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Original Article

Efficacy of dupilumab for airway hypersecretion and airway wall thickening in patients with moderate-to-severe asthma: A prospective, observational study

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Abbreviations:

ACQ-7, 7-item Asthma Control Questionnaire; Ai, airway inner luminal area; AQLQ, Asthma Quality of Life Questionnaire; AX, low-frequency reactance area; BD, bronchodilator; BSA, body surface area; CANO, alveolar concentration of nitric oxide; CASA-Q, Cough and Sputum Assessment Questionnaire; CRSwNP, chronic rhinosinusitis with nasal polyps; CT, computed tomography; Dmin, cumulative dose of inhaled methacholine at the inflection point at which respiratory resistance begins to increase; ERS, European Respiratory

ABSTRACT

Background: Dupilumab has clinical effects in patients with moderate-to-severe asthma. When considering interleukin (IL)-4 and IL-13 signaling, effects of dupilumab on airway mucus hypersecretion and airway remodeling are expected, but they have been reported in only a few short-term studies. Its efficacy for airway hyperresponsiveness (AHR) remains unknown. We comprehensively assessed the efficacy of dupilumab, especially for subjective and objective measures of airway mucus hypersecretion and airway dimensions in moderate-to-severe asthmatic patients.

Methods: In 28 adult patients with moderate-to-severe uncontrolled asthma, the comprehensive efficacy of 48-week dupilumab treatment, including the Cough and Sputum Assessment Questionnaire (CASA-Q), radiological mucus scores and airway dimensions on computed tomography (CT), was assessed prospectively. Treatment responsiveness to dupilumab was analyzed.

Results: With 48-week dupilumab treatment, all four cough and sputum domain scores of CASA-Q improved significantly. Radiological mucus scores and airway wall thickening on CT were significantly decreased. The decreases in mucus scores were significantly associated with improvements in Asthma Control Questionnaire scores, Asthma Quality of Life Questionnaire (AQLQ) overall scores, airway obstruction, and airway type 2 inflammation. When defined by > 0.5 improvement in AQLQ overall scores, 18 patients (64%) were identified as responders.

Conclusions: Dupilumab reversed subjective and objective measures of airway mucus hypersecretion and some aspects of airway remodeling in patients with moderate-to-severe uncontrolled asthma.

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Society; FEF25–75, forced expiratory flow between 25% and 75% of FVC; Fres, resonant frequency; FVC, forced vital capacity; HRCT, high-resolution CT; IOS, impulse oscillometry; LAMA, long-acting muscarinic antagonists; LCQ, Leicester Cough Questionnaire; N-ERD, non-steroidal anti-inflammatory drug-exacerbated respiratory disease; OCS, oral corticosteroid; R5, respiratory resistance at 5 Hz; R20, respiratory resistance at 20 Hz; Rrs, respiratory resistance; SRrs, slope of the methacholine respiratory resistance dose-response curve; VAS, Visual Analog Scale; WA, wall area; X5, respiratory reactance at 5 Hz

Introduction

Dupilumab is a fully humanized monoclonal antibody that binds to the α subunit of the interleukin (IL)-4 receptor. By inhibiting IL-4 and IL-13 signaling, key drivers of type 2 inflammation, dupilumab has various clinical effects, such as decreasing the annualized rate of asthma exacerbations and type 2 biomarkers, improving asthma control and pulmonary function,^{1–3} and reducing oral corticosteroid (OCS) use^{4,5} in patients with uncontrolled, moderate-to-severe asthma. IL-4 induces mast cell activation, T helper 2 differentiation in T cells, and immunoglobulin (Ig) E class switching in B cells.⁶ IL-13 facilitates airway smooth muscle proliferation, goblet cell hyperplasia, increased airway mucus secretion, increased extracellular matrix secretion by fibroblasts, subepithelial basal membrane thickening, and airway hyperresponsiveness (AHR),^{6,7} all of which, except for AHR, are features of airway remodeling.⁶ Therefore, the effects of dupilumab on airway mucus hypersecretion and airway remodeling are expected, but only a case report⁸ and a randomized, double-blind, placebo-controlled trial,⁹ both of which involved 16-week treatment, have examined them. The effects of dupilumab on mucus symptoms and AHR remain unknown.¹⁰

Airway mucus hypersecretion is one of the cardinal features of chronic airway inflammatory diseases including asthma. It is associated with airway obstruction,¹¹ asthma severity,¹² and asthma control.¹³ Despite this, it had been difficult to quantify airway mucus hypersecretion, until the radiological mucus score measured by multidetector computed tomography (CT) was introduced.¹⁴ The mucus score is representative of mucus plugs, and mucus plugs are associated with airway obstruction and eosinophilic airway inflammation;¹⁴ as well as the pathophysiology of acute fatal asthma.¹⁵ Conventional therapies, including high-dose inhaled corticosteroids (ICS) and OCS, do not consistently reverse airway mucus hypersecretion and airway mucus plugs.^{14,16}

Airway remodeling is another predominant feature of asthma. It is responsible for structural changes of airways and lung parenchyma, determining fixed airflow obstruction.¹⁷ The correlation between airway wall thickening measured by CT and AHR was suggested in patients with asthma in previous studies.^{18,19} Evidence of the beneficial effects of high-dose ICS on airway remodeling has been inconsistently reported. Twelve-month treatment with high-dose fluticasone propionate decreased reticular basement membrane thickness, but not subepithelial collagen deposition, in patients with mild-to-moderate asthma.²⁰ Meanwhile, eight-week treatment with high-dose fluticasone propionate did not change subepithelial collagen deposition in patients with mild asthma.²¹ When considering the side effects of long-term use of high-dose

ICS, targeted therapeutics to modulate airway remodeling are required. Recently, some studies have evaluated the effects of biological therapies targeting IgE,^{22,23} IL-5,²⁴ and IL-5 receptor α ²⁵ on airway remodeling by using high-resolution CT (HRCT). However, the effect of dupilumab on airway remodeling has been reported in only one study,⁹ and long-term effect remains unknown.

The aim of this study was to comprehensively assess the clinical effects of 48-week treatment with dupilumab, especially on subjective and objective measures of airway mucus hypersecretion and airway dimensions in adult patients with uncontrolled, moderate-to-severe asthma.

