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Further evidence for association of YKL-40 with severe asthma airway remodeling

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Conflicts of interest

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Clinical Trial Registration

This study was registered in the University hospital Medical Information Network (UMIN) Clinical Trials Registry system https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000003917

Keywords

airflow limitation; airway remodeling; asthma; YKL-40; severe asthma

List of abbreviations

AFD, airway fractal dimension

ATS, American Thoracic Society

CFE, consistent frequent exacerbators

CNE, consistent non-exacerbators

CT, computed tomography

ELISA, enzyme-linked immunosorbent assay

FeNO, fractional exhaled nitric oxide

FEV_1, forced expiratory volume in one second

FVC, forced vital capacity

Hi-CARAT, Hokkaido-based Investigative Cohort Analysis for Refractory Asthma

HR, hazard ratio

HU, Hounsfield Unit

IE, intermittent exacerbators

JRS, Japanese Respiratory Society

LA, luminal area

LAC, low-attenuation cluster

OCS, oral corticosteroids

V1, visit 1-year

V2, visit 2-year

V6, visit 6-year

WA%, wall area percentage

WA, wall area

WT, wall thickness

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Authorship

Hiroka.K.: conception and design of the study, acquisition and interpretation of data, statistical analysis, and drafting of the manuscript; K.S.: conception and design of the study, acquisition and interpretation

of data, CT analysis, and editing of the manuscript; Nao.T.: acquisition and interpretation of data, CT analysis, and editing of the manuscript; H.M.: conception and design of the study, acquisition and interpretation of data; Nat.T., Hiroki.K.: conception and design of the study, acquisition and interpretation of data; Mas.S.: conception and design of the study, acquisition and interpretation of data, and critical revision of the manuscript; Y.A., Mac.S. Mi.S., No.T., M.M., G.H.: interpretation of data; A.O.: acquisition and interpretation of data, CT analysis; S.S, T.H.: interpretation of data, and critical revision of the manuscript; J.O., K.I.: acquisition and interpretation of data, and critical revision of the manuscript; M.N., S.K.: conception and design of the study, acquisition and interpretation of data, and finalizing of the manuscript.

Introduction

The chitinase-like protein YKL-40, also called human cartilage glycoprotein 39 (HCgp-39) and chitinase 3-like 1 (CHI3L1), is a prototypic mammalian chitinase-like protein that induces the proliferation of mesenchymal cells, such as human chondrocytes, synovial cells, skin tissue, and fetal lung fibroblasts, as well as the migration and adhesion of vascular smooth muscle cells [1, 2]. In cross-sectional studies, the expression of YKL-40 has been associated with airflow limitation on spirometry and airway remodeling on histology in patients with asthma [3-5]. However, it remains unclear whether YKL-40 is associated with morphological changes in the lumens of the central and peripheral airways and parenchyma, and whether YKL-40 is associated with future progression of airflow limitation.

Spirometry is the gold standard for assessing the physiological status of asthma. Clinically, a low post-bronchodilator forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) ratio indicates fixed airflow limitation and allows indirect estimation of irreversible airway narrowing. Additionally, computed tomography (CT) allows direct quantification of wall thickening of the proximal airways [6, 7]. CT also allows quantification of 3D morphological complexity, such as fractal property of the lumen of the entire airway tree, comprising both proximal and peripheral airways [8]. Furthermore, our recent CT study evaluated parenchymal destruction by identifying a low- attenuation cluster (LAC), defined as neighboring voxels <-910 Hounsfield Unit (HU), and showed that exponent D of the size distribution of LACs was a marker of parenchymal destruction that was associated with

asthma with fixed airflow limitation and predicted the future progression of airflow limitation [9]. Therefore, with respect to the mechanistic link between YKL-40 levels and progression of airflow limitation in asthma, we hypothesized that YKL-40 levels could reflect not only wall remodeling of the proximal airways but also the complexity of the entire airway lumen tree and parenchymal destruction, all of which presumably underlie the longitudinal development of airflow limitation in patients with asthma.

To test this hypothesis, this study evaluated data from the Hokkaido-based Investigative Cohort Analysis for Refractory Asthma (Hi-CARAT), a multicenter observational research study aimed at characterizing severe asthma [10]. Following the baseline chest CT, spirometry was performed annually after inhalation of bronchodilators for a total of 6 years in all subjects. Using these data, this study attempted to examine first, whether YKL-40 at the baseline examination was cross-sectionally associated with clinical and physiological parameters related to severe asthma and airway structural changes on CT, and second, whether YKL-40 was associated with lung function decline over the subsequent 5 years.

Materials and Methods

This study was approved by the ethics committees of Hokkaido University Hospital (approval number, 009-0205). All subjects provided written informed consent. This study was registered in the University

39 hospital Medical Information Network (UMIN) Clinical Trials Registry system
40 (https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000003917). Details of the
41 materials and methods used in this study have been described in our previous report [10].
42 The Hi-CARAT is a multicenter observational cohort study that primarily aims to characterize patients
43 with severe asthma, including smokers. We did not exclude subjects with coexisting chronic
44 obstructive pulmonary disease if they had dominant asthma features so as to reflect the real-world
45 situation. Subjects were enrolled at Hokkaido University Hospital and its 29 affiliated hospitals and
46 clinics between February 2010 and September 2012. The diagnosis of severe asthma was based on the
47 American Thoracic Society (ATS) criteria for refractory asthma published in 2000 [11], with slight
48 modifications. In brief, we used the following additional criteria. Patients whose asthma was well-
49 controlled under the current medications were asked if they experienced episodic deterioration of
50 symptoms, urgent care visits, and rescue use of short-acting bronchodilators within 1 year after the
51 dose of the current medication was reduced.
52
53 A total of 127 severe asthma patients were selected for the baseline analyses [11]. A flow chart
54 demonstrating the study process from the initial screening at entry to Visit 1-year (V1), Visit 2-year
55 (V2) and the end of the 6-year follow-up period (Visit 6-year, V6) is depicted in Figure E1.
56
57 **Measurement of biomarkers**
58 After collection, blood samples were immediately frozen and stored at −80°C until assayed. Serum

59 YKL-40 levels were measured using an enzyme-linked immunosorbent assay (ELISA) (R&D Systems,
60 Minneapolis, MN, USA). In this study, the minimum limit of detection of the YKL-40 assay was 3.55
61 pg/mL. Serum periostin levels were measured using ELISA at Shino-test (Kanagawa, Japan), as
62 previously described [10, 12, 13].
63
64 **Pulmonary function tests**
65 Annual spirometry was performed before and after inhalation of 400 μg oxitropium and 400 μg
66 salbutamol.
67 Because of the severity of asthma in all subjects, no respiratory medicines were prohibited, except for
68 the use of short-acting bronchodilators for at least 12 hours before all measurements. A quality-control
69 protocol was developed based on the criteria applied in the Lung Health Study [14] and the Japanese
70 Respiratory Society (JRS) guidelines [15] to increase the accuracy and decrease intraindividual
71 variability. Spirometry was performed in triplicate. Acceptable measurements required more than two
72 reproducible measurements from up to eight forced expirations, in accordance with the JRS guidelines
73 [15]. The best FEV_1 and FVC values were subsequently recorded from acceptable maneuvers.
74 Spirometric data with flow-volume curves were transferred to the central study office and assessed for
75 acceptability by an independent investigator who was blinded to any other information. Further details
76 of the pulmonary function tests are described in our previous report [10]. Baseline data (at entry) were
77 obtained at a 2-day stay at Hokkaido University Hospital. Later at V1, data were obtained from the

78 outpatient clinic of Hokkaido University Hospital.

79

80 **CT imaging**

81 All participants underwent multidetector row spiral CT scan with a 64-detector array (Aquilion Multi,
82 TSX-101A/6A; Toshiba Medical Systems, Tochigi, Japan) in the supine position, at full inspiration,
83 at Hokkaido University Hospital at entry. The acquisition parameters were as follows, 120 kVp, 300
84 mA, 64 detectors, 0.5 mm collimation, slice thickness of 0.5 mm, 0.5 s/rotation, helical pitch of 41,
85 and FC03 and FC52 reconstruction kernels. Images of FC52 were used for airway analysis, while those
86 of FC03 was used for parenchymal analysis.

