

ORIGINAL ARTICLE

A Chitinase-like Protein in the Lung and Circulation of Patients with Severe Asthma

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ABSTRACT

BACKGROUND

The evolutionarily conserved 18-glycosyl-hydrolase family contains true chitinases and chitinase-like proteins that lack enzymatic activity. Acidic mammalian chitinase has recently been associated with animal models of asthma. The related chitinase-like protein, YKL-40 (also called human cartilage glycoprotein 39 [HCgp-39] and chitinase 3–like 1), can be readily measured in the serum. However, its relationship to asthma has not been evaluated.

METHODS

We quantified serum YKL-40 levels in three cohorts of patients with asthma — one recruited from the patient population at Yale University, one from the University of Paris, and one from the University of Wisconsin — as well as in controls from the surrounding communities. In the Paris cohort, immunohistochemical analysis and morphometric quantitation were used to evaluate the locus of expression of YKL-40 in the lung. The clinical characteristics of the patients with high serum or lung YKL-40 levels were also evaluated.

RESULTS

Serum YKL-40 levels were significantly elevated in patients with asthma as compared with controls. In the Paris cohort, lung YKL-40 levels were elevated and were correlated with circulating YKL-40 levels ($r=0.55$, $P<0.001$) and with airway remodeling (measured as the thickness of the subepithelial basement membrane) ($r=0.51$, $P=0.003$). In all three cohorts, serum YKL-40 levels correlated positively with the severity of asthma and inversely with the forced expiratory volume in 1 second. Patients with elevated levels of YKL-40 had significantly more frequent rescue-inhaler use, greater oral corticosteroid use, and a greater rate of hospitalization than patients with lower levels.

CONCLUSIONS

YKL-40 is found in increased quantities in the serum and lungs in a subgroup of patients with asthma, in whom expression of chitinase in both compartments correlates with the severity of asthma. The recovery of YKL-40 from these patients indicates either a causative or a sentinel role for this molecule in asthma.

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CHITINASES ARE A FAMILY OF EVOLUTIONARILY conserved hydrolases characterized by the ability to cleave the environmentally abundant polysaccharide chitin.¹⁻³ Mammals do not synthesize chitin, but they do synthesize chitinases.⁴ We recently showed, in an animal model of asthma, that chitinases are important effector molecules in the airway inflammation that occurs in the disease.^{5,6} In those studies, a true chitinase, acidic mammalian chitinase, was shown to play a critical role in inflammation driven by type 2 helper T (Th2) cells and was expressed in an exaggerated manner in tissues from patients with asthma. Those experiments also demonstrated that the level of expression of the chitinase-like protein YKL-40 (a chitinase homolog, also called human cartilage glycoprotein 39 [HCgp-39] and chitinase 3-like 1, that lacks chitinase activity) was increased during Th2-type inflammation.⁷ Although a number of studies have suggested that YKL-40 has a role in inflammation and tissue remodeling in human disease,⁸⁻¹² its expression in human asthma has not been investigated.

We hypothesized that the level of expression of YKL-40 would be increased in patients with asthma and would correlate with the severity of the asthma. To test this hypothesis, we quantitated the levels of circulating YKL-40 in three cohorts of patients with asthma and controls and compared these findings with the severity of asthma, levels of expression of YKL-40 in the airway, and measures of airway remodeling.

METHODS

STUDY DESIGN AND SUBJECTS

We performed a cross-sectional analysis of serum samples from an established cohort of patients with asthma from Yale University, in New Haven, Connecticut, plus controls. On the basis of the results of this exploratory analysis, we analyzed sets of serum samples from a cohort of patients with asthma from the University of Paris, plus controls, and from a cohort of patients with asthma from the University of Wisconsin in Madison, plus controls. In addition, the levels of expression of YKL-40 in the airway and their relationship to serum YKL-40 levels was investigated in the Paris cohort. The methods of recruitment of patients with asthma and controls from the surrounding community were similar at each of the three centers. Each center had its own inclusion and exclu-

sion criteria for controls and patients with asthma and its own criteria for the severity of asthma, based on established guidelines (see the Supplementary Appendix, available with the full text of this article at www.nejm.org).¹³

In the Yale and Wisconsin cohorts, patients with asthma were stratified according to the severity of asthma as defined by medication requirements to achieve asthma control (the use of a rescue inhaler ≤ 2 days per week). The case definition for mild asthma was asthma requiring the use of 200 μg or less of fluticasone per day, or the equivalent, to achieve asthma control. Moderate asthma was defined as that requiring the use of 200 to 800 μg of fluticasone per day, or the equivalent, to achieve control. In the Paris cohort, the case definition for mild asthma was asthma requiring exclusive treatment with short-acting inhaled β_2 -agonists only when needed for asthma symptoms. The definition of moderate asthma was asthma requiring treatment with 250 to 500 μg of fluticasone per day, or the equivalent, in addition to long-acting β_2 -agonists to achieve asthma control. In contrast to the Yale and Wisconsin cohorts, for the Paris cohort, the evaluation of pulmonary function was also used in the case definition for asthma: patients with mild or moderate disease had a prebronchodilator forced expiratory volume in 1 second (FEV_1) of 80% or more of the predicted value and a ratio of FEV_1 to forced vital capacity (FVC) of 0.70 or more; patients with severe asthma had a prebronchodilator FEV_1 of less than 70% of the predicted value and a ratio of FEV_1 to FVC of less than 0.70. In all three cohorts, patients with severe asthma fulfilled the definition of severe asthma adopted by the American Thoracic Society Workshop on Refractory Asthma.¹⁴

Our studies were approved by the human investigation committees at all three institutions. All subjects gave written informed consent.

