract 2018; 6: 1982–1988. e1981.
494 33. Krings JG, Goss CW, Lew D, Samant M, McGregor MC, Boomer J , et.al.
495 Quantitative CT metrics are associated with longitudinal lung function decline and
496 future asthma exacerbations: results from SARP-3. J Allergy Clin Immunol.
497 2021;S0091-6749(21)00176-7. doi: 10.1016/j.jaci.2021.01.029.
498 34. Tuder RM. Bringing light to chronic obstructive lung disease pathogenesis and
499 resilience. Ann Am Thorac Soc 2018;15:S227-S233.
500 35. Bhatt SP, Bodduluri S, Newell JD, et al; COPDGene Investigators. CT-derived
501 biomechanical metrics improve agreement between spirometry and emphysema.
502 Acad Radiol 2016 Oct;23:1255-1263.
503 36. Bhatt SP, Bodduluri S, Hoffman EA, Newell JD Jr, Sieren JC, Dransfield MT ,et.al.

Computed tomography measure of lung at risk and lung function decline in chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2017 Sep;196:569-76

37. Grainge CL, Lau LC, Ward JA, Dulay V, Lahiff G, Wilson S et.al.. Effect of bronchoconstriction on airway remodelling in asthma. N Engl J Med. 2011 May;364:2006-2015.

FIGURE LEGENDS

Figure 1. The representative asthmatics with or without fixed airflow obstruction.

Coronal images (A) and three-dimensional imaging (B) on CT of asthmatics with FAO and without FAO. Larger clusters of low attenuation area < −910 HU were found in asthmatics with FAO (the exponent D = 0.84, regression line in red (C)) compared to those without FAO (the exponent D = 1.35, regression line in blue (C)).

Figure 2. Comparisons of D, LAA%910 in asthma with or without fixed airflow obstruction, and COPD and in non-severe and severe asthmatics

(A) Exponent D was the lowest, and LAA%910 was the highest in COPD, followed by asthma with fixed airflow obstruction (FAO), asthma without FAO. (B) There were no significant differences in exponent D and LAA%910 between non-severe and severe asthmatics.

Figure 3. Comparisons of D, LAA%910 between asthmatics with or without fixed airflow obstruction in non-smokers and smokers.

D decreased and LAA%910 increased in cases of fixed airflow obstruction both in non-smokers and smokers.

Figure 4. Comparisons of D, LAA%910 between non-severe and severe asthmatics with or without fixed airflow obstruction.

D decreased and LAA%910 increased in cases of fixed airflow obstruction both in non-severe and severe asthmatics.

TABLES

Table 1. Characteristics of subjects with asthma with or without fixed airflow obstruction and those with COPD

	Asthma without FAO	Asthma with FAO	COPD
Patients, N	88	101	42
Male, N (%)	25 (28.4)	50 (49.5)	36 (85.7) §
Age, years	55.9 ± 14.6*†	65.2 ± 9.9	69.3 ± 7.6
BMI, kg/m^2	25.6 ± 5.8†	24.5 ± 4.2†	22.6 ± 3.4
Severe, N (%)	54 (61.4)	72 (71.3)	
Smokers, N (%)	25 (28.4)	45 (44.6)	42 (100) §
Pack-years	10.4 (0–11.5)†	16.1 (0–26.8)†	60.0 (40.8-68.9)
Duration of asthma, years	14.5 ± 12.7*	25.1 ± 15.5	
Atopy, N (%)	62 (70.5)	64 (63.4)	
AQLQ	5.6 (5.0–6.4)	5.7 (5.0–6.4)	
Daily ICS dose, mg	1235.2 ± 628.9	1371.0 ± 765.5	
Maintenance OCS use, N (%)	16 (18.2)	30 (29.7)	
Eo,µL	289.4 ± 0.46*	350.8 ± 0.45†	175.7± 0.31
IgE, IU/ml	414.1 ± 0.68	414.8 ± 0.60	
FeNO, ppb	37.4 (0.35)	39.6 (0.32)	
%FEV$_1$, %	117.5 ± 19.7*†	97.6 ± 21.0 †	81.2 ± 19.9
FEV$_1$/FVC, %	78.7 ± 5.6*†	58.1 ± 7.7	60.3 ± 11.6
%RV, %	105.2 ± 18.6*†	116.9 ± 22.3†	130.9 ± 28.3
RV/TLC, %	33.8 ± 6.1*†	38.7 ± 6.6	38.6 ± 7.8
%TLC, %	110.5 ± 12.9	114.3 ± 14.1	111.1 ± 16.8
% D$_{Lco}$, %	99.4 ± 20.9†	107.3 ± 22.8 †	81.6 ± 23.1
%Kco, %	110.1 ± 18.8†	107.1 ± 25.0 †	70.4 ± 19.5
%CT-LV, %	88.1 ± 17.4	92.4 ± 17.0	89.6 ± 15.3
LA/BSA, mm^2/m^2	14.7 ± 7.5*†	11.6 ± 6.2	11.4 ± 5.8
WA%, %	56.6 ± 6.7*†	61.0 ± 6.5	60.2 ± 5.8
WA/BSA, mm^2/m^2	18.1 ± 5.8	17.0 ± 6.1	16.2 ± 5.6
AFD	1.95 ± 0.05*	1.92 ± 0.05	1.93 ± 0.04
Exponent D	1.08 (0.07)*†	1.02 (0.06)†	0.97 (0.08)
LAA%910, %	10.0 (0.59)*†	19.8 (0.40)†	29.1 (0.36)
LAA%950, %	0.47 (0.58)*†	2.65 (0.68)†	9.37 (0.68)

Data are shown as the mean ± standard deviation, median (interquartile range), geometric mean (log10 SD), or number (%).

*; $p < 0.05$, compared with asthma with FAO.

†; $p < 0.05$, compared with COPD.

§; $p<0.05$, between asthma without FAO, asthma with FAO and COPD.

FAO : fixed airflow obstruction, BMI : body mass index, AQLQ : Asthma Quality of Life Questionnaire, ICS : inhaled corticosteroids, OCS : oral corticosteroids, Eos : blood eosinophil count, FeNO : fractional exhaled nitric oxide, FEV_1 : forced expiratory volume in 1 sec, RV : residual volume, TLC : total lung capacity, D_{Lco} : diffusing capacity for carbon monoxide, Kco : carbon monoxide transfer coefficient, %CT-LV : CT-derived lung volume adjusted by predicted value of total lung capacity, LA : airway luminal area, WA : airway wall area, BSA : body surface area, AFD : airway fractal dimension, LAA : low attenuation area.

551 **Table 2.** Comparisons between asthmatics with or without fixed airflow obstruction in

552 non-smokers and smokers

	Non-smokers		Smokers	
	Without FAO	**With FAO**	**Without FAO**	**With FAO**
Patients, N	63	56	25	45
Male, N (%)	12 (19.1)	13 (23.2)	13 (52.0) *	37(82.2)
Age, years	56.1 ± 15.5*	65.1 ± 10.3	55.4 ± 12.4*	65.4 ± 9.4
BMI, kg/m^2	25.1 ± 4.9	24.7 ± 4.7	26.8 ± 7.6	24.2 ± 3.6
Severe, N (%)	40 (63.5)	34 (60.7)	14 (56.0) *	38 (84.4)
Pack-years	1.7 (0-3.8)	1.1 (0−1.1)	32.2 (11.7-41.5)	34.9 (16.1−46)
Duration of asthma, years	14.0 ± 12.5*	27.8 ± 15.2	14.9 ± 13.5*	21.7 ± 15.4
AQLQ	5.6 (5.0-6.4)	5.8 (5.4-6.4)	5.6 (5.0-6.5)	5.5 (4.8-6.5)
%FEV$_1$, %	120.3 ± 22.5*	104.3 ± 21.8	116.4 ± 18.6*	92.2 ± 18.9
FEV$_1$/FVC, %	79.1 ± 5.7*	58.9 ± 6.7	77.7 ± 5.4*	57.1 ± 8.8
%RV, %	103.2 ± 17.7*	114.1 ± 21.1	110.4 ± 20.1	120.4 ± 23.4
RV/TLC, %	33.5 ± 6.3*	39.1 ± 6.4	34.5 ± 5.8*	38.2 ± 6.9
%TLC, %	110.8 ± 12.8	113.8 ± 13.9	109.6 ± 13.2	114.5 ± 14.6
% D$_{Lco}$, %	100.3 ± 19.8	107.3 ± 21.0	97.3 ± 23.7	107.4 ± 25.2
%Kco,%	112.7 ± 19.5	117.1 ± 22.0	103.7 ± 15.5	94.7 ± 22.9
%CT-LV, %	87.0 ± 17.9	93.2 ± 16.0	90.6 ± 16.1	91.3 ± 18.4
LA/BSA, mm^2/m^2	14.1 ± 6.5*	10.5 ± 5.7	16.2 ± 9.6	13.1 ± 6.5
WA%, %	56.7 ± 7.2*	61.8 ± 6.9	56.4 ± 5.2*	60.0 ± 5.8
WA/BSA, mm^2/m^2	17.3 ± 4.5*	15.9 ± 5.2	20.0 ± 8.0	18.4 ± 6.9
AFD	1.95 ± 0.05*	1.91 ± 0.06	1.95 ± 0.05	1.93 ± 0.04
LAA%910, %	9.36 (0.62)*	17.6 (0.43)	11.8 (0.50)*	22.5 (0.35)
LAA%950, %	0.29 (0.54)*	1.56 (0.66)±	0.91 (0.67)*	4.00 (0.66)

553 Data are shown as the mean ± standard deviation, median (interquartile range),

554 geometric mean ($\log_{10}$ SD), or number (%).