87

88 To evaluate the central airway dimensions, the luminal area (LA), wall area (WA), and wall thickness
89 (WT) were measured at the right apical (RB1) segmental airways, and wall area percentage (WA%)
90 was calculated as the ratio of the WA to the sum of the LA and WA. LA, WA and WT were divided
91 by body surface area to normalize inter-subject variations. Moreover, the entire airway tree was
92 automatically segmented without manual modification and exported as DICOM files using the
93 SYNAPSE VINCENT volume analyzer Ver 5.4 (FUJIFILM Medical, Tokyo, Japan). Custom-made
94 software written in Python 3 was used to calculate the airway fractal dimension (AFD) based on the
95 box-counting method, as reported previously [8, 9, 16].
96 Following interpolation to generate cubic voxels from the original rectangular voxels, the segmented

97 airway tree was binarized using a threshold of -800 HU and the largest 3D connected region including
98 the trachea was extracted. Then, different sized grids were sequentially overlaid on the extracted
99 airway trees, and for a given grid size (s), the number of voxels covering the airway tree was counted
100 as N(s). The s increased by a factor of 2 (s = 2, 4, 8, 16, 32, 64, 128, 256). After completion of repeated
101 counting, linear regression was performed by plotting log (s) and log (N(s)) on x-axis and y-axis,
102 respectively, and the absolute slope of the regression line was calculated as AFD. A lower AFD
103 indicates lower complexity of the branching patterns of the airway tree. Moreover, we calculated the
104 fractal dimension of the low-attenuation cluster at a threshold of -910 HU (exponent D) to evaluate the
105 parenchyma complexity as previously described [9]. In brief, a lower exponent D reflects greater extent
106 of parenchymal destruction (see the Online Supplement).
107

108 **Assessment of exacerbation**

109 Asthma exacerbation was defined based on the need for systemic corticosteroids for more than 3 days
110 and/or hospital admission. Frequent exacerbators were defined as patients who experienced two or
111 more exacerbations within 1 year. In our previous study, we categorized the subjects into three groups
112 based on their exacerbation status from entry to V3. The three groups were, (1) consistent frequent
113 exacerbators (CFE); (2) consistent non-exacerbators (CNE), and (3) intermittent exacerbators (IE).
114 Further details are described in our previous report [17].
115

116 **Study protocol**

117 This study consisted of two parts: Analysis 1 and Analysis 2 (Figures 1 and E2). Both analyses were
118 conducted in the same population, but with different time phases.

119

120 ***Analysis 1 (Cross-sectional analysis, at entry, N = 97)***

121 Analysis 1 was conducted using the cross-sectional data obtained at entry into the study. As shown in
122 Figure E2, 20 subjects were excluded from the analysis because of a lack of sufficient data, including
123 CT indices. Finally, 97 subjects were included in this analysis.

124

125 ***Analysis 2 (5-year FEV1 change, from V1 to V6, N = 103)***

126 Table E1 depicts the particularly sharp annual FEV_1 decline from entry to V1, as compared with other
127 periods. As mentioned above, at entry, spirometry was performed at a 2-day stay at Hokkaido
128 University Hospital, whereas data were obtained at an outpatient clinic later at V1. In addition, at the
129 entry of this study, we carefully evaluated the patients' asthma condition and clinically confirmed that
130 the condition remained stable in each subject. We perceived that the spirometry that was conducted
131 upon admission, with careful evaluation for patients' stable condition, contributed to the satisfying
132 values of FEV_1 at entry. Hence, we considered that using the FEV_1 value at entry would not provide
133 an accurate baseline for further decline in FEV_1 in this cohort. Consequently, we used the data obtained
134 from V1 to V6 (5 years' follow-up) for Analysis 2. In this analysis, we evaluated several indices,

135 including the serum YKL-40 level at V1.

136

137 **Statistical analyses**

138 For univariable analyses, we used chi-square tests for categorical variables and one-way analysis of
139 variance for parametric continuous variables. Several biomarkers with log-normal distribution were
140 $\log_{10}$ transformed before parametric tests. Pearson's correlation coefficient (r) or Spearman's rank
141 correlation coefficient (rho) was used to evaluate the correlation between two parametric or
142 nonparametric parameters, respectively. A linear mixed-effects model was used for subjects who had
143 at least three spirometric measurements, including V1, to accommodate loss-to-follow-up subjects,
144 regardless of whether they dropped out during the study period. The best linear unbiased prediction of
145 the annual changes in the maximum pre- and post-bronchodilator FEV_1 (mL/year) was estimated using
146 the random coefficient regression model as previously reported [18]. Multiple regression analyses were
147 conducted to calculate standardized partial regression coefficient (β) and 95% confidence intervals
148 (CIs). We calculated the hazard ratio (HR) and 95% CIs using the Cox proportional hazards model.
149 Statistical analyses were performed using the statistical software package SYSTAT for Windows,
150 version 13.2 (SYSTAT, San Jose, CA, U.S.A.) and EZR, version 1.54 (Saitama Medical Center, Jichi
151 Medical University, Saitama, Japan), which is a graphical user interface for the R software (The R
152 Foundation for Statistical Computing, Vienna, Austria) [19]. For all analyses, statistical significance was
153 set at $p < 0.05$.

154

155 # Results

156 **Subject demographics**

157 Table 1 shows the characteristics of subjects who were conducted in Analysis 1 (n=97). Mean age was
158 57.7 ± 12.1 (range: 29–83) years. Men comprised 44.3% (n = 43) and atopic patients constituted 63.9%
159 (n = 62) of the study population. Nine (9.3%) patients were current smokers, and 51 (52.6%) were
160 former smokers; 35 (36.1%) patients used oral corticosteroids (OCS) daily. Only four patients were
161 treated with omalizumab, and none received anti-IL-5, anti-IL-5R, or anti-IL-4/13R antibodies during
162 the follow-up period. Sputum data were available in 88 subjects out of 97, median value of the sputum
163 eosinophil and neutrophil was 10.2% and 54.3%, respectively. Figure 2 shows the variable distribution
164 of circulating YKL-40 levels ($\log_{10}$ transformed) at entry. Results show varying levels of serum YKL-
165 40. Geometric mean value of YKL-40 was 44.0 ng/mL. Serum YKL-40 values at VE and V1 were
166 significantly correlated ($r = 0.84$, $P < 0.001$; Figure E3).

167

168 **Analysis 1**

169 Several clinical indices related to airflow limitation and airway remodeling were measured by
170 pulmonary function tests and CT imaging. Serum YKL-40 levels showed modest association with
171 FEV_1 ($r = -0.19$, $P = 0.06$) and was significantly associated with WA% ($r = 0.25$, $P = 0.01$) and AFD
172 ($r = -0.22$, $P = 0.04$) (Table 2). However, exponent D was not significantly associated with serum

173 YKL-40 (Table 2).

174 The association of circulating YKL-40 levels with several inflammatory biomarkers and the sinus
175 score (Lund Mackay Score), which has been reported to be associated with high blood eosinophil
176 counts and serum periostin in our previous report [10], was analyzed. As shown in Table 3, circulating
177 YKL-40 levels did not show any significant association with inflammatory biomarkers, except for the
178 proportion of sputum eosinophil and neutrophil, which was negatively and positively associated with
179 serum YKL-40 levels, respectively.

180

181 **Analysis 2**

182 The characteristics of the 103 subjects at V1 are shown in Table E2. First, we calculated the individual
183 annual change in FEV_1 (mL/year) for 5 years (from V1 to V6) using a linear mixed-effects model as
184 described in the Statistical analyses section. Figure 3 shows the distribution of annual changes in FEV_1,
185 and Figure E3 shows the spaghetti plot for 103 subjects, with a follow-up of 5 years. The mean annual
186 change in FEV_1 was −33.7 ± 23.3 mL/year.

187 Table 4 shows that serum YKL-40 and blood neutrophils at V1 were significantly correlated with the
188 annual change in FEV_1 ($r = -0.24$, $P = 0.01$; Figure 4, and $r = -0.21$, $P = 0.03$, respectively), while other
189 indices including fractional exhaled nitric oxide (FeNO), serum periostin, and pulmonary functions,
190 were not. In multiple regression analysis, serum YKL-40 was significantly associated with the annual
191 change in FEV_1, even after adjustment for age and sex (model 1; $\beta = -0.24$, $P = 0.02$; Table E3).