MEASUREMENT OF SERUM YKL-40 AND SERUM IgE LEVELS

Measurement of serum YKL-40 and serum IgE levels was performed in duplicate with the use of commercially available enzyme-linked immunosorbent assay (ELISA) kits (for YKL-40, Quidel; for IgE, Pharmacia or Dade-Behring); median values are presented. The minimum detection limit of the YKL-40 assay is 20 ng per milliliter. We evaluated the specificity of the YKL-40 ELISA by

Table 1. Baseline Characteristics of the Patients.*

Characteristic	Controls			Patients with Asthma			P Value for Controls vs. All Patients	P Value for Mild vs. Moderate vs. Severe†
		All	Mild	Moderate	Severe			
Yale cohort								
No. of patients	38	97	24	29	44			
Age — yr	42±11	44±13	39±12	48±14	44±12	0.38		0.07
Male sex — no. (%)	11 (29)	24 (25)	9 (38)	5 (17)	10 (23)	0.67		0.22
Race or ethnic group — no. (%)								
White	22 (58)	59 (61)	20 (83)	23 (79)	16 (36)			
Black	5 (13)	22 (23)	1 (4)	5 (17)	16 (36)			
Latino	8 (21)	14 (14)	2 (8)	1 (3)	11 (25)			
Other	3 (8)	2 (2)	1 (4)	0	1 (2)			
History of atopy — no. (%)	8 (21)	79 (81)	15 (62)	28 (97)	41 (93)	0.001		0.02
BMI	27.1±4.1‡	31.4±8.3‡	27.9±5.9	30.6±7.6	33.8±8.8	0.01		0.03
Serum IgE — IU/ml	69.3±84.9‡	142.1±143.3	110.8±180.9	155.5±126.7	150.4±131.3	0.001		0.06
Asthma history								
Age at onset — yr			20	19	18			0.59
Median			1–50	1–54	1–50			
Range								
Duration — yr								0.04
Median			18	26	26			
Range			1–54	3–71	6–49			
Hospitalized for asthma — no. (%)			6 (26)‡	12 (41)	35 (80)			<0.001
History of intubations — no. (%)			2 (9)‡	2 (7)	15 (34)			0.001
No. of days of rescue use/wk								
Median			1.5	2.2	6.1			<0.001
Range			0.0–7.0	0.0–7.0	0.0–7.0			
ICS used/day — µg								
Median			376	505	871			<0.001
Range			0–1000	0–1000	0–1000			
OCS used/day — mg								
Median			0	0.17	6.36			<0.001
Range				0.00–5.00	0.00–40.00			

No. of OCS tapering periods in previous year					<0.001
Median	0.42	0.66	4.21		
Range	0.00–2.00	0.00–3.00	0.00–12.00		
FEV ₁ — % of predicted value	107±16	93±18	73±23§		<0.001
FEV ₁ :FVC	0.79±0.1	0.71±0.1	0.65±0.13§		<0.001
FEF _{25–75} — % of predicted value	96±37	59±27‡	41±23§		<0.001
Paris cohort					
No. of patients	12	10	15		
Age — yr	51.7±9.6	43.1±13.9	50.7±14.4	0.38	
Male sex — no. (%)	6 (50)	4 (40)	7 (47)		
Race or ethnic group — no. (%)					
White	8 (67)	11 (73)	9 (90)	10 (67)	
Black	3 (25)	4 (27)	0	5 (33)	
Other	1 (8)	0	1 (10)	0	
History of atopy — no. (%)	0	13 (87)	7 (70)	11 (73)	
Serum IgE — IU/ml	22.6±17.8	298.4±271.6	332.5±401.1	652.7±801.7	<0.001
Asthma history					
Age at onset — yr		25.3±19.9	28.4±19.0	28.7±16.0	<0.001
Duration — yr		17.9±12.9	22.4±19.6	21.9±15.4	0.24
FEV ₁ — % of predicted value					
Before β_2 -agonist use		96.6±15.1	87.5±11.4	54.3±11.9	<0.001
After β_2 -agonist use		102.0±14.8	94.6±12.8	62.8±13.5	<0.001
FEV ₁ :FVC before β_2 -agonist use		0.75±0.10	0.73±0.12	0.54±0.11	<0.001
ICS used/day — μ g		0	950±158	3200±1000	
OCS used/day — mg		0	0	10.0±19.6¶	
Wisconsin cohort					
No. of patients	9	15	15	27	
Age — yr	22±2	26±7	26±8	39±12	<0.001
Male sex — no. (%)	2 (22)	7 (47)	9 (60)	12 (44)	0.35
Race or ethnic group — no. (%)					0.17
White	9 (100)	13 (87)	11 (73)	22 (81)	
Black	0	2 (13)	2 (13)	5 (19)	
Other	0	0	2 (13)	0	
Serum IgE — IU/ml	26.1±24.8	316.1±409.1	294.7±283.7	236.0±309.0	0.57

Table 1. (Continued.)

Characteristic	Controls			P Value for Controls vs. All Patients	P Value for Mild vs. Moderate vs. Severe ^j
	All	Patients with Asthma	Severe		
Asthma history					
Age at onset — yr					0.03
Median		16	9	18	
Range		4–35	1–19	1–43	
Duration — yr					0.045
Median		10	19	16	
Range		2–20	5–40	1–44	
Hospitalized for asthma — no. (%)		1 (7)	5 (33)	15 (56)	0.005
History of urgent care — no. (%)		5 (33)	6 (40)	18 (67)	0.009
ICS used/day — μ g					<0.001
Median		0	500	1000	
Range		0–880	400–640	0–1880	
FEV ₁ — % of predicted value		100 $\pm$ 13	88 $\pm$ 17	69 $\pm$ 23 [‡]	<0.001
FEV ₁ :FVC		0.80 $\pm$ 0.10	0.71 $\pm$ 0.10	0.66 $\pm$ 0.14 [‡]	<0.001

* Plus-minus values are means $\pm$ SD. Continuous data were compared with the use of the Kruskal–Wallis test, and categorical data were compared with the use of Pearson's chi-square test. Race or ethnic group was assigned by study investigators. The body-mass index (BMI) is the weight in kilograms divided by the square of the height in meters. ICS denotes inhaled corticosteroids, OCS oral corticosteroids, FEV₁ forced expiratory volume in 1 second, FVC forced vital capacity, and FEV₁:FVC forced expiratory flow between 25 and 75% of the FVC.