555 *: $P < 0.05$, compared with asthmatics with FAO

556 FAO : fixed airflow obstruction, BMI : body mass index, AQLQ : Asthma Quality of

557 Life Questionnaire, FEV_1 : forced expiratory volume in 1 sec, FVC : forced vital

capacity, RV : residual volume, TLC : total lung capacity, D_{Lco} : diffusing capacity for carbon monoxide, Kco : carbon monoxide transfer coefficient, %CT-LV : CT-derived lung volume adjusted by predicted value of total lung capacity. LA : airway luminal area, WA : airway wall area, BSA : body surface area, AFD : airway fractal dimension, LAA : low attenuation area.

563 **Table 3.** Multivariable analysis to explore factors associated with FEV_1 in asthma at the

564 baseline evaluation

	Model 1			Model 2		
	Estimate	95% CI	p-value	Estimate	95% CI	p-value
D	9.48	5.47-13.5	<0.0001			
LAA%910				-5.75	-11.0 - -0.52	0.03
WA%	-0.39	-0.77- -0.01	0.046	-0.55	-0.94 - -0.16	0.006
AFD	10.8	5.78 -15.8	<0.0001	11.5	6.32 - 16.8	<0.0001
Age	-0.01	-0.24 - 0.22	0.91	-0.18	-0.40 - 0.05	0.12
Female	-8.43	-11.4 - -5.50	<0.0001	-9.48	-12.6- -6.31	<0.0001
BMI	-0.32	-0.87 - 0.23	0.25	-0.03	-0.59 - 0.52	0.90
Pack-years	-0.01	-0.24 - 0.03	0.14	-0.13	-0.28 - 0.01	0.07
Severe asthma	-5.12	-8.03 - -2.22	0.0006	-5.78	-8.81 - -2.75	<0.0001
Atopy	0.53	-2.21 - 3.28	0.70	0.80	-2.08 - 3.67	0.55
Eo	-6.03	-11.5 - -0.59	0.03	-7.25	-12.9- -1.59	0.01

565 Odds, Chi-squared test and estimated values were calculated for 0.1 increase in D and

566 AFD, for 1 increase in other continuous variables.

567 LAA : low attenuation area, WA : wall area, AFD : airway fractal dimension, BMI : body

568 mass index, Eo : blood eosinophil count,

569 Eo and LAA%910 were log10 transformed.

570

571

572 **Table 4.** Multivariable analysis to explore baseline factors associated with subsequent

573 longitudinal decline in FEV_1 in asthma

	Model 1			Model 2		
	Estimate	95% CI	p-value	Estimate	95% CI	p-value
D	8.89	1.48 - 16.3	0.02			
LAA%910				-9.34	-19.2 - 0.56	0.06
WA%	0.13	-0.68 - 0.94	0.75	-0.07	-0.86 - 0.72	0.86
AFD	-1.74	-11.4 - 7.86	0.72	-0.28	-9.90 - 9.33	0.95
Age	-0.03	-0.49 - 0.43	0.91	-0.15	-0.61 - 0.31	0.51
Female	2.34	-3.54 - 8.22	0.43	0.79	-5.30 - 6.88	0.79
BMI	-0.04	-1.12 - 1.03	0.94	0.18	-0.88 - 1.23	0.74
Pack-years	0.09	-0.18 - 0.36	0.51	0.03	-0.24 - 0.30	0.82
Atopy	-3.54	- 8.92 -1.83	0.19	-2.19	-7.60 - 3.22	0.42
Eo	4.14	-5.67 - 13.9	0.40	3.12	-6.70 - 12.9	0.53

574 Estimated values were calculated for 0.1 increase in D and AFD, for 1 increase in other

575 continuous variables.

576 LAA : low attenuation area, WA :wall area, AFD : airway fractal dimension. BMI : body

577 mass index, Eo : blood eosinophil count

578 Eo and LAA%910 were log10 transformed.

Online repository

Parenchymal destruction in asthma : Fixed airflow obstruction and lung function trajectory

Kaoruko Shimizu, M.D., Ph.D.[1]*, Naoya Tanabe, M.D., Ph.D.[2]*, Akira Oguma, M.D.[1], Hirokazu Kimura, M.D., Ph.D.[1], Masaru Suzuki, M.D., Ph.D.[1], Isao Yokota, Ph. D, M.P.H..[3], Hironi Makita, M.D., Ph. D.[1,4], Susumu Sato, M.D., Ph. D.[2], Toyohiro Hirai, M.D., Ph. D.[2], Masaharu Nishimura, M.D., Ph. D.[1,4], Satoshi Konno, M.D., Ph.D.[1], The Hi-CARAT investigators

*These two authors contributed equally to the study

[1]Department of Respiratory Medicine, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan

[2]Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[3]Department of Biostatistics, Graduate School of Medicine, Hokkaido University, Sapporo, Japan

[4]Hokkaido Institute of Respiratory Diseases, Sapporo, Japan

Correspondence: Kaoruko Shimizu

Department of Respiratory Medicine, Faculty of Medicine and Graduate, School of Medicine, Hokkaido University, North 15 West 7, Kita-ku, Sapporo 060-8638, Japan

Tel: +81-11-706-5911

24 Fax: +81-11-706-7899

25 E-mail: okaoru@med.hokudai.ac.jp

Contents

1. Supplemental Methods

In the Hokkaido-based investigative cohort analysis for refractory asthma (Hi-CARAT), patients with severe asthma were recruited from Hokkaido University Hospital and 29 affiliated hospitals and clinics between February 2010 and September 2012 [E1]. Respiratory physicians diagnosed asthma according to the Global Initiative on Asthma criteria [E2]. The definition of severe asthma was based on the American Thoracic Society criteria of refractory asthma in 2000 [E1], requiring one or two major and two minor criteria.

Major criteria

In order to achieve asthma control ,

1. Treatment with continuous or near continuous (>50% of year) oral corticosteroids
2. Requirement for treatment with high-dose inhaled corticosteroids:

We modified the inhaled corticosteroid doses due to differences in their availability in Japan as follows:

Flutide®≥800 μg, Pulmicort®≥1200 μg, QVAR®≥600 μg, Alvesco®≥600 μg, Asmanex® ≥600 μg, Adoair® ≥1000 μg, Symbicort® ≥960 μg

Minor criteria

1. Requirement for daily treatment with a controller medication in addition to inhaled corticosteroids, e.g., long-acting β-agonist, theophylline, or leukotriene antagonist
2. Asthma symptoms requiring short-acting β-agonist use on a daily or near daily basis
3. Persistent airway obstruction (FEV_1<80% predicted; diurnal PEF variability>20%)
4. One or more urgent care visits for asthma per year
5. Three or more oral steroid “bursts” per year

6. Prompt deterioration with <25% reduction in oral or inhaled corticosteroid dose
7. Near-fatal asthma event in the past. Additionally, we also recruited mild to moderate asthma in stable condition for at least 6 months, without high doses of inhaled or oral corticosteroids.

Asthma patients

Subjects were participants of Hi-CARAT. The subjects whose CT data were available at baseline were included and classified into non-smokers (<10 pack-years) and smokers (≥10 pack-years). Following the protocol of the Hi CARAT study, the subjects underwent four times of baseline spirometry such as pre-bronchodilator and post-bronchodilator (400 μg of salbutamol) examinations on the first day and pre-bronchodilator and post-bronchodilator (400 μg of oxytropium followed by 400 μg of salbutamol) examinations on the second day. According to the previous papers of the Hi-CARAT study, we had applied the best FEV_1 among the four spirometries to the present analysis. We defined the forced expiratory volume in 1 s/forced vital capacity (FEV_1/FVC) ratio < 0.7 when the best FEV_1, was obtained, as fixed airflow obstruction (FAO).

Participants stayed at Hokkaido University Hospital for 2 days for initial screening, which corresponds to the baseline visit (year 0), and were consecutively followed up yearly on an outpatient basis (Visit 1-6) for 5 years as outpatients. Participants underwent pulmonary function tests and CT on the same day.