192 Moreover, the association between serum YKL-40 and the annual change in FEV_1 was also detected
193 even when exponent D, which reflects the extent of parenchymal destruction, was included (model 2;
194 $\beta = -0.26$, $P = 0.01$).
195
196 **Association with exacerbation status**
197 Finally, we compared circulating YKL-40 levels among the three groups (CNE [n = 36], IE [n = 49],
198 and CFE [n = 14]) categorized based on the exacerbation status over a 3-year follow-up period. Of the
199 103 subjects, four were excluded due to unavailability of exacerbation data for the first 3 years. We
200 did not observe significant differences in serum YKL-40 levels ($\log_{10}$ transformed) or annual FEV_1
201 changes among the groups (Table E4). Results from the Cox proportional hazards model revealed that
202 circulating YKL-40 levels were not significantly associated with the numbers of days to first
203 exacerbation (HR 0.53, 95% CI 0.26–1.11, P = 0.09).
204
205 **Discussion**
206 In the present study, based on results from both cross-sectional and longitudinal study designs, we
207 provided further evidence that YKL-40 plays a significant role in the development of airway
208 remodeling in severe asthma. To the best of our knowledge, no previous study has presented cross-
209 sectional and longitudinal data to reveal the relationship between a molecule and the development of
210 airflow limitation in a single cohort study.

211

212 In the cross-sectional analysis, serum YKL-40 levels were significantly associated with WA% (r =
213 0.25; 95% CI, 0.05 to 0.43) and AFD values (r = -0.22; 95% CI, -0.40 to -0.02) and modestly associated
214 with FEV_1 (r = -0.19; 95% CI, -0.38 to 0.01). Clinically, the measurement of FEV_1/FVC after
215 inhalation of a bronchodilator has been thought to be a useful indicator of fixed airflow limitation,
216 which reflects the irreversible aspect of airway narrowing. However, these indices could also reflect
217 other factors, such as airway inflammation and airway collapse due to reduced elastic recoil,
218 considering that subjects with emphysema were also included in this study. On the other hand,
219 bronchial wall thickening measured by high-resolution CT has been hypothesized to reflect airway
220 remodeling more accurately; thus, the fact that the association was stronger for WA% than ffor
221 FEV_1/FVC was consistent with our hypothesis. Of particular interest in our findings is that, in addition
222 to WA%, which is a regional measure of airways, there was a modest negative association between
223 YKL-40 levels and AFD. Airway fractal geometry is the study of entire airway structures that seem
224 chaotic and yet exhibit a hierarchal self-similar pattern [16]. Fractal dimensions have been used to
225 quantify airway remodeling in digitized airway casts in patients with asthma [20]. Moreover, no
226 association was found between YKL-40 and exponent D, an index reflecting the parenchymal
227 destruction [9]. Taken together, the associations of parameters obtained from both cross-sectional (WA%
228 and AFD) and longitudinal (ΔFEV_1) analyses provided further evidence that YKL-40 plays a
229 significant role in the development of airway remodeling but not parenchymal destruction in asthma.

230

231 In Analysis 2, the mean annual change in FEV_1 was −33.7 ± 23.3 mL/year. Although we only focused
232 on patients with severe asthma, based on the definition mentioned above, the mean value of annual
233 FEV_1 decline was similar to that previously reported in studies that recruited patients with even mild
234 and/or moderate levels of asthma [21-24]. This may be somewhat surprising; however, it should be noted
235 that all the participants in this study were regularly seen and received optimal therapy from each
236 respiratory physician. Moreover, although a report from Kanemitsu et al. demonstrated a significant
237 impact of serum periostin levels on the prediction of rapid FEV_1 decliners [25], we did not find similar
238 results in our study. Additionally, a retrospective analysis by Matsunaga et al. reported a significant
239 relationship between FEV_1 decline and severe exacerbations [26], and FeNO [27, 28]. However, neither
240 asthma exacerbation nor FeNO was associated with FEV_1 decline in our study. We speculate that this
241 might be due to our selection of subjects including smokers and asthma severity.

242

243 It should be noted that, although the relationship between YKL-40 and rate of FEV_1 decline was
244 statistically significant in this study, the overall strength of the association was relatively weak. In fact,
245 there were other associated factors besides the YKL-40, one of which was exponent D. However, both
246 YKL-40 levels and exponent D were independently associated with annual FEV_1 decline, which is
247 consistent with the findings of our recent report [9] that showed an association between parenchymal
248 destruction and FEV_1 decline in patients with asthma. Thus, it is unlikely that YKL-40 can be used as

249 a standalone biomarker for selecting rapid FEV_1 decliners. In this study, FEV_1 decline was also
250 associated with blood neutrophils, which is consistent with the findings of Backman et al.'s study that
251 demonstrated the association between FEV_1 decline and blood neutrophil counts in patients with
252 asthma, including smokers [29]. However, based on our multivariable analysis, we believe that YKL-40
253 plays a more important role in airway remodeling.
254
255 According to our Cox proportional hazards model results, serum YKL-40 levels were modestly
256 associated with lower risk of exacerbation although it was not significant (HR 0.53; 95% CI 0.26 to
257 1.11). Several previous studies showed that subjects with frequent exacerbations had rapid decline in
258 FEV_1 [26, 30, 31]; however, our current study did not show a similar association. In our previous study [17],
259 we found that high FeNO level was significantly associated with frequent exacerbations, but it was not
260 associated with decline in FEV_1 in this study. Regarding periostin, some studies showed association
261 with decline in FEV_1 [25] and impaired lung function [32, 33] but our previous studies showed no association
262 with exacerbation [17]. These results are not surprising and indicate the differential role of biomarker(s)
263 in exacerbation and lung function decline.
264
265 In this study, serum YKL-40 levels were positively associated with the proportion of sputum
266 neutrophils and negatively associated with sputum eosinophils. Although the specific role and detailed
267 mechanisms of YKL-40 in asthma are unclear, we considered that YKL-40 may be directly involved

268 in or may indirectly reflect neutrophilic airway inflammation. Previous studies have shown that YKL-
269 40 is rather a biomarker of non-Th2 or neutrophilic inflammation [34-38]. Our results seem to conflict
270 with some reports showing that YKL-40 is associated with Th2 biomarkers, such as blood eosinophils
271 and total IgE [39-41], and with asthma exacerbation [39]. We speculate that this discrepancy is due to the
272 characteristics of the population and the study inclusion criteria. Our study focused only on severe
273 asthma, all of which require high doses of ICS and/or oral steroids. Additionally, in the present study,
274 we intentionally included asthma patients with a smoking history, considering the high smoking rate
275 in Japan [42, 43]. We believe that this strategy of focusing only on severe asthma, which is an economical
276 and medical burden for management of asthma, and of including smoking subjects, which more
277 precisely reflects the real-world situation, would be clinically relevant and would provide the most
278 useful evidence for understanding the heterogeneity of severe asthma.
279
280 This study had some limitations. First, the sample size may have been too small, despite the recruitment
281 of patients from 29 affiliated hospitals/pulmonary clinics. Nevertheless, all patients were carefully
282 followed up, and the final follow-up rate at the end of the 6th year was high (Table E1). Spirometry
283 was performed before and after the inhalation of oxitropium and salbutamol annually, and the rigorous
284 criteria were used to select the best flow-volume curve (see Material and Methods). Second, we used
285 the older definition of severe asthma, which was developed during the ATS workshop in 2000, because
286 the new definition reported in the European Respiratory Society/ATS guidelines in 2014 had not been

287 officially announced when this study was underway. Nevertheless, as mentioned in the Methods
288 section, we were certain that all patients were under the appropriate treatment with high-dose ICS or
289 OCS for asthma control. Third, as we did not perform serial measurements of YKL-40, we could not
290 determine intraindividual variation in serum YKL-40 levels over time. However as shown in Figure
291 E4, the serum YKL-40 values at entry and V1 were significantly correlated.