† The P value was calculated for the comparison among the patients with mild, moderate, or severe asthma (by the Kruskal–Wallis test).

‡ Data are missing for one subject.

§ Data are missing for two subjects.

¶ Data are from the four patients with severe asthma who were treated with oral corticosteroids.

confirming that the capture and detection antibodies lacked cross-reactivity to other human chitinases, including acidic mammalian chitinase, YKL-39, and chitotriosidase.

FIBER-OPTIC BRONCHOSCOPY AND SAMPLE COLLECTION

At the University of Paris, bronchoscopy was performed to obtain specimens for biochemical and immunohistochemical analysis. The bronchoscopy was performed on the same day as phlebotomy, according to the guidelines of the American Thoracic Society.^{15,16} The participating patients with asthma had not had an exacerbation within 2 months before bronchoscopy. Bronchoalveolar lavage was performed with the use of 50 ml of sterile saline, and the samples were immediately centrifuged (at 150×g for 10 minutes at 4°C). Cytospin preparations from cell pellets were fixed in acetone for 10 minutes at room temperature. Four bronchial-biopsy specimens were taken from the bifurcations of the right middle or lower lobes, immediately immersed in Tissue-Tek compound (Sakura Finetek), snap-frozen in liquid nitrogen, and kept at -80°C until they were processed.

IMMUNOHISTOCHEMICAL AND MORPHOMETRIC ANALYSES

Serial 5-μm sections of bronchial-biopsy specimens were mounted on glass slides coated with γ-methacryloxypropyltrimethoxysilane (Sigma) and fixed in acetone for 10 minutes at room temperature. Immunohistochemical analysis was performed as previously described.¹⁶ The primary antibody was rabbit anti-human YKL-40 antibody (1 μg per milliliter) (MedImmune). Control antibodies were rabbit IgG (DakoCytomation) and anti-YKL-40 antibody coincubated with 1 μg of recombinant human acidic mammalian chitinase and chitotriosidase per milliliter. The secondary antibody was biotin-conjugated antirabbit antibody. Detection was accomplished with the use of the avidine-alkaline phosphatase complex (Vector), fast red (DakoCytomation), and a light nuclear Mayer's hematoxylin counterstain.

The number of YKL-40-positive cells was counted per cubic millimeter of bronchial epithelium and subepithelium in tissue sections. The total biopsy area (in cubic millimeters) and thickness of the subepithelial basement membrane were determined by means of morphometry and computer-assisted image analysis (Microvision In-

struments).¹⁶ The areas of biopsy were similar among patients with mild asthma, those with moderate asthma, those with severe asthma, and controls (median, 0.433 to 0.496 mm²; *P*=0.40 by the Kruskal-Wallis test). Immunohistochemical measurements were made at a magnification of 400, and the total biopsy area and thickness of the subepithelial basement membrane were assessed at a magnification of 60.

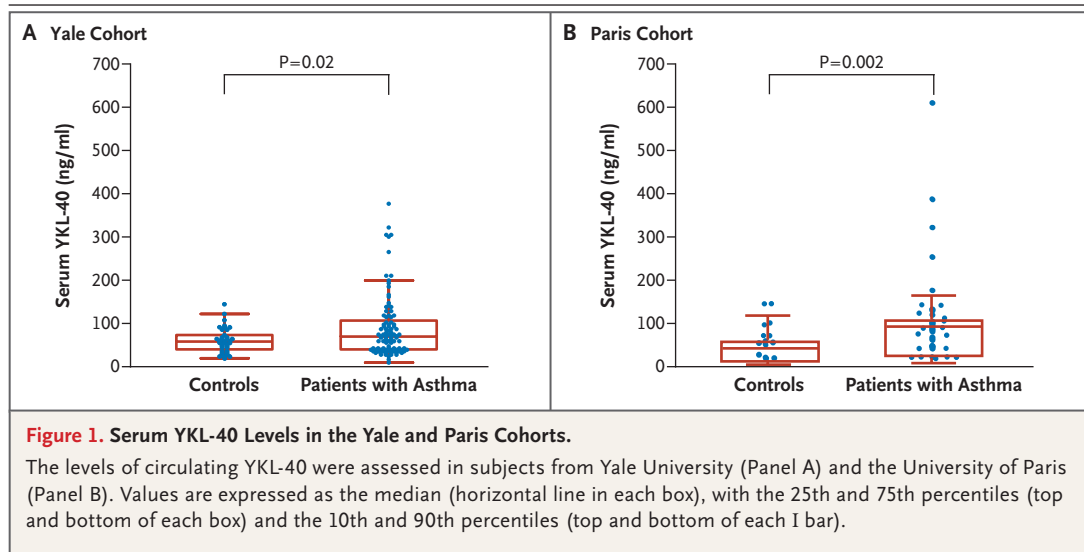
STATISTICAL ANALYSIS

Analyses were performed with the use of SPSS software, version 6.1.3, or MedCalc. We compared the characteristics of subjects using the Kruskal-Wallis test and Pearson's chi-square test. Simple associations were assessed with the use of Spearman's rank-correlation analysis. YKL-40 levels were not normally distributed and therefore were logarithmically transformed. The assumption of normality of the log-transformed values was verified by means of the Kolmogorov-Smirnov test, and the values were compared among the study groups with the use of the independent-samples *t*-test and analysis of covariance with a test for linear trend. The YKL-40 levels are expressed as medians and interquartile ranges. Post hoc comparisons of pairwise differences between patients with mild asthma, those with moderate asthma, and those with severe asthma were evaluated with significance tests corrected with the use of Tukey's test. Multivariable linear regression analysis was performed to determine the associations of variables with YKL-40 in the entire study sample (patients with asthma and controls). The coefficient of variation representing the intrasubject variability was calculated as the square root of the mean squared coefficient of variation for each subject's measurements over the study period.

