Sputum YKL-40 Levels and Pathophysiology of Asthma and Chronic Obstructive Pulmonary Disease

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Key Words

YKL-40 · Chitinase-like protein · Induced sputum · Asthma · Chronic obstructive pulmonary disease · Neutrophilic inflammation

Abstract

Background: Recent evidence suggests that YKL-40, also called chitinase-3-like-1 protein, is involved in the pathogenesis of asthma and chronic obstructive pulmonary disease (COPD). Details of sputum YKL-40 in asthma and COPD, however, remain unknown. **Objectives:** To clarify associations of sputum YKL-40 levels with clinical indices in asthma and COPD. **Methods:** Thirty-nine patients with asthma, 14 age-matched never-smokers as controls, 45 patients with COPD, and 7 age-matched smokers as controls were recruited for this study. Sputum YKL-40 levels were measured and YKL-40 expression in sputum cells was evaluated by immunocytochemistry. **Results:** Sputum YKL-40 levels were higher in patients with COPD (346 ± 325 ng/ml) than in their smoker controls (125 ± 122 ng/ml; $p < 0.05$), but were not significantly different between patients with asthma (117 ± 170 ng/ml) and their controls (94 ± 44 ng/ml; $p = 0.15$). In pa-

tients with asthma only, sputum YKL-40 levels were positively correlated with disease severity ($r = 0.34$, $p = 0.034$) and negatively correlated with pre- and postbronchodilator %FEV₁ ($r = -0.47$ and -0.42 , respectively; $p < 0.01$) and forced mid-expiratory flow ($r = -0.48$ and -0.46 , respectively, $p < 0.01$). Sputum YKL-40 levels were positively correlated with sputum neutrophil counts in asthma ($r = 0.55$, $p < 0.001$) and with neutrophil and macrophage counts in COPD ($r = 0.45$ and 0.65 , respectively, $p < 0.01$). YKL-40 was expressed in the cytoplasm of sputum neutrophils and macrophages in all groups. **Conclusions:** Elevated sputum YKL-40 reflects air-flow obstruction in asthma whereas the roles of YKL-40 in the proximal airways in COPD remain to be elucidated.

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Introduction

YKL-40, also known as chitinase-3-like-1 protein (CHI3L1) or human cartilage glycoprotein-39, is classified as a 'mammalian chitinase-like protein' although it does not exhibit chitinase activity. YKL-40 is produced in response to inflammatory stimuli and is secreted by sev-

eral types of cells, including neutrophils [1], macrophages [2], chondrocytes, synovial cells [3] and vascular smooth muscle cells [4]. YKL-40 exhibits potent proliferative activity in skin and fetal lung fibroblasts [5], and stimulates the migration of vascular smooth muscle cells and vascular endothelial cells [4, 6]. Serum YKL-40 levels are elevated in patients with various diseases, such as hepatic fibrosis, systemic sclerosis, osteoarthritis and idiopathic pulmonary fibrosis [7], suggesting the involvement of YKL-40 in inflammatory processes and tissue remodeling [8].

Asthma and chronic obstructive pulmonary disease (COPD) are characterized by airway inflammation and remodeling that lead to reversible or irreversible airflow obstruction. Recent studies have shown that YKL-40 is involved in the pathophysiology of asthma [9–12] and COPD [13]. Serum YKL-40 levels were higher in patients with asthma [9] and COPD [13] than in healthy controls, and were correlated with airflow obstruction and disease severity. Serum YKL-40 levels were higher in patients with asthma with exacerbations than in those in a stable condition [11]. In patients with COPD, elevated YKL-40 levels in the bronchoalveolar lavage fluid (BALF) have been reported to be associated with airflow obstruction. In the case of asthma, Kuepper et al. [12] showed that YKL-40 levels in the BALF of patients with allergic asthma were increased after administration of segmental allergen challenges. However, associations of YKL-40 levels in the airways with clinical indices in asthma remain largely unknown.

