ORIGINAL ARTICLE

Increased YKL-40 and Chitotriosidase in Asthma and Chronic Obstructive Pulmonary Disease

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Abstract

Rationale: Serum chitinases may be novel biomarkers of airway inflammation and remodeling, but less is known about factors regulating their levels.

Objectives: To examine serum chitotriosidase activity and YKL-40 levels in patients with asthma and chronic obstructive pulmonary disease (COPD) and evaluate clinically relevant factors that may affect chitinase levels, including genetic variability, corticosteroid treatment, disease exacerbations, and allergen exposure.

Methods: Serum chitotriosidase (*CHIT1*) activity and YKL-40 (*CHI3L1*) levels, as well as the *CHIT1* rs3831317 and *CHI3L1* rs4950928 genotypes, were examined in subsets of patients with mild to moderate asthma (n = 76), severe asthma (n = 93), and COPD (n = 64) taking part in the European multicenter BIOAIR (Longitudinal Assessment of Clinical Course and Biomarkers in Severe Chronic Airway Disease) study. Blood was obtained at baseline, before and after a 2-week oral steroid intervention, up to six times during a 1-year period, and during exacerbations. Baseline chitinase levels were also measured in 72 healthy control subjects. The effect of allergen inhalation on blood and sputum YKL-40 levels was measured in two separate groups of patients with mild atopic asthma; one group underwent repeated low-dose allergen challenge (n = 15), and the other underwent high-dose allergen challenge (n = 16).

Measurements and Main Results: Serum chitotriosidase and YKL-40 were significantly elevated in patients with asthma and those with COPD compared with healthy control subjects. Genotype and age strongly affected both YKL-40 and chitotriosidase activity, but associations with disease remained following adjustment for these factors. Correlations were observed with lung function but not with other biomarkers, including exhaled nitric oxide, blood eosinophils, periostin, and IgE. Generally, acute exacerbations, allergen-induced airway obstruction, and corticosteroid treatment did not affect circulating chitinase levels.

Conclusions: YKL-40 and chitotriosidase are increased in asthma and more so in COPD. The data in the present study support these substances as being relatively steroid-insensitive, non–T-helper cell type 2–type biomarkers distinctly related to chronic inflammatory disease processes.

Keywords: asthma; chitotriosidase; chronic obstructive pulmonary disease; YKL-40

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*A complete list of members may be found before the beginning of the References.

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At a Glance Commentary

Scientific Knowledge on the Subject: The chitinases and related proteins have shown promise as novel circulating markers of inflammation in patients with respiratory disease.

What This Study Adds to the Field: We contribute knowledge to this field regarding factors that regulate levels of chitinases and related proteins in the blood. We conclude that these proteins are relatively steroid-insensitive, non–T-helper cell type 2–specific markers of chronic rather than acute disease processes.

To realize the concept of precision medicine in patients with severe airway inflammation, who are notoriously difficult to treat, there is a great need for novel biomarkers of this disease. A relatively unexpected group of proteins has been proposed to show potential for this purpose—the chitinases.

Chitin is an abundant, tough structural polysaccharide used by a variety of organisms, including insects, crustaceans, parasites, fungi, and bacteria, to protect against external threats (1). Although in humans chitin seems absent, enzymes capable of its degradation are expressed. The chitinase family includes the true chitinases—acidic mammalian chitinase (AMCase) and chitotriosidase—as well as the structurally related chitinase-like protein YKL-40, which lacks enzymatic activity (2). In recent years, these proteins have been linked to airway inflammation (2). Although AMCase has been associated with airway inflammation predominantly in animal models (3), chitotriosidase activity is increased in the airways of smokers and subjects with chronic obstructive pulmonary disease (COPD) compared with healthy control subjects (4, 5), as well as in the serum of patients with asthma (6). YKL-40 levels are increased in the serum and lungs of patients with asthma compared with healthy control subjects, and they correlate with markers of disease severity (7, 8).

However, information is still lacking regarding the suitability of these proteins as biomarkers of airway inflammation. It was therefore our aim in this study to provide a more detailed evaluation of serum chitinase levels in airway disease by investigating both serum YKL-40 levels and chitotriosidase activity in parallel in patients taking part in the European multicenter BIOAIR (Longitudinal Assessment of Clinical Course and Biomarkers in Severe Chronic Airway Disease) study (9–11). The design of this study enabled us, for the first time, to examine in the same study the repeatability of chitinase measurements over a 1-year period, the effect of oral steroid treatment, and the effect of spontaneous exacerbations in both patients with asthma and patients with COPD. YKL-40 levels and chitotriosidase activity are subject to genetic regulation and were therefore also analyzed, taking into account the effect of common polymorphisms in the *CHI3L1* and *CHIT1* genes, respectively. *CHI3L1* rs4950928 (−131C>G) has been associated with features of asthma and increased serum YKL-40 (8, 12), and individuals homozygous for a relatively common 24-bp duplication in exon 10 of *CHIT1* (rs3831317) display a complete lack of chitotriosidase activity (13). To assess whether acute airway obstruction and inflammation affect chitinase levels in blood or sputum, we also examined the effect of inhaled allergen on YKL-40 levels in two separate subsets of patients with mild atopic asthma following high-dose or repeated low-dose allergen challenge. Some of the results of these studies have been reported previously in the form of abstracts (14–16).

Methods

Subjects

BIOAIR cohort. BIOAIR is a European multicenter study (9–11). Briefly, subjects were between 18 and 80 years of age and were divided into three groups: patients with mild to moderate asthma, patients with severe asthma, and patients with COPD (Table 1). Further details and definitions regarding the BIOAIR study and patient groups are provided in the online supplement. To examine the repeatability of biomarker measurements, patients attended up to six scheduled clinic visits over the 1-year study period. Patients who had exacerbations had additional visits. Most patients taking part in the BIOAIR study (88% of all subjects) underwent a 2-week, double-blind, placebo-controlled oral steroid intervention consisting of a standard course of prednisolone (0.5 mg/kg body weight/d) added to regular treatment. For certain comparisons, a positive response to therapy was defined as an increase in FEV_1 greater than 12% following steroid treatment.

Healthy subjects. Baseline chitinase measurements were obtained from a group of up to 72 healthy volunteers aged 21–77 years (average age, 40 yr) recruited at the Academic Medical Center, University of Amsterdam, who have been examined in previous studies of chitinase levels (17).

Allergen-challenged subjects with asthma. Plasma and sputum samples were collected from well-characterized patients with mild atopic asthma taking part in two separate allergen challenge studies (*see* online supplement for details). Patients underwent either (*1*) high-dose allergen challenge (n = 16) to produce a 20% acute fall in FEV_1 (18) or (*2*) repeated low-dose allergen challenge (n = 15) leading to minimal bronchoconstriction but eosinophilic airway inflammation and increased bronchial hyperresponsiveness to methacholine (19).