RESULTS

CHARACTERISTICS OF THE PATIENTS

In the Yale cohort, there were significant differences between controls and patients with asthma in factors associated with asthma; for example, patients with asthma had a higher mean body-mass index (BMI) (*P*=0.01), a history of atopy (*P*=0.001), and elevated IgE levels (*P*=0.001) (Table 1).¹⁷ More black and Latino patients had severe asthma as compared with mild or moderate asthma. As compared with patients with mild or moderate asthma, those with severe asthma had a history of more



hospitalizations, intubations, use of rescue medication, and periods of tapering of oral corticosteroids; a longer duration of asthma; and more severely compromised pulmonary function.

In the Paris cohort, there were significant differences in the levels of IgE, dose of inhaled corticosteroids used for the treatment of asthma, and lung function among patients with mild asthma, those with moderate asthma, and those with severe asthma (Table 1). In the Wisconsin cohort, the severity of asthma was significantly associated with age at asthma onset, asthma duration, number of patients hospitalized for asthma, history of urgent care visits, inhaled-corticosteroid dose, and pulmonary function; these characteristics were generally similar to those in the Yale cohort (Table 1).

SERUM YKL-40 LEVELS

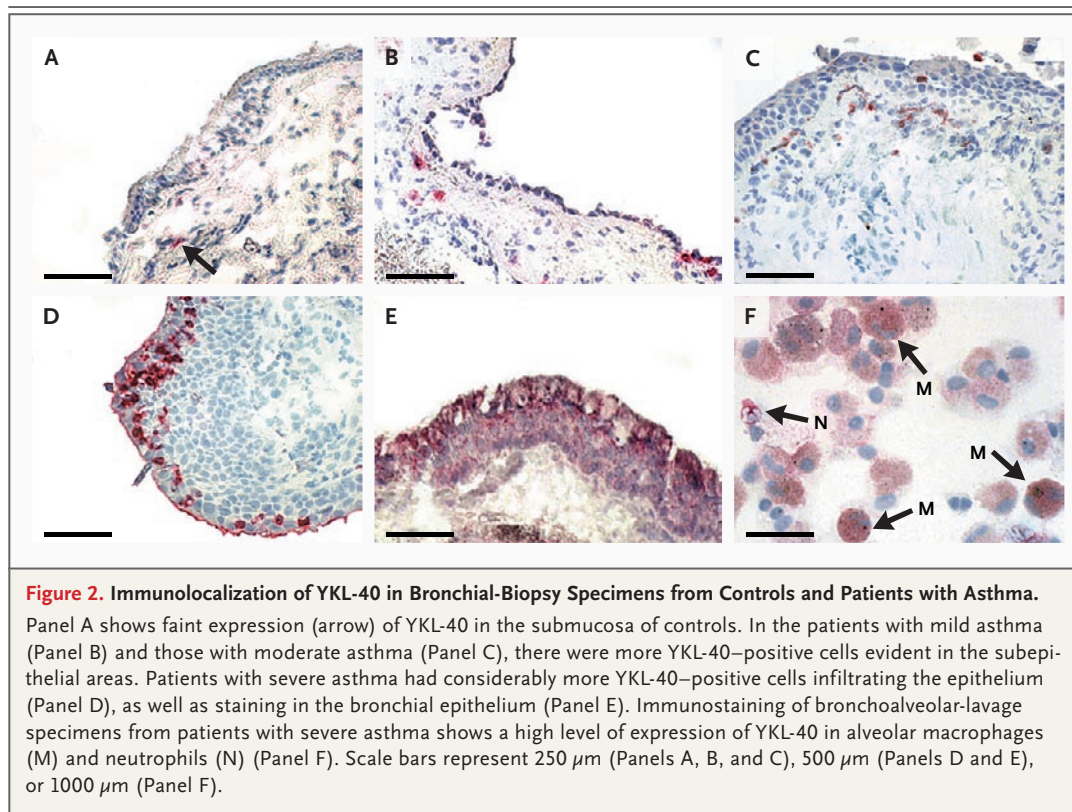
In the Yale cohort, the serum YKL-40 levels in patients with asthma were higher than those in controls (median, 69.7 ng per milliliter [interquartile range, 40.0 to 107.1] vs. 58.3 ng per milliliter [40.0 to 73.3]; $P=0.02$) (Fig. 1A). Among 40 patients with stable asthma and controls who had a total of 114 repeat measurements during the 4-year study period (20 subjects had two measurements, 10 had three measurements, 8 had four measurements, 1 had five measurements, and 1 had seven measurements), the mean coefficient of variation per subject was 37% (data not shown).

The findings in the Yale cohort were subse-

quently confirmed in the Paris cohort (Fig. 1B). Although this population was smaller than the Yale population, serum YKL-40 levels were significantly elevated in patients with asthma as compared with controls (median, 97.7 ng per milliliter [interquartile range, 26.8 to 103.5] vs. 41.5 ng per milliliter [11.5 to 57.8]) ($P=0.002$).