Methods

Patients and study design

This was a prospective, single-arm, observational study. Adult patients with moderate-to-severe asthma who had visited our respiratory clinic from September 2019 to August 2022 were enrolled. Asthma diagnosis and severity were based on the Global Initiative for Asthma guideline.²⁶ All patients were poorly controlled with medium-to-high-dose ICS and controller medications, including long-acting β_2 -agonists (LABA), leukotriene receptor antagonists, theophylline, and long-acting muscarinic antagonists, with or without OCS. Chronic rhinosinusitis with nasal polyps (CRSwNP) and allergic rhinitis were diagnosed by an experienced otorhinolaryngologist (MS). The exclusion criteria were as follows: 1) active lung diseases other than asthma; 2) smoking history of >10 pack-years or within the previous one year; and 3) pregnant or nursing women.

After a one-week run-in period, all patients received 300 mg (loading dose, 600 mg) of subcutaneous dupilumab every two weeks for 48 weeks. Baseline asthma-controller medication and treatments for comorbidities, including CRSwNP and allergic rhinitis, were continued throughout the study.

The study protocol was approved by the ethics committee of Nagoya City University (60-19-0124), and written informed consent was obtained from all participants. This study was registered in the UMIN Clinical Trials Registry (UMIN000038669).

Endpoints

The primary efficacy endpoint was the serial changes in cough and sputum symptoms evaluated by the Cough and Sputum Assessment Questionnaire (CASA-Q)²⁷ during 48-week treatment with dupilumab.

The secondary efficacy endpoints were the changes from baseline in radiological mucus scores¹⁴ and airway dimensions²⁸ on

HRCT at weeks 24 and 48. Other secondary endpoints were the annualized rate of asthma exacerbations that required OCS for at least three days, unscheduled physician visits, and hospitalization during the 48 weeks before and after treatment; the changes from baseline in fractional exhaled nitric oxide (FeNO) levels, pre- and post-bronchodilator (BD) pulmonary function [spirometry and impulse oscillometry (IOS)], blood biomarkers [absolute numbers of eosinophils, neutrophils, and basophils, serum total IgE, and serum periostin], questionnaire scores [the 7-item Asthma Control Questionnaire (ACQ-7),²⁹ the Asthma Quality of Life Questionnaire (AQLQ),³⁰ the cough Visual Analog Scale (VAS),³¹ the Leicester Cough Questionnaire (LCQ)³²] at weeks 8, 16, 24, and 48; the changes from baseline in AHR to methacholine, capsaicin cough sensitivity, and induced sputum cell counts at weeks 24 and 48; and the achievement rates of clinical and deep remission at 48-week follow-up.^{33,34} The final secondary endpoint was the treatment responsiveness to dupilumab.

Details of each measurement are provided in [Supplementary Methods](#).

Measurements

Questionnaires

The CASA-Q²⁷ is a tool for measuring cough and sputum symptoms and their impact in patients with chronic airway diseases. It consists of two cough domains: cough symptoms (COUS) with three items and cough impact (COUI) with eight items; and two sputum domains: sputum symptoms (SPUS) with three items and sputum impact (SPUI) with six items. All items were scored from 0 to 4. Within each domain, items were summed and rescaled using the following algorithm: (sum rescored items)/(range of rescored item sum) $\times$ 100. This resulted in CASA-Q domain scores that ranged from 0 to 100, with higher scores associated with fewer symptoms/less impact due to cough or sputum.²⁷

Details of other questionnaires (the ACQ-7,²⁹ the AQLQ,³⁰ the cough VAS,³¹ the LCQ³²) are provided in [Supplementary Methods](#).

CT analysis of mucus plugs and central airway dimensions

All patients underwent CT using a CT scanner (SOMATOM Force, Siemens Healthineers, Erlangen, Germany) with a high-frequency algorithm. HRCT images were obtained with the patients in the supine position at full inspiration. Whole-lung HRCT images of 1-mm-thick slices over 1-mm intervals were used for the analysis. The pixel sizes were as follows: X and Y axes, 0.68 ± 0.5 mm; and Z axis, 0.5 mm.³⁵

Mucus plugs were assessed in all segments in both lungs (19 segments total) by an experienced chest radiologist (YO) according to the scoring system for quantifying mucus plugs introduced by Dunican *et al.*¹⁴ The mucus score was based on the number of bronchopulmonary segments with mucus plugs (from 0 to 19). Mucus plugs were defined as present when the bronchus was completely occluded in an area of more than 2 cm from the costal and diaphragmatic pleura. The radiologist was blinded to the clinical characteristics and outcomes of the patients.

Airway dimensions of the apical segmental bronchus (B¹) of the right upper lobe²⁸ were measured using SYNAPSE VINCENT (Fuji-film Medical, Tokyo, Japan). Airway inner luminal area (Ai) and absolute wall thickness (T) were measured automatically. The total diameter of the bronchus (D) was calculated as follows: $D = 2\sqrt{Ai/\pi} + 2T$. The airway outer area of the bronchus (Ao) and airway wall area (WA) were calculated as follows: $Ao = \pi (D/2)^2$ and $WA = Ao - Ai$. The percentage wall area (WA%) was calculated as follows: $WA\% = (WA/Ao) \times 100$. All airway dimensions except for WA% were corrected for body surface area (BSA).

FeNO analysis

FeNO levels were determined using a Sievers NOA71 280i chemiluminescence analyzer (GE Analytical Instruments, Boulder, CO, USA) according to the guideline.³⁶ FeNO levels were determined at expiratory flows of 50, 100, 150, and 200 mL/s. Alveolar concentration of NO (CANO) was calculated using the following equation: $CANO = \text{slope} - \text{intercept}/740$, after plotting NO output (i.e., eNO level $\times$ expiratory flow).³⁶

Pulmonary function

Respiratory impedance was measured using an IOS analyzer (Erich Jaeger GmbH, Hochberg, Germany) according to European Respiratory Society (ERS) standards.³⁷ Respiratory resistance at 5 Hz (R5), resistance at 20 Hz (R20), difference between R5 and R20 (R5–R20), respiratory reactance at 5 Hz (X5), resonant frequency (Fres) and low-frequency reactance area (AX) were measured.

After IOS measurements, spirometry was performed according to American Thoracic Society/ERS guidelines.³⁸ Pre- and post-BD forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), and forced expiratory flow between 25% and 75% of FVC (FEF_{25–75}) were evaluated using a Chestac-8900 (Chest, Tokyo, Japan).