Clinical Measurements

Lung function measurements, reversibility testing, fraction of exhaled nitric oxide (Fe_{NO}) measurements, skin prick testing, and sputum induction and processing were all performed as described in the online supplement. Serum periostin levels were measured by ELISA using two rat antihuman periostin monoclonal antibodies (clones SS18A and SS17B) as described previously (20).

YKL-40 assay. Serum YKL-40 levels were measured by ELISA according to the manufacturer's instructions (Human Chitinase 3-like 1 DuoSet ELISA Development Kit; R&D Systems, Abingdon, UK). Two different dilutions were made for each sample, from which an average was obtained. All samples were analyzed in duplicate in random order. Within-assay variability was 3%, and between-assay variability was 14%.

Chitotriosidase assay. Chitotriosidase activity in the serum was detected fluorometrically using the substrate 4-methylumbelliferyl-(4-deoxy)chitobiose as described elsewhere (21, 22) and as outlined in more detail in the online supplement. To discriminate between chitotriosidase activity and that of AMCase in serum samples, a neutralizing antibody against AMCase was used.

Table 1. Baseline Subject Characteristics (BIOAIR Study)

	Mild to Moderate Asthma	Severe Asthma	COPD	*P* Value
Total number of patients included	76	93	64	ND
Age, yr, mean ± SD (min–max)	43.4 ± 1.6 (21–70)	50.2 ± 1.4 (18–72)	64.3 ± 1.1 (47–79)	<0.0001*
Females, %	59.7	57.1	22.6	<0.0001†
FEV_1, % predicted	89.9 ± 2.5	73.2 ± 2.3	48.04 ± 1.9	<0.0001*
FEV_1, L	2.76 ± 0.09	2.08 ± 0.08	1.40 ± 0.08	<0.0001*
FEV_1/FVC	0.70 ± 0.01	0.67 ± 0.01	0.51 ± 0.01	<0.0001†
Reversibility, ΔFEV_1 % predicted	10.5 ± 0.7	8.7 ± 0.7	3.4 ± 0.5	<0.0001†
ICS (beclomethasone equivalent), μg, median (mean ± SD)	775 (606 ± 223)	1,600 (2,044 ± 912)	800 (1,062 ± 631)	<0.0001†
OCS (prednisolone equivalent),‡ mg, median (mean ± SD)	—	10 (14.15 ± 11.8)		ND
BMI, kg/m^2	25.2 ± 0.5	28.3 ± 0.6	27.3 ± 0.7	0.0004§
St. George's Respiratory Questionnaire score	23.2 ± 2.2	44.8 ± 2.1	44.5 ± 2.3	<0.0001§
Asthma Control Questionnaire score	1.03 ± 0.7	2.03 ± 0.1	—	<0.0001‖
CRP, mg/L	4.6 ± 1.2	5.6 ± 0.8	10.4 ± 2.2	0.01†
Atopy, %	46.9	37.6	—¶	1.0**
F_{ENO}, ppb	39.3 ± 4.4	46.9 ± 6.6	17.0 ± 2.9	0.002*
Serum periostin, ng/ml	86.0 ± 25.75	93.5 ± 45.21	80.1 ± 24.02	0.05†
Sputum eosinophils, %	5.9 ± 1.8	17.9 ± 3.7	3.3 ± 1.7	0.001*
Sputum neutrophils, %	45.8 ± 4.4	40.4 ± 3.8	62.7 ± 5.1	0.004†
Blood eosinophils, 10^8/L	3.20 ± 0.4	3.75 ± 0.4	2.72 ± 0.4	0.22
Blood neutrophils, 10^9/L	3.78 ± 0.15	5.43 ± 0.29	5.03 ± 0.23	<0.0001§

Definition of abbreviations: BIOAIR = Longitudinal Assessment of Clinical Course and Biomarkers in Severe Chronic Airway Disease; BMI = body mass index; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; F_{ENO} = fraction of exhaled nitric oxide; ICS = inhaled corticosteroids; ND = not determined; OCS = oral corticosteroids.
Data are mean ± SD unless otherwise noted.
*Three-way comparison significant; all groups are different.
†Significant difference between patients with severe asthma and those with COPD.
‡One patient with COPD received 60 mg/d of prednisolone.
§Significant difference between patients with severe and those with mild to moderate asthma.
‖Mann-Whitney *U* test.
¶Atopy was an exclusion criterion for the COPD group.
**χ^2 test.

Genotyping

The *CHIT1* 24-bp duplication (rs3831317) and −131C>G polymorphism in the *CHI3L1* gene (rs4950928) were genotyped as described in the online supplement. DNA was available from subjects taking part in the BIOAIR study (n=179), as well as from the healthy control subjects (n=58 for *CHI3L1* and n=72 for *CHIT1*).

Statistics

Basic comparisons were performed using GraphPad Prism statistical software (GraphPad Software, La Jolla, CA), and *P* values <0.05 were accepted as significant. The majority of variables in this study were not normally distributed according to the Kolmogorov-Smirnov test and therefore were analyzed using nonparametric tests. The results are presented as median (interquartile range) unless otherwise stated. Further analyses were performed using Stata 13 software (StataCorp, College Station, TX). Linear regression was used to adjust for age, sex, and genotype, and a linear mixed model was used for dependent samples (e.g., before vs. after treatment). For all regression analyses, logarithmic transformations of chitinase and YKL-40 measurements were performed before analysis. Fisher's exact test was used to examine genotype frequencies between groups. Multiple regression models were constructed to examine the effects of age, sex, genotype, smoking, disease, and lung function on chitinase levels. Some variables showed clear deviations from linearity, and these were fitted using cubic splines with three knots (23).

Results

Baseline Serum YKL-40 Levels and Chitotriosidase Activity

The results are presented as median (interquartile range). Compared with serum YKL-40 levels in healthy control subjects (23.0 [17.1–26.5] ng/ml; n = 48), levels were significantly elevated in patients with mild to moderate asthma (33.3 [22.9–43.5] ng/ml; n = 61; $P < 0.001$) and those with severe asthma (43.3 [31.1–75.9] ng/ml; n = 76; $P < 0.001$) and were highest in patients with COPD (64.0 [37.4–142.2] ng/ml; n = 45; $P < 0.001$) (Figure 1A). All patient groups were significantly different compared with each other (mild to moderate asthma vs. severe asthma, $P = 0.002$; mild to moderate asthma vs. COPD, $P < 0.001$; severe asthma vs. COPD, $P = 0.006$).