Sputum YKL-40 levels may provide more relevant and specific information on asthma than the levels found in blood samples. Therefore, we investigated the relationships of sputum YKL-40 levels with clinical indices in asthma, and assessed the similarities and differences in sputum YKL-40 associations with disease pathophysiology between asthma and COPD.

Methods and Materials

Subjects

For this cross-sectional study, 39 patients with stable asthma, who regularly visited our outpatient Asthma and Cough Clinic, were enrolled. Asthma was diagnosed according to the American Thoracic Society criteria [14] based on a history of recurrent episodes of wheezing and chest tightness, with or without cough, and documented airway reversibility with a bronchodilator or hyperresponsiveness to inhaled methacholine. Severity was defined according to the step classification of the Global Initiative for Asthma guidelines, as revised in 2002 [15], and classified as follows: mild intermittent (step 1), mild persistent (2), moderate persistent

(3), and severe persistent (4). All patients with asthma were lifelong never-smokers.

Patients with COPD ($n = 45$) as defined by the Global Initiative for Chronic Obstructive Lung Disease guidelines (GOLD) 2003 [16], who had a history of chronic respiratory symptoms, such as cough and sputum with or without breathlessness, had a post-bronchodilator forced expiratory volume in 1 s (FEV_1)/forced vital capacity (FVC) ratio of less than 0.7 and regularly visited our outpatient COPD clinic were recruited. Patients were either current ($n = 10$, 62.9 ± 26.3 pack-years) or former smokers ($n = 35$; 62.7 ± 28.8 pack-years). Typical emphysematous changes were observed in all patients with COPD on chest computed tomography scans. Among these, 6 were considered to have chronic bronchitis that was defined by the presence of sputum production for 3 consecutive months within the last 2 years. The conditions of both asthma and COPD patients were stable, and they had been free of exacerbations for 4 weeks or more. Patients who had any active malignant diseases within 5 years, connective tissue diseases, infectious diseases, or active respiratory disorders other than asthma or COPD were excluded.

We recruited 14 age-matched healthy never-smokers as controls for patients with asthma and 7 age-matched former or current smokers without COPD as controls for patients with COPD from our hospital. The research protocol was approved by the Ethics Committee of Kyoto University, and written informed consent was obtained from all subjects.

Sputum Induction and Processing

Sputum induction and processing were performed as described by Pin et al. [17], with slight modifications [18]. In brief, the subjects were premedicated with inhaled salbutamol (200 μ g). They then inhaled hypertonic (3%) saline solution, administered with an ultrasonic nebulizer (MU-32; Azwell Inc., Osaka, Japan) for 15 min. Adequate sputum plugs were separated from saliva and first treated with 0.1% dithiothreitol (Sputasol; Oxoid Ltd., UK), followed by the same volume of Dulbecco's phosphate-buffered saline (PBS). After centrifugation, sputum supernatants were stored at -80°C . Cell differentials were determined by counting at least 400 nonsquamous cells stained by the May-Grünwald-Giemsa method.

Measurement of YKL-40 Levels in Sputum Supernatants

YKL-40 levels in sputum supernatants were measured using an enzyme-linked immunosorbent assay kit (Quidel, San Diego, Calif., USA) following the manufacturer's instructions. The detection limit of this assay was 10 ng/ml. Values below this threshold were assigned values of 10 ng/ml before adjusting for the dilution with dithiothreitol and PBS. A spike-back analysis that used exogenous YKL-40 resulted in greater than 80% recovery.

Specific IgE Measurement

In patients with asthma and COPD, serum allergen-specific IgE antibodies for mixed moulds, house-dust mite, cat dander, dog dander, Japanese cedar pollen, mixed grass pollens and mixed weed pollens were detected with a capsulated hydrophilic carrier polymer radioallergosorbent test fluoroenzyme immunoassay (Phadia, Uppsala, Sweden) in an external laboratory (Mitsubishi Kagaku Bio-Clinical Laboratories, Kyoto, Japan). Atopy was determined based on the detection of at least one allergen-specific IgE antibody.