IMMUNOHISTOCHEMICAL ANALYSIS OF YKL-40 IN THE LUNG

The expression of YKL-40 in bronchial-biopsy specimens was evaluated in the Paris cohort (Fig. 2). These studies showed that there were few YKL-40-expressing cells in controls, with significantly increased numbers in patients with asthma (median, 3.1 YKL-40-positive cells per cubic millimeter [interquartile range, 2.1 to 7.4] vs. 16.2 per cubic millimeter [9.1 to 30.2]) ($P=0.005$). YKL-40 staining was seen in subepithelial cells from the majority of patients with asthma (Fig. 2). Among patients with severe asthma, the number of subepithelial cells with YKL-40 staining was increased, and staining of the bronchial epithelium was also evident (Fig. 2D and 2E). In cytospin preparations of bronchoalveolar-lavage specimens from the patients with severe asthma, YKL-40 was also noted in the cytoplasm of macrophages and neutrophils (Fig. 2F). In patients with asthma, lung YKL-40 levels correlated with serum YKL-40 levels ($r=0.55$, $P<0.001$) (Fig. 3). No correlation was found between the number of YKL-40-positive cells in biopsy specimens and the number of in-



flammatory cells (macrophages, eosinophils, lymphocytes, or neutrophils) in bronchoalveolar-lavage specimens (data not shown).

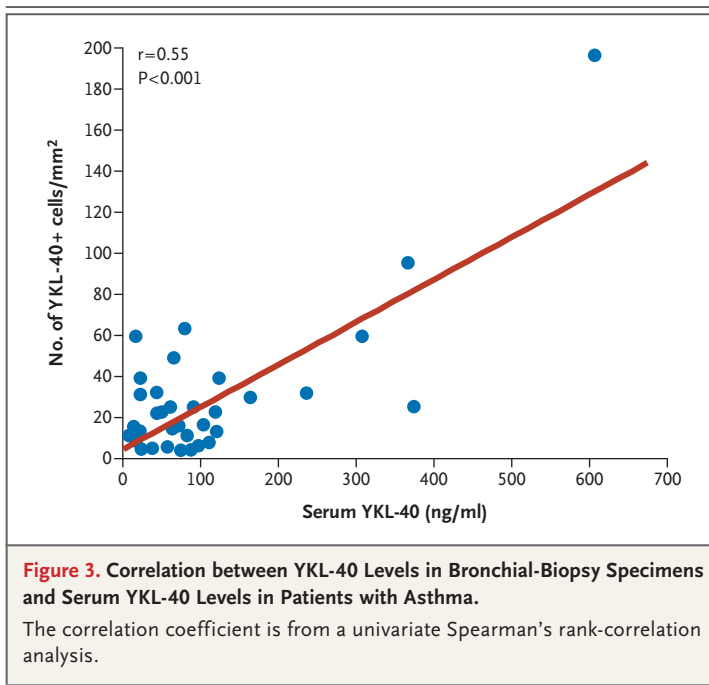
YKL-40 EXPRESSION, SEVERITY OF ASTHMA, AND THICKNESS OF THE SUBEPITHELIAL BASEMENT MEMBRANE

On the basis of the findings described above, the relationship between YKL-40 levels in the airway and the severity of asthma was evaluated (Fig. 4). These studies showed that, in the Paris cohort, the median number of tissue cells expressing YKL-40 correlated positively with the severity of asthma, with 3.1 cells per cubic millimeter (interquartile range, 2.1 to 7.4) in controls, 13.7 per cubic millimeter (3.0 to 28.3) in patients with mild asthma, 11.3 (7.9 to 17.5) in those with moderate asthma, and 23.1 per cubic millimeter (15.2 to 54.1) in those with severe asthma (P for trend = 0.003) (Fig. 4A).

We then studied the relationships among the severity of asthma, the thickness of the subepithelial basement membrane, or YKL-40 levels by comparing these variables in patients in the Paris cohort. The subepithelial basement membrane was

thicker in patients with mild or moderate asthma (median, 9.4 μm [interquartile range, 7.0 to 10.9] and 9.2 μm [8.8 to 10.8], respectively), than in controls (4.7 μm [3.9 to 4.9]; $P < 0.001$ for both comparisons) and was thickest in the patients with severe asthma (12.4 μm [11.5 to 13.4]; $P < 0.001$, $P < 0.001$, and $P = 0.003$ for the comparison with controls, patients with mild asthma, and patients with moderate asthma, respectively). There was a significant correlation between the thickness of the subepithelial basement membrane and the serum YKL-40 levels in this cohort ($r = 0.51$, $P = 0.003$) (Fig. 4B).

The circulating YKL-40 levels and the severity of asthma were also evaluated among patients with asthma in all three cohorts. In the Yale cohort, the median YKL-40 levels were 49.1 ng per milliliter (interquartile range, 36.7 to 94.2) among patients with mild asthma, 68.4 ng per milliliter (38.0 to 88.0) among those with moderate asthma, and 77.0 ng per milliliter (44.6 to 158.4) among those with severe asthma (P for trend = 0.003) (Fig. 4C). This association between the severity of asthma and the circulating YKL-40 levels was also



evident in the Paris cohort, with a median of 45.5 ng per milliliter (interquartile range, 24.5 to 78.5) among patients with mild asthma, 41.0 ng per milliliter (25.0 to 67.0) among those with moderate asthma, and 94.0 ng per milliliter (72.0 to 181.5) among those with severe asthma (P for trend = 0.007) (Fig. 4D), and in the Wisconsin cohort, with 37.5 ng per milliliter (25.1 to 60.9), 62.5 ng per milliliter (42.8 to 80.0), and 64.1 ng per milliliter (45.8 to 82.2), respectively (P for trend = 0.048) (Fig. 4E). Serum YKL-40 levels also correlated inversely with the FEV_1 in all three cohorts (Yale: $r = -0.22$, $P = 0.01$; Paris: $r = -0.21$, $P = 0.005$; and Wisconsin: $r = -0.33$, $P = 0.009$). Thus, the circulating YKL-40 levels correlated with the severity of asthma, the thickness of the subepithelial basement membrane, and pulmonary function in these cohorts.