Compared with healthy control subjects (62.5 [46.0–90.0] nmol/ml/h; n = 72), serum chitotriosidase activity was greatest in patients with COPD (169.5 [90.0–269.6] nmol/ml/h; n = 52; $P < 0.001$) but was also significantly elevated in subjects with mild to moderate asthma (96.3 [61.3–137.6] nmol/ml/h; n = 63; $P <$

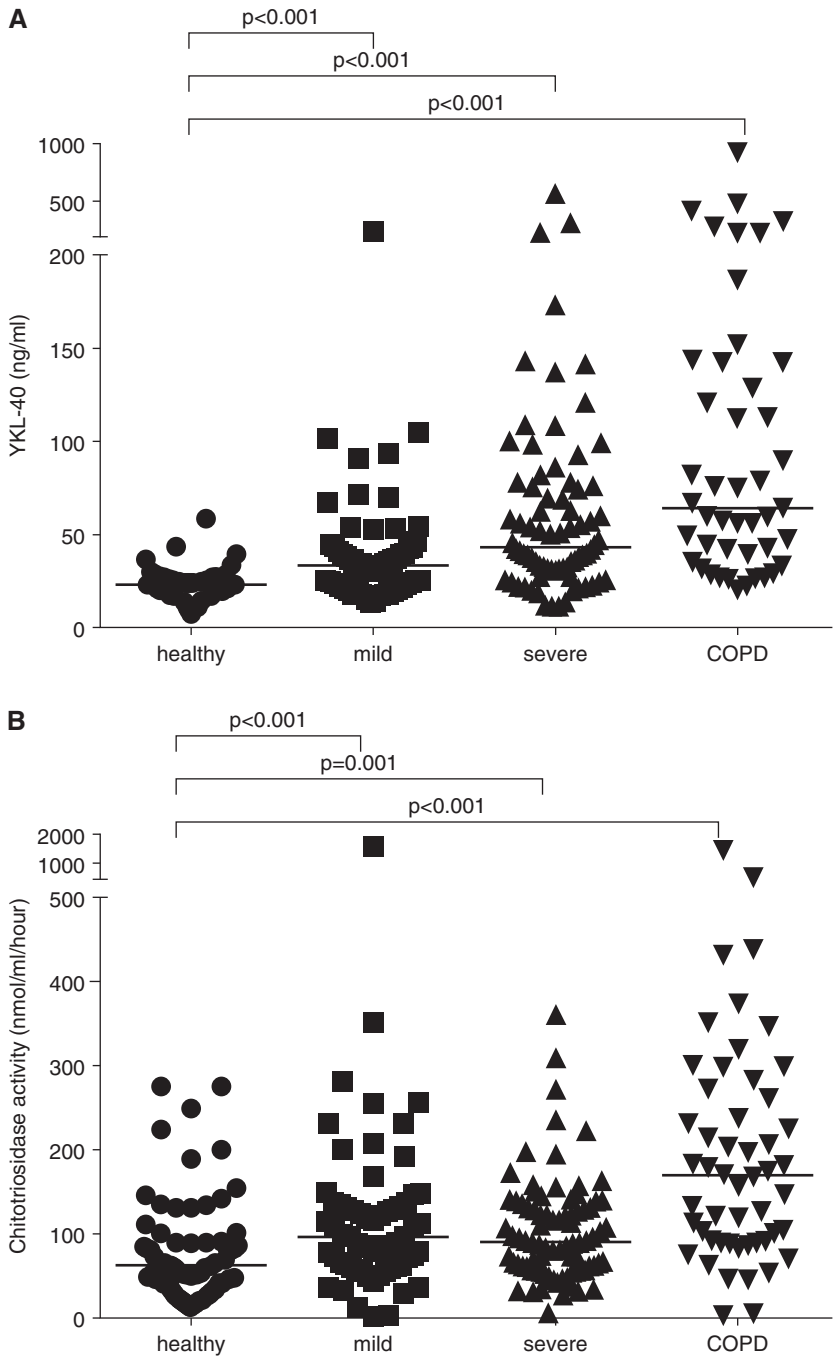

Figure 1. Serum levels of YKL-40 and chitotriosidase activity. (*A*) YKL-40 levels are shown in healthy control subjects and patients with mild to moderate or severe asthma and patients with chronic obstructive pulmonary disease (COPD). (*B*) Chitotriosidase activity is shown in healthy control subjects, patients with mild to moderate or severe asthma, and patients with COPD. *P* values are shown for comparisons with healthy control subjects. The results are presented as individual data points with median *bars*.

0.001) and those with severe asthma (90.7 [58.1–134.8] nmol/ml/h; n = 84; $P = 0.001$) (Figure 1B). Chitotriosidase levels in both asthma groups were lower than those in patients with COPD ($P < 0.001$).

Effect of *CHI3L1* rs4950928 and *CHIT1* rs3831317 Genotype on YKL-40 Levels and Chitotriosidase Activity

The frequencies of the *CHIT1* rs3831317 and *CHI3L1* rs4950928 genotypes in the different subject groups are shown in Table 2. The occurrence of the different genotypes was not different between the subject groups ($P = 0.976$ for *CHI3L1* rs4950928 and $P = 0.421$ for *CHIT1* rs3831317).

YKL-40 levels were related to *CHI3L1* rs4950928 genotype (Figure 2A). When all subjects were analyzed together, those with the CC genotype showed higher levels of serum YKL-40 (39.0 [25.5–75.5] ng/ml; n = 120) than those with the CG genotype (32.2 [20.2–47.5] ng/ml; n = 54; $P = 0.009$) and those with the GG genotype, in whom levels were lowest (18.1 [11.5–28.3] ng/ml; n = 11; $P < 0.001$).

Following regression analyses with adjustment for *CHI3L1* rs4950928 genotype, age, and sex, patients with mild and severe asthma and patients with COPD all still had significantly higher YKL-40 levels than healthy control subjects ($P < 0.001$). A significant difference also remained between patients with mild asthma and those with COPD ($P = 0.018$), but not between patients with mild asthma and those with severe asthma ($P = 0.062$) or between patients with severe asthma and those with COPD ($P = 0.283$).

Serum chitotriosidase activity showed a strong relationship with *CHIT1* rs3831317 genotype (Figure 2B). Subjects homozygous for a 24-bp duplication in exon 10 were completely lacking in chitotriosidase activity, although this genotype was rare among subjects (2.3% in total) and therefore the data were too few to be included in statistical comparisons. Comparing all subjects, those lacking the 24-bp duplication had significantly greater levels of chitotriosidase than heterozygotes (respectively, 113.6 [74.5–156.6] [n = 146] vs. 59.2 [36.0–86.2] nmol/ml/h [n = 76]; $P < 0.0001$).

When between-group differences in chitotriosidase activity were examined in regression analyses with adjustment for age, sex, and genotype, mild asthma and COPD were significantly different compared with healthy control subjects ($P < 0.001$), whereas severe asthma was not ($P = 0.106$). There were also significant differences in chitotriosidase activity between COPD and severe asthma ($P = 0.007$) and between mild and severe asthma ($P = 0.051$), but not between mild asthma and COPD ($P = 0.416$).