Blood biomarkers

Serum total IgE and serum-specific IgE antibodies against common aeroallergens were measured using radioimmunosorbent tests (ImmunoCAP®, Phadia K.K., Tokyo, Japan). Serum periostin levels were measured using an enzyme-linked immunosorbent assay kit from Shino-Test (Kanagawa, Japan).³⁹

AHR to methacholine

AHR to methacholine was determined by measuring respiratory resistance (Rrs; cmH₂O/L/s) (Astograph™; Chest, Tokyo, Japan) under continuous methacholine inhalation.^{19,40} Briefly, twofold increasing concentrations of methacholine chloride diluted in physiological saline in 10 dose steps (49–25,000 µg/mL) were prepared. They were inhaled during tidal breathing from nebulizers with an output of 0.15 mL/min. After recording the baseline Rrs during inhalation of physiological saline for 1 min, methacholine was inhaled sequentially, beginning with the lowest concentration, at 1-min intervals. Dmin, the cumulative dose of inhaled methacholine at the inflection points at which Rrs began to increase, was used as the index of airway sensitivity. This variable was measured in terms of a unit defined as 1-min inhalation of methacholine (one mg/mL). Lower Dmin indicated increased airway sensitivity. Methacholine inhalation was continued until Rrs reached twice the baseline value. The slope of the methacholine-Rrs dose-response curve (SRrs) was used as the index of airway reactivity. Higher SRrs indicated increased airway reactivity.

Capsaicin cough sensitivity

After AHR to methacholine measurement, capsaicin cough sensitivity was tested by continuous inhalation of a capsaicin solution using Astograph™, as described previously.⁴¹ Capsaicin concentrations causing ≥ 2 and ≥ 5 coughs are referred to as C2 and C5, respectively.

Sputum induction and processing

Sputum was induced and processed as described previously.⁴² Cell differentials were determined by counting at least 400 non-squamous cells after staining with the May-Grünwald-Giemsa method.

Clinical and deep remissions

Clinical remission was defined as the absence of significant asthma symptoms (ACQ < 1.5) and no use of maintenance OCS at

48-week follow-up, and no exacerbation during the 48-week period after the introduction of dupilumab.³⁴ Deep remission was defined as clinical remission plus normalized pulmonary function ($FEV_1 \geq 80\%$ predicted) and suppressed type 2 inflammation (blood eosinophils $< 300/\mu\text{L}$ and $FeNO < 35$ ppb) at 48-week follow-up.³⁴ Complete remission was defined as deep remission plus negative AHR at 48-week follow-up.³³

Treatment responsiveness to dupilumab

Response to dupilumab treatment was assessed in all patients. Patient demographic and disease characteristics, including pre-determined factors (baseline blood eosinophil counts and $FeNO$ levels) and pre-evaluated factors in previous studies,^{2,43,44} were compared between responders and non-responders. Treatment responsiveness to dupilumab was defined as ≥ 0.5 improvement in the AQLQ overall scores³⁰ at 48 weeks.

Statistical analysis

Data were analyzed using JMP Pro14.0 software (SAS Institute, Inc., Tokyo, Japan). Data are presented as medians (range), means $\pm$ standard deviation or numbers (%). To analyze serial changes of variables through 48 weeks, repeated-measures analysis of variance was used. To compare variables from baseline to each time point, a paired t-test or Wilcoxon signed-rank test was used, as appropriate. To compare indices between responders and non-responders, the chi-squared test or Wilcoxon rank sum test was used, as appropriate. Correlations between variables were analyzed using Spearman's correlation coefficients. Values of $P < 0.05$ were considered significant.

Results

Patient characteristics

Thirty adult patients with uncontrolled moderate-to-severe asthma (28 with severe asthma and two with moderate asthma) were enrolled. In Japan, dupilumab is approved for patients with moderate-to-severe uncontrolled asthma.⁴⁵ Of the 30 patients, two changed their residential areas before completing 48-week treatment. In total, 28 patients completed 48-week treatment. The baseline characteristics of the 28 patients are shown in Table 1. All patients had poorly controlled asthma despite receiving moderate-to-high-dose ICS and concomitant controller medications. Two patients could not use LABA because of arrhythmias and used other controllers instead. Of the 28 patients, 18 (64%) had CRSwNP, 15 (54%) had allergic rhinitis, six (21%) had non-steroidal anti-inflammatory drug-exacerbated respiratory disease, and five (18%) had atopic dermatitis or psychiatric disorders (Table 1).

Primary outcome

Cough and sputum symptoms

With dupilumab treatment, all four domain scores of CASA-Q (COUS, COUI, SPUS, and SPUI) improved significantly and continuously from baseline to completion of 48-week treatment (all $P < 0.05$) (Fig. 1 and Table 2).

Secondary outcomes

Radiological mucus scores on CT

Regarding radiological mucus plugs on CT, 12 (43%) of 28 patients had mucus plugs in at least one lung segment at baseline. With dupilumab treatment, mucus scores decreased significantly

Table 1
Patients' baseline demographic characteristics.

	n = 28
Age, years	50.5 (37–83)
Females n (%)	18 (64)
Body mass index, kg/m^2	24.0 (17.9–33.0)
Smoking status, never/former	21/7
Atopic status, atopic (%)	21 (75)
Age of asthma onset, years	38 (2–67)
Duration of asthma, years	17 (2–53)
Comorbidities, n (%)	
Chronic rhinosinusitis with nasal polyps	18 (64)
Allergic rhinitis	15 (54)
Atopic dermatitis	5 (18)
Psychiatric disorders	5 (18)
N-ERD	6 (21)
Inhaled corticosteroid dose [†] , $\mu\text{g}/\text{day}$	750 (400–1400)
Concomitant controller medications, n (%)	
Long-acting β_2 -agonists [‡]	26 (93)
Leukotriene receptor antagonists	26 (93)
Sustained release theophylline	15 (54)
Long-acting muscarinic antagonists	13 (46)
Anti-allergic medicine	15 (54)
Oral corticosteroids	3 (11)
History of other biologics, n (%)	4 (14)
Fractional exhaled nitric oxide, ppb	48.6 (10.2–174.5)
Prebronchodilator FEV_1 , %predicted	94 (41–138)
Blood eosinophil count, $/\mu\text{L}$	372 (0–1159)
Serum total IgE, IU/mL	289 (5–16,000)
Exacerbation in the previous year, times	2.8 ± 4.9
Unscheduled physicians visit in the previous year, times	1.0 ± 3.0
Hospitalization in the previous year, times	0.5 ± 0.9

Values are given medians (range), number (%), or mean $\pm$ SD.