Statistical analyses

Student-T test or Wilcoxon signed rank test were used for multiple comparisons of asthma with and without FAO in non-smokers and smokers, and in non-severe and severe asthmatics. Parametric and nonparametric continuous variables were compared between asthma with and without FAO, and COPD using ANOVA followed by Tukey's multiple comparison test, and Wilcoxson signed rank test followed by Dunn's multiple comparison test, respectively. Chi-square test were used for categorical variables. Spearman's correlation analysis was used to investigate the associations between exponent D, low attenuation area (LAA) %910, and $\%FEV_1$. Longitudinal changes in FEV_1 were calculated using data from the first-year visit (one year after the screening examination including CT) to the sixth-year visit. Patients whose FEV_1 was measured more than 3 times were included to calculate the FEV1 change (N = 102). Values from pulmonary function test at the screening examination were excluded because the data obtained at the screening were disproportionately higher than those obtained in following years. (please see Online supplementary table E1).

Moreover, association between exponent D or LAA%910 and FAO was examined using multivariable logistic regression models, and association between exponent D or LAA%910 and $\%FEV_1$ was also tested using multivariable linear regression models adjusted for categorical variables including sex, asthma severity and atopic status, and continuous variables of age, body mass index (BMI), pack-years, blood eosinophil counts, AFD and WA%. Additionally, associations between exponent D or LAA%910 and the longitudinal FEV_1 changes were tested using multivariable linear regression models adjusted for the abovementioned factors other than asthma severity.

2. Supplemental Results

Of 189 eligible asthma patients, 101 were categorized as having FAO and were compared with COPD patients (Online supplementary Figure E1).

Associations of D and LAA%910 with FAO in asthmatics depending on severity and smoking status

Since smoking could affect airway and parenchyma structure, comparisons of physiological and CT indices between asthmatics with and without FAO were performed in non-smokers and smokers, separately. Table 2 and Figure 3 show that D decreased, LAA%950 and LAA%910 increased in asthma with FAO compared with in asthma without FAO both in non-smokers and smokers while no difference in CT derived lung volume adjusted by predicted TLC value was found. Furthermore, in subgroup analysis of severe or non-severe asthmatics (Supplementary Table E2), decreased D and increased LAA%910 were found in non-severe and severe asthmatics with FAO (Figure 4). Meanwhile, D and LAA%910 showed no differences between severe asthma and non-severe asthma both in non-smokers and smokers. (Online supplementary Figure E2)

Associations of D and LAA%910 with FAO, %FEV_1 at baseline, and longitudinal FEV_1 decline

Multivariable analyses were performed to explore whether the parenchymal destruction estimated using LAA% and exponent D and airway disease estimated using WA% and AFD on CT were associated with FAO (Table E3) and FEV_1 independent of demographics, and smoking history (Table 3). Due to a close association between D and LAA%910, these were separately included in models. Decreased D and AFD as well as

increased LAA%910 and WA% were independently associated with FAO and %FEV_1 after adjustment for age, sex, BMI, pack-years, asthma severity, atopy, and blood eosinophil count.

Furthermore, Table 4 shows that D, but not LAA%910, was significantly associated with FEV_1 decline (-33.8±23.4 ml/year, (mean±SD)) after adjustment for age, sex, BMI, pack-years, atopy, and blood eosinophil count in severe asthma patients (N = 102).

Online supplementary Figure E3 shows no significant correlations between D and blood eosinophil count or the percentage of eosinophils or neutrophils in sputum. (rho=-0.04, p=0.54, rho=-0.08, p=0.31, rho=-0.02, p=0.75, respectively)

3. Supplemental Discussion

No significant difference in D between severe and non-severe asthmatics was found in this study. This is consistent with a study by Gupta *et al.* [E3], but not with a study by Mitsunobu *et al.*, who showed a significant reduction in exponent D in non-smokers with severe asthma compared to those with mild to moderate asthma [E4] . While Mitunobu *et al.* defined the exponent D using two-dimensional low attenuation clusters, the present study and Gupta *et al.* used three-dimensional low attenuation clusters. Moreover, this difference presumably arises from the differences in severity of airflow between the studies. In this study, D decreased when FAO occurred in non-severe and severe asthma. Therefore, parenchymal destruction could have physiological effects and induce FAO regardless of asthma severity.

This study defined low attenuation regions using CT values of −910HU and −950 HU as cut-offs to calculate LAA% and of -910HU as cut-off to calculate the exponent D. Because the number of low attenuation clusters was too small to calculate the regression line slope on the log-log plot (the exponent D), especially in asthmatics with almost normal CT findings when using −950HU as the cut-off, we decided to use the −910 HU cut-off to take more clusters of low attenuation for the rigorous calculation of the exponent D (D). Since previous reports [E5] used a −910HU cut-off to detect mild emphysema and that the extent of lung density reduction is generally milder in asthmatics than COPD, we believe that LAA%910 and D are valid to detect subtle parenchymal disorders in asthmatics.

This study also used fractals to evaluate airway structure using AFD [E6]. Few papers have performed combinational analysis using the two power law indicators [E7],

158 D and AFD. The finding that lower AFD and D were independently associated
159 with %FEV_1 in asthma suggests that airflow limitation is determined by the
160 parenchymal destruction and airway structure in asthmatics. Considering that airflow
161 limitation in COPD is affected by emphysema and airway disease, further comparisons
162 of airflow limitation determinants between COPD and asthma should be performed in
163 future studies.

4. References

E1. Kimura H, Konno S, Nakamaru Y, Makita H, Taniguchi N, Shimizu K, et.al. Sinus computed tomographic findings in adult smokers and nonsmokers with asthma. Analysis of Clinical Indices and Biomarkers. Ann Am Thorac Soc. 2017 Mar;14:332-341.

E2. Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA). Date last updated: April 2015. Available from: http://www.ginasthma.org 20 Mar 2021

E3. Gupta S, Hartley R, Khan UT, Singapuri A, Hargadon B, Monteiro W , et.al. .Quantitative computed tomography-derived clusters: redefining airway remodelling in asthmatic patients. J Allergy Clin Immunol. 2014 Mar;133:729-738.e18.

E4. Mitsunobu F, Ashida K, Hosaki Y, et.al. Complexity of terminal airspace geometry assessed by computed tomography in asthma Am J Respir Crit Care Med. 2003 Feb;167:411-417.

E5. Bodduluri S, Reinhardt JM, Hoffman EA, Newell JD Jr, Nath H , et.al. Signs of gas trapping in normal lung density regions in smokers. Am J Respir Crit Care Med. 2017 Dec;196:1404-1410.

E6. Bodduluri S, Puliyakote ASK, Gerard SE, Reinhardt JM, Hoffman EA, Newell JD Jr, et.al. Airway fractal dimension predicts respiratory morbidity and mortality in COPD. J Clin Invest. 2018 Dec;128:5374-5382.

E7. Tanabe N, Sato S, Suki B, Hirai T. Fractal analysis of lung structure in chronic obstructive pulmonary disease. Front Physiol. 2020 Dec;11:603197

5. Supplemental Figure Legends

Figure E1. Flowchart of the participants with asthma and chronic obstructive pulmonary disease.

Patients with asthma who participated in the initial study were enrolled. One severe asthma patient was excluded because of the missing post-bronchodilator spirometry data on the same day as CT exam. Patients with non-severe asthma, 18 patients without CT data required for parenchymal and airway indices, and 5 patients examined using a different CT scanner were excluded. Patients with COPD, of whom %FEV1 was 50% or more were included.

COPD, chronic obstructive lung disease; CT, computed tomography; FEV_1, forced expiratory volume in 1 sec

Figure E2. Comparisons of D, LAA%910 between non-severe and severe asthmatics in non-smokers and smokers.

D did not differ between non-severe and severe asthmatics in non-smokers and smokers.

Figure E3. Relationships between D and blood eosinophil counts, eosinophil%, and neutrophil% in sputum.

No significant correlations were found between D and blood eosinophil counts, eosinophil%, and neutrophil% in sputum. (rho = -0.04, p = 0.54, rho = -0.08, p = 0.31, rho = -0.02, p = 0.75, respectively)

6. Supplemental Tables

Table E1. %FEV_1 at baseline and follow-up visits

	baseline	**First year**	**Second year**	**Third year**	**Fourth year**	**Fifth year**	**Sixth year**
%FEV_1, %	102.7±20.6	88.1±19.0	87.6±20.4	86.5±19.4	81.9±27.3	85.5±21.0	85.4±21.4

Data are shown as the mean ± standard deviation.

FEV_1, forced expiratory volume in 1 sec.

Table E2. Comparisons between patients with or without fixed airflow obstruction in severe and non-severe asthmatics.

