Serum levels of Clara cell secretory protein, asthma, and lung function in the adult general population

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TO THE EDITOR:

Clara cell secretory protein (CC-16) has been proposed as a biological marker of lung epithelial injury and pulmonary permeability. It has anti-inflammatory properties and protective effects from oxidative stress on the respiratory tract.[1] Acute exposures to pulmonary irritants such as smoke, chlorine, or ozone induce transient increases in serum CC-16 levels. In contrast, chronic exposures, such as smoking, occupational exposure to silica, and firefighting, are associated with decreased serum CC-16 levels.[2 and 3] Clinical studies have shown decreased serum levels of CC-16 in asthmatic subjects[4 and 5] and in patients with chronic obstructive pulmonary disease (COPD).[6] A positive association between serum CC-16 levels and FEV_1 was recently observed in a large clinical study on patients with COPD.[7] However, there is a lack of epidemiological studies addressing the relation of serum CC-16 levels with asthma and lung function in the general population.

In this study, we evaluated serum CC-16 level as a biomarker relevant in the study of asthma and lung function phenotypes in adults from the Spanish branch of the population-based multicenter European Community Respiratory Health Survey.
We used a commercially available ELISA kit (BioVendor Laboratory, Inc, Modrice, Czech Republic) to measure CC-16 levels in serum samples from 859 participants (see Table E1 in this article's Online Repository at www.jacionline.org), of whom 642 (75%) came from a random sample of the general population and 217 (25%) from an enriched sample of subjects reporting asthma medication and/or respiratory symptoms. Ethical approval and written consent were obtained.
Subjects were defined as having physician-confirmed current asthma if they received an asthma diagnosis by a physician and they either experienced respiratory symptoms or used asthma medications in the previous 12 months.[8] Prebronchodilator lung function (FEV_1 and forced vital capacity [FVC]) was measured by using standard methods. Percent predicted values were calculated according to reference equations from the third National Health and Nutrition Examination Survey, as published by Hankinson et al.[9] Airflow limitation was defined and classified according to the Global Initiative for Obstructive Lung Disease based on FEV_1/FVC value of less than 0.70. A second definition of airflow limitation based on the statistically defined lower limit of normal based on reference equations by Hankinson et al[9] was used. A restrictive spirometric pattern was defined as FVC% predicted value of less than 80% in the absence of airflow limitation.
Associations between serum CC-16 levels (as an independent variable) and asthma, spirometric patterns, the modified prebronchodilator Global Initiative for Obstructive Lung Disease COPD stages, and lung function parameters (as outcomes) were estimated with logistic, multinomial, or linear regression models, where appropriate. Estimates were adjusted for center and type of sample, sex, age, body mass index, smoking status and pack-years, and height (when needed).
Mean serum CC-16 levels were 5.8 ± 2.9 μg/L, ranging from 0.37 to 19.7 μg/L. Serum CC-16 levels were higher in males than in females, varied with age, and decreased with increasing body mass index, current smoking, and pack-years smoked (see Table E2 in this article's Online Repository at www.jacionline.org). Serum CC-16 levels did not vary with current asthma in the total population (Table I). However, lower serum CC-16 levels were associated with current asthma among never smokers (see Table E3 in this article's Online Repository at www.jacionline.org). Decreased CC-16 levels were observed in subjects with airflow limitation and, in particular, in subjects with moderate/severe GOLD COPD stages as compared to subjects with no airflow limitation (Table I). These results were confirmed when serum CC-16 levels were categorized according to quartiles (see Fig E1 in this article's Online Repository at www.jacionline.org). The spirometric restrictive pattern was not associated with serum CC-16 levels, but it should be noted that the sample size for this spirometric pattern was small in our study.