Pulmonary Function

We measured FVC, FEV₁ and forced mid-expiratory flow (FEF_{25–75%}) using a Chestac-65V (Chest MI Corp., Tokyo, Japan). Spirograms were obtained in triplicate; the best of 3 reproducible measurements was recorded as recommended by the American Thoracic Society/European Respiratory Society [19].

Immunostaining

Sputum cells from at least 3 samples obtained from patients with asthma, patients with COPD and their age-matched controls were used for immunostaining. After adjusting for cell number, sputum cells were mounted on slides by cytocentrifugation, air-dried, fixed in acetone/methanol (60:40) and stored at –20°C until immunostaining. For double immunostaining, samples were first blocked with 5% BSA in PBS for nonspecific binding. The slides were then incubated either with a rabbit polyclonal antibody against human YKL-40 (33 µg/ml) (Quidel) or a rabbit IgG (Dako, Glostrup, Denmark) at the same concentration as the control and either a monoclonal mouse antibody against human neutrophil elastase (NE; Dako), CD68 (Dako), or major basic protein (MBP; Chemicon, Temecula, Calif., USA) or mouse IgG (Sigma-Aldrich, Tokyo, Japan) in PBS containing 1% BSA. Concentrations of mouse IgG used for negative controls are shown in table 1. After rinsing in PBS, samples were incubated with Alexa Fluor 488 donkey anti-rabbit IgG (Invitrogen Corp., Carlsbad, Calif.,

USA) and Alexa Fluor 546 goat anti-mouse IgG (Invitrogen). A fluorescence microscope was used for immunocytochemical evaluations.

Positive staining was detected as green for the YKL-40 antigen and red for the NE, CD68 and MBP antigens.

Statistical Analysis

A Mann-Whitney U test was used to compare 2 groups. For comparisons of nominal data, a χ^2 test or Fisher's exact test was used. Correlations were analyzed using Spearman's rank correlation test. p values <0.05 were considered significant. Differences among 3 groups were first examined using a Kruskal-Wallis test. Results are given as means $\pm$ SDs, unless otherwise stated. Statistical analysis was performed using JMP 6.0 (SAS Campus Drive, Cary, N.C., USA).

Table 1. Concentrations of mouse IgG used for negative controls

Mouse monoclonal antibody	Concentration of mouse IgG
Anti-neutrophil elastase	0.07 µg/ml
Anti-CD68	1.25 µg/ml

Table 2. Characteristics and findings of patients with asthma and their age-matched controls

Characteristics	Asthma (n = 39)	Control (n = 14)	p value
Male, n	12	5	0.75
Age, years	58 (14)	51 (14)	0.47
Body mass index	23.7 (3.3)	22.0 (2.7)	0.083
Disease duration, years	16.3 (17.2)	–	–
Disease severity ^a	1/17/12/9	–	–
Comorbidity of chronic sinusitis	7	–	–
Atopy ^b , n	22	–	–
Serum IgE, IU/ml	118 (8–1,276)	–	–
Dose of inhaled steroids, µg/day ^c	897 (607)	–	–
Before bronchodilation			
FEV ₁ /FVC, %	67.9 (11.8)	81.3 (5.9)	<0.001
FEV ₁ , % predicted	87.6 (22.6)	105.8 (12.6)	0.003
FEF _{25–75%} , % predicted	52.1 (29.9)	93.3 (27.9)	<0.001
After bronchodilation			
FEV ₁ /FVC, %	69.5 (11.6)	82.2 (6.3)	<0.001
FEV ₁ , % predicted	90.3 (21.9)	108.4 (12.6)	0.006
FEF _{25–75%} , % predicted	57.3 (32.1)	96.7 (31.6)	<0.001
Induced sputum			
Macrophages, $\times 10^5 \cdot g^{-1}$	4.6 (5.3)	6.4 (7.3)	0.76
Neutrophils, $\times 10^5 \cdot g^{-1}$	12.0 (22.5)	6.6 (4.5)	0.55
Eosinophils, $\times 10^5 \cdot g^{-1}$	2.6 (6.4)	0.1 (0.3)	<0.001

Results are means (SD) except for IgE, medians (ranges).