CHARACTERISTICS OF PATIENTS WITH HIGH YKL-40 LEVELS

To further investigate high circulating YKL-40 levels, we performed a post hoc analysis of the data for correlations between serum YKL-40 levels and asthma characteristics in the Yale cohort (Table 2). The YKL-40 levels correlated positively with a number of clinical measurements associated with severe asthma, including the number of oral corticosteroid tapering periods in the previous year, the

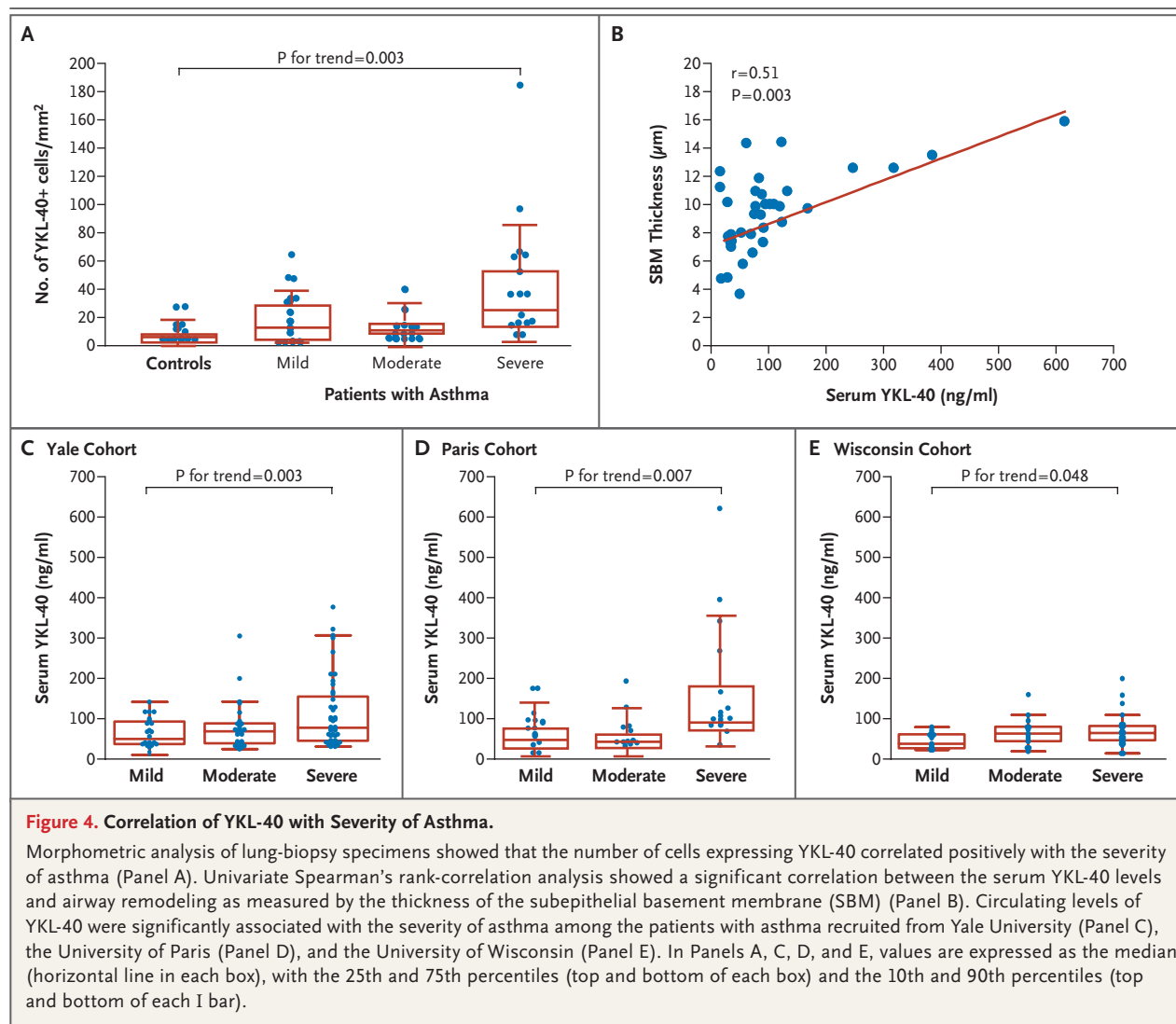
dose of oral corticosteroids, and the frequency of rescue-inhaler use, and correlated negatively with the percentage of the predicted FEV_1 . YKL-40 levels did not correlate with the age at the onset of asthma, the duration of asthma, the number of intubations or hospitalizations, the serum IgE level, or the inhaled-corticosteroid dose.

A post hoc multivariable analysis of the data for the Yale cohort was also undertaken to determine whether the correlation between YKL-40 levels and the severity of asthma persisted after adjustments for confounders that differed significantly according to the severity of asthma and affected YKL-40 levels — including age, sex, race or ethnic group, history of atopy, BMI, and serum IgE level (Table 2). In accordance with our initial observations, this analysis showed that the severity of asthma was independently associated with YKL-40 levels after adjustment for these factors (P for trend = 0.02).

A pairwise examination of differences among the three asthma-severity groups revealed that patients with severe asthma had significantly higher YKL-40 levels than either those with mild asthma or those with moderate asthma ($P = 0.006$ and $P = 0.004$, respectively). Furthermore, patients with asthma who had serum YKL-40 levels above the 50th percentile (60.94 ng per milliliter) had more impaired lung function and required higher doses of corticosteroids than those with YKL-40 levels below this threshold ($P = 0.04$ and $P = 0.007$, respectively) (data not shown).