[TABLE I]
A positive association was observed between serum CC-16 levels and FEV_1% predicted and FEV_1/FVC (Table I). When analyses were restricted to the 634 subjects from the random sample, we found very similar estimates of serum CC-16 level effects on FEV_1% predicted, FVC% predicted, and FEV_1/FVC (adjusted beta coefficients [*P*], 1.71 [.002], 0.61 [.23], and 0.89 [<.001], respectively). When serum

CC-16 levels were categorized into quartiles, the lowest quartile was consistently associated with the lowest levels of FEV_1, FVC, and FEV_1/FVC (Fig 1).

[FIG 1]

Analyses were repeated after stratification by asthma and smoking (see Fig E2 and Fig E3 in this article's Online Repository at www.jacionline.org). The interaction term between current asthma and serum CC-16 levels was borderline significant for FEV_1% predicted (P = .08) and statistically significant for FEV_1/FVC (P = .007), suggesting that the effects of serum CC-16 levels on lung function may be stronger among asthmatic subjects. However, these interactions should be interpreted with caution because of the relatively small number of asthmatic subjects. The association between serum CC-16 levels and lung function parameters did not vary with smoking habits.

In this study, we observed decreased serum CC-16 levels to be associated with airflow limitation and lower lung function in the general population. We did not find serum CC-16 levels to be associated with asthma. To date, most evidence of an association between serum CC-16 levels and asthma comes from clinical studies. A possible explanation for this apparent discrepancy is that participants from clinical studies are likely to have more severe asthma than participants from population-based studies. This scenario is supported by the positive correlation that we found between serum CC-16 levels and lung function among asthmatic subjects. In addition, in our study, the association between serum CC-16 levels and asthma may have been affected by the complex interrelationships between smoking, serum CC-16 levels, and asthma, as suggested by the finding that lower serum CC-16 levels were associated with asthma among never smokers but not among ever smokers. Larger studies with well-characterized phenotypic information on asthma are required to determine conclusively its relation with serum CC-16 levels. A positive association between serum CC-16 levels and lung function has been reported in clinical studies[7] and in occupationally exposed subjects.[3 and 6] We extended these findings to adults from the general population by showing a positive association between serum CC-16 levels and FEV_1% predicted and FEV_1/FVC ratio and a borderline positive association with FVC% predicted. All the associations were confirmed after adjustment for relevant covariates, including smoking. Thus, serum CC-16 levels may provide additional information that is complementary to and partially independent of demographics and smoking in relation to lung function parameters at the population level. Consistently, we observed a low level of serum CC-16 in subjects with airflow limitation, particularly in those in the moderate to severe COPD stages, in line with the association between serum CC-16 levels and COPD and COPD severity shown by Lomas et al.[6] Because associations of serum CC-16 levels with lung function held true after adjusting for smoke and pack-years and after stratification by smoking, we concluded that these associations were at least partly independent of smoking.

A strength of this analysis is that it was conducted on a large and well-characterized sample of the general population. Detailed phenotypic data were available from questionnaires and spirometric tests. Asthma cases were population-based, and associations with lung function parameters were confirmed both in the total and in the random sample of the study. Thus, our findings indicate that the serum CC-16 level is a potential biomarker of lung function deficits in the general population.

However, because of the cross-sectional approach of the study, it was not possible to evaluate the temporal relationship of the biomarker to lung function or disease status and longitudinal studies are warranted to resolve the temporality of this association and to evaluate any potential role of this biomarker in the prevention or clinical settings.

In summary, we found reduced serum CC-16 levels to be associated with airflow limitation and lower lung function in the general population after adjusting for the effects of cigarette smoking and other covariates. These data warrant evaluation of serum CC-16 level as a potential biomarker of lung function deficits and obstructive lung disease in the longitudinal setting.

