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To the Editor:

Poor asthma control and recurrent exacerbations have been shown to be a phenotypic counterpart of asthma with predominantly small-airway involvement.¹ Biomarkers are not always accurate in asthmatic patients, especially in serum, because compartmentalization can occur between the blood and airways. Blood eosinophil counts do not represent an overall view of airway inflammation, and exhaled nitric oxide measurements at different flow rates (fraction of exhaled nitric oxide [FENO] and alveolar nitric oxide [CalvNO]) have been developed and validated to reflect more accurately proximal and distal airway inflammation.²

Club cell secretory protein (CCSP) serum concentration has been shown to be associated with chronic obstructive pulmonary disease, bronchiolitis obliterans syndrome, and sarcoidosis, which are all predominantly diseases involving the small airways. Ranges of CCSP concentrations in healthy subjects, reproducibility, and relationships between serum and airway levels are known and can be used as potential surrogate markers. Our aim was to assess small-airway disease in asthmatic patients and to find a related biomarker. We used a dynamic assessment of gas trapping using computed tomographic (CT) imaging of the chest during methacholine challenge as a marker of small-airway disease.

Thirty-two nonsmoking asthmatic women treated with inhaled corticosteroids (Global Initiative for Asthma steps 2-3) and known to have methacholine hyperresponsiveness were included. All patients were asked to avoid taking control medication of any kind for at least 72 hours according to guidelines. Before the bronchial provocation test coupled with thoracic CT scanning, FENO and CalvNO concentrations were measured (HypairFeNO; Medisoft, Sorinnes, Belgium). CCSP concentrations were measured in serum by using an ELISA test, according to the manufacturer's instructions (validated range, 80-4000 pg/mL; intra-assay coefficient of variation, 3.4%; and interassay coefficient of variation, 4.7%; Biovendor, Brno, Czech Republic).

Methacholine challenges were performed according to American Thoracic Society guidelines with the 5-breath dosimeter method (ADD Aerodoser with Atomisor NL11AD nebulizer, Atomisor, France, CE 0459, Dir. Eur. 93/42/CEE) in the CT room, with the patient lying on the table in the supine

TABLE I. Relationship between log (E/I MLD DRS) and clinical and respiratory parameters

Variables: Y = log(E/I MLD DRS)	Univariate analyses			Multivariate analysis		
	Coefficient	SE	P value	Coefficient	SE	P value
Age (y)	-0.054	0.018	.007			
BMI (kg/m ²)	-0.031	0.041	.449			
ACQ-6 score	-0.093	0.387	.812			
Baseline FEV ₁ (% predicted)	-2.705	2.799	.342			
Baseline FVC (% predicted)	-1.285	2.745	.644			
Baseline FEV ₁ /FVC ratio	-0.081	0.045	.086			
FVC decrease at PC ₂₀ (%)	-7.289	5.560	.201			
Log (FVC DRS)	1.237	0.221	<.001	1.18409	0.15398	<.001
FENO (ppb)	0.017	0.013	.197			
Corrected CalvNO (ppb)	0.098	0.060	.120			
CCSP (ng/mL)	-0.593	0.126	<.001	-0.45119	0.07423	<.001
Pi10	-0.208	0.285	.472			

Variables with *P* values of less than .15 (shown in boldface) were considered for the multivariate analysis.
ACQ-6, Asthma Control Questionnaire; BMI, body mass index.

position. For each methacholine dose from baseline to PC₂₀, expiratory slices were acquired by using a 64-detector row CT scanner (LightSpeed VCT; GE Healthcare, Waukesha, Wis). Expiratory acquisition of 2 small volumes of 10 consecutive slices of 1 mm each at standardized sites (first volume between the apices and the carina and second volume between the carina and the diaphragm) were acquired to limit irradiation.³ Each chest CT examination was reconstructed by using a standard filter, and images were acquired by means of spirometric gating (WinspiroPRO; Medical International Research, Waukesha, Wis) to monitor lung volumes and determine respiratory end points. CT air trapping was quantified by using expiratory/inspiratory ratios of the mean lung density (E/I MLD), and airway wall thickness as a reflection of proximal airway remodeling was assessed by computing the square root of the wall area of a 10-mm luminal perimeter (Pi10) index.⁴ To quantify the progression of air trapping during the course of the bronchial provocation test, the dose-response slope (DRS) of E/I MLD was computed as follows:

$$\frac{(\text{Last MLD value} - \text{Baseline MLD value})}{\