^a Step classification of the Global Initiative for Asthma (1/2/3/4). ^b Data are missing for 4 patients with asthma. ^c Dose equivalent to chlorofluorocarbon beclomethasone. The dose for patients not treated with inhaled corticosteroids was assigned 0 µg/day.

Table 3. Characteristics and findings of patients with COPD and their age-matched smoker controls

Characteristics	COPD (n = 45)	Smoker control (n = 7)	p value
Male, n	45	6	0.13
Age, years	72 (9)	67 (5)	0.13
Body mass index	22.0 (2.7)	22.6 (2.3)	0.82
Lifetime smoking, former:current	35:10	5:2	0.66
Pack-years	62.7 (27.9)	36.3 (12.8)	0.004
Disease duration, years	6.2 (5.2)	–	–
Disease severity ^a	20/20/5/0	–	–
Comorbidity of chronic sinusitis	4	–	–
Atopy, n	14	–	–
Serum IgE, IU/ml	130 (5–1,500)	–	–
Dose of inhaled steroids, µg/day ^b	364 (637)	–	–
Before bronchodilation			
FEV ₁ /FVC, %	51.5 (10.7)	73.8 (9.1)	<0.001
FEV ₁ , % predicted	77.3 (21.1)	96.1 (8.6)	0.018
FEF _{25–75%} , % predicted	23.9 (9.3)	65.3 (22.7)	<0.001
After bronchodilation			
FEV ₁ /FVC, %	52.9 (11.3)	76.7 (7.4)	<0.001
FEV ₁ , % predicted	83.4 (19.9)	100.2 (8.1)	0.033
FEF _{25–75%} , % predicted	27.1 (11.3)	74.0 (17.4)	<0.001
Induced sputum			
Macrophages, × 10 ⁵ ·g ⁻¹	9.4 (11.3)	2.9 (3.2)	0.033
Neutrophils, × 10 ⁵ ·g ⁻¹	26.9 (38.0)	8.8 (8.7)	0.046
Eosinophils, × 10 ⁵ ·g ⁻¹	1.5 (2.6)	0.5 (0.6)	0.13

Results are means (SD) except for IgE, medians (ranges).

^a Stages of GOLD criteria for COPD (I/II/III/IV). ^b Dose equivalent to chlorofluorocarbon beclomethasone. The dose for patients untreated with inhaled corticosteroids was assigned 0 µg/day.

Results

Characteristics of Patients with Asthma and COPD and Their Age-Matched Controls

Characteristics, results of pulmonary function tests and sputum cell differentials of 39 patients with asthma and their age-matched controls are shown in table 2. In the asthma group, differences in patient characteristics other than serum IgE levels between atopic (median serum IgE = 120 IU/ml) and nonatopic patients (median serum IgE = 39 IU/ml; $p = 0.037$) were not statistically significant. The findings for 45 patients with COPD and their age-matched smoker controls are shown in table 3. Differences in patient characteristics between COPD patients with and without chronic bronchitis were not statistically significant. When patients with asthma and COPD were compared, patients with COPD were predominantly males and older than those with asthma, and more patients with asthma ($n = 38$) received inhaled corticosteroids than did COPD patients ($n = 12$; $p <$

0.001), and more patients with asthma used theophylline (9 patients with asthma vs. 1 patient with COPD; $p = 0.005$). Patients with COPD showed more severe air-flow limitation (for FEV₁/FVC and %FEF_{25–75%}, $p < 0.001$; for %FEV₁, $p = 0.042$) and showed a greater number of macrophages and neutrophils in induced sputum ($p = 0.020$ and $p < 0.001$, respectively) than those with asthma.