APPENDIX

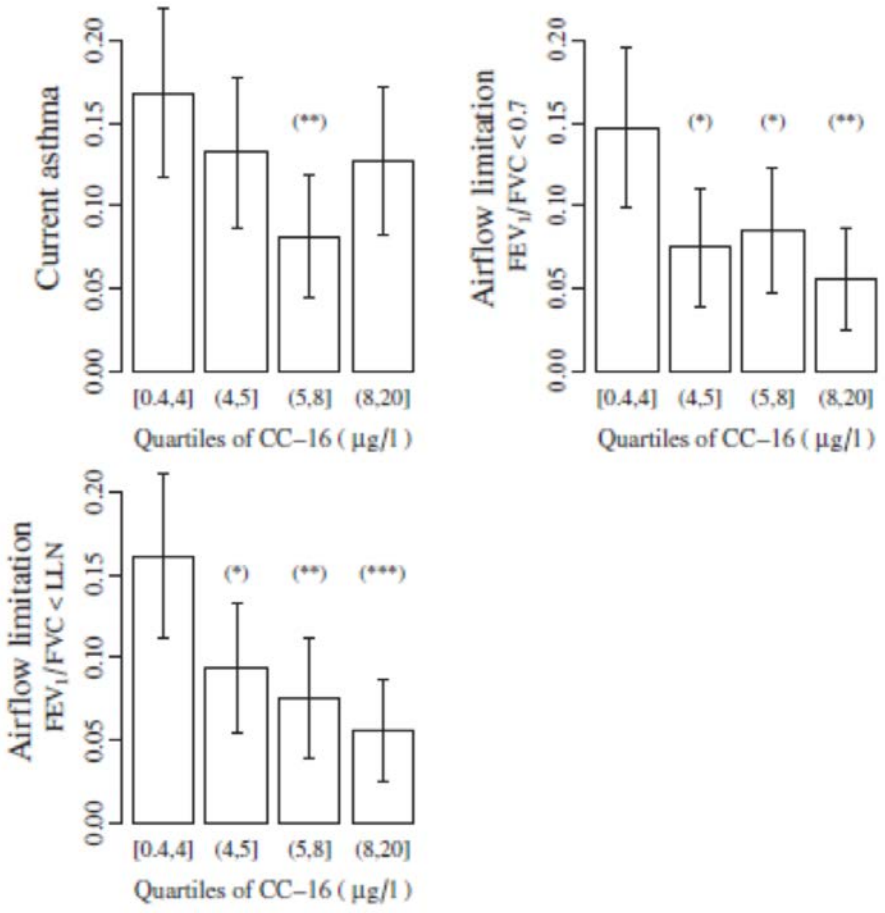

FIG E1. Prevalence of asthma and airflow limitation by quartiles of serum CC-16 levels. Asterisks represent *P* values obtained by comparing the unadjusted odds ratio of having the outcome for each quartile of the serum CC-16 level, using the first quartile as reference. *LLN*, Lower limit of normal. **P* < .10, ***P* < .05, and ****P* <.001.