ACQ-7, 7-item Asthma Control Questionnaire; IU, international unit; N-ERD, non-steroidal anti-inflammatory drug-exacerbated respiratory disease.

[†]Equivalent to fluticasone propionate.

[‡]Two patients could not use a long-acting β_2 -agonist because of arrhythmia.

and continuously from baseline to completion of 48-week treatment ($P = 0.02$; Fig. 2 and Table 3).

The improvement in mucus scores was significantly associated with improvements in AQLQ overall scores ($\rho = -0.40$, $P = 0.04$) (Fig. 3A), FVC (%predicted) ($\rho = -0.40$, $P = 0.04$) (Fig. 3B), FEV_1 (%predicted) ($\rho = -0.52$, $P = 0.004$) (Fig. 3C), FEF_{25-75} (%predicted) ($\rho = -0.47$, $P = 0.01$) (Fig. 3D), $FeNO$ ($\rho = 0.62$, $P = 0.0006$) (Fig. 3E), and sputum eosinophil proportion ($\rho = 0.56$, $P = 0.04$) (Fig. 3F), from baseline to 48 weeks. Meanwhile, there were no correlations of mucus scores with SPUS or SPUI (both $P > 0.10$).

Airway dimensions on CT

WA% (Supplementary Fig. 1 and Table 3), WA, and WA/BSA were significantly and continuously decreased throughout 48-week treatment (all $P < 0.05$) (Table 3). Ai and Ai/BSA did not change.

There were no significant associations between the improvement in WA% or WA/BSA and the improvements in other clinical indices including pulmonary function.

Annualized rate of asthma exacerbations

The annualized rates of asthma exacerbations (2.8 ± 4.9 vs 0.9 ± 2.2 ; $P = 0.0001$) (Supplementary Fig. 2A), unscheduled physician visits (1.0 ± 3.0 vs 0.5 ± 1.8 ; $P = 0.01$) (Supplementary Fig. 2B), and hospitalization (0.5 ± 0.9 vs 0.1 ± 0.4 ; $P = 0.006$) (Supplementary Fig. 2C) decreased significantly after treatment.

FeNO and pulmonary function

$FeNO$ decreased significantly and continuously throughout 48-week treatment period ($P < 0.0001$) (Table 4). CANO did not change.

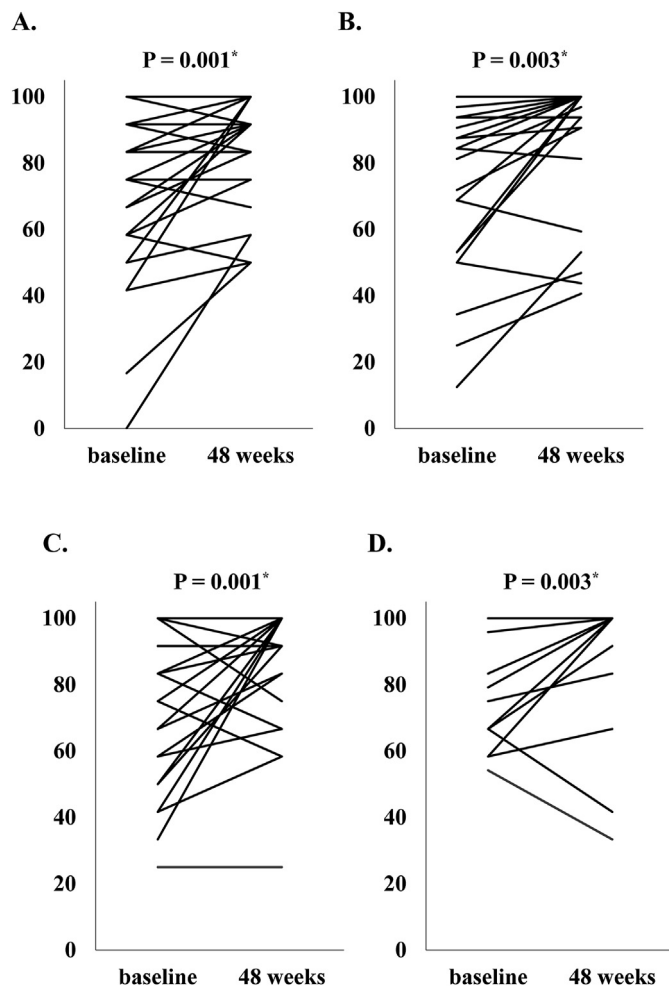


Fig. 1. The changes in Cough and Sputum Assessment Questionnaire scores in four domains: cough symptoms (A); cough impact (B); sputum symptoms (C); and sputum impact (D) from baseline to completion of 48-week treatment with dupilumab. *P value by repeated-measures analysis of variance.

Table 2
Effects of dupilumab on questionnaire scores (n = 28).