DISCUSSION

Our data from three independently recruited populations of patients with asthma show that circulating levels of YKL-40 are increased in patients with asthma, as compared with healthy persons, and correlate with the severity of disease. In addition, the serum YKL-40 levels correlate positively with the level of expression of YKL-40 in the airway, the thickness of the subepithelial basement membrane, and clinical indexes of disease severity and correlate inversely with lung function. Although these findings do not allow us to determine whether YKL-40 is a link in the chain of pathogenesis of more severe asthma or a sentinel molecule indicating more severe disease, they support the hypothesis that YKL-40 plays an important role in determining the pathobiologic characteristics of severe asthma.



Multivariable analysis showed that the association between severe asthma and elevated serum YKL-40 levels persisted even after adjustments were made for other variables associated with the severity of asthma, including race or ethnic group, sex, and BMI. Although this finding suggests that elevated circulating YKL-40 levels are a general feature of patients with severe asthma, the association was largely due to increases in the serum YKL-40 levels in the subpopulation of patients with severe asthma. One interpretation of this finding is that severe asthma is a heterogeneous disorder and that elevated YKL-40 levels indicate a pathobiologically distinct form of the disease.

Our studies showed that the association between YKL-40 and asthma could be generalized

across populations of patients with asthma of varying severity, as defined with the use of various criteria, and during treatment with their usual therapeutic regimens. This allowed for the evaluation of multiple cohorts of patients with asthma under "real world" conditions. However, as a result of this design, the studies were cross-sectional, the populations were not strictly controlled in terms of medication use, an exacerbation study was not conducted, and biopsy specimens were available for only one cohort. These limitations need to be kept in mind in interpreting our findings.

Chitin, a polymer of *N*-acetylglucosamine, is the second most abundant polysaccharide in nature. It is an essential part of the exoskeleton of crustaceans and insects, the walls of fungi, and the mi-

Table 2. Correlations of Asthma Measures and Patient Characteristics with Serum YKL-40 Levels.*

Measure or Characteristic	Spearman's Rank Correlation	Adjusted Mean YKL-40 Level (95% CI)†	P Value
Severity of asthma			0.02 for trend
None (controls)		58.6 (45.4–75.6)	
Mild		59.0 (43.6–79.9)	
Moderate		69.2 (50.1–95.8)	
Severe		87.4 (66.6–114.6)	
Serum IgE (IU/ml)	0.45		0.07
Age at asthma onset	0.07		0.52
Duration of asthma	0.11		0.39
Lifetime no. of hospitalizations	0.12		0.27
Lifetime no. of intubations	–0.07		0.53
No. of OCS taper periods in the past yr	0.27		0.01
Micrograms of ICS/day	0.16		0.13
Milligrams of OCS/day	0.24		0.02
No. of days of rescue use/wk	0.22		0.03
FEV ₁	–0.22		0.01
FEV ₁ :FVC	–0.08		0.36
FEF _{25–75}	–0.15		0.10

* OCS denotes oral corticosteroids, ICS inhaled corticosteroids, FEV₁ forced expiratory volume in 1 second, FVC forced vital capacity, and FEF_{25–75} forced expiratory flow between 25 and 75% of the FVC.

† Levels are reported as nanograms per milliliter of serum. Adjustments were made for age, sex, race or ethnic group, history of asthma, body-mass index, severity of asthma, and serum IgE level.

crofilarial sheath of nematodes.^{2,3,18–20} In these settings, chitin confers protection against the harsh conditions in the environment of the organism. Although mammals do not produce chitin, studies have shown that they do express true chitinases, including acidic mammalian chitinase and chitotriosidase.^{1,21–23} These studies also identified the chitinase-like protein YKL-40, which binds chitin but lacks chitinase activity.¹² Although the biologic characteristics of YKL-40 and other chitinase-like proteins remain incompletely understood, a role in inflammation and tissue remodeling has been suggested by the findings that YKL-40 and its murine homolog (breast regression protein 39) regulate stromal-cell proliferation, differentiation, and survival and that elevated circulating YKL-40 levels are present in patients with meningitis, pneumonia, rheumatoid arthritis, osteoarthritis, breast or lung cancer, and hepatic fibrosis.^{10,24,25} Our studies add to the understanding of the biologic characteristics of chitinase-like proteins by defining important relationships between circulating YKL-40 levels and severe asthma.

In conclusion, we have shown that YKL-40 is found in increased quantities in the circulation and lungs of a subpopulation of patients with asthma, in whom the protein levels correlate positively with the severity of disease and the thickness of the subepithelial basement membrane and inversely with lung function. Although our data suggest that YKL-40 either participates in the pathogenesis of asthma or is a biomarker of severity, prospective studies will be required to determine the setting in which serum YKL-40 levels increase in relation to the development of severe disease, whether YKL-40 levels are stable or increase during exacerbations of asthma, and the potential role of serum YKL-40 levels in the management of asthma and asthma research. Our data do, however, indicate that the biologic characteristics of YKL-40 should be incorporated into the understanding of the pathobiologic characteristics of asthma.

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(no. 7,214,373) entitled "Methods, compositions and kits relating to chitinases and chitinase-like molecules and inflammatory disease" was issued on May 8, 2007, and is exclusively licensed to MedImmune (now owned by AstraZeneca); Dr. Elias is one of the inventors. Dr. Elias also reports receiving consulting fees from Eli Lilly, Pfizer, Promedior, MedImmune, Roche, and Millenium. No other potential conflict of interest relevant to this article was reported.