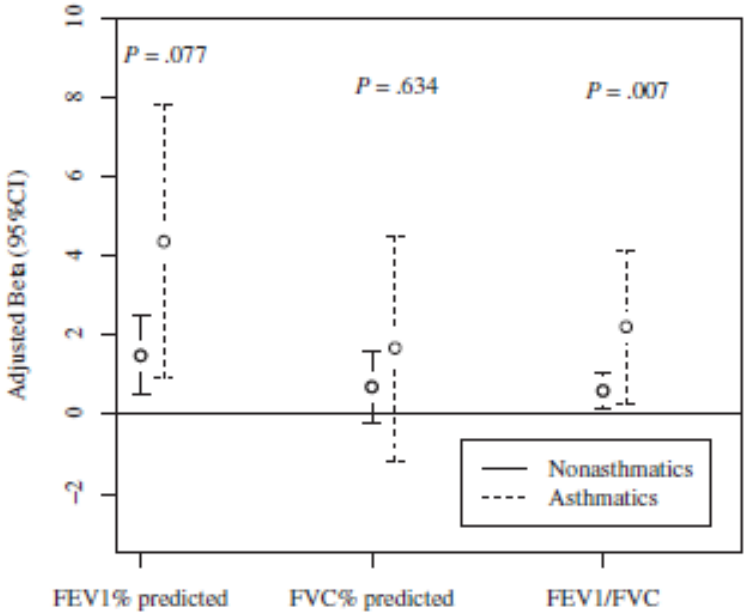

FIG E2. Adjusted regression coefficients (beta) and 95% CI for the association between serum CC-16 levels and lung function parameters, estimated through a multivariate linear regression model, stratified by asthma status. The *P* values refer to the interaction between asthma status and serum CC-16 levels.

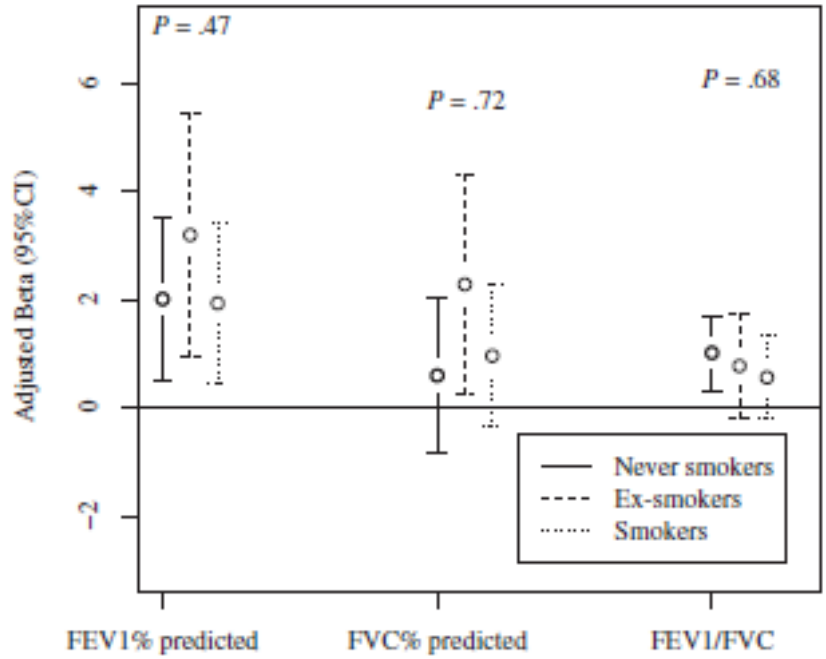

FIG E3. Adjusted regression coefficients (beta) and 95% CI for the association between serum CC-16 levels and lung function parameters, estimated through a multivariate linear regression model, stratified by smoking status. The *P* values refer to the interaction between smoking status and serum CC-16 levels.

Table E1. Sociodemographic and clinical characteristics of the study subjects

	All subjects (N = 859)
Subjects characteristics, n (%)	
Center	
Barcelona	199 (23.2)
Galdakao	343 (39.9)
Albacete	317 (36.9)
Enriched sample	217 (25.4)
Sex (men)	422 (49.4)
Age (y)	
28.0-32.4	132 (15.5)
32.5-39.9	263 (30.8)
40.0-47.4	257 (30.1)
≥47.5	202 (23.7)
BMI (kg/m^2)	
<20	25 (2.9)
20-24.9	324 (38.1)
25-29.9	343 (40.3)
≥30	159 (18.7)
Smoking status and intensity	
Never smokers	288 (34.3)
Ex-smokers ≤20 pack-years	112 (13.5)
Ex-smokers >20 pack-years	66 (7.9)
Current smokers ≤20 pack-years	194 (23.4)
Current smokers >20 pack-years	171 (20.6)
Respiratory diseases/phenotypes, n (%)	
Current asthma	108 (12.7)
Obstructive/restrictive spirometric patterns	
Definition based on $FEV_1/FVC < 70\%$*	
Normals	746 (87.7)
Spirometric restrictive pattern	28 (3.3)
Airflow limitation	77 (9.0)
Definitions based on $FEV_1/FVC <$ LLN†	
Normals	741 (87.1)
Spirometric restrictive pattern	28 (3.3)