	Non-severe asthma		Severe asthma	
	Without FAO	**With FAO**	**Without FAO**	**With FAO**
Patients, N	34	29	54	72
Male, N (%)	13 (38.2)	12 (41.4)	12 (22.2)	38 (52.8)
Age, years	63.4 ± 12.3*	70.8 ± 7.8	51.2 ± 14.0*	63.0 ± 9.7
BMI, kg/m^2	24.1 ± 4.4	23.4 ± 3.0	26.6 ± 6.4	24.9 ± 4.6
Smokers, N (%)	11 (32.4)	7 (24.1)	14 (25.9) *	38 (52.8)
Pack-years	13.4 (0-17.9)	7.1 (0-8.3)	8.5 (0-10.6)*	19.8 (0-31.1)
Duration of asthma, years	15.6 ± 12.5*	27.1 ± 17.5	14.0 ± 13.0*	24.3 ± 14.7
AQLQ	6.1 (5.8-6.7)	6.2 (5.8-6.6)	5.3 (4.8-6.1)	5.4 ()
%FEV$_1$, %	124.5 ±21.4*	104.6 ± 23.8	113.1 ±17.5*	94.8 ± 19.3
FEV$_1$/FVC, %	77.3 ± 4.5*	59.3 ±7.5 20.6	79.6 ± 6.1*	57.6 ± 7.8
%RV, %	100.5 ± 16.5*	109.5 ± 20.6	108.2 ± 19.4*	119.9 ± 22.4
RV/TLC, %	34.1 ± 5.9*	37.8 ± 6.0	33.6 ± 6.3*	39.0 ± 6.9
%TLC, %	111.0 ± 10.5	115.9 ± 13.1	110.1 ± 14.2	113.4 ± 14.5
%DL$_{co}$, %	106.2 ± 22.6	116.4 ± 27.2	95.1 ± 18.8*	103.7 ± 19.9
%Kco ,%	107.6 ± 18.0	111.9 ± 17.6	111.7 ± 19.3	105.1 ± 27.2
%CT-LV, %	90.0 ± 15.5	94.1 ± 17.5	86.8 ± 18.5	91.7 ± 16.9
LA/BSA, mm^2/m^2	14.6 ±5.8*	11.2 ± 6.7	14.8 ± 8.5*	11.8 ±6.0
WA%, %	56.1 ±7.5*	61.9 ± 6.7	57.0 ± 6.1*	60.7 ± 6.4
WA/BSA, mm^2/m^2	17.7 ± 3.9	16.7 ± 6.1	18.3 ± 6.8	17.1 ± 6.1
AFD	1.95 ± 0.05	1.94 ± 0.05	1.95 ± 0.06	1.91 ± 0.05
LAA%910, %	12.2(0.59)*	20.1(0.31)	8.7(0.58)*	19.7(0.43)
LAA%950, %	0.77(0.66)*	1.78(0.51)	0.27(0.52)*	3.009(0.74)

Data are shown as the mean ± standard deviation (SD), median (interquartile range), geometric mean (log10 SD), or number (%). *: $P < 0.05$, compared with asthmatics with FAO. FAO : fixed airflow obstruction, BMI : body mass index, AQLQ : Asthma Quality of Life Questionnaire, FEV_1 : forced expiratory volume in 1 sec, FVC : forced vital capacity, RV : residual volume, TLC : total lung capacity, D_{Lco} : diffusing capacity for carbon monoxide, Kco : carbon monoxide transfer coefficient, %CT-LV : CT-derived lung volume adjusted by predicted value of total lung capacity. LA : airway

luminal area, WA : airway wall area; BSA : body surface area, AFD : airway fractal dimension, LAA : low attenuation area.

Table E3. Multivariable analysis to explore factors associated with fixed airflow obstruction in asthma at the baseline examination

	Model 1			Model 2		
	Odds (95%CI)	Chi-squared test	p-value	Odds(95%CI)	Chi-squared test	p-value
D	0.20 (0.08 - 0.43)	19.8	<0.0001			
LAA%910				11.4 (3.74–35.0)	25.8	<0.0001
WA%	1.11 (1.04 - 1.18)	10.2	0.001	1.12 (1.05–1.19)	14.0	0.0002
AFD	0.28 (0.12 - 0.61)	10.1	0.002	0.26 (0.11–0.59)	10.8	0.001
Age	1.06 (1.02 - 1.11)	8.98	0.003	1.10 (1.05–1.15)	23.3	<0.0001
Female	0.15 (0.06 - 0.42)	15.4	<0.0001	0.36 (0.13–0.97)	4.26	0.04
BMI	1.53 (0.92 - 1.09)	0.01	0.92	0.99 (0.90–1.08)	0.10	0.76
Pack-years	0.58 (0.06 - 5.65)	0.22	0.64	0.99 (0.97–1.02)	0.20	0.65
Severe asthma	2.66 (1.05 - 6.72)	4.41	0.04	4.25 (1.60–11.3)	9.26	0.002
Atopy	0.88 (0.37 - 2.13)	0.07	0.78	0.79 (0.31–1.99)	0.25	0.62
Eo	1.42 (0.61 - 3.47)	0.65	0.42	1.56 (0.65–3.74)	1.01	0.31

Odds, Chi-squared test and estimated values were calculated for 0.1 increase in D and AFD, for 1 increase in other continuous variables.

LAA : low attenuation area, WA : wall area, AFD : airway fractal dimension. BMI : body mass index, Eo : blood eosinophil count

Eo and LAA%910 were log10 transformed.

Parenchymal destruction in asthma: Fixed airflow obstruction and lung function trajectory

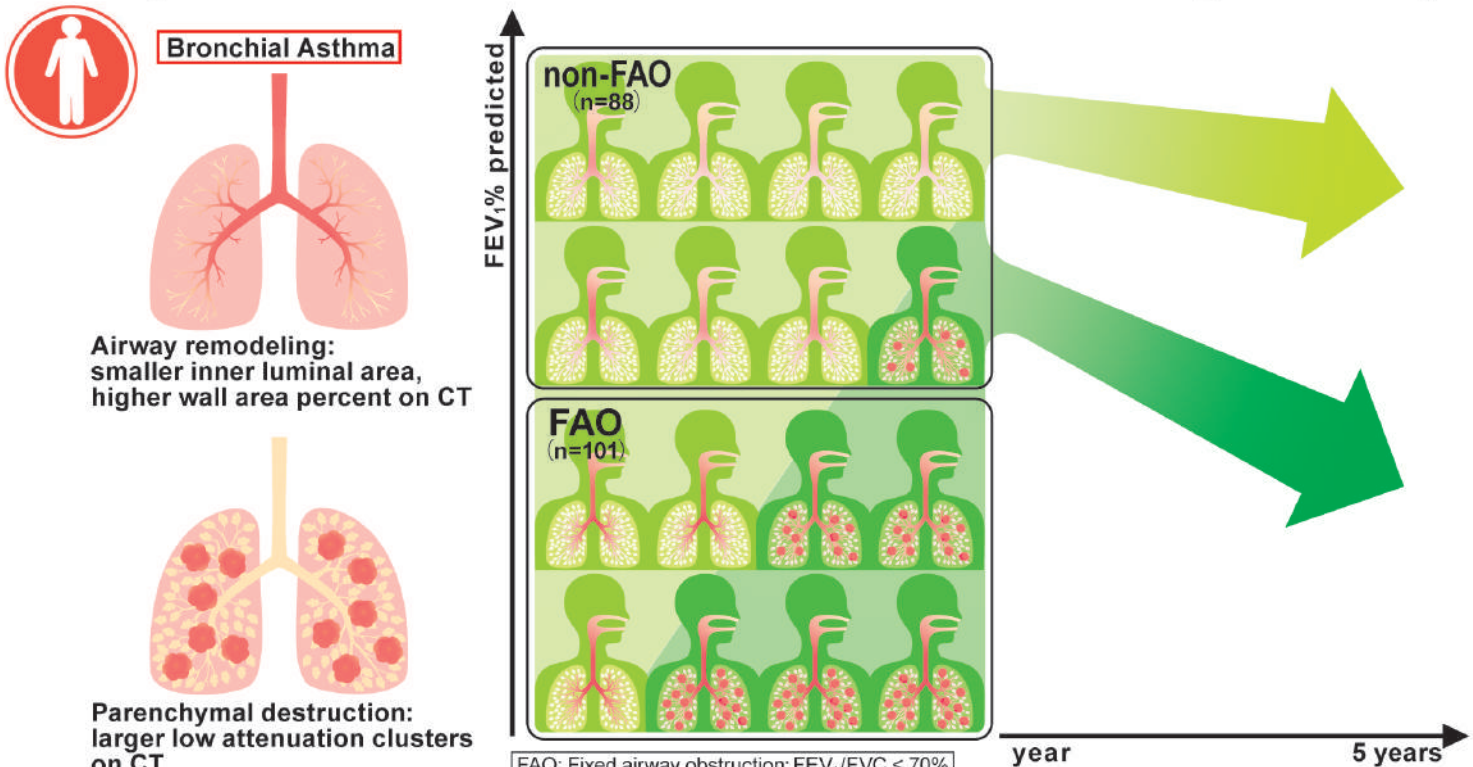
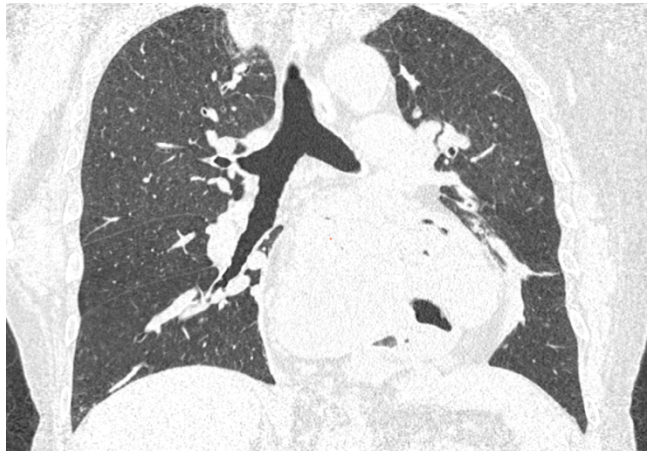