Questionnaire	Time points					P-value*
	Baseline	8 weeks	16 weeks	24 weeks	48 weeks	
CASA-Q						
Cough symptom	75 (0–100)	75 (25–100) [†]	83 (50–100) [†]	83 (25–100) [†]	88 (50–100) [†]	0.001
Cough impact	84 (13–100)	89 (31–100) [†]	94 (38–100) [†]	91 (28–100) [†]	98 (41–100) [†]	0.003
Sputum symptom	75 (25–100)	83 (17–100) [†]	92 (42–100) [†]	83 (0–100) [†]	96 (25–100) [†]	0.001
Sputum impact	96 (54–100)	100 (29–100) [†]	100 (54–100) [†]	100 (25–100)	100 (33–100) [†]	0.003
ACQ-7	1.7 (0.1–3.9)	0.9 (0–2.7) [†]	0.7 (0–3.1) [†]	0.6 (0–2.9) [†]	0.6 (0–2.6) [†]	<0.0001
AQLQ						
Symptoms	5.0 (1.1–6.8)	5.8 (3.3–7.0) [†]	6.0 (2.9–7.0) [†]	6.0 (2.8–7.0) [†]	6.2 (3.4–7.0) [†]	0.0002
Activities	4.8 (1.9–7.0)	5.6 (2.6–7.0) [†]	5.9 (2.8–7.0) [†]	6.0 (1.9–7.0) [†]	6.1 (2.2–7.0) [†]	0.003
Emotions	4.8 (1.6–7.0)	6.0 (3.0–7.0) [†]	6.2 (2.2–7.0) [†]	6.0 (2.2–7.0) [†]	6.4 (3.0–7.0) [†]	0.0001
Environment	5.3 (1.0–7.0)	6.0 (1.5–7.0) [†]	6.0 (2.8–7.0)	5.8 (2.8–7.0) [†]	6.0 (2.3–7.0) [†]	0.03
Overall	5.0 (1.4–6.9)	5.8 (3.0–7.0) [†]	6.0 (2.8–7.0) [†]	6.0 (2.8–7.0) [†]	6.0 (3.5–7.0) [†]	0.0005
Cough VAS	24 (0–98)	16 (0–68) [†]	9 (0–78) [†]	13 (0–65) [†]	10 (0–73) [†]	0.008
LCQ						
Physical	5.2 (2.8–7.0)	5.7 (3.8–7.0) [†]	6.0 (3.6–7.0) [†]	6.1 (3.1–7.0) [†]	6.3 (3.4–7.0) [†]	<0.0001
Mental	5.4 (1.4–7.0)	6.0 (4.0–7.0) [†]	6.6 (3.3–7.0) [†]	6.5 (3.3–7.0) [†]	6.6 (3.7–7.0) [†]	<0.0001
Social	5.0 (2.5–7.0)	6.0 (3.3–7.0) [†]	6.0 (3.5–7.0) [†]	6.3 (2.0–7.0) [†]	6.5 (4.0–7.0) [†]	<0.0001
Total	15.8 (7.4–21.0)	17.7 (11.3–21.0) [†]	18.3 (10.4–21.0) [†]	18.6 (10.4–21.0) [†]	19.5 (11.5–21.0) [†]	<0.0001

Values at baseline are medians (range).

ACQ-7, 7-item Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; CASA-Q, Cough and Sputum Assessment Questionnaire; LCQ, Leicester Cough Questionnaire; VAS, Visual Analog Scale.

*P-value: by repeated-measures analysis of variance.

[†]P < 0.05 for the comparison between baseline and each time point by a paired t-test or Wilcoxon signed-rank test.

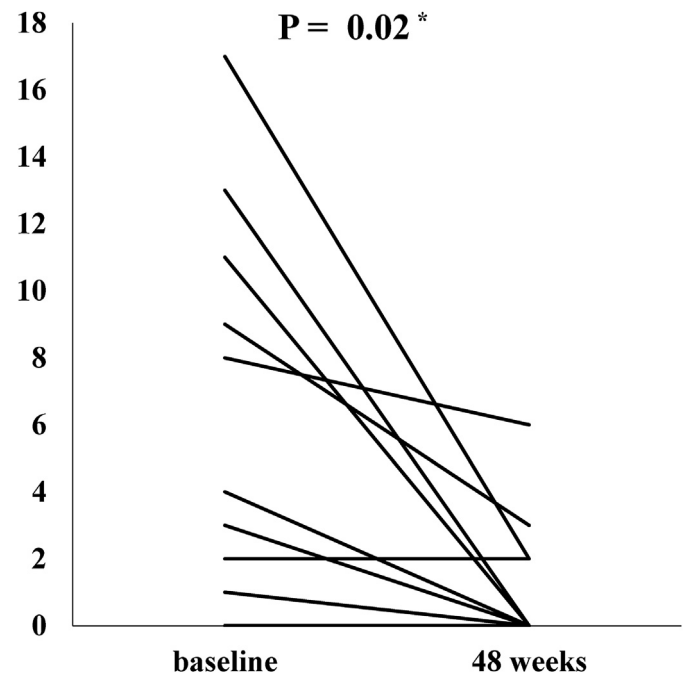


Fig. 2. Changes in mucus scores in all patients from baseline to completion of 48-week treatment with dupilumab. *P value by repeated-measures analysis of variance.

Pre-BD FVC (%predicted) (P = 0.02) and FEV₁ (%predicted) (P = 0.03) improved significantly and continuously from baseline to completion of 48-week treatment. Post-BD FEV₁ (%predicted) tended to improve from baseline to completion of 48-week treatment (P = 0.05). R5 (P = 0.04) and R20 (P = 0.006) decreased significantly from baseline to completion of 48-week treatment (Table 4). FEF_{25–75} (%predicted) and other IOS indices did not change.

AHR to methacholine

AHR results were obtained in 17 (61%) of the 28 patients. Airway sensitivity as indicated by Dmin improved significantly and

Table 3
Effects of dupilumab on computed tomography indices (n = 28).

	Time points			P-value*
	Baseline	24 weeks	48 weeks	
Mucus scores	0 (0–17)	0 (0–5) [†]	0 (0–6) [†]	0.02
WA%, %	62 (38–77)	54 (38–69) [†]	55 (34–71) [†]	0.02
WA, mm ²	15.2 (3.8–32.1)	14.3 (4.4–25.4) [†]	12.1 (4.4–24.0) [†]	0.04
WA/BSA, mm ² /m ²	8.9 (2.2–20.3)	8.7 (3.0–16.0) [†]	8.3 (3.0–15.4) [†]	0.04
Ai, mm ²	10.7 (1.6–32.8)	11.4 (3.0–31.6)	10.0 (3.0–27.8)	0.46
Ai/BSA, mm ² /m ²	6.2 (0.9–21.5)	7.2 (2.1–20.7)	6.3 (1.9–18.2)	0.44

Values are medians (range).

Ai, airway inner luminal area; BSA, body surface area; WA, wall area.

*P-value: by repeated-measures analysis of variance.

[†]P < 0.05 for the comparison between baseline and each time point by a paired t-test or Wilcoxon signed-rank test.

continuously from baseline to completion of 48-week treatment (P = 0.03) (Supplementary Fig. 3 and Table 4). Airway reactivity did not change.

Capsaicin cough sensitivity

C2 and C5 did not change from baseline to completion of 48-week treatment (Table 4).

Blood biomarkers

Blood eosinophil counts were increased at 24-weeks, and they then subsequently decreased at 48 weeks. Blood neutrophil and basophil counts did not change. The levels of serum total IgE (P = 0.04) and serum periostin (P = 0.002) decreased significantly and continuously from baseline to completion of 48-week treatment (Table 4).

Induced sputum cells

Induced sputum samples were obtained in 16 (57%) of 28 patients. Sputum eosinophils numerically decreased but did not reach statistical significance. Neutrophil proportion did not change (both P > 0.10) (Table 4).