Sputum YKL-40 Levels in Patient and Control Groups

Sputum YKL-40 levels were significantly higher in patients with COPD (346 ± 325 ng/ml) than in their smoker controls (125 ± 122 ng/ml; $p = 0.011$) (fig. 1a) whereas there was no significant difference between patients with asthma ($n = 39$, 117 ± 170 ng/ml) and their controls (94 ± 44 ng/ml; $p = 0.15$). In 14 patients with asthma and 2 smoker controls, sputum YKL-40 levels were below the detection limit. An atopic status in patients with asthma did not affect sputum YKL-40 levels (atopic asthma 105 ± 125 ng/ml, nonatopic asthma 155 ± 271 ng/ml; $p =$

Fig. 1. Logarithmic results for sputum YKL-40 levels. Horizontal bars indicate mean values. $p = 0.34$ by the Kruskal-Wallis test. **a** Sputum YKL-40 levels in patients with COPD and their age-matched smoker controls. **b** Sputum YKL-40 levels in patients with atopic asthma and nonatopic asthma and their age-matched controls.

0.88) (fig. 1b). For patients with COPD, differences in sputum YKL-40 levels between those who had chronic bronchitis ($n = 6$; 471 ± 384 ng/ml) and those who did not (327 ± 316 ng/ml; $p = 0.19$) were not statistically significant. When patients with asthma and COPD were compared, patients with COPD showed higher sputum YKL-40 levels than those with asthma ($p < 0.001$).

Relationships between YKL-40 Sputum Levels and Clinical Indices in Patients with Asthma and COPD

In patients with asthma, YKL-40 sputum levels were positively correlated with disease severity ($r = 0.34$, $p = 0.034$) (fig. 2) and maintenance doses of inhaled corticosteroids ($r = 0.33$, $p = 0.045$) whereas in patients with COPD, there was no significant correlation between YKL-40 sputum levels and the GOLD stages ($r = -0.24$, $p = 0.11$) or maintenance doses of ICS ($r = 0.23$, $p = 0.13$). In patients with asthma, sputum YKL-40 levels were not associated with gender (males 148 ± 238 ng/ml, females 93 ± 74 ng/ml; $p = 0.88$). In either patient group, sputum YKL-40 did not associate with age (asthma, $r = -0.09$, $p = 0.50$; COPD, $r = 0.22$, $p = 0.15$). Moreover, body mass index, serum IgE levels, concurrent chronic sinusitis and use of theophylline did not affect sputum YKL-40 levels (data not shown). In patients with asthma, sputum YKL-40 levels were negatively correlated with both pre- and postbronchodilator FEV₁ (fig. 3a, b) and FEF_{25-75%} values (fig. 4a, b). In contrast, in COPD patients and the controls of both patient groups, no correlations were observed between YKL-40 sputum levels and measures of pulmonary function (fig. 3c, d, 4c, d for COPD; data not shown for controls).

Fig. 2. Correlation between sputum YKL-40 levels and disease severity in asthma ($r = 0.34$, $p = 0.034$).

Relationships between YKL-40 Sputum Levels and Sputum Inflammatory Cells: Immunocytochemical Examinations of Sputum Inflammatory Cells

In patients with asthma, sputum YKL-40 levels were correlated only with the numbers of sputum neutrophils, while in COPD patients, sputum YKL-40 levels were correlated with the numbers of sputum macrophages and neutrophils (fig. 5). When the 14 patients with asthma who showed sputum YKL-40 levels under the detection limit were compared to the remaining 25 patients with asthma, the former showed fewer sputum neutrophils ($10.6 \pm 29.9 \cdot 10^5 \cdot g^{-1}$), in addition to higher prebronchodilator FEV₁ ($97.0 \pm 20.1\%$) and FEF_{25-75%} ($61.9 \pm 26.5\%$) values than the remaining 25 patients with asthma (12.8

Fig. 3. Correlations between sputum YKL-40 levels and pre- and postbronchodilator %FEV₁ in patients with asthma (**a**, **b**) and COPD (**c**, **d**).