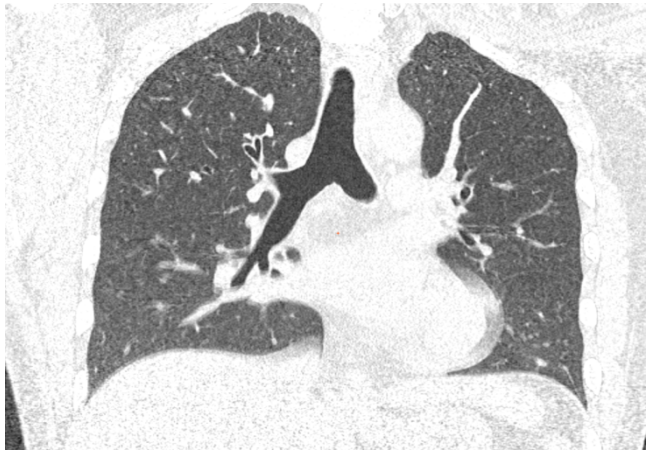
Figure 1.

[A]

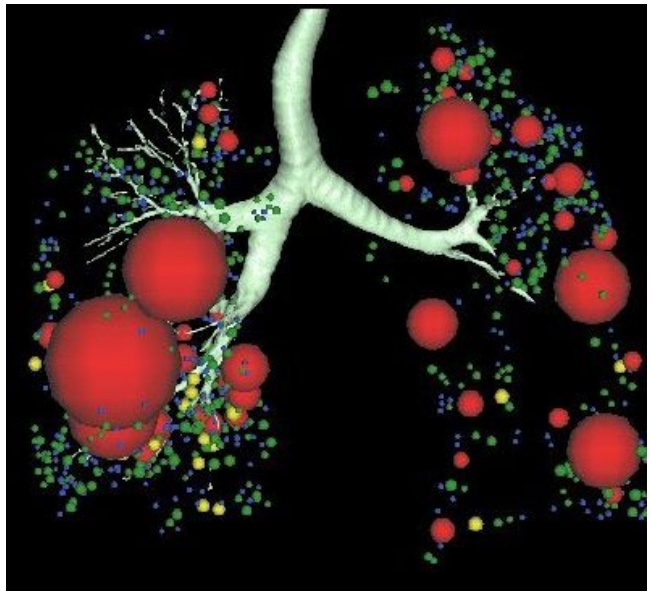
Asthma with FAO

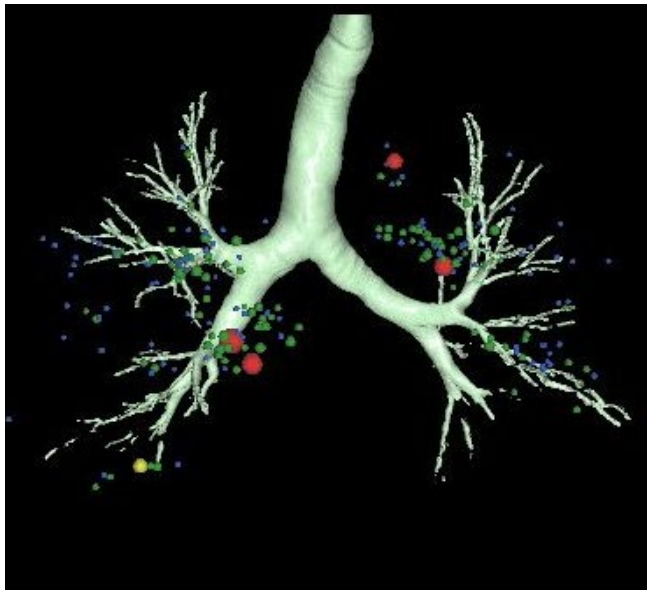
Asthma without FAO





[B]





[C]