Questionnaire scores

The ACQ-7 scores, the AQLQ scores in all four domains and overall scores, the cough VAS, and the LCQ scores in all three domains and total scores improved significantly and continuously throughout 48-week treatment period (all P < 0.05) (Table 2). In addition, clinically beneficial improvements were achieved in the ACQ-7 (≤ -0.5 points),²⁹ the AQLQ (≥ 0.5 points),³⁰ and the LCQ (> 1.3 points)^{32,46} at completion of 48-week treatment.

Clinical and deep remissions

After 48-week follow-up, the achievement rates of clinical, deep, and complete remission were 71%, 14%, and 7% respectively.

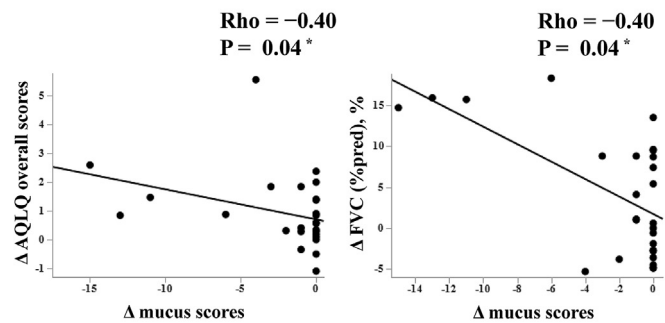
Treatment responsiveness to dupilumab

Based on > 0.5 improvement in the AQLQ, 18 (64%) of 28 patients were identified as responders at 48-week treatment with dupilumab. The comparisons of patient demographic and disease characteristics between responders and non-responders are shown in Table 5. The ratio of comorbid CRSwNP (P = 0.046), blood eosinophil counts (P = 0.03), serum total IgE (P = 0.02) and mucus scores (P = 0.04) were significantly higher in responders than in non-responders.

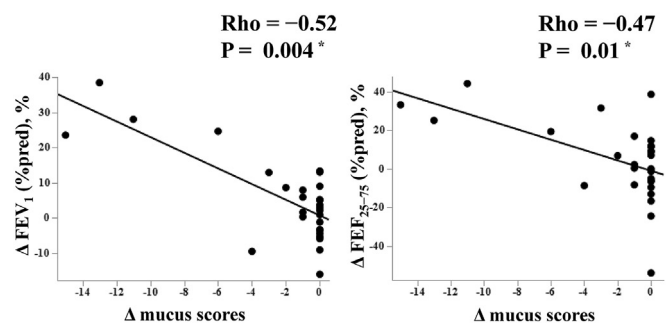
Discussion

In this study of patients with uncontrolled moderate-to-severe asthma treated with a 48-week course of dupilumab, the

A. Δ AQLQ overall scores B. Δ FVC (%pred), %



C. Δ FEV₁ (%pred), % D. Δ FEF₂₅₋₇₅ (%pred), %



E. Δ FeNO, ppb F. Δ Sputum eosinophil, %

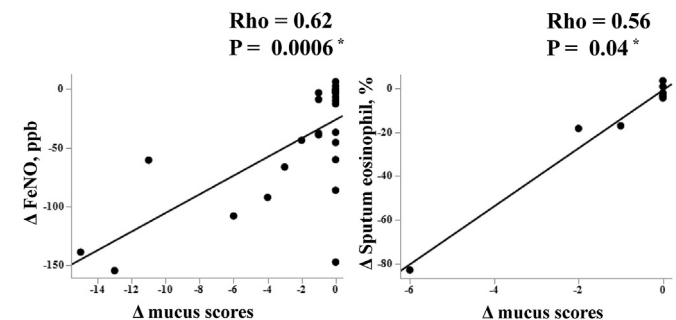


Fig. 3. Correlations between the changes (Δ) in mucus scores and Δ Asthma Quality of Life Questionnaire overall scores (A), Δ Forced vital capacity (FVC) (%predicted) (B), Δ FEV₁ (%predicted) (C), Δ FEF₂₅₋₇₅ (%predicted) (D), Δ Fractional exhaled nitric oxide (E), and Δ sputum eosinophil proportion (F). *P value by Spearman's correlation coefficients. FEF₂₅₋₇₅, forced expiratory flow between 25% and 75% of FVC.

following were observed: 1) cough and sputum symptoms evaluated by CASA-Q improved significantly; 2) mucus scores on CT decreased significantly; 3) airway wall thickness on CT decreased significantly; and 4) AHR to methacholine improved significantly. To the best of our knowledge, this is the first study to show that dupilumab improved subjective and objective measures of airway mucus hypersecretion and AHR in patients with asthma.

Airway mucus hypersecretion is one of the fundamental features of asthma. It is associated with a greater decline in FEV₁,⁴⁷ more severe disease,¹² chronic persistent airflow obstruction,¹¹ and increased exacerbation rates¹³ in patients with asthma. Control of airway mucus hypersecretion is therefore essential, but conventional therapies, including high-dose ICS and OCS, have

Table 4

Effects of dupilumab on FeNO, pulmonary function, AHR, blood biomarkers, and induced sputum cells (n = 28).