292

293 In conclusion, through a 5-year follow-up and repeated measurements of FEV_1 after inhalation of both
294 β2-agonist and anticholinergics, we demonstrated that serum YKL-40 levels at baseline examination
295 were significantly associated with annual FEV_1 decline. We also demonstrated that serum YKL-40
296 levels were associated with not only WA% at one segmental airway of right B1 but also AFD, which
297 is a comprehensive structural measure of airways. This cross sectional and longitudinal study provide
298 further evidence for association of YKL-40 with the pathogenesis of airway remodeling in severe
299 asthma.

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Figure legends

Figure 1.

Study protocol of this study. (A) Analysis 1 (cross-sectional analysis, at entry, n = 97). (B) Analysis 2 (5-year FEV_1 change, from V1 to V6, n = 103).

Figure 2.

Distribution of serum YKL-40 levels ($\log_{10}$ transformed) at entry (n = 97).

Figure 3.

Distribution of individual annual changes in FEV_1 (mL/year) with a follow-up of 5 years (n = 103). These values were estimated from a linear mixed effects model.

Figure 4.

Association between individual annual changes in FEV_1 and serum YKL-40 levels ($\log_{10}$ transformed) at V1 (n = 103).

Table 1. Characteristics of subjects at entry in Analysis 1 (n = 97).

Male sex, n (%)	43 (44.3%)
Age at enrollment, years	57.7 ± 12.1
Asthma duration, years	19.2 ± 13.9
Smoking status (Current/Ex/Never), %	9.3/52.6/38.1
Pack years	5.5 (0-23.4)
Body mass index, kg/m^2	25.5 ± 4.9
Daily ICS dose, μg *	1636.3 ± 484.7
Maintenance OCS use, n (%)	35 (36.1%)
Daily OCS dose, mg	0 (0-5)
Atopy, n (%)	62 (63.9%)
Nasal polyp, n (%)	26 (26.8%)
ACT	21 (17-23)
AQLQ	5.5 (4.8-6.3)
Number of exacerbations in 3 years	1 (0-4)
Blood eosinophil, cells/μL	206.4 (0.51)
Blood neutrophil, cells/μL	4460.2 (0.16)
Serum IgE, IU/mL	152.4 (0.71)
Serum periostin, ng/mL	81.8 (0.21)
Serum YKL-40, ng/mL	44.0 (0.35)
FeNO, ppb	31.5 (0.36)
Sputum eosinophil, % †	10.2 (1.2-31.2)
Sputum neutrophil, % †	54.3 (35.2-71.6)
FEV_1, L ‡	2.33 ± 0.75
FEV_1, %predicted ‡	92.0 ± 19.1
FEV_1/FVC, % §	66.7 ± 13.0

ACT, asthma control test; AQLQ, asthma quality of life questionnaire; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; ICS, inhaled corticosteroid; OCS, oral corticosteroid

Data are shown as the mean ± standard deviation, median (interquartile range), geometric mean (log_{10} SD), or number (%).

* Equivalent to budesonide dose

† N=88

‡ Maximum value of FEV_1 among four procedures (see Methods)

§ FEV_1/FVC was applied the value corresponding to the maximum FEV_1

Table 2. Association between YKL-40 ($\log_{10}$ transformed) and pulmonary function and airway CT indices at entry in Analysis 1 (n = 97).

	r	95% CI	P-value
Pulmonary function			
FEV_1	-0.19	-0.38 to 0.01	0.06
%predicted FEV_1	-0.12	-0.31 to 0.09	0.26
FEV_1/FVC	-0.12	-0.32 to 0.08	0.23
Airway CT			
WT*	0.13	-0.07 to 0.32	0.20
WT/BSA*	0.12	-0.09 to 0.31	0.26
WA*	-0.01	-0.20 to 0.20	0.96
WA/BSA*	<0.01	-0.20 to 0.20	0.99
WA%*	0.25	0.05 to 0.43	0.01
LA*	-0.19	-0.38 to 0.01	0.07
LA/BSA*	-0.18	-0.37 to 0.02	0.08
Airway fractal dimension †	-0.22	-0.40 to -0.02	0.04
Exponent D†	-0.10	-0.29 to 0.11	0.35

BSA, body surface area; CI, confidence interval; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; LA, luminal area; WA, wall area; WT, wall thickness

Pearson product-moment correlation coefficient, unless otherwise stated

*: Rt B1

†: n=96

Table 3. Association between YKL-40 (log $_{10}$ transformed) and inflammation markers at entry in Analysis 1 (n = 97).

	r/rho	95% CI	P-value
Blood eosinophil *	-0.06	-0.25 to 0.14	0.57
Blood neutrophil *	0.07	-0.14 to 0.26	0.52
Serum total IgE *	0.01	-0.19 to 0.21	0.89
FeNO *	-0.17	-0.35 to 0.03	0.10
Serum periostin *	0.02	-0.18 to 0.22	0.85
Sputum eosinophil †	-0.24	-0.43 to -0.02	0.03
Sputum neutrophil †	0.27	0.06 to 0.46	0.01
LMS †	-0.10	-0.30 to 0.11	0.34

CI, confidence interval; FeNO, fractional exhaled nitric oxide; LMS, Lund Mackay Score

Pearson product-moment correlation coefficient, unless otherwise stated

*: Log $_{10}$ transformed

†: Spearman's rank correlation coefficient

Table 4. Association between annual FEV_1 change and several asthma indices at Visit 1 (n = 103).

	r/rho	95% CI	P-value
Age	-0.07	-0.26 to 0.13	0.50
Asthma duration	-0.13	-0.32 to 0.06	0.18
Body mass index	0.08	-0.11 to 0.27	0.41
Pack-years *	-0.10	-0.29 to 0.11	0.34
Blood eosinophil †	0.02	-0.18 to 0.21	0.87
Blood neutrophil †	-0.21	-0.39 to -0.02	0.03
Serum total IgE †	-0.01	-0.20 to 0.19	0.93
Serum periostin †	-0.01	-0.20 to 0.19	0.94
Serum YKL-40 †	-0.24	-0.42 to -0.05	0.01
FeNO †	0.04	-0.16 to 0.23	0.70
FEV_1	0.05	-0.14 to 0.24	0.61
%predicted FEV_1	0.02	-0.17 to 0.22	0.81
FEV_1/FVC	0.05	-0.14 to 0.24	0.60

CI, confidence interval; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity

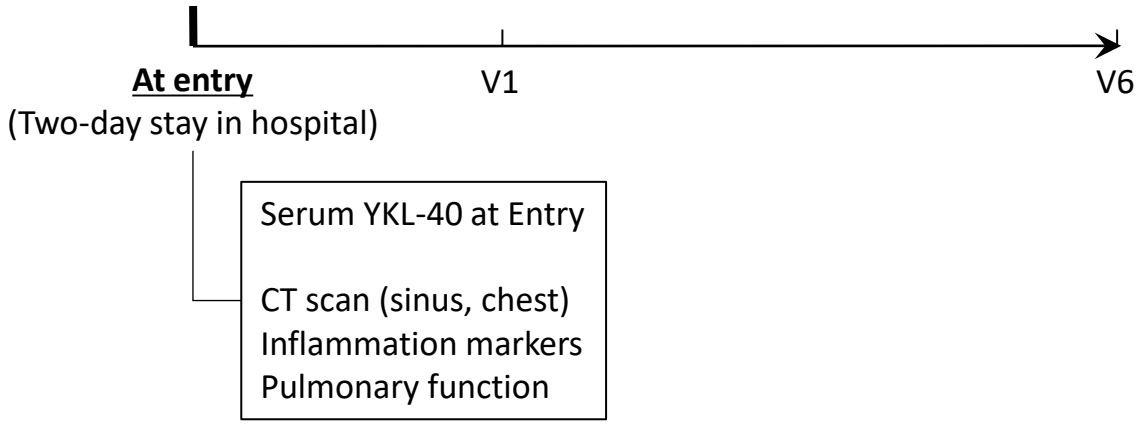
Pearson product-moment correlation coefficient, unless otherwise stated

*: Spearman's rank correlation coefficient

†: Log_{10} transformed

Figure 1

(A) Analysis 1 (Cross-sectional analysis, at entry, n = 97)



(B) Analysis 2 (5-year FEV_1 change, from V1 to V6, n = 103)