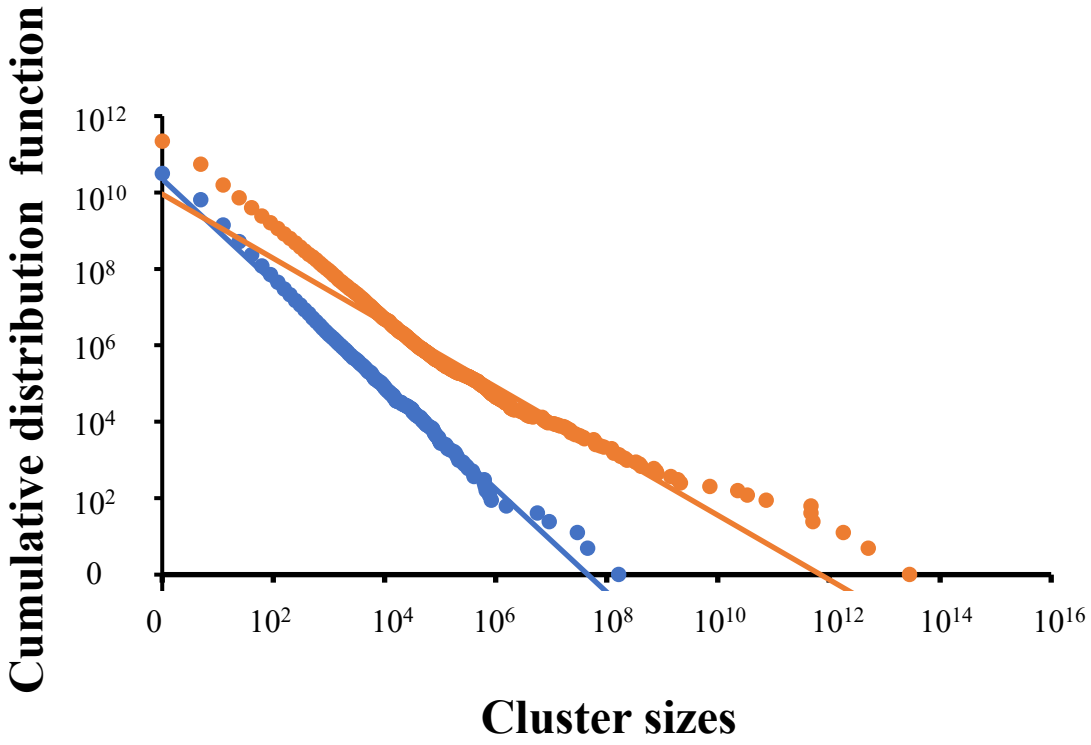


Figure 2

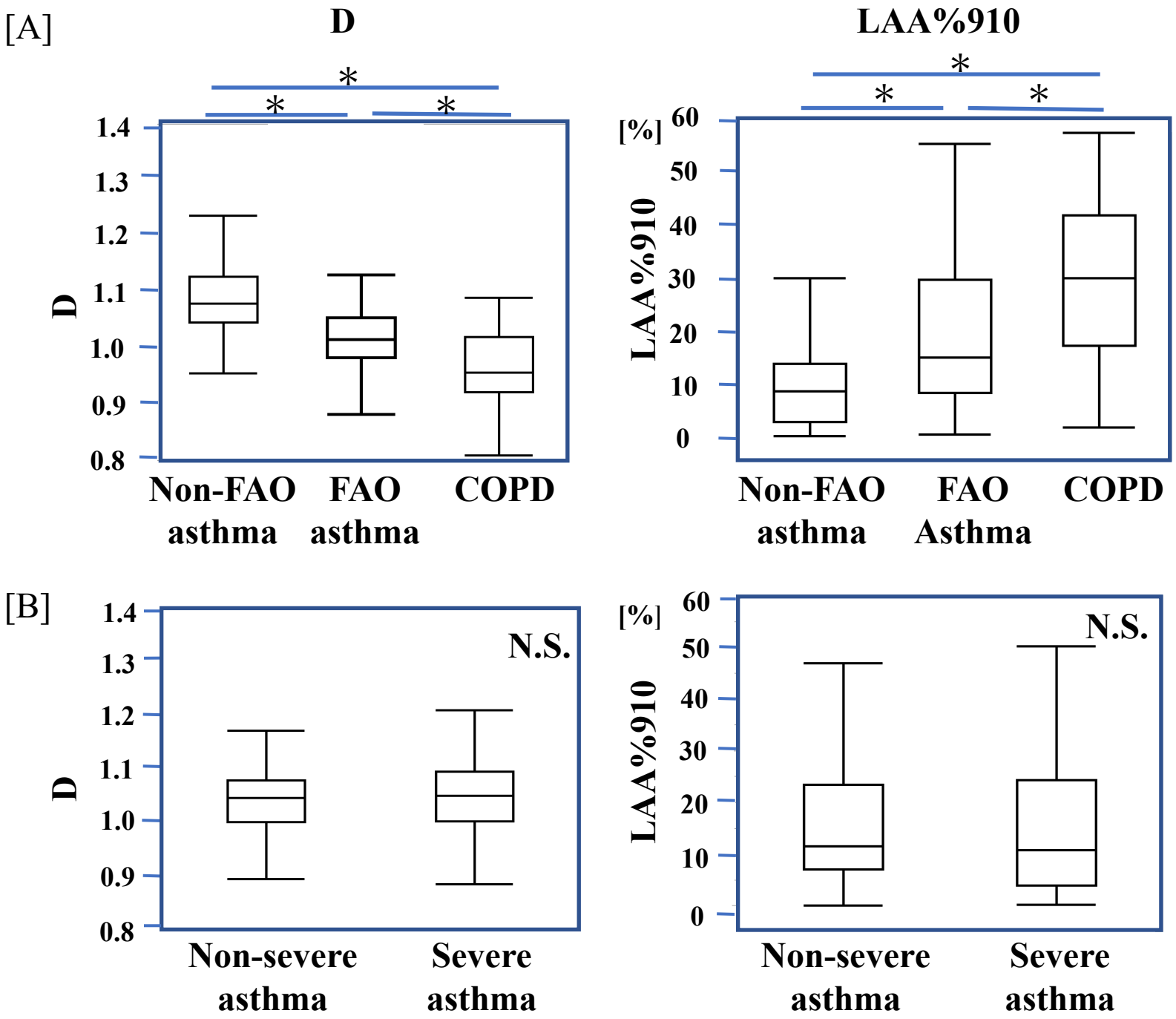

[A]
D
*
*
*
1.4
1.3
1.2
1.1
1.0
0.9
0.8
D
Non-FAO asthma
FAO asthma
COPD
LAA%910
*
*
*
[%]
60
50
40
30
20
10
0
LAA%910
Non-FAO asthma
FAO Asthma
COPD
[B]
N.S.
1.4
1.3
1.2
1.1
1.0
0.9
0.8
D
Non-severe asthma
Severe asthma
N.S.
[%]
60
50
40
30
20
10
0
LAA%910
Non-severe asthma
Severe asthma

Figure 3.

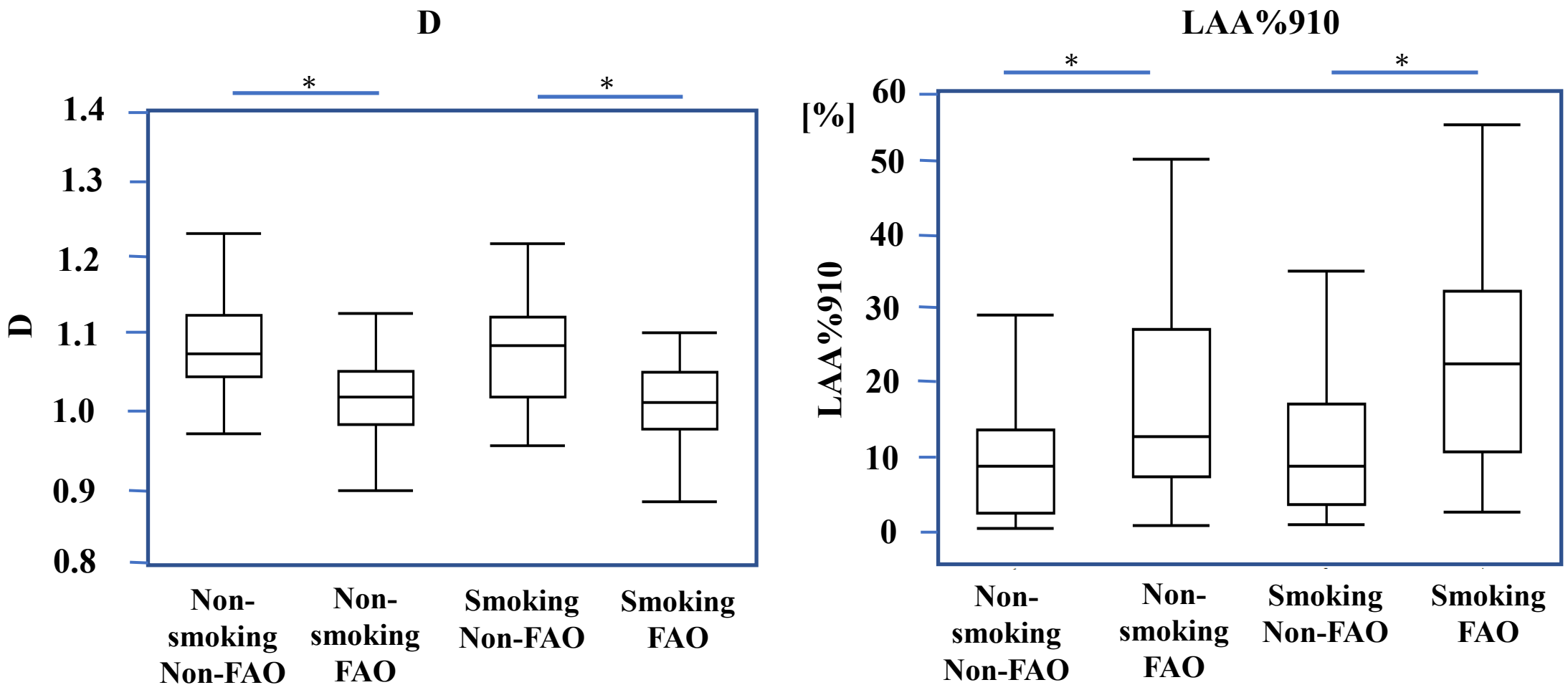

D
*
*
1.4
1.3
1.2
1.1
1.0
0.9
0.8
D
Non-smoking Non-FAO
Non-smoking FAO
Smoking Non-FAO
Smoking FAO
LAA%910
*
*
[%]
60
50
40
30
20
10
0
LAA%910
Non-smoking Non-FAO
Non-smoking FAO
Smoking Non-FAO
Smoking FAO

Figure 4.

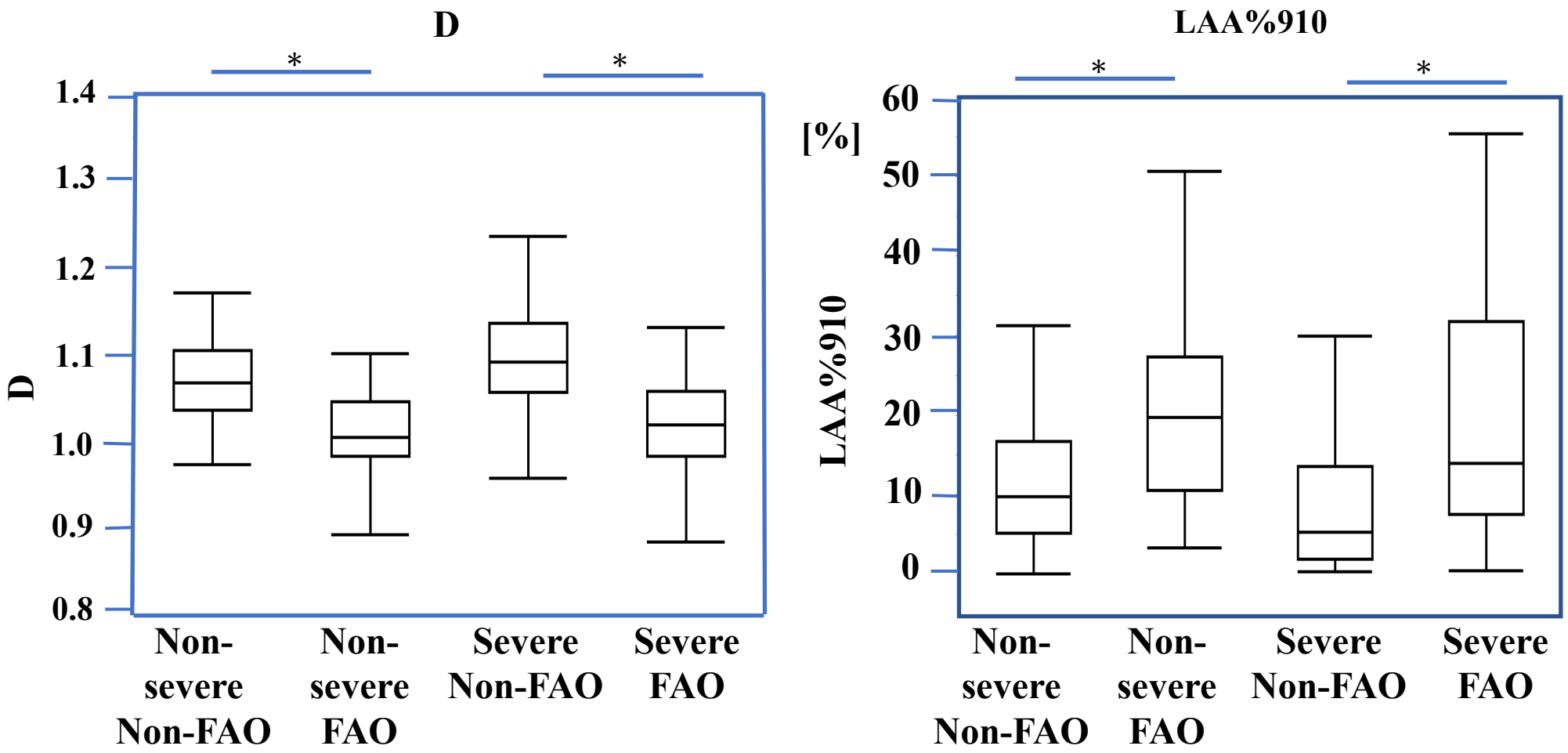

D
*
*
1.4
1.3
1.2
1.1
1.0
0.9
0.8
D
Non-severe Non-FAO
Non-severe FAO
Severe Non-FAO
Severe FAO
LAA%910
*
*
60
50
40
30
20
10
0
[%]
LAA%910
Non-severe Non-FAO
Non-severe FAO
Severe Non-FAO
Severe FAO

Figure E1.