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REFERENCES

1. Elias JA, Homer RJ, Hamid Q, Lee CG. Chitinases and chitinase-like proteins in T(H)2 inflammation and asthma. *J Allergy Clin Immunol* 2005;116:497-500.
2. Meibom KL, Li XB, Nielsen AT, Wu CY, Roseman S, Schoolnik GK. The *Vibrio cholerae* chitin utilization program. *Proc Natl Acad Sci U S A* 2004;101:2524-9.
3. Fuhrman JA, Piessens WF. Chitin synthesis and sheath morphogenesis in *Brugia malayi* microfilariae. *Mol Biochem Parasitol* 1985;17:93-104.
4. Kawada M, Hachiya Y, Arihiro A, Mizoguchi E. Role of mammalian chitinases in inflammatory conditions. *Keio J Med* 2007;56:21-7.
5. Zhu Z, Zheng T, Homer RJ, et al. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. *Science* 2004;304:1678-82.
6. Couzin J. Immunology: unexpectedly, ancient molecule tied to asthma. *Science* 2004;304:1577.
7. Lee CG, Homer RJ, Lee GR, Flavell RA, Elias JA. Generation and use of breast regression protein (BRP)-39 null mice to define the role(s) of BRP-39 in Th2 inflammation and IL-13-induced inflammation and remodeling. *Am J Respir Crit Care Med* 2007;175:A253. abstract.
8. Johansen JS, Jensen HS, Price PA. A new biochemical marker for joint injury: analysis of YKL-40 in serum and synovial fluid. *Br J Rheumatol* 1993;32:949-55.
9. Johansen JS, Williamson MK, Rice JS, Price PA. Identification of proteins secreted by human osteoblastic cells in culture. *J Bone Miner Res* 1992;7:501-12.
10. Kelleher TB, Mehta SH, Bhaskar R, et al. Prediction of hepatic fibrosis in HIV/HCV co-infected patients using serum fibrosis markers: the SHASTA index. *J Hepatol* 2005;43:78-84.
11. Johansen JS. Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibrosis and cancer. *Dan Med Bull* 2006;53:172-209.
12. Hakala BE, White C, Recklies AD. Human cartilage gp-39, a major secretory product of articular chondrocytes and synovial cells, is a mammalian member of a chitinase protein family. *J Biol Chem* 1993;268:25803-10.
13. National Asthma Education and Prevention Program Expert Panel report: guidelines for the diagnosis and management of asthma update on selected topics — 2002. *J Allergy Clin Immunol* 2002;110: Suppl 5:S141-S219. [Erratum, *J Allergy Clin Immunol* 2003;111:466.]
14. American Thoracic Society. Proceedings of the ATS workshop on refractory asthma: current understanding, recommendations, and unanswered questions. *Am J Respir Crit Care Med* 2000;162:2341-51.
15. Summary and recommendations of a workshop on the investigative use of fiberoptic bronchoscopy and bronchoalveolar lavage in asthmatics. *Am Rev Respir Dis* 1985;132:180-2.
16. Benayoun L, Druilhe A, Dombret MC, Aubier M, Pretolani M. Airway structural alterations selectively associated with severe asthma. *Am J Respir Crit Care Med* 2003;167:1360-8.
17. de Marco R, Marcon A, Jarvis D, et al. Prognostic factors of asthma severity: a 9-year international prospective cohort study. *J Allergy Clin Immunol* 2006;117:1249-56.
18. Araujo AC, Souto-Padrón T, de Souza W. Cytochemical localization of carbohydrate residues in microfilariae of *Wuchereria bancrofti* and *Brugia malayi*. *J Histochem Cytochem* 1993;41:571-8.
19. Debono M, Gordee RS. Antibiotics that inhibit fungal cell wall development. *Annu Rev Microbiol* 1994;48:471-97.
20. Shahabuddin M, Kaslow DC. Plasmodium: parasite chitinase and its role in malaria transmission. *Exp Parasitol* 1994;79:85-8.
21. Boot RG, Blommaert EF, Swart E, et al. Identification of a novel acidic mammalian chitinase distinct from chitotriosidase. *J Biol Chem* 2001;276:6770-8.
22. Suzuki M, Morimatsu M, Yamashita T, Iwanaga T, Syuto B. A novel serum chitinase that is expressed in bovine liver. *FEBS Lett* 2001;506:127-30.
23. Renkema GH, Boot RG, Muijsers AO, Donker-Koopman WE, Aerts JM. Purification and characterization of human chitotriosidase, a novel member of the chitinase family of proteins. *J Biol Chem* 1995;270:2198-202.
24. Østergaard C, Johansen JS, Benfield T, Price PA, Lundgren JD. YKL-40 is elevated in cerebrospinal fluid from patients with purulent meningitis. *Clin Diagn Lab Immunol* 2002;9:598-604.
25. Nordenbaek C, Johansen JS, Junker P, Borregaard N, Sørensen O, Price PA. YKL-40, a matrix protein of specific granules in neutrophils, is elevated in serum of patients with community-acquired pneumonia requiring hospitalization. *J Infect Dis* 1999;180:1722-6.

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Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Chupp GL, Lee CG, Jarjour N, et al. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007;357:2016-27.

Supplement to the Text for NEJM #07-3600

Table 1. Inclusion/Exclusion Criteria for the three Asthma Cohorts

Yale University	University of Paris	University of Wisconsin
<u>All Subjects</u> ≥ 18 years of age ≤ 10 pack years of smoking <u>Normals</u> No history of respiratory disease <u>Asthmatics</u> History, physical, and physiologic testing consistent with a diagnosis of asthma	<u>All Subjects</u> ≥ 18 years of age ≤ 5 pack years of smoking <u>Normals</u> No history of allergies Normal skin prick tests Normal lung function without bronchodilator reversibility <u>Asthmatics</u> History, physical, and physiologic testing consistent with a diagnosis of asthma	<u>All Subjects</u> 18-55 years of age ≤ 10 pack years of smoking <u>Normals</u> No history of allergies Normal skin prick tests Normal lung function without bronchodilator reversibility <u>Asthmatics</u> Historical evidence of bronchodilator reversibility indicated by a 12% (and 200 ml) increase in FEV1 after: 1) Administration of a short acting β-agonist 2) Treatment with an inhaled corticosteroid 3) Oral prednisone burst OR Methacholine reactivity (PC20) of < 8 mg/ml.