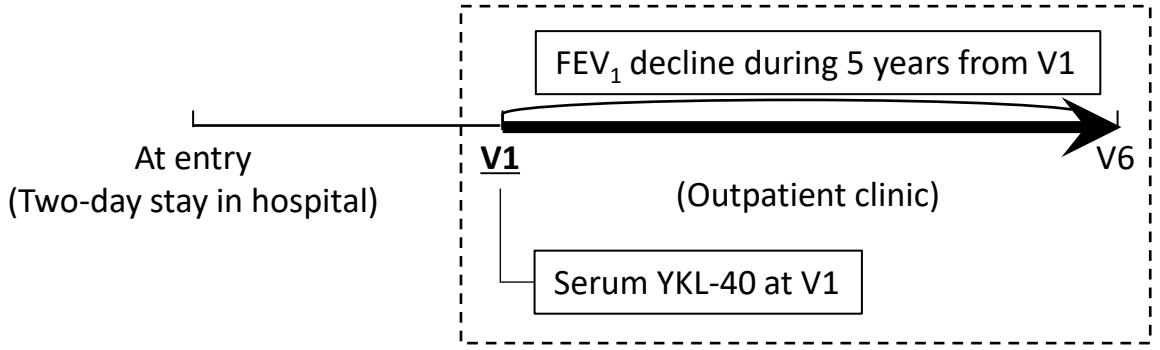


Figure 2

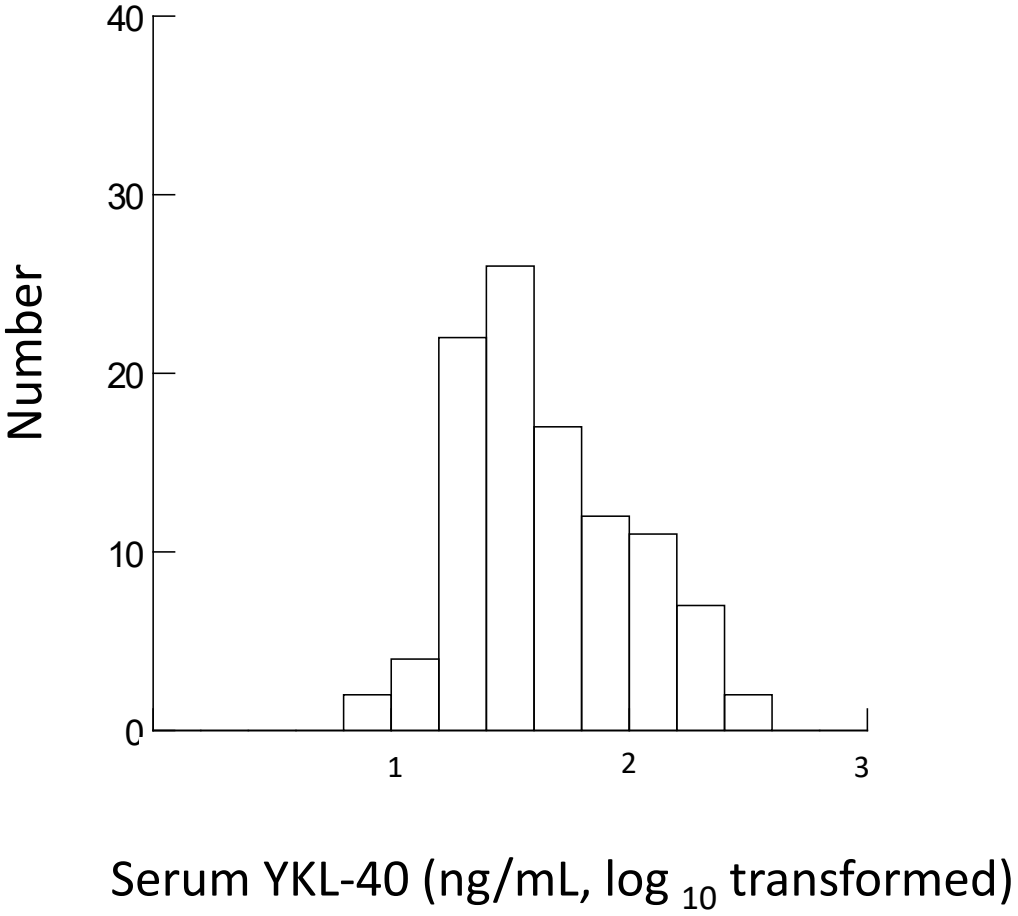


Figure 3

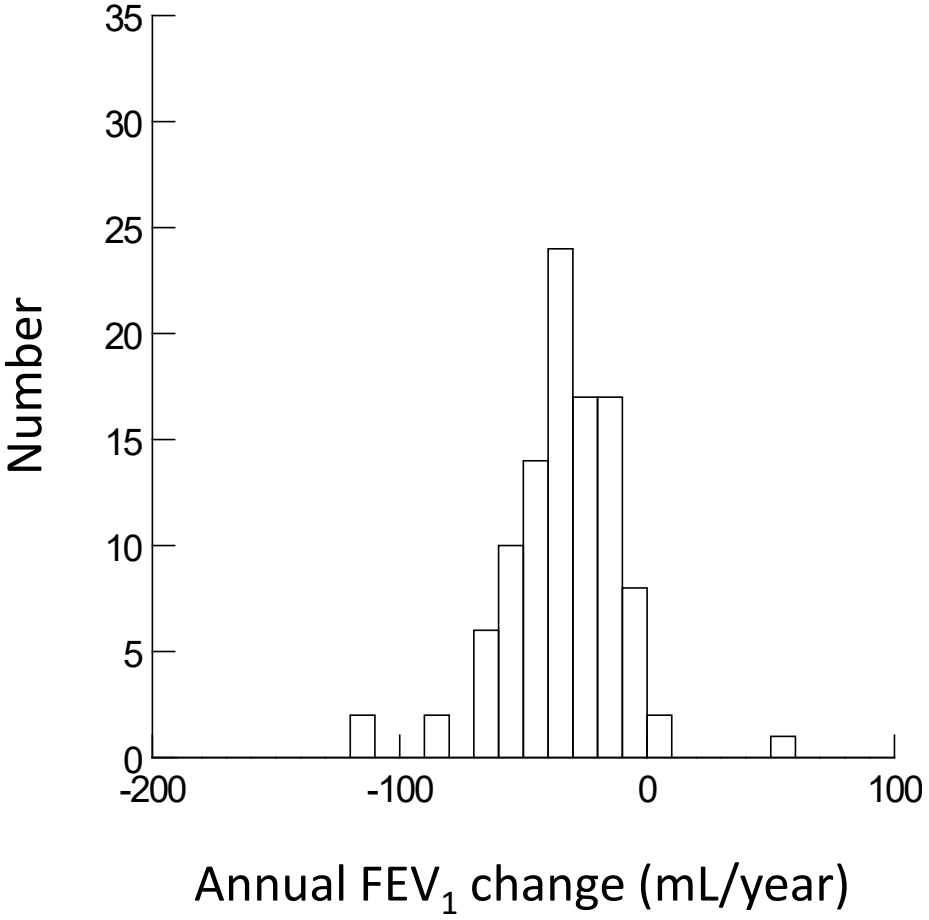
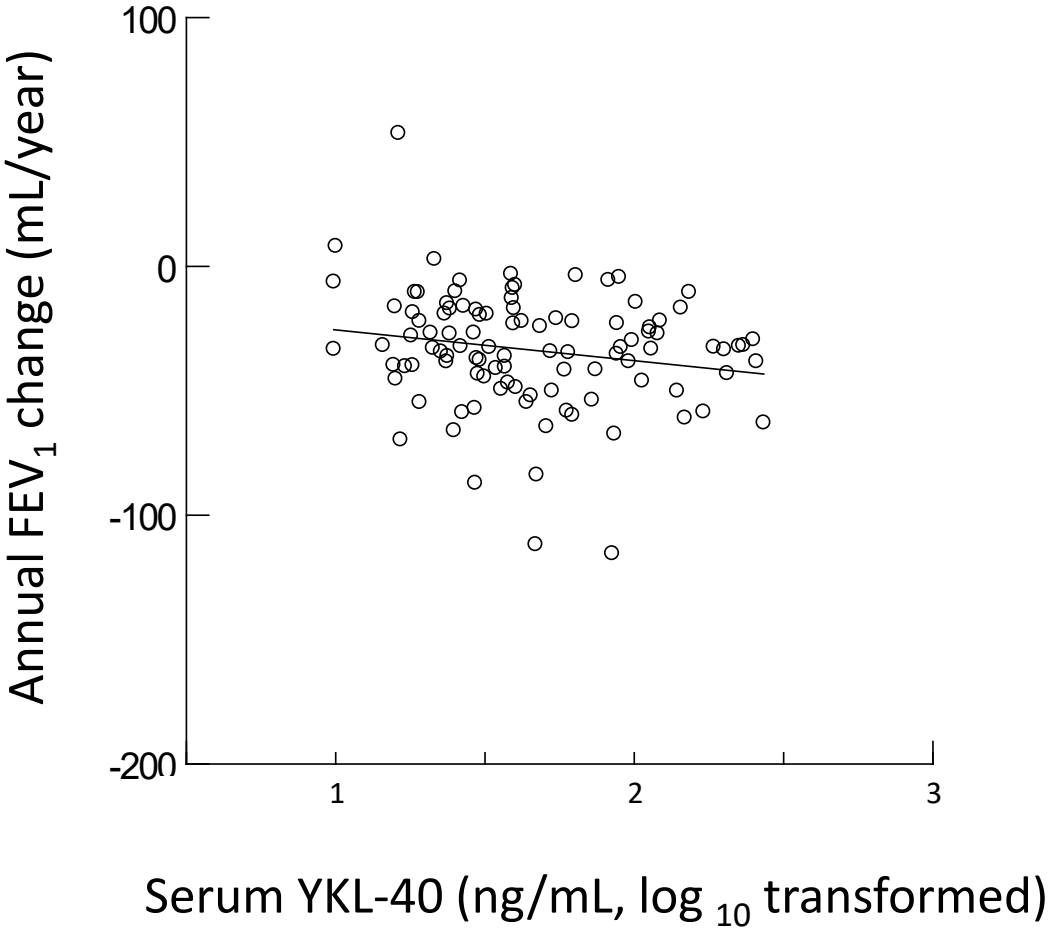