Overall, there were no associations between *CHI3L1* rs4950928 or *CHIT1* rs3831317 genotype and subject characteristics (*see* Tables E4 and E5 in the

Table 2. Frequencies of *CHI3L1* rs4950928 and *CHIT1* rs3831317 Genotypes

CHI3L1 rs4950928	CC	CG	GG
Mild to moderate asthma (n = 49)	61.2	30.6	8.2
Severe asthma (n = 62)	63.0	30.6	6.4
COPD (n = 40)	67.5	27.5	5
Healthy control subjects (n = 57)	70.2	24.5	5.3

CHIT1 rs3831317	No 24-bp Duplication	Heterozygous	Homozygous
Mild to moderate asthma (n = 58)	65.5	31.0	3.5
Severe asthma (n = 72)	63.9	34.7	1.4
COPD (n = 49)	67.3	28.6	4.1
Healthy control subjects (n = 64)	60.9	39.1	0

Definition of abbreviation: COPD = chronic obstructive pulmonary disease.
Results are presented as percentages.

online supplement), apart from lower periostin levels in patients with asthma with the rs4950928 GG genotype (CC = 88 [71–109] ng/ml; CG = 83 [67.8–102.3] ng/ml; GG = 57.5 [50.3–68.8] ng/ml; $P = 0.003$) and a higher body mass index (BMI) in patients with asthma lacking the 24-bp duplication at rs3831317, or wild type, compared to those heterozygous for the duplication (wild type = 27 [24–30] kg/m^2; heterozygous = 26 [23–27] kg/m^2; $P = 0.034$). However, it should also be noted that the present study is underpowered to examine clinical characteristics between different genotypes in the resulting subgroups.

Relationships between the Chitinases and Subject Characteristics

Possible correlations between levels of serum YKL-40 and chitotriosidase activity and subject characteristics were examined (Table 3). In all BIOAIR patients, age was found to correlate positively with both YKL-40 ($r = 0.49$; $P < 0.001$) and chitotriosidase activity ($r = 0.47$; $P < 0.001$). Measures of lung function showed negative correlations with both YKL-40 levels (FEV_1 % predicted, $r = -0.42$, $P < 0.0001$; FVC % predicted, $r = -0.40$, $P < 0.0001$; FEV_1/FVC, $r = -0.34$, $P < 0.001$) and chitotriosidase levels (FEV_1, $r = -0.25$, $P < 0.001$; FVC, $r = -0.16$, $P = 0.03$; FEV_1/FVC, $r = -0.23$, $P = 0.001$). In addition, there was a weak but statistically significant correlation between serum YKL-40 level and chitotriosidase activity ($r = 0.221$; $P = 0.003$). Correlations in the separate subject groups are shown in Tables E6 and E7. YKL-40 also showed a weak though significant association with BMI, but only in patients with asthma ($r = 0.21$; $P = 0.01$).

Chitinase Levels in T-Helper Cell Type 2–driven Asthma

In patients with asthma, no significant correlations were observed between either YKL-40 level or chitotriosidase activity and proposed markers of T-helper cell type 2 (Th2)-type inflammation, including serum periostin, blood eosinophils, F_{ENO}, and total IgE (Table E6). To further examine possible relationships between chitinase levels and Th2-type inflammation, patients with asthma were divided into a Th2-high group, based on the presence of both blood eosinophil counts and periostin levels above the group median (median eosinophil count, 0.25×10^9/L; median periostin level, 84 ng/ml), and a Th2-low group, in whom levels of both biomarkers were below the median (Table 4). No differences in chitinase levels were observed between the Th2-low and Th2-high groups.

Effect of Steroid Treatment on Chitinase Levels

The effect of a 2-week, placebo-controlled oral steroid intervention was examined in subjects taking part in the BIOAIR study. No significant reductions in YKL-40 levels were observed following corticosteroid treatment (Figure 3A).

When the effect of steroid treatment was analyzed in all patient groups, serum chitotriosidase activity was slightly reduced following treatment with oral prednisolone (106 [66–168] vs. 101 [63–143] nmol/ml/h; n = 157; $P < 0.001$). However, when patient groups were analyzed separately (Figure 3B), oral steroid treatment caused a reduction in chitotriosidase activity only in patients with COPD (186 [111–279] vs. 128 (86–189) nmol/ml/h; n = 38; $P < 0.001$). When interactions between treatment and age were taken into account, the interaction between treatment and subject groups ceased to be significant. The interaction between treatment and age in this model was negative ($P = 0.001$); that is, the reduction in chitotriosidase activity was greater in older people. There were no interactions between the examined genotypes and effect of corticosteroid therapy on chitinase levels.

In patients with asthma, neither baseline YKL-40 level nor chitotriosidase activity was associated with response to corticosteroid therapy, defined as an increase in FEV_1 greater than 12% following treatment with oral prednisolone. YKL-40 levels in nonresponders were 39 (26–69) ng/ml (n = 102) compared with 33 (25–53) ng/ml in responders (n = 22) ($P = 0.276$), whereas chitotriosidase activity was 94 (61–132) nmol/ml/h in nonresponders compared with 94 (60–158) nmol/ml/h in responders ($P = 0.594$).

Effect of Exacerbations on Chitinase Levels

Compared with baseline, neither YKL-40 level nor chitotriosidase activity was significantly different during exacerbations (Table E8). Exacerbations occurred in 7 patients with mild to moderate asthma, 27 patients with severe asthma, and 11 patients with COPD. Chitinase levels before and during exacerbations showed no significant interactions with age or genotype.

Repeatability of Chitinase Measurements

In the BIOAIR study, patients were followed for 1 year, during which time up to six blood samples were obtained at different time points (not during exacerbations). The data for average between-visit variability for YKL-40 levels and serum chitotriosidase activity, as well as the variability of F_{ENO} and percentage of sputum eosinophils for comparison, are shown in Table 5.

Chitinase Levels and Smoking Status

Of 76 patients with mild asthma, 1 was a current smoker and 14 were former smokers. Of 93 patients with severe asthma, 29 were former smokers and 2 were current