$\pm 17.7 \cdot 10^5 \cdot g^{-1}$; $82.3 \pm 22.6\%$; $46.6 \pm 30.8\%$, respectively; $p < 0.05$ for all comparisons). No significant correlations were observed between sputum YKL-40 levels and sputum eosinophil counts in patients with asthma or in patients with COPD ($r = 0.12$, $p = 0.47$; $r = 0.25$, $p = 0.10$, respectively). There were no significant correlations between sputum YKL-40 levels and neutrophil, macrophage or eosinophil counts in the controls of both patient groups (data not shown).

The presence or absence of YKL-40 in CD68- or NE-positive cells in at least 3 samples obtained from patients with asthma and patients with COPD, and their controls was examined immunocytochemically. The presence or absence of YKL-40 in MBP-positive cells was examined in patients with asthma. In all examined subjects, YKL-40 was positive for cells that were positive for NE or CD68 antigens but was negative for cells that were positive for MBP (fig. 6–12). There were no apparent qualitative dif-

ferences in the expression of YKL-40 in neutrophils or macrophages between patients with asthma and COPD and their controls. There were no apparent effects of age, gender and medications on YKL-40 expression.

Discussion

To our knowledge, this is the first study to examine sputum YKL-40 levels in patients with asthma and COPD. Sputum YKL-40 levels were elevated in patients with COPD compared with their age-matched smoker controls but did not differ between patients with asthma and their age-matched controls. In patients with asthma, sputum YKL-40 levels were positively correlated with disease severity and sputum neutrophil counts and were negatively correlated with measures of pulmonary function. In patients with COPD, no significant associations

Fig. 4. Correlations between sputum YKL-40 levels and pre- and postbronchodilator %FEF_{25-75%} in patients with asthma (**a, b**) and COPD (**c, d**).

were found, except for sputum YKL-40 levels with macrophage and neutrophil counts.

Recent evidence suggests that chitinases [20] and chitinase-like proteins including YKL-40 [21] are involved in the pathophysiology of asthma. Chupp et al. [9] reported that serum YKL-40 levels were increased in patients with asthma and that these levels were positively correlated with disease severity (p value for trend = 0.02) and sub-basement membrane thickness in bronchial biopsy ($r = 0.51, p = 0.003$) and were negatively, although weakly, correlated with the levels of FEV₁ ($r = -0.22, p = 0.01$). The same researchers showed that the *CHI3L1* gene encoding YKL-40 had a single-nucleotide polymorphism in its promoter region that was associated with elevated YKL-40 protein levels, asthma susceptibility, airway hyperresponsiveness and impaired lung function [10]. Our results confirmed and expanded upon previous findings.

In agreement with the results of the serum sample analysis performed by Chupp et al. [9], sputum YKL-40 levels in patients with asthma correlated with disease severity and degree of airflow obstruction. To evaluate irreversible functional changes, we recruited patients who were in a stable condition and also evaluated postbronchodilator indices of pulmonary function. Moreover, in contrast to previous studies, we examined sputum samples; these samples provide more direct information on airway conditions than serum samples. Consequently, correlations of YKL-40 levels with both pre- and post-FEV₁ values as well as FEF_{25-75%} values were stronger than those reported in a previous study on asthma [9], showing that YKL-40 in the airways was associated with airway remodeling in asthma.