35
30
25
20
15
10
5
0
Number
-200
-100
0
100
Annual FEV1 change (mL/year)

Figure 4


100
0
-100
-200
1
2
3
Annual FEV1 change (mL/year)
Serum YKL-40 (ng/mL, log 10 transformed)

Online supplement

Methods

Assessment of Exponent D

The neighboring voxels < -910 Hounsfield Unit (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 [1]. A lower D indicates a greater extent of parenchymal destruction. More detailed information can be found in our previous report [2].

E-Figure legends

Figure E1.

The protocol of the Hokkaido Severe Asthma Cohort Study.

Figure E2.

Flow chart for selection of eligible subjects in this study.

Figure E3.

The spaghetti plot of individual FEV_1 changes (mL) for 5 years (from V1 to V6).

Figure E4.

Association between serum YKL-40 levels ($\log_{10}$ transformed) at entry and V1.

E-References

1. Shimizu K, Tanabe N, Tho NV, et al. Per cent low attenuation volume and fractal dimension of low attenuation clusters on CT predict different long-term outcomes in COPD. *Thorax.* 2020;75:116-122.

2. Shimizu K, Tanabe N, Oguma A, et al. Parenchymal destruction in asthma: Fixed airflow obstruction and lung function trajectory. *J. Allergy Clin. Immunol.* 2021, in press.

Table E1. Average of FEV_1 (L) in annual visits.

	At Entry	V1	V2	V3	V4	V5	V6
	(2-day stay in hospital)	(Outpatinet clinic)					
Number of cases	103	103	102	102	89	82	87
Mean±SD, L	2.32±0.76	2.19±0.67	2.19±0.73	2.10±0.72	2.07±0.69	2.12±0.76	2.07±0.74

V1, Visit 1 year; V2, Visit 2 year; V3, Visit 3 year; V4, Visit 4 year; V5, Visit 5 year; V6, Visit 6 year

Table E2. Characteristics of subjects at Visit 1 year in Analysis 2 (n = 103).

Smoking status (Current/Ex/Never), %	10.6/51.5/37.9
Pack years	5.5 (0-23.3)
Body mass index, kg/m2	25.5 ± 4.8
Daily ICS dose, μg *	1578.6 ± 583.1
Maintenance OCS use, N (%)	35 (34.0%)
Blood eosinophil, cells/μL	216.8 (0.45)
Blood neutrophil, cells/μL	4617.7 (0.17)
Serum IgE, IU/mL	138.1 (0.68)
Serum periostin, ng/mL	91.1 (0.19)
Serum YKL-40, ng/mL	60.3 (0.31)
FeNO, ppb	26.7 (0.34)
FEV_1, L †	2.19 ± 0.67
FEV_1, %predicted †	87.9 ± 18.1
FEV_1/FVC, % ‡	65.8 ± 13.2

FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; ICS, inhaled corticosteroid; OCS, oral corticosteroid

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

* Equivalent to budesonide dose

† Maximum value of FEV_1 among two procedures (see Methods)

‡ FEV_1/FVC was applied the value corresponding to the maximum FEV_1

Table E3. Multivariable analysis of association between annual FEV_1 change (mL/year) and several asthma indices at Visit 1 (n = 103).

	Model 1			Model 2		
	β	95% CI	P-value	β	95% CI	P-value
Age	0.03	-0.18 to 0.25	0.76	0.14	-0.08 to 0.36	0.24
Asthma duration	-0.14	-0.34 to 0.05	0.15	-0.13	-0.33 to 0.07	0.19
Male sex	-0.13	-0.34 to 0.09	0.25	-0.15	-0.36 to 0.07	0.18
%predicted FEV1	-0.10	-0.33 to 0.13	0.40	-0.16	-0.39 to 0.07	0.16
Blood neutrophil *	-0.17	-0.36 to 0.02	0.08	-0.15	-0.34 to 0.04	0.12
Serum YKL-40 *	-0.24	-0.45 to -0.04	0.02	-0.26	-0.46 to -0.06	0.01
Exponent D†				0.27	0.06 to 0.48	0.01

CI, confidence interval; FEV_1, forced expiratory volume in one second

Multiple regression analysis.

β: standardized partial regression coefficient

*: Log_{10} transformed

†: At entry, n = 102

Table E4. Characteristics of subjects according to three exacerbation status at entry.

	Exacerbation group			P-value
	CNE (n = 36)	IE (n = 49)	CFE (n = 14)	
Male sex, n (%)	14 (38.9%)	25 (51.0%)	4 (28.6%)	0.26
Age at enrollment, years	56.5 ± 2.0	59.9 ± 1.7	55.7 ± 3.2	0.33
Body mass index, kg/m^2	25.6 ± 0.8	25.7 ± 0.7	24.9 ± 1.4	0.88
Maintenance OCS use, n (%)	12 (33.3%)	16 (32.7%)	9 (64.3%)	0.08
Blood eosinophil, cells/μL	152.3 (0.51)	226.0 (0.50)	370.2 (0.39)	0.04
Blood neutrophil, cells/μL	4537.1 (0.17)	4525.6 (0.16)	4168.9 (0.17)	0.76
Serum YKL-40, ng/mL	51.6 (0.35)	44.3 (0.38)	39.9 (0.30)	0.54
FeNO, ppb	23.4 (0.36)	35.2 (0.34)	44.1 (0.34)	0.02
FEV_1, L *	2.37 ± 0.13	2.28 ± 0.11	2.23 ± 0.21	0.81
FEV_1, %predicted *	93.6 ± 3.2	90.2 ± 2.8	91.0 ± 5.2	0.72
FEV_1/FVC, % †	67.9 ± 2.1	65.1 ± 1.8	67.3 ± 3.4	0.58
Annual FEV_1 change, mL/year	-32.8 ± 4.0	-34.1 ± 3.4	-37.5 ± 6.4	0.83

CFE, consistent frequent exacerbators; CNE, consistent non-exacerbators; FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; ICS, inhaled corticosteroid; IE, intermittent exacerbators; OCS, oral corticosteroid

Data are shown as the mean ± standard deviation, median (interquartile range), geometric mean ($\log_{10}$ SD), or number (%).