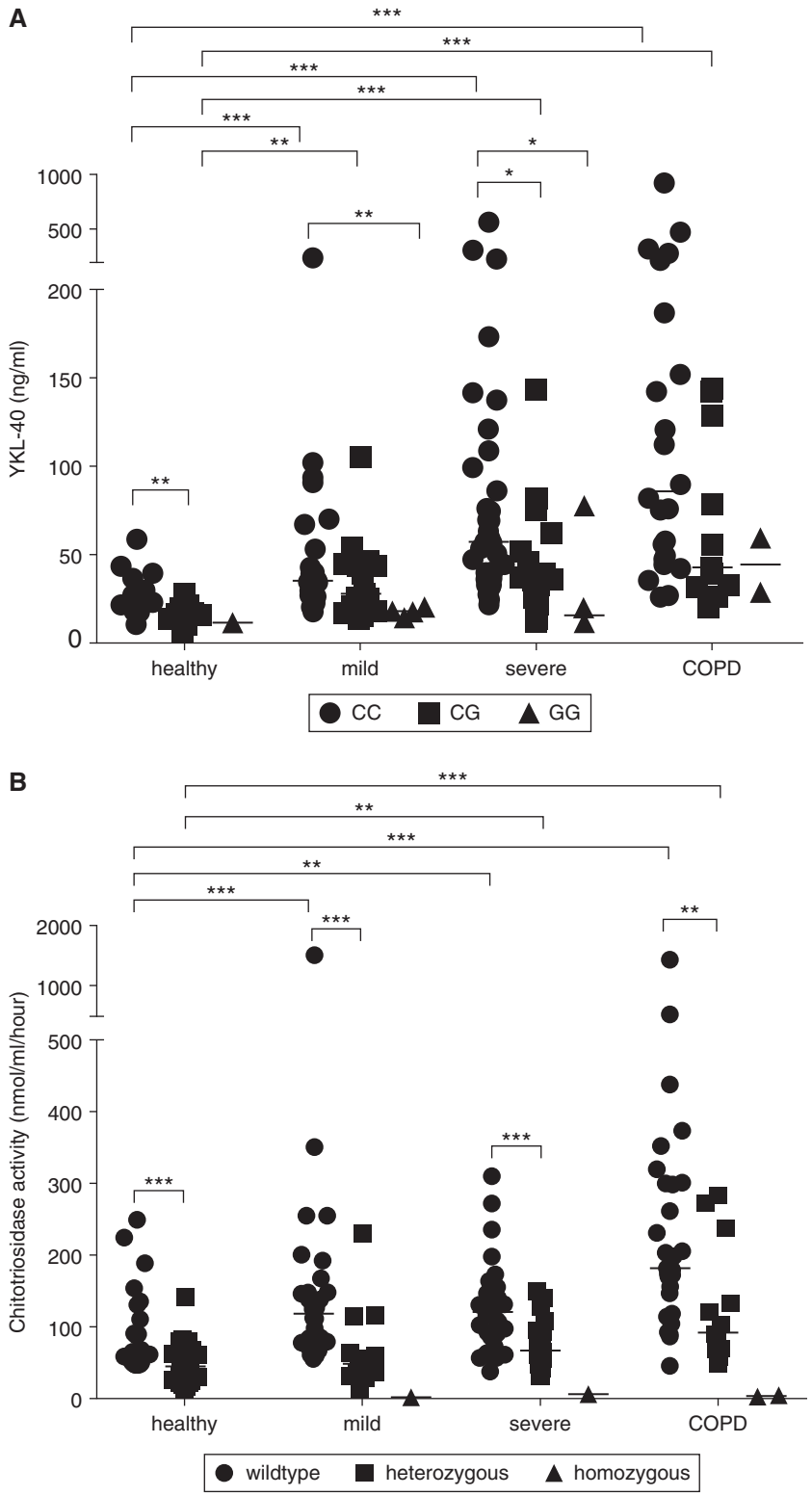

Figure 2. Serum levels of YKL-40 and chitotriosidase activity according to *CHI3L1* rs4950928 and *CHIT1* rs3831317 genotype. (*A*) YKL-40 levels and (*B*) chitotriosidase activity are shown in the different subject groups according to relevant genotype. YKL-40 is shown in subjects with the rs4950928 −131 CC, CG, and GG genotypes. Information regarding *CHI3L1* genotype and YKL-40 levels was available for 39 healthy control subjects and 49 subjects with mild to moderate asthma, 60 with severe asthma, and 37 with chronic obstructive pulmonary disease (COPD). Chitotriosidase activity is shown in subjects lacking a 24-bp

smokers. All 64 patients with COPD were either former (n = 45) or current (n = 19) smokers. We found no significant differences in either YKL-40 or chitotriosidase levels when we compared the patients with asthma who were never smokers with those who had smoked previously, nor did we find such differences when we compared patients with COPD who were former versus current smokers (Figure E2). When univariate correlations were performed on the BIOAIR group as a whole, a significant relationship with number of pack-years smoked was observed (Table 3), although this association was not apparent when the disease groups were analyzed separately (Tables E6 and E7).

Multiple Regression Analyses

As the findings above implied that YKL-40 levels and chitotriosidase activity are affected by several factors, we performed multiple regression analyses to examine the effects of the independent variables age, sex, genotype, smoking status, disease group, and lung function on chitinase levels.

In the BIOAIR patient group as a whole, YKL-40 levels were significantly associated with, in descending order of importance, lung function (FVC % predicted), rs4950928 genotype, age, and sex (Tables 6 and 7). FVC showed a nonlinear relationship with YKL-40 levels, as demonstrated in Figure E3. For the whole patient group model, smoking (in pack-years) and disease group were without significant effects. Multiple regression analyses in the separate groups are shown in Tables E9–E11. Generally similar associations were observed when the groups were analyzed separately, although there was no effect of lung function in patients with mild asthma (Table E9).

In descending order of importance in the BIOAIR group as a whole, genotype, age, and smoking status (in pack-years) were found to have significant effects on chitotriosidase activity. The difference between mild asthma and COPD was significant, but the difference between mild and severe asthma was not. No significant relationships with lung function were observed (Tables 6 and 7). The relationship between chitotriosidase activity and number of pack-years of smoking was not linear, as shown in Figure E4. When the patient groups were

Table 3. Correlations between Subject Characteristics and Chitinase Levels in BIOAIR Subjects

Subject Characteristic	YKL-40 (*ng/ml*)		Chitotriosidase Activity (*nmol/ml/h*)	
	Spearman's Rank Correlation Coefficient	*P* Value	Spearman's Rank Correlation Coefficient	*P* Value
Age, yr	0.49	<0.0001	0.47	<0.0001
BMI, kg/m^2	0.13	0.08	0.02	0.80
FEV_1, L	−0.41	<0.0001	−0.25	<0.001
FEV_1, % predicted	−0.42	<0.0001	−0.17	0.02
FEV_1/FVC	−0.34	<0.0001	−0.23	0.001
FVC, L	−0.31	<0.0001	−0.16	0.03
FVC, % predicted	−0.40	<0.0001	−0.07	0.33
Reversibility, ΔFEV_1 % predicted	−0.20	0.01	−0.19	0.01
ICS, μg/d	0.12	0.11	−0.05	0.51
St. George's Respiratory Questionnaire score	0.21	0.01	0.04	0.58
Asthma Control Questionnaire score	0.22	0.01	0.03	0.74
Periostin, ng/ml	0.12	0.11	−0.04	0.59
Smoking, pack-years	0.32	<0.001	0.34	<0.001
F_{ENO}, ppb	−0.05	0.68	−0.08	0.42
CRP, mg/L	0.17	0.04	0.08	0.33
IgE, kU/L	−0.05	0.51	−0.10	0.16
Sputum eosinophils, %	−0.15	0.16	0.05	0.62
Sputum neutrophils, %	0.16	0.14	0.15	0.13
Sputum macrophages, %	−0.15	0.11	−0.16	0.06
Blood eosinophils, 10^9/L	−0.15	0.05	−0.05	0.54
Blood neutrophils, 10^9/L	0.08	0.28	0.11	0.16

Definition of abbreviations: BIOAIR = Longitudinal Assessment of Clinical Course and Biomarkers in Severe Chronic Airway Disease; BMI = body mass index; CRP = C-reactive protein; F_{ENO} = fraction of exhaled nitric oxide; ICS = inhaled corticosteroids.

analyzed separately (Tables E9–E11), we found that the factors affecting chitotriosidase activity were age and genotype. A relationship with pack-years of smoking was observed only in patients with mild asthma, in whom it should be noted that the number of patients with a positive smoking history was also relatively low (15 of 76).