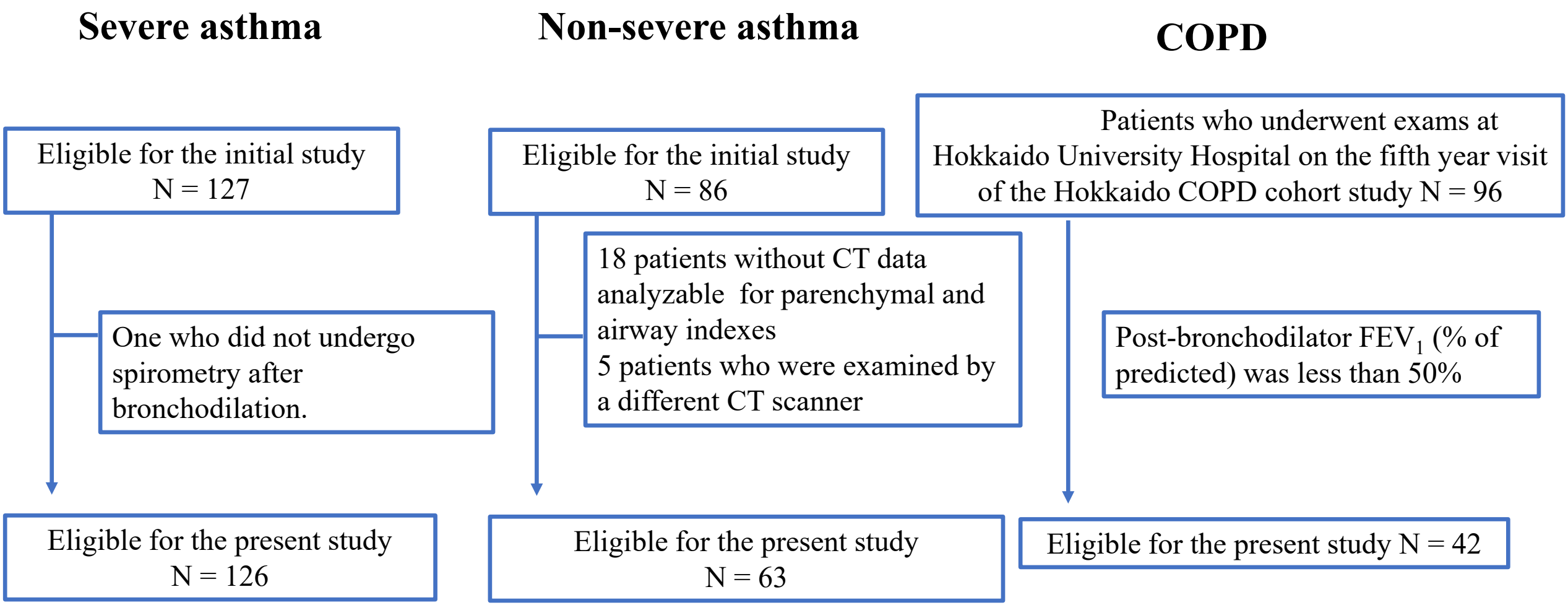
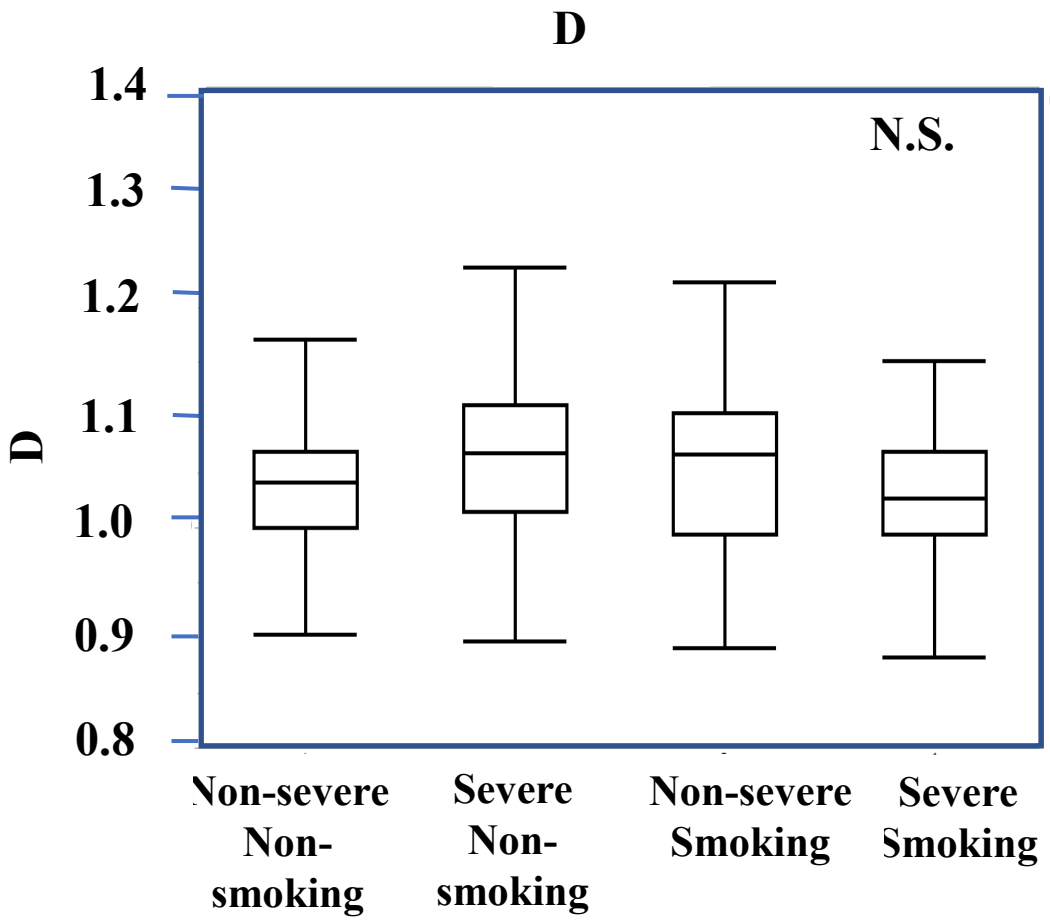

Severe asthma
Non-severe asthma
COPD
Eligible for the initial study N = 127
Eligible for the initial study N = 86
Patients who underwent exams at Hokkaido University Hospital on the fifth year visit of the Hokkaido COPD cohort study N = 96
One who did not undergo spirometry after bronchodilation.
18 patients without CT data analyzable for parenchymal and airway indexes
5 patients who were examined by a different CT scanner
Post-bronchodilator FEV1 (% of predicted) was less than 50%
Eligible for the present study N = 126
Eligible for the present study N = 63
Eligible for the present study N = 42

Figure E2.