	All subjects (N = 859)
Airflow limitation	82 (9.6)
Modified GOLD COPD stages‡	
No	774 (91.0)
Mild	35 (4.1)
Moderate/severe	42 (4.9)
Lung function, mean ± SD	
FEV_1% predicted	100.2% ± 14.8%
FVC % predicted	101.6% ± 12.2%
FEV_1/FVC	79.5% ± 7.2%
CC-16 (μg/L)	
Mean ± SD	5.79 ± 2.9
Median (Q1-Q3)	5.34 (3.64, 7.52)

BMI, Body mass index; *GOLD*, Global Initiative for Obstructive Lung Disease; *LLN*, lower limit of normal; *Q*, quartile.
∗ Airflow limitation defined as $FEV_1/FVC < 0.70$, and restrictive spirometric pattern defined as FVC% predicted < 80% and $FEV_1/FVC \geq 0.70$.
†Airflow limitation defined as $FEV_1/FVC <$ LLN, and restrictive spirometric pattern defined as FVC% predicted < 80% and $FEV_1/FVC \geq$ LLN.
‡Stages are based on lung function tests with no bronchodilator.

Table E2. Association between sociodemographic characteristics and serum CC-16 levels

	n	Serum CC-16 level, mean ± SD	Beta∗
Sex			
Female	433	5.52 ± 2.89†	
Male	422	6.07 ± 2.92	1.05‡
Age (y)			
28-32.4	132	6.61 ± 3.38§	
32.5-39.9	263	5.68 ± 2.67	−0.87†
40.0-47.4	257	5.51 ± 2.86	−0.76§
≥47.5	202	5.74 ± 2.90	−0.65§
BMI (kg/m^2)			
<20	25	6.39 ± 3.57†	0.72
20-24.9 (reference)	324	6.12 ± 3.09	
25-29.9	343	5.67 ± 2.69	−0.57§
≥30	159	5.31 ± 2.86	−1.01‡
Smoking status and intensity (pack-years)			
Never smokers	288	6.45 ± 2.59‡	
Ex-smokers ≤20 pack-years	112	6.26 ± 2.56	−0.08
Ex-smokers >20 pack-years	66	5.75 ± 2.55	−0.84†
Current smokers ≤20 pack-years	194	5.73 ± 3.26	−0.94‡
Current smokers >20 pack-years	171	4.31 ± 2.88	−2.42‡

BMI, Body mass index.

∗ Coefficients and *P* values from 1 multivariate regression model with center and type of sample, sex, age, BMI, and smoking status (5-level variable) as covariates and the serum CC-16 level as the outcome variable.

† Overall *P* value for the association between each subject characteristic and the serum CC-16 level: $P < .05$.

‡ Overall *P* value for the association between each subject characteristic and the serum CC-16 level: $P < .001$.

§ Overall *P* value for the association between each subject characteristic and the serum CC-16 level: $P < .10$.

Table E3. Adjusted odds ratios (ORs, 95% CI) for the association between a 1-SD increase in the serum CC-16 level (μg/L) and asthma stratified by smoking status∗

Current asthma	n (asthmatic subjects/nonasthmatic subjects)	Serum CC-16 average levels (in asthmatic subjects/in nonasthmatic subjects)	Adjusted OR† (95% CI)
Never smokers	35/250	5.52/6.58	0.5 (0.3-0.9)‡
Ex-smokers	23/155	5.86/6.13	0.8 (0.4-1.5)
Current smokers	47/323	5.40/5.04	1.2 (0.9-1.7)

BMI, Body mass index.
∗ *P* value for interaction between serum CC-16 levels and smoking status = .006.
†ORs (95% CI) were estimated with logistic regression models adjusted for center and type of sample (6-level variable), sex, age (categorical), and BMI (categorical).
‡*P* value for the association between asthma and the serum CC-16 level: $P < .05$.