Effect of Allergen Challenge on YKL-40 Levels

The effects of high-dose and repeated low-dose allergen challenges on plasma and sputum YKL-40 levels were examined in patients with mild atopic asthma (Figure 4). Plasma YKL-40 was not affected by either high-dose allergen challenge or repeated low-dose allergen challenge, although both challenges resulted in significant physiological responses (i.e., reductions in FEV_1 and increased responsiveness toward inhaled methacholine) (18, 19). Sputum YKL-40 levels were significantly higher following high-dose allergen challenge (before challenge, 68 [48–96] ng/g sputum; after challenge, 113 [69–258] ng/g sputum; n = 15; $P = 0.007$), but they were not affected by repeated low-dose allergen challenge. Sputum YKL-40 levels correlated with number of sputum neutrophils in both studies ($r = 0.5$; $P < 0.05$).

Discussion

The main findings of the present study are that serum YKL-40 levels and chitotriosidase activity are elevated in patients with asthma and in patients with COPD compared with healthy control subjects. Both YKL-40 and chitotriosidase were higher in subjects with COPD than in patients with mild to moderate asthma or those with severe asthma, and the increased levels correlated with reduced lung function and were relatively steroid insensitive. We demonstrate that genetic variability and age strongly affect both YKL-40 and chitotriosidase activity but that the increased levels compared with healthy control subjects mostly persisted after adjustment for these factors. Acute changes in airway inflammation caused by allergen exposure or naturally occurring exacerbations were without effect on the circulating chitinases measured, and the variability of serum YKL-40 and chitotriosidase activity over time was relatively low. Taken together, these findings confirm that the chitinases represent a distinct class of biomarkers of severe airway disease and provide important information regarding their use as such.

Serum YKL-40 levels and chitotriosidase activity have not previously been examined in parallel in patients with COPD and patients with asthma. The finding of increased serum YKL-40 in patients with asthma confirms previous observations (7, 8, 24, 25). The higher levels in patients with severe asthma than in patients with mild asthma, along with the correlation between YKL-40 and reduced lung function, strengthen the association of serum YKL-40 with disease severity (7, 8). The exact biological role of YKL-40 remains unclear, but it consistently correlates with airway obstruction in studies of patients with asthma (7, 8, 24). YKL-40 associates with measures of airway remodeling, such as bronchial wall thickness and subepithelial

Figure 2. (Continued). duplication in exon 10 of *CHIT1* rs3831317 (wild type), those heterozygous for the 24-bp duplication, and those homozygous for the 24-bp duplication. Information regarding *CHIT1* genotype and chitotriosidase activity were available from 64 healthy control subjects and 51 patients with mild to moderate asthma, 67 with severe asthma, and 44 with COPD. The results are presented as individual data points with median *bars*. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 4. YKL-40 Levels and Chitotriosidase Activity in Th2-Low and Th2-High Patients with Asthma

	Th2-Low	Th2-High	*P* Value
YKL-40, ng/ml	36.9 (21.9–53.4) (n = 43)	36.5 (25.5–55.6) (n = 46)	0.49
Chitotriosidase activity, nmol/ml/h	84.7 (56.5–122.8) (n = 47)	96.6 (56.0–156.9) (n = 48)	0.19

Definition of abbreviation: Th2 = T-helper cell type 2.
Asthma patients were grouped according to having both serum periostin levels and blood eosinophil numbers above (Th2-high) or below (Th2-low) the group median (median eosinophil count, 0.25×10^9/L; median periostin level, 84 ng/ml). Results are presented as median (interquartile range).

fibrosis (7, 8), which is in line with reports that YKL-40 increases the proliferation of bronchial smooth muscle cells (26, 27) and is involved in fibrotic lung diseases (28). Also of relevance to a role in airway remodeling is the discovery that a high serum YKL-40 level is associated with a greater decline in lung function over time (29). The increased YKL-40 levels in patients with COPD compared with patients with asthma may therefore reflect a greater degree of airway remodeling.

It is widely recognized that asthma is a heterogeneous disorder of multiple phenotypes characterized by differing clinical characteristics and underlying pathobiology. Patients with Th2-driven asthma have been described as being more atopic and more sensitive to corticosteroid therapy and as having more airway eosinophils and a greater degree of bronchial hyperresponsiveness (30). Blood eosinophils, F_{ENO}, and serum periostin levels have been described as potential biomarkers of Th2-type airway inflammation (30). In the present study, we did not observe any relationships between either YKL-40 or chitotriosidase with atopic status, response to corticosteroid therapy, blood eosinophil numbers, or serum periostin levels. Dividing patients into Th2-high and Th2-low groups based on having both high blood eosinophil and high periostin levels did not reveal any differences in chitinase levels.

In accordance with our findings, Jia and colleagues showed that whereas periostin may be the most sensitive and specific indicator of airway eosinophilia, there were no correlations between YKL-40 with periostin, eosinophils (in blood, sputum, or tissue), or F_{ENO} (31). Regarding atopy, the reported findings are variable. On one hand, Chupp and colleagues (7) and Ober and coworkers (12) reported no associations between YKL-40 with atopy or IgE. On the other hand, Tang and colleagues (24) found higher circulating YKL-40 levels in Chinese patients with higher IgE levels, and Specjalski and coworkers (32) made the same observation in patients with atopic versus nonatopic asthma. In the present study, we also observed the highest levels of YKL-40 and chitotriosidase in patients with COPD, further suggesting that the chitinases are not Th2-specific markers of airway disease. Interestingly, a weak association with increased BMI was observed, which has also been described previously (32). In contrast to our findings, initial studies in mice did suggest an involvement in Th2-type processes, as the expression of chitinase proteins (AMCase, Ym1, and Ym2) is dependent on IL-13 (3, 33, 34) and mice lacking Brp39 (YKL-40) have defective IL-13–induced inflammation (35).

Chitotriosidase activity was significantly elevated in patients with mild to moderate asthma compared with healthy control subjects, but not in patients with severe asthma, following adjustment for age, sex, and genotype. Published reports regarding chitotriosidase activity in asthma are few and varied, with differences based on the matrix studied, the severity of patients' disease, and/or patients' current medications. Studies have shown that, compared with healthy control subjects, there may be reduced chitotriosidase activity in bronchoalveolar lavage (BAL) fluid from patients with mild asthma not being treated with steroids (4), no difference in serum from patients with asthma (36), and increased levels in the serum of patients with asthma with or without allergies (6). The lack of association with disease severity, alongside conflicting previous findings, suggests that the involvement of chitotriosidase in asthma is less clear than that of YKL-40.