Although the biologic functions of YKL-40 have not been completely elucidated, YKL-40 may be involved in persistent airway inflammation as well as tissue repair in

Fig. 5. Correlations between sputum YKL-40 levels and sputum neutrophil counts (**a, c**) and macrophage counts (**b, d**) in patients with asthma (**a, b**) and COPD (**c, d**). Logarithmic results are given for sputum YKL-40 levels, and sputum neutrophil and macrophage counts.

asthma as described below. Within 10 min after administration of segmental allergen challenges, the YKL-40 levels in BALF samples obtained from patients with allergic asthma increased and remained elevated for up to 24 h [12]. YKL-40 is induced by the proinflammatory cytokines tumor necrosis factor- α and interleukin-1 [22] as well as by interleukin-13 [23], a potential key regulator of asthma [24], and COPD [25]. Lee et al. [23] showed that mice with null mutations of BRP-39 (BRP-39 $^{-/-}$), a mouse homologue of YKL-40, showed markedly diminished antigen-induced Th2 responses and a decrease in the ability of IL-13 to induce tissue inflammation and fibrosis. YKL-40 also binds to collagen I and regulates collagen fibril formation [26]. These findings suggest that YKL-40 may play a biological role in airway inflammation and tissue remodeling in asthma.

In all groups, YKL-40 was expressed in the cytoplasm of sputum neutrophils and macrophages. This finding is

consistent with previous findings showing the presence of YKL-40 in neutrophils and macrophages in BALF samples obtained from patients with COPD [13] and severe asthma [9]. We found new associations of sputum YKL-40 levels with sputum cell types. Sputum YKL-40 levels were correlated with sputum neutrophil counts in patients with asthma and with both macrophage and neutrophil counts in patients with COPD. In addition, patients with asthma who showed sputum YKL-40 levels below the detection limit had lower sputum neutrophil counts than the remaining asthmatic patients. This association of sputum YKL-40 levels with neutrophil counts and expression of YKL-40 in sputum neutrophils suggests that neutrophils are the major cell source of sputum YKL-40 in asthma; this may partly explain the lack of a difference in sputum YKL-40 levels between asthmatic patients and their age-matched controls, and the fact that a significant number of patients with asthma showed

Fig. 6–12. Representative micrographs of sputum cytospin preparations. At least 3 samples obtained from patients and their age-matched controls are presented. F = Female; M = male; y.o. = years old. **6** Healthy controls. **7, 9** Atopic asthma. **8, 10** Nonatopic asthma. **11** Smoker controls. **12** COPD. **6–8, 11, 12** Double stained with antibody against CD68 and negative control using rabbit IgG (**a**), with antibodies against CD68 and YKL-40 (**b**), with antibody

against NE and negative control using rabbit IgG (**c**), with antibodies against NE and YKL-40 (**d**) and negative control using mouse IgG and rabbit IgG (**e**). **9, 10** Double stained with antibody against MBP and negative control using rabbit IgG (**a**), with antibodies against MBP and YKL-40 (**b**) and negative control using mouse IgG and rabbit IgG (**c**). Red indicates NE, CD68 and MBP; green indicates YKL-40; orange results for merged images.

Fig. 6–12. Representative micrographs of sputum cytospin preparations. At least 3 samples obtained from patients and their age-matched controls are presented. F = Female; M = male; y.o. = years old. **6** Healthy controls. **7, 9** Atopic asthma. **8, 10** Nonatopic asthma. **11** Smoker controls. **12** COPD. **6–8, 11, 12** Double stained with antibody against CD68 and negative control using rabbit IgG (**a**), with antibodies against CD68 and YKL-40 (**b**), with antibody

against NE and negative control using rabbit IgG (**c**), with antibodies against NE and YKL-40 (**d**) and negative control using mouse IgG and rabbit IgG (**e**). **9, 10** Double stained with antibody against MBP and negative control using rabbit IgG (**a**), with antibodies against MBP and YKL-40 (**b**) and negative control using mouse IgG and rabbit IgG (**c**). Red indicates NE, CD68 and MBP; green indicates YKL-40; orange results for merged images.

sputum YKL-40 levels below the detection limit. Neutrophilic airway inflammation plays an important role in a subgroup of patients with asthma [27] and is correlated with fixed airflow obstruction [28, 29] but is not a predominant feature in this patient population as a whole. In fact, sputum neutrophil counts were similar between patients with asthma and their age-matched controls in our study. Although correlations do not imply causation, in the case of asthma, YKL-40 in the airways may contribute to airflow obstruction in association with neutrophilic inflammation.