D
N.S.
D
1.4
1.3
1.2
1.1
1.0
0.9
0.8
Non-severe Non-smoking
Severe Non-smoking
Non-severe Smoking
Severe Smoking

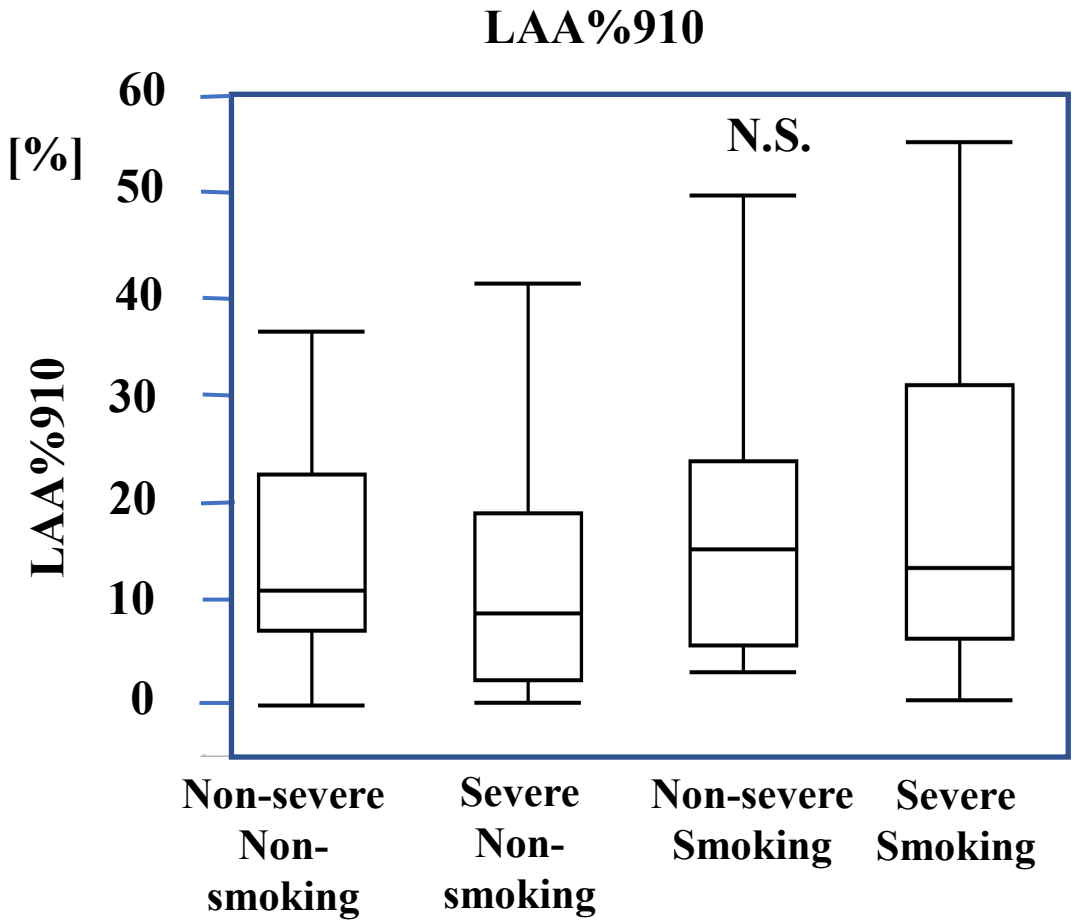
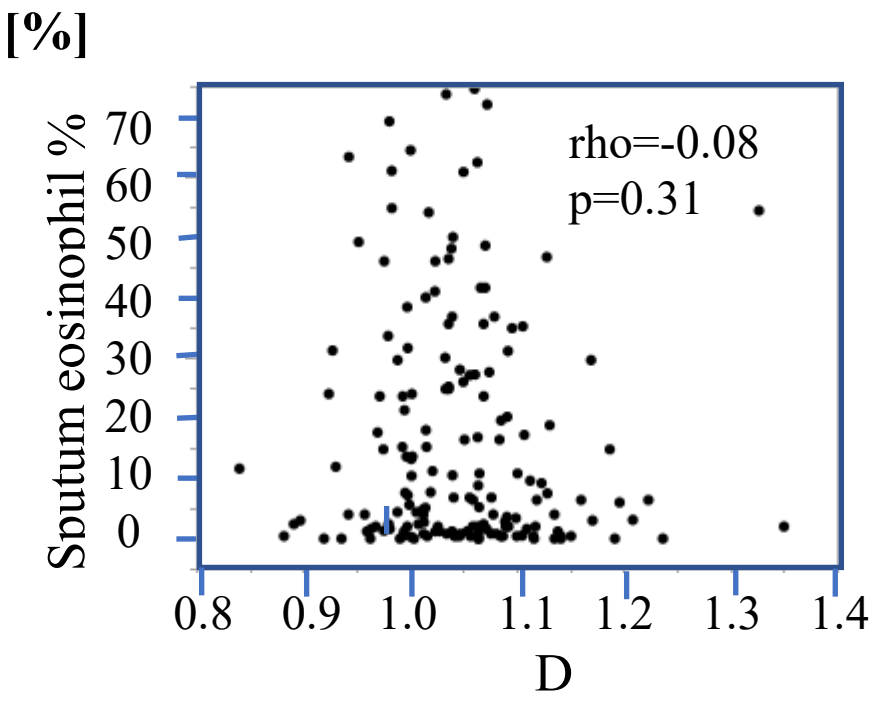
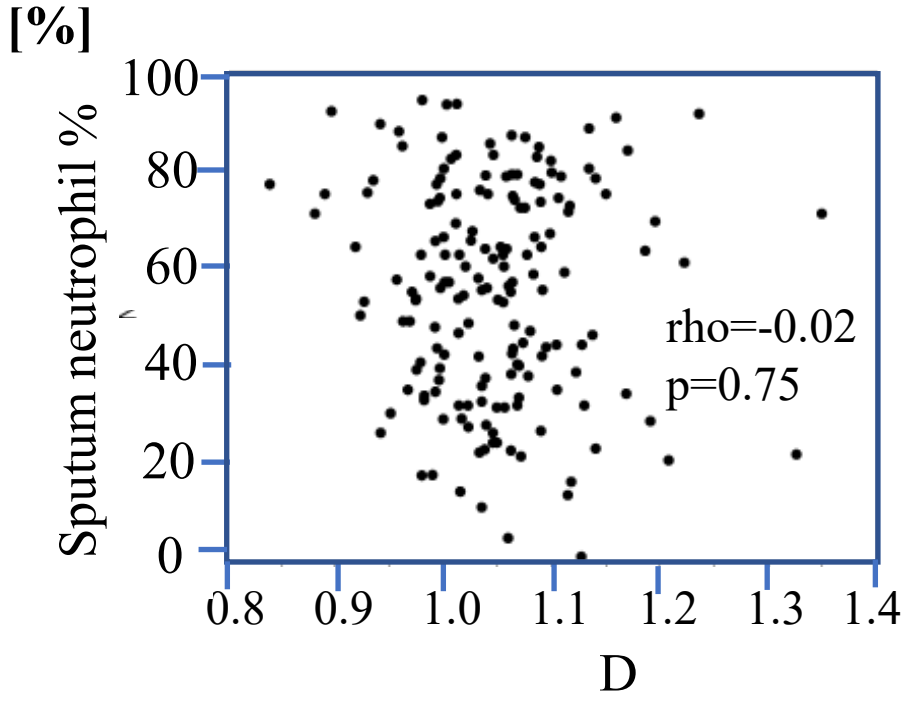
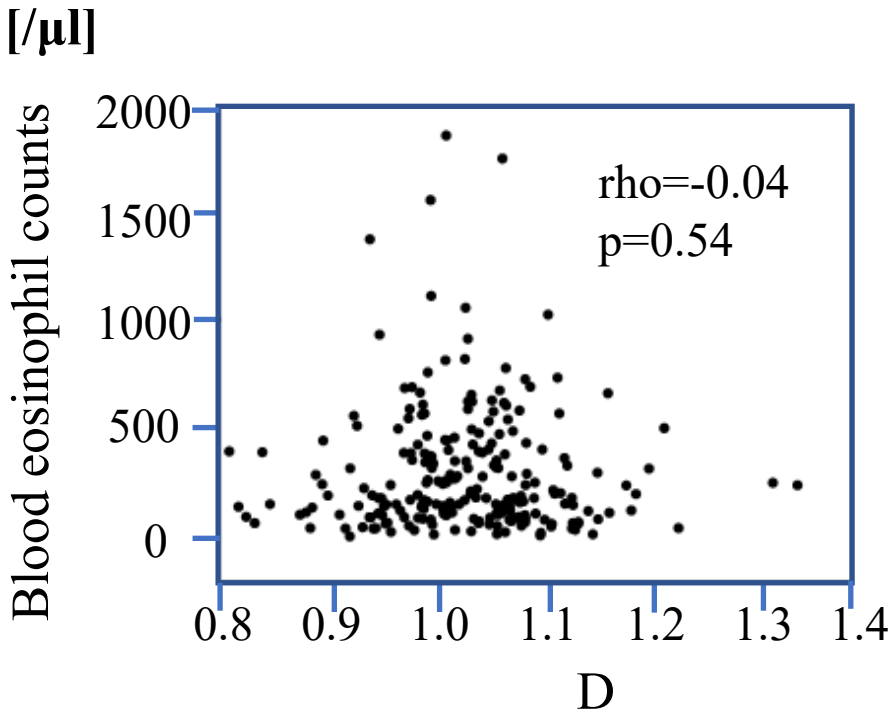

LAA%910
N.S.
[%]
LAA%910
60
50
40
30
20
10
0
Non-severe Non-smoking
Severe Non-smoking
Non-severe Smoking
Severe Smoking

Figure E3


[%]
Sputum eosinophil %
70
60
50
40
30
20
10
0
rho=-0.08
p=0.31
0.8
0.9
1.0
1.1
1.2
1.3
1.4
D


[%]
Sputum neutrophil %
100
80
60
40
20
0
rho=-0.02
p=0.75
0.8
0.9
1.0
1.1
1.2
1.3
1.4
D


[/µl]
Blood eosinophil counts
2000
1500
1000
500
0
rho=-0.04
p=0.54
0.8
0.9
1.0
1.1
1.2
1.3
1.4
D