Variables	Time points					P-value*
	Baseline	8 weeks	16 weeks	24 weeks	48 weeks	
FeNO, ppb	48.6 (10.2–174.5)	21.0 (4.7–78.1) [†]	20.9 (3.4–78.1) [†]	22.8 (4.9–45.1) [†]	18.6 (9.1–47.7) [†]	<0.0001
CANO, ppb	3.3 (0.5–21.3)	1.9 (1.0–7.7)	1.5 (0.2–3.6)	1.5 (0.2–6.4)	2.0 (0.6–4.4)	0.12
Pulmonary function						
Pre-BD FVC, %predicted	97 (66–152)	100 (73–157) [†]	96 (78–156)	98 (80–147)	98 (80–153) [†]	0.02
Pre-BD FEV ₁ , %predicted	94 (41–138)	95 (68–137)	97 (69–146) [†]	96 (72–148) [†]	98 (63–151) [†]	0.03
Pre-BD FEF _{25–75} , %predicted	70 (7–126)	73 (17–110)	77 (20–118)	70 (20–117)	70 (25–120)	0.15
Post-BD FEV ₁ , %predicted	96 (47–148)	97 (72–146)	99 (73–149)	97 (67–159)	99 (74–151)	0.05
Impulse oscillometry						
R5, kPa/L/s	0.32 (0.19–0.66)	0.31 (0.18–1.15)	0.26 (0.13–0.45) [†]	0.32 (0.19–0.63)	0.31 (0.13–0.47)	0.04
R20, kPa/L/s	0.26 (0.17–0.51)	0.27 (0.16–0.57)	0.24 (0.12–0.41) [†]	0.26 (0.17–0.57)	0.25 (0.12–0.42) [†]	0.006
R5–R20, kPa/L/s	0.05 (0.01–0.26)	0.06 (0–0.58)	0.05 (0–0.13)	0.04 (0–0.12)	0.05 (0–0.13)	0.39
X5, kPa/L/s	–0.11 (–0.3–0.04)	–0.11 (–0.37–0.05)	–0.1 (–0.18–0.01)	–0.1 (–0.19–0.06)	–0.11 (–0.18–0)	0.57
Fres, Hz	11.9 (7.4–27.1)	11.5 (7.7–31.7)	11.4 (7.2–16.4)	10.8 (8.1–20.4)	10.8 (7.2–18.8)	0.15
AX, kPa/L	0.32 (0.07–3.12)	0.26 (0.07–6)	0.28 (0.01–0.92)	0.25 (0.09–1.03)	0.26 (0.01–0.97)	0.35
AHR (n = 17)						
Dmin, units	0.40 (0.02–25)	N/A	N/A	5.5 (0.002–25) [†]	5.8 (0.15–25) [†]	0.03
SRrs, cmH ₂ O/L/s	0.05 (0–0.19)	N/A	N/A	0.06 (0–0.34)	0.03 (0–0.23)	0.91
Cough sensitivity						
C2, μ M	4.88 (0.61–156.16)	N/A	N/A	9.76 (0.61–312.32)	19.52 (0.61–78.08)	0.53
C5, μ M	19.52 (0.61–156.16)	N/A	N/A	19.52 (1.22–312.32)	19.52 (0.61–312.32)	0.29
Blood biomarkers						
Eosinophil counts, / μ L	372 (0–1159)	342 (11–3584)	471 (23–3636)	436 (0–4499)	376 (34–1849)	0.08
Neutrophil counts, / μ L	3802 (1789–7571)	3362 (1975–9566)	3144 (2047–8893)	3490 (1927–10,077)	3319 (2192–9131)	0.54
Basophil counts, / μ L	56 (11–145)	61 (15–167)	51 (22–165)	50 (0–167)	60 (14–101)	0.36
Serum total IgE, IU/mL	289 (5–16,000)	161 (5–7710) [†]	112 (5–6550) [†]	94 (5–5750) [†]	58 (5–2870) ^{††}	0.04
Periostin, ng/mL	107 (43–390)	N/A	N/A	66 (37–116) [†]	64 (36–156) [†]	0.002
Induced sputum (n = 16)						
Eosinophil proportion, %	4.3 (0–83)	N/A	N/A	1.0 (0–17)	0 (0–8.6)	0.12
Neutrophil proportion, %	67 (4–97)	N/A	N/A	80 (27–98)	82 (23–93)	0.87

Values are medians (range).

*P-value: by repeated-measures analysis of variance.

AX, low-frequency reactance area; BD, bronchodilator; C2 and C5, capsaicin concentrations causing ≥ 2 and ≥ 5 coughs; CANO, alveolar concentration of nitric oxide; Dmin, airway sensitivity; FEF_{25–75}, forced expiratory flow between 25% and 75% of FVC; Fres, resonant frequency; FVC, forced vital capacity; IU, international unit; N/A, not assessed; R5 and R20, respiratory resistance at 5 and 20 Hz; SRrs, airway reactivity; X5, respiratory reactance at 5 Hz.[†]P < 0.05 for comparison between baseline and each time point by a paired t-test or Wilcoxon signed-rank test.^{††}P < 0.05 for comparison between 24 and 48 weeks by a paired t-test or Wilcoxon signed-rank test.

been ineffective.^{14,16,48} Mucus plugs can persist for over three years despite ongoing use of these therapies in patients with asthma.¹⁶ In the present study, dupilumab significantly improved sputum symptoms and radiological mucus scores, subjective and objective measures of airway mucus hypersecretion. The improvement in mucus scores was significantly associated with the improvements in AQLQ, airway obstruction, and airway type 2 inflammation. Mucus scores, as well as type 2 biomarker levels were significantly higher in responders than in non-responders. Regarding the mechanisms of mucus score improvement with dupilumab, blockade of IL-13 signaling may be involved. IL-13 plays a pivotal role in mucus hypersecretion by driving goblet cell metaplasia and enhancing the production of mucin 5AC.⁴⁹ Gene expression for IL-13 and the ratio of mucin 5AC to mucin 5B were significantly increased in asthmatic patients with high mucus scores.¹⁴ Dupilumab may improve mucus scores by inhibiting these effects of IL-13. When considering these results, dupilumab could be a promising treatment for uncontrolled moderate-to-severe asthma in patients with airway mucus hypersecretion and airway type 2 inflammation. Despite the improvement in mucus symptoms and mucus scores, there was no significant correlation between them. Consistent with these results, the previous study showed that 40% of asthmatic patients with high mucus scores did not have symptoms of chronic mucus hypersecretion.¹⁴ The reason for the dissociation between mucus symptoms and mucus scores remains unknown. The fourth- and fifth-generation bronchi where mucus plugs are frequently observed^{14,50} during asthma exacerbation as well as in the stable phase of asthma may lack receptors for coughing that are located in larger bronchi.⁵¹