The increased YKL-40 and chitotriosidase seen in patients with COPD is in accordance with previous observations. Increased BAL chitotriosidase activity has been found in smokers (4, 5), and elevated circulating YKL-40 has been found in patients with COPD compared with control subjects, in whom levels may relate to smoking history (37, 38). In the present study, no differences were observed between current smokers and ex-smokers, suggesting that current smoke exposure *per se* is not responsible for increased chitinase levels. However, a significant relationship between chitotriosidase activity and pack-years of smoking was observed in the multiple regression analyses, suggesting that the degree of airway damage may affect

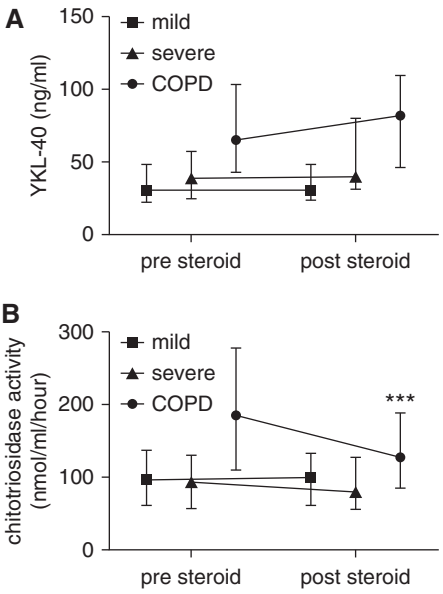

Figure 3. Effect of oral corticosteroid treatment on serum chitotriosidase activity and YKL-40 levels. (*A*) Serum YKL-40 levels and (*B*) chitotriosidase activity were measured before and after 2 weeks of treatment with oral prednisolone. The results are presented as median values (with interquartile range) for patients with chronic obstructive pulmonary disease (COPD), severe asthma, and mild to moderate asthma. Paired comparisons were possible for 55 (YKL-40) and 53 (chitotriosidase) patients with mild to moderate asthma, 67 (YKL-40) and 66 (chitotriosidase) patients with severe asthma, and 38 patients with COPD. ***$P < 0.001$ compared with presteroid values.

Table 5. Biomarker Stability in the BIOAIR Study

	Mild to Moderate Asthma	Severe Asthma	COPD
Chitotriosidase activity	19	16	17
YKL-40	32	36	38
F_{ENO}	45	46	52
Sputum eosinophil percentage	95	91	106

Definition of abbreviations: BIOAIR = Longitudinal Assessment of Clinical Course and Biomarkers in Severe Chronic Airway Disease; COPD = chronic obstructive pulmonary disease; F_{ENO} = fraction of exhaled nitric oxide.
Data represent average between-visit coefficient of variation (as a percentage) of up to six repeat visits.

chitotriosidase activity. There may be involvement of airway neutrophils and macrophages, as these were more abundant in COPD sputum compared with asthma sputum. Both neutrophils and macrophages are known sources of YKL-40 and chitotriosidase (39–41), although no obvious relationships were observed between airway neutrophils and macrophages and either YKL-40 or chitotriosidase in BIOAIR study patients.

We and others have demonstrated that serum levels of both YKL-40 and chitotriosidase increase with age (42, 43). The patient groups in the BIOAIR study were not age matched, but significant between-group differences remained following statistical adjustment for age, suggesting that age alone is not responsible for any of the observed increases in chitinase levels.

YKL-40 and chitotriosidase levels were both strongly affected by variations in the *CHI3L1* and *CHIT1* genes, respectively. Genetic variants in the *CHI3L1* gene have been associated with asthma and related phenotypes (12, 44–46). Ober and colleagues (12) observed a relationship between one particular *CHI3L1* polymorphism (−131C>G rs4950928), circulating YKL-40 levels, and asthma. We confirm that the rs4950928 CC genotype was associated with greater levels of circulating YKL-40; in contrast to Ober and colleagues, however, we did not observe any associations with markers of asthma severity, which has also been the case in other recent investigations (46). As DNA methylation can affect transcription factor binding, we investigated the effect of DNA methylation at three sites located in the *CHI3L1* promoter region near rs4950928 because this single-nucleotide polymorphism affects MYC and MAX binding (12). However, no clear trends were observed between DNA methylation in this region and circulating YKL-40 levels (Figure E1 and Table E3). The *CHIT1* 24-bp duplication (rs3831317) results in a nonfunctional protein lacking enzymatic activity (13). This genetic variant shows no association with asthma and atopy (47, 48), and in the present study no differences in the frequencies of the *CHIT1* rs3831317 variants were observed in the different disease groups.

Table 6. Multiple Regression Analyses in BIOAIR Patients

	β (95% CI)	*P* Value	Adjusted R^2
YKL-40, n = 140			
Age	0.017 (0.009–0.025)	<0.001	
FVC, % predicted, spline 1	−0.032 (−0.044 to −0.021)	<0.001	
FVC, % predicted, spline 2	0.021 (0.010–0.032)	<0.001	
CHI3L1 rs4950928, CC vs. CG	−0.457 (−0.668 to −0.246)	<0.001	
CHI3L1 rs4950928, CC vs. GG	−0.959 (−1.360 to −0.558)	<0.001	
Sex	−0.331 (−0.541 to −0.120)	0.002	
Smoking pack-years	−0.003 (−0.012 to 0.005)	0.419	
Group, MA vs. SA	0.046 (−0.211 to 0.303)	0.725	
Group, MA vs. COPD	0.136 (−0.330 to 0.601)	0.566	
Combined model			0.512
Chitotriosidase, n = 152			
Age	0.019 (0.012–0.025)	<0.001	
CHIT1 rs3831317, WT vs. HET	−0.746 (−0.925 to −0.567)	<0.001	
Pack-years, spline 1	0.042 (0.015–0.068)	0.002	
Pack-years, spline 2	−0.066 (−0.111 to −0.021)	0.005	
Group, MA vs. COPD	−0.569 (−1.080 to −0.057)	0.030	
Group, MA vs. SA	−0.147 (−0.359 to 0.065)	0.174	
Sex	−0.084 (−0.260 to 0.092)	0.347	
FVC, % predicted	0.002 (−0.002 to 0.007)	0.301	
Combined model			0.473

Definition of abbreviations: BIOAIR = Longitudinal Assessment of Clinical Course and Biomarkers in Severe Chronic Airway Disease; CI = confidence interval; COPD = chronic obstructive pulmonary disease; HET = heterozygous for 24-bp duplication at rs3831317; MA = mild to moderate asthma; SA = severe asthma; WT = wild type lacking 24-bp duplication at rs3831317.