P-values were obtained by one-way ANOVA or chi-square tests.

* Maximum value of FEV_1 among four procedures (see Methods)

† FEV_1/FVC was applied the value corresponding to the maximum FEV_1

Figure E1

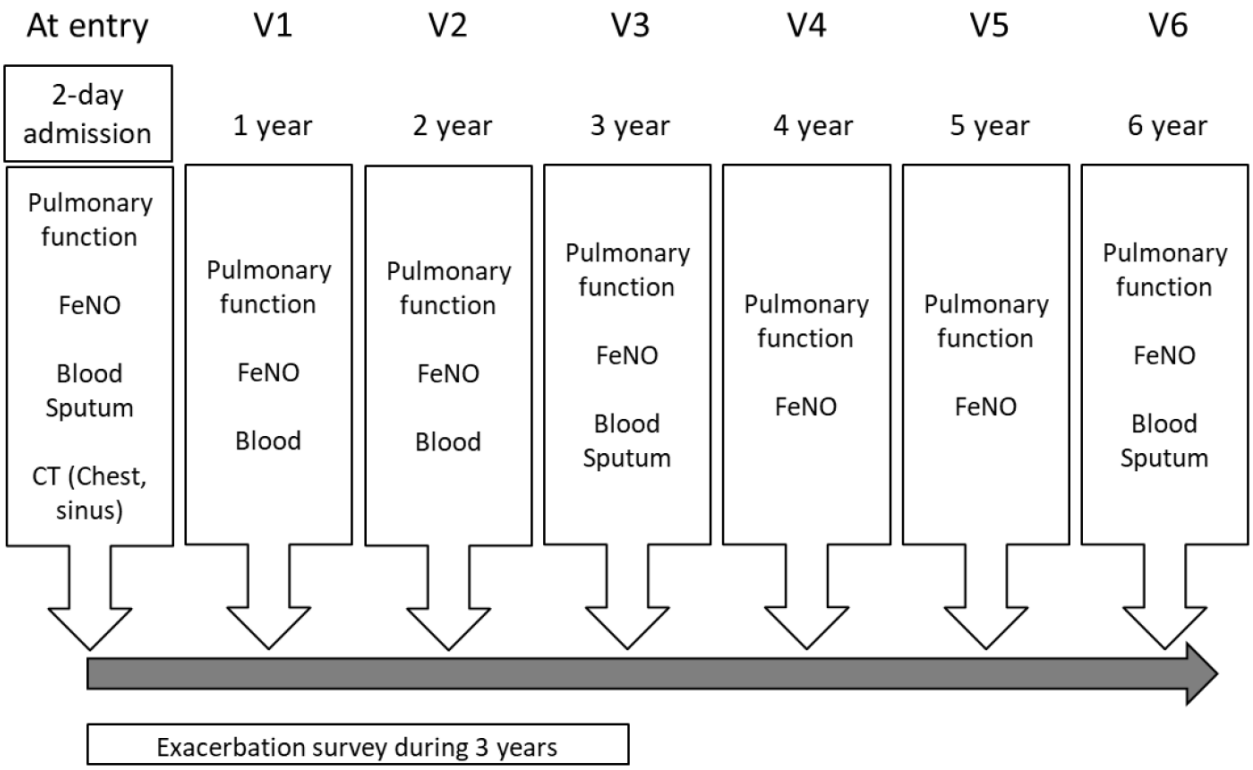

At entry
V1
V2
V3
V4
V5
V6
2-day admission
1 year
2 year
3 year
4 year
5 year
6 year
Pulmonary function
FeNO
Blood
Sputum
CT (Chest, sinus)
Pulmonary function
FeNO
Blood
Pulmonary function
FeNO
Blood
Pulmonary function
FeNO
Blood
Sputum
Pulmonary function
FeNO
Pulmonary function
FeNO
Pulmonary function
FeNO
Blood
Sputum
Exacerbation survey during 3 years

Figure E2

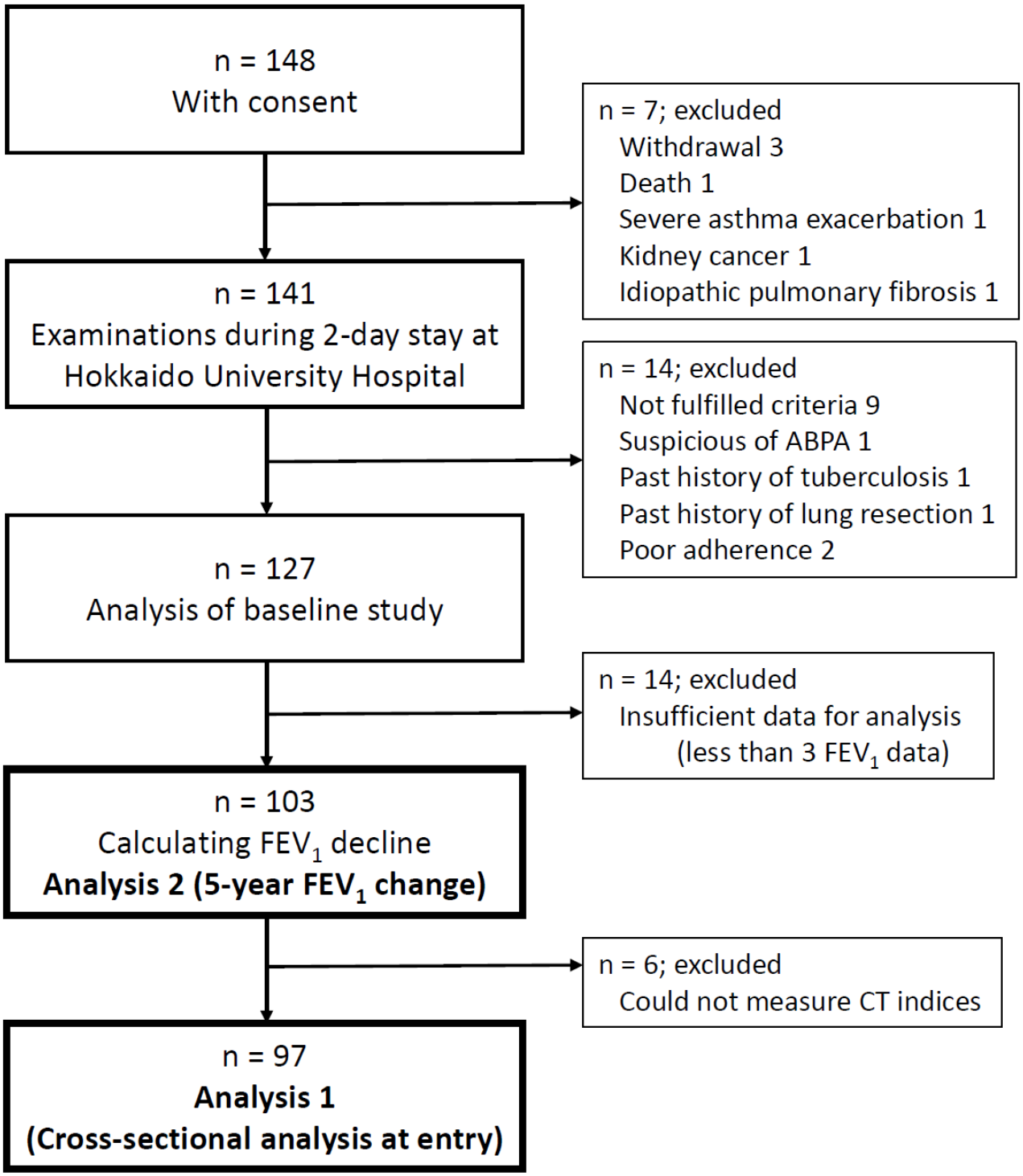

n = 148
With consent
n = 7; excluded
Withdrawal 3
Death 1
Severe asthma exacerbation 1
Kidney cancer 1
Idiopathic pulmonary fibrosis 1
n = 141
Examinations during 2-day stay at Hokkaido University Hospital
n = 14; excluded
Not fulfilled criteria 9
Suspicious of ABPA 1
Past history of tuberculosis 1
Past history of lung resection 1
Poor adherence 2
n = 127
Analysis of baseline study
n = 14; excluded
Insufficient data for analysis
(less than 3 FEV1 data)
n = 103
Calculating FEV1 decline
Analysis 2 (5-year FEV1 change)
n = 6; excluded
Could not measure CT indices
n = 97
Analysis 1
(Cross-sectional analysis at entry)