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TABLES AND FIGURES

Table I. Unadjusted and adjusted estimates (95% CI and *P*) for the association between a 1-SD increase in serum CC-16 level (μg/L) and asthma, spirometric patterns, and lung function

Outcome	n	Unadjusted		Adjusted	
		Est (95% CI)	*P* value	Est (95% CI)	*P* value
Asthma					
Current asthma*	108/848	OR = 0.90 (0.73-1.10)	.32	Adjusted OR = 0.93 (0.72-1.17)	.53
Spirometric patterns					
Definition based on $FEV_1/FVC < 70\%$ and					
Normal	746	RRR = 1		Adjusted RRR = 1	
Restrictive	28	0.94 (0.64-1.38)	.74	1.10 (0.71-1.69)	.68
Airflow limitation	77	0.66 (0.51-0.87)	.003	0.71 (0.52-0.96)	.03
Definition based on $FEV_1/FVC <$ LLN and					
Normal	741	RRR = 1		Adjusted RRR = 1	
Restrictive	28	0.93 (0.64-1.37)	.73	1.10 (0.71-1.69)	.67
Airflow limitation	82	0.65 (0.50-0.85)	.002	0.72 (0.54-0.97)	.029
Modified GOLD COPD stages and					
No (reference)	774	RRR = 1		Adjusted RRR = 1	
Mild	35	0.95 (0.67-1.34)	.76	0.88 (0.60, 1.30)	.52
Moderate/severe	42	0.45 (0.30-0.68)	<.001	0.52 (0.32, 0.85)	.01
Lung function¶					
FEV_1% predicted	851	Beta = 2.83 (1.85-3.80)	<.001	Adjusted beta = 1.92 (0.96-2.88)	<.001
FVC% predicted	851	Beta =	<.001	Adjusted beta =	.06

Outcome	n	Unadjusted		Adjusted	
		Est (95% CI)	*P* value	Est (95% CI)	*P* value
		1.50 (0.65-2.35)		0.84 (−0.02 to 1.71)	
FEV_1/FVC	851	Beta = 1.16 (0.68-1.63)	<.001	Adjusted beta = 0.84 (0.38-1.30)	<.001

BMI, Body mass index; *GOLD*, Global Initiative for Obstructive Lung Disease; *LLN*, lower limit of normal; *OR*, odds ratio; *RRR*, relative risk ratio.

∗ ORs (95% CI) were estimated with logistic regression models unadjusted and adjusted for center and type of sample (6-level variable), sex, age (categorical), BMI (underweight, <20 kg/m^2; normal weight, 20-25 kg/m^2; overweight, 25-30 kg/m^2; or obese, >30 kg/m^2), smoking status and pack-years (never smokers, ex-smokers who smoked ≤20 pack-years, ex-smokers who smoked >20 pack-years, current smokers with ≤20 pack-years, and current smokers with >20 pack-years), and height (when needed).

† RRRs (95% CI) were estimated with multinomial regression models unadjusted and adjusted for center and sample, sex, age (categorical), BMI (categorical), and smoking status (5-level variable).

‡ Airflow limitation defined as $FEV_1/FVC < 0.70$, and restrictive spirometric pattern defined as FVC% predicted < 80% and $FEV_1/FVC \geq 0.70$.

§ Airflow limitation defined as $FEV_1/FVC <$ LLN, and restrictive spirometric pattern defined as FVC% predicted < 80% and $FEV_1/FVC \geq$ LLN.

⁝ Stages are based on lung function tests with no bronchodilator.

¶ Estimates obtained with a linear regression model, adjusted for center and sample, age (categorical), BMI (categorical), and smoking status (5-level variable); estimates for FEV_1/FVC were also adjusted for sex and height.