Airway remodeling is another important feature of asthma that is characterized by loss of epithelial integrity, goblet cell and sub-mucosal gland enlargement, basement membrane thickening, subepithelial fibrosis, increased smooth muscle mass, angiogenesis, and decreased cartilage integrity.¹⁷ Airway remodeling leads to fixed airflow obstruction,¹⁷ and airway wall thickening measured by CT has been reported to correlate with AHR in previous studies.^{18,19} Because direct assessment of human airway remodeling by bronchial biopsies is invasive and thus limited, HRCT²⁸ has been used as an indirect but non-invasive technique to examine airway remodeling. So far, biological therapies targeting IgE,^{22,23} IL-5,²⁴ IL-5R α ,²⁵ and IL-4R α ⁹ can improve airway wall thickening on HRCT. Consistent with the previous study,⁹ the present study showed that dupilumab significantly improved airway wall thickening on HRCT. Additionally, post-BD FEV₁, one of the markers of airway remodeling, tended to improve in the present study. The mechanisms underlying the improvement in airway wall thickening caused by dupilumab remain unclear. IL-4 and IL-13 are involved in airway remodeling by inducing transforming growth factor β by bronchial epithelial cells.⁵² IL-13 also contributes to goblet cell metaplasia, fibroblast proliferation, and increased synthesis of α -smooth muscle actin and collagen III.^{52,53} Dupilumab may reverse airway remodeling by inhibiting these effects of IL-4 and IL-13. One previous study using a mouse model treated with anti-IL-4R α or anti-IL-5 antibody reported that targeting multiple factors of type 2 inflammation and remodeling, such as endothelial cells, B cells, T helper 2 cells, macrophages, goblet cells, and bronchial epithelial cells, as well as eosinophils, was required to affect airway remodeling.⁵⁴ In this study,⁵⁴ dual blockade of IL-4 and IL-13

Table 5
Comparisons of responders and non-responders.

	Responders (n = 18)	Non-responders (n = 10)	P-value*
Age, years	50 (37–77)	54 (43–83)	0.46
Females, n (%)	13 (72)	5 (50)	0.24
Body mass index, kg/m ²	23.5 (17.9–29.7)	24.0 (21.1–33.0)	0.55
Smoking, never/ex, n	15/3	6/4	0.17
Age of asthma onset, years	39 (7–67)	37 (2–57)	0.90
Duration of asthma, years	17 (2–50)	20 (2–53)	0.90
Comorbidities, n (%)			
CRSwNP	14 (78)	4 (40)	0.046
Allergic rhinitis	11 (61)	4 (40)	0.28
Atopic dermatitis	4 (22)	1 (10)	0.42
N-ERD	4 (22)	2 (20)	0.89
History of other biologics, n (%)	3 (17)	1 (10)	0.63
FeNO, ppb	58.2 (10.2–174.5)	22.8 (14.0–108.0)	0.12
Pre-BD FEV ₁ , %predicted	94 (41–138)	95 (66–110)	0.98
Blood eosinophil counts, /μL	455 (0–1159)	172 (9–428)	0.03
Serum total IgE, IU/mL	396 (8–16,000)	165 (5–799)	0.02
Mucus scores	1 (0–17)	0 (0–1)	0.04
Number of exacerbations in the previous year, times	0 (0–14)	0 (0–15)	0.87

Values are given medians (range) or number (%).

*P-value by the chi-squared test or Wilcoxon rank sum test.

BD, bronchodilator; CRSwNP, chronic rhinosinusitis with nasal polyps; IU, international unit; N-ERD, non-steroidal anti-inflammatory drug-exacerbated respiratory disease.

improved lung function by impacting these type-2 inflammation and remodeling-related factors, except blood eosinophil reduction, whereas, blocking IL-5 reduced blood eosinophils, but affected neither these factors nor lung function.

Cough is a major symptom of asthma and is associated with disease severity,¹² control status,⁵⁵ and worse clinical outcomes.¹³ Bronchoconstriction and allergen exposure sensitize and/or provoke cough in asthma by neurobiological processes originating from cough receptors.⁵⁶ Despite standard treatment with ICS and bronchodilators, cough persists as a prominent symptom in asthma.^{48,57} In the present study, dupilumab was found for the first time to significantly improve cough symptoms, evaluated by the CASA-Q or the cough VAS, and cough-related QOL, evaluated by the LCQ. These improvements were associated with the improvement in mucus scores (data not shown), but not with capsaicin cough reflex, which reflects cough receptor sensitivity. Dupilumab may ameliorate asthmatic cough by improving airway mucus hypersecretion, not by inhibiting neural pathways.

Regarding other clinical indices, several studies^{1–5} have reported that dupilumab exerts various clinical effects in patients with uncontrolled moderate-to-severe asthma. Consistent with these studies, the present study confirmed the clinical effects of dupilumab, including the improvements in asthma control, asthma-related QOL, and pulmonary function, and the decreases in the annualized rate of asthma exacerbations and airway type 2 inflammation. In addition, the present study observed for the first time a significant improvement in AHR to methacholine,¹⁰ a key feature of asthma. Though the effects of omalizumab and tezepelumab on AHR have been reported, no dupilumab studies were identified.¹⁰ AHR is related to baseline airflow obstruction, the changes in the infiltration of smooth muscle by mast cells,⁵⁸ and the amount of airway smooth muscle. Inhibition of the mast cell-activated pathway, as well as the type 2-specific pathway (targeting eosinophils, IL-4 and IL-13), seems to be necessary to improve AHR.⁵⁹ The present results may reflect the diverse effects of dupilumab on various hematopoietic/immune cells and non-hematopoietic/immune cells and may raise the potential of dupilumab to achieve asthma remission in at least a subset of patients.⁶⁰

This study has several limitations. First, there was no randomization and no placebo control. Setting up a placebo control arm was considered ethically problematic, especially in patients with

uncontrolled moderate-to-severe asthma, because several studies have reported the clinical effects of dupilumab. Second, the sample size of this study was small. Despite this, the present study confirmed several new clinical effects of dupilumab, including on sputum and cough symptoms and AHR, and the achievement rates of clinical, deep, and complete remission. A prospective study with a larger sample size is needed to confirm these effects. Third, not all patients could undergo AHR to methacholine testing and sputum induction due to poor pulmonary function and dyspnea. It was ethically unacceptable for us to perform tests that might have deteriorated the patients' condition.

In conclusion, the results of the present study demonstrated that dupilumab could reverse subjective and objective measures of airway mucus hypersecretion and airway remodeling in adult patients with uncontrolled moderate-to-severe asthma.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2024.02.002>.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

TT had contributed to conception of the study, recruitment of patients, disease diagnosis and management, acquisition and interpretation of data, and writing and drafting manuscript. MS had contributed conception of the study, recruitment of patients, disease diagnosis and management, acquisition and interpretation of data, and revision of the manuscript. YO had contributed to radiological analysis and revision of the manuscript. NT, KF, and AN had contributed to recruitment of patients, disease diagnosis and management. HN, RK, KIt, YK, YM, SF, TU, HO, MT, KM, YI, and TO had contributed to performance of the tests. Klz. had contributed to measurement of blood biomarkers and revision of the manuscript. AN had contributed to critical revision of the manuscript.

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