Figure E3

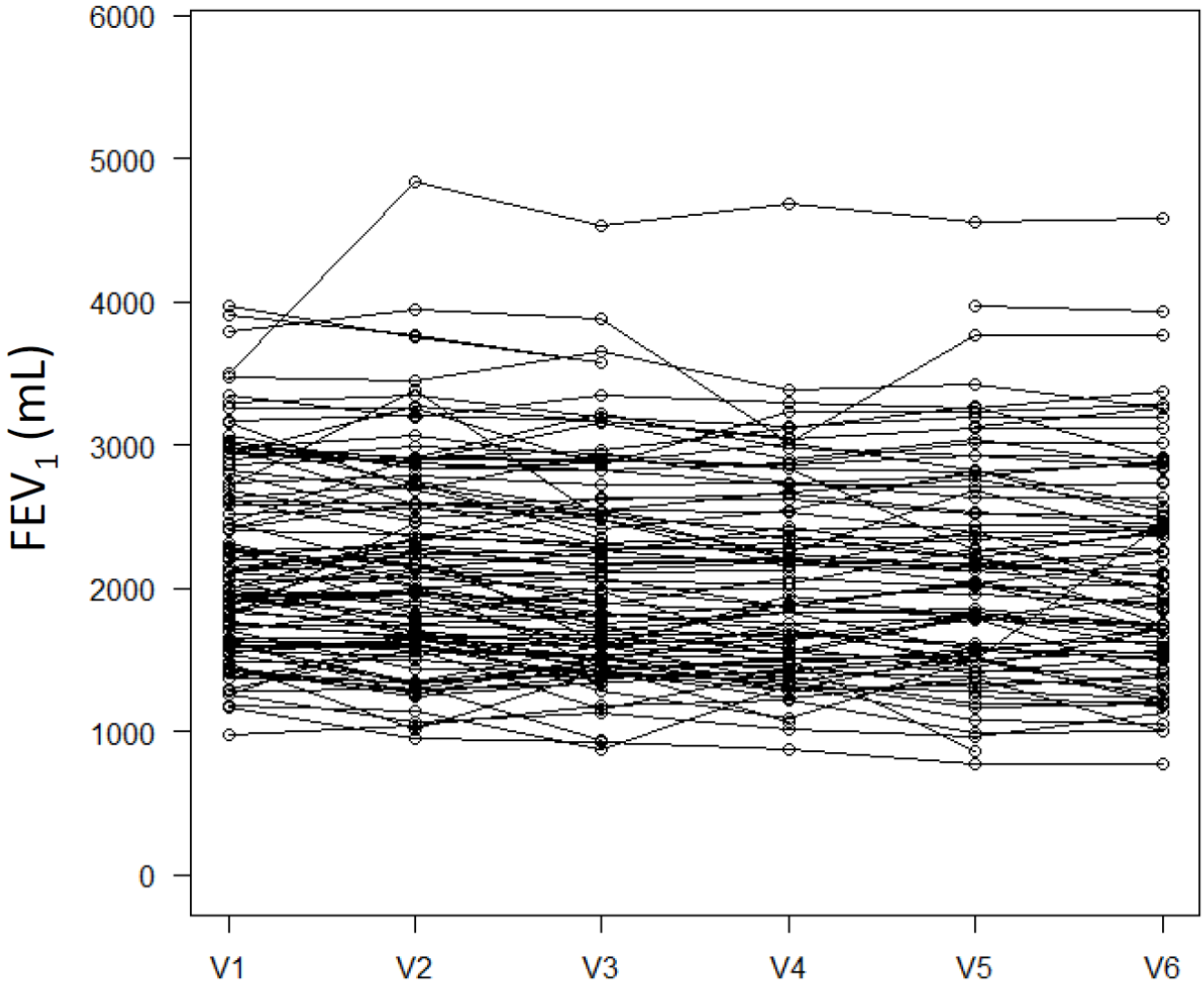
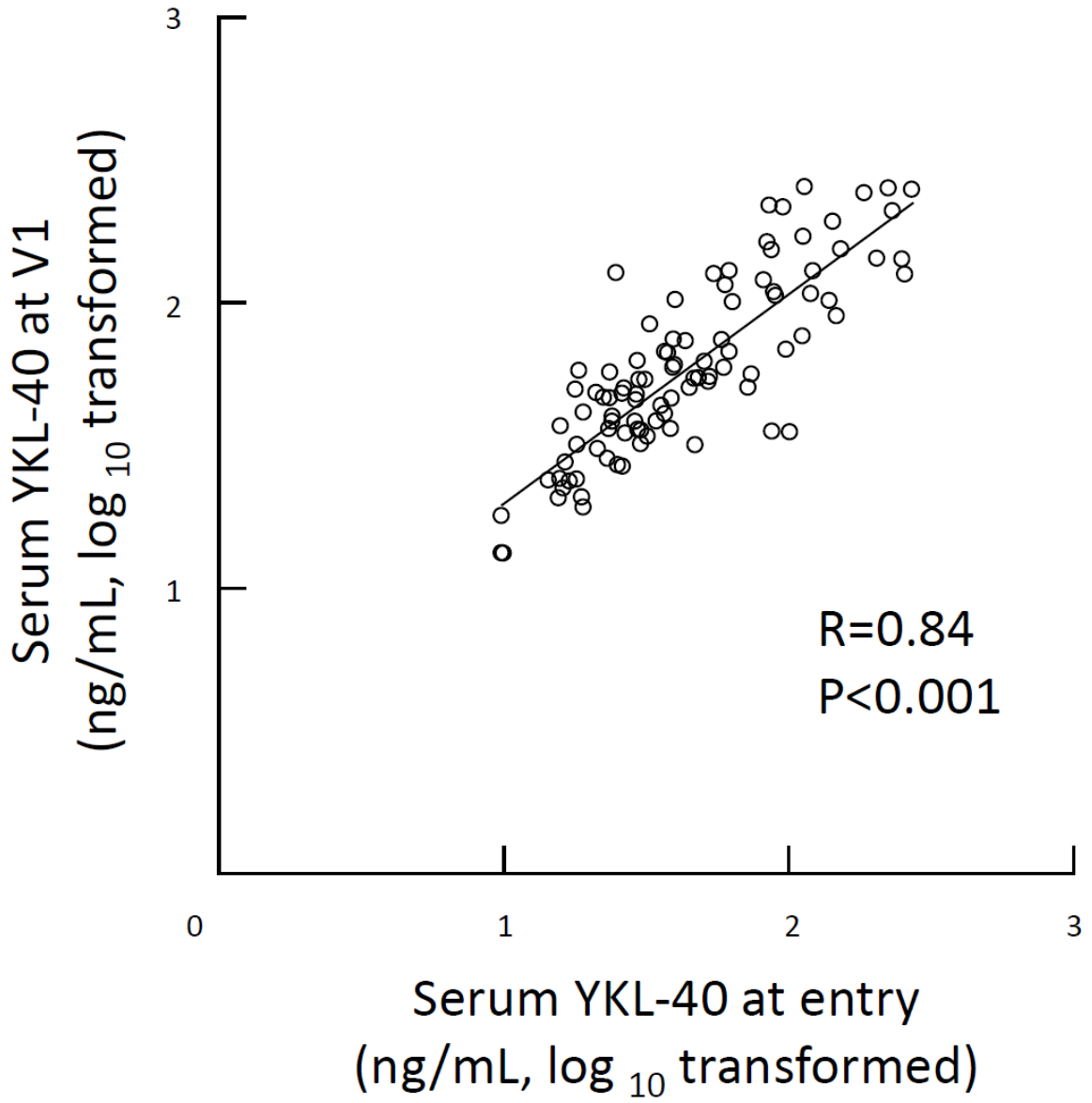


Figure E4


3
2
1
0
1
2
3
Serum YKL-40 at V1
(ng/mL, log 10 transformed)
R=0.84
P<0.001
Serum YKL-40 at entry
(ng/mL, log 10 transformed)