Recently, Tang et al. [11] reported a moderate negative correlation of serum YKL-40 levels with %FEV₁ ($r = -0.44$, $p = 0.001$) and a mild correlation with peripheral blood eosinophil percentages ($r = 0.27$, $p = 0.032$) in patients with asthma. In addition, Kuepper et al. [12] reported that YKL-40 levels in BALF of patients with allergic asthma after administration of segmental allergen challenges were positively correlated with eosinophil counts in the BALF. In our study, sputum YKL-40 levels were not correlated with sputum eosinophil counts. Our results, however, do not contradict previous findings because the asthmatic patients in our study were in a stable condition whereas 74% of the patients in the study by Tang et al. [11] had exacerbations. Moreover, neutrophilic and eosinophilic airway inflammations are not reciprocally exclusive in asthma, particularly in patients with poor asthma control [30].

In our study, the atopic status of patients with asthma did not affect sputum YKL-40 levels. Studies on the association of the *CHI3L1* gene with atopy have shown inconsistent findings: some single-nucleotide polymorphisms were associated with risks of atopy [31] whereas others showed protective effects [32]. Possible associations of YKL-40 with atopy should be further clarified.

Unexpectedly, we found no correlations between sputum YKL-40 levels and clinical indices, including the presence of chronic bronchitis, in patients with COPD. This finding was in contrast to the findings of Ltue et al. [13], who reported negative correlations between BALF YKL-40 levels and FEV₁ values and carbon monoxide diffusion capacity in patients with COPD. They showed that YKL-40 contributed to the synthesis of proinflammatory and fibrogenic chemokines by alveolar macrophages in COPD. The discrepancies between our findings and the findings of Ltue et al. cannot be fully explained but may be attributed to better %FEV₁ values in our study than in the study of Ltue et al. (the median %FEV₁ was 78.8% in our study and 61.5% in theirs) [13] and different sample sources, i.e. sputum versus BALF. Sputum is de-

rived from more proximal airways and contains fewer macrophages than those present in BALF. In addition to the lack of differences in sputum YKL-40 levels between COPD patients with and without chronic bronchitis, the discrepancy between our findings in COPD and asthma may suggest that YKL-40 in the airways is differently involved in the pathogenesis of COPD and asthma, in particular in terms of the locations that are predominantly involved, although the findings in asthma and COPD in this study cannot be compared directly because there were significant differences in patients' characteristics, such as age and gender, between the two patient groups.

Our study has several limitations. First, the number of age-matched smoker controls was small because older smokers without airflow limitation were difficult to find and recruit. However, the difference in sputum YKL-40 levels between patients with COPD and smoker controls was significant. Second, we did not assess possible relationships between clinical indices and the degrees of YKL-40 expression in sputum cells because cells obtained from sputum samples were inadequate for quantifying the extent of YKL-40 expression. The number of epithelial cells, which also express YKL-40 in severe asthma [9], was not assessed because the epithelial cells in the sputum were too few to be analyzed. Last, assigning values of 10 ng/ml when sputum YKL-40 levels were below this threshold may have overestimated actual sputum YKL-40 levels. Our findings, however, did not change even when values of 0.1 ng/ml were assigned (data not shown). The associations of sputum YKL-40 levels with clinical indices in asthma were robust.

In conclusion, elevated sputum YKL-40 levels reflect airflow obstruction only in asthma. Further analysis may be required to determine the roles of YKL-40 in the proximal airways in COPD.

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Financial Disclosure and Conflicts of Interest

None.

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