The BIOAIR study included a 2-week, placebo-controlled steroid intervention with oral prednisolone. In patients with asthma, steroid treatment did not reduce serum YKL-40 or chitotriosidase activity. There was a reduction in serum chitotriosidase activity, but not of YKL-40, after steroid treatment in patients with COPD, those who were oldest, and those who had the highest levels of chitotriosidase activity. It should be noted, however, that subjects in the BIOAIR study used inhaled corticosteroids. That could potentially have affected basal chitinase levels, as Lai and colleagues recently showed that serum YKL-40 was reduced following 8 weeks of inhaled corticosteroid treatment (49). Nevertheless, as we and others have shown, patients taking the highest doses of ICS are also those with the highest levels of YKL-40, suggesting that YKL-40 release is relatively refractory to steroid treatment (7, 49). One may speculate that the reason why chitinase levels are highest in patients with the most severe disease and taking the highest levels of corticosteroids is that corticosteroids are able to alternatively activate macrophages, a cell type known to release mediators involved in tissue repair (50).

To investigate the repeatability of chitinase measurements, up to six serum samples were collected from the same patients over a 1-year period. The mean coefficients of variation were 16–19% for chitotriosidase and 32–38% for YKL-40. In comparison with other biomarkers measured at the same time points, such as F_{ENO} (coefficient of variation, 45–56%) and sputum eosinophil percentage (coefficient

Table 7. Variable Importance of Multiple Regression Analyses in BIOAIR Patients

Variables	Conditional importance	Unadjusted R^2
YKL-40		
FVC, % predicted	0.12362347	0.543417
CHI3L1 rs4950928 genotype	0.12105902	0.543417
Age	0.05651369	0.543417
Sex	0.03374412	0.543417
Smoking pack-years	0.00231065	0.543417
Group	0.00118992	0.543417
Chitotriosidase		
CHIT1 rs3831317 genotype	0.23784618	0.5007306
Age	0.10398267	0.5007306
Smoking pack-years	0.03569944	0.5007306
Group	0.01845461	0.5007306
FVC, % predicted	0.00375439	0.5007306
Sex	0.00310467	0.5007306

Definition of abbreviation: BIOAIR = Longitudinal Assessment of Clinical Course and Biomarkers in Severe Chronic Airway Disease.

of variation, 91–95%), both chitotriosidase and YKL-40 were relatively stable. Naturally occurring exacerbations, most commonly caused by viral infections, in patients with severe asthma and patients with COPD were without effect on the levels of the chitinases measured, suggesting that they do not reflect acute changes in airway inflammation.

In the present study, circulating YKL-40 remained unchanged following high-dose allergen challenge or repeated low-dose allergen challenge. Sputum YKL-40 was increased by a high-dose allergen challenge. Lee and colleagues also showed that sputum, but not serum, YKL-40 levels increased at 7 and 24 hours after a single high-dose allergen challenge (51). Similarly, Gavala and colleagues found that BAL fluid YKL-40 levels were increased 48 hours after *in vivo* segmental high-dose allergen challenge (52). Taken together, these data seem to show that sputum (and BAL) may reflect a local release of YKL-40 during allergen-induced inflammation that is not reflected in the blood. A single high dose of allergen may be considered a greater challenge for the airways than repeated low doses, and, as the latter is considered a more realistic model of natural allergen exposure, YKL-40 levels may remain relatively stable during such exposures in everyday life. In both studies, sputum YKL-40 correlated with neutrophil numbers, confirming previous reports of the cellular origin of YKL-40 (41).

As the BIOAIR study was designed to compare severe asthma and COPD with mild asthma, the study cohort lacks an internal healthy control group, which is a limitation. To compare chitinase levels with those in healthy subjects, we used a previously described control group (17). However, clinical characteristics of relevance to respiratory disease were not available for these control subjects, thus preventing their inclusion in multiple regression analyses. Nevertheless, performing such analyses for the groups with respiratory disease only was able to further highlight the importance of age, genotype, lung function, and sex on YKL-40 levels and similarly the effects of age, genotype, and smoking history on chitotriosidase activity.

In conclusion, we demonstrate that YKL-40 and chitotriosidase are relatively steroid-insensitive biomarkers that are distinctly elevated in well-characterized patients with asthma and patients with COPD. Taking into account age and genetic variability, the chitinases qualify for measurement as part of new biomarker panels for the assessment of severe, chronic lung inflammation rather than acute lung inflammation. Furthermore, it will be of interest in future longitudinal studies to test the prognostic value of monitoring chitinases as biomarkers of decline in lung function (29), be it in patients with asthma, COPD, fibrosis, or infections or in pediatric conditions such as bronchopulmonary dysplasia. ■

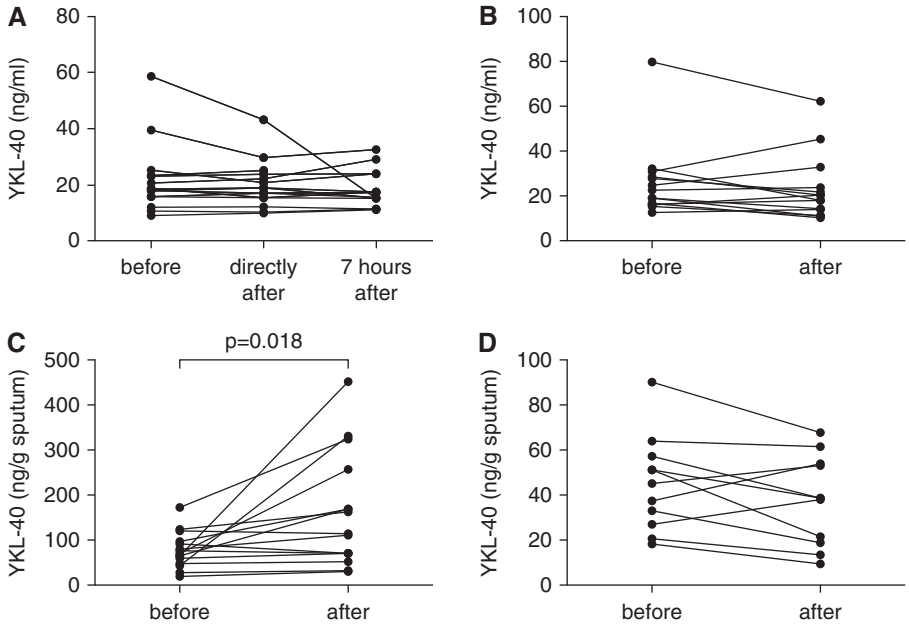

Figure 4. Plasma and sputum levels of YKL-40 following high-dose and repeated low-dose allergen challenges. Plasma levels of YKL-40 were measured (*A*) before, directly after, and 7 hours after high-dose allergen challenge (n = 16) and (*B*) before and after a 7-day repeated low-dose allergen challenge (n = 14). No significant differences were observed. Sputum supernatant levels of YKL-40 were measured (*C*) before and 6 hours after high-dose allergen challenge (n = 15) and (*D*) before and after a 7-day repeated low-dose allergen challenge (n = 11). The results are expressed as nanograms of YKL-40 per gram of processed sputum. High-dose allergen challenge significantly increased sputum YKL-40 ($P = 0.018$), but low-dose allergen challenge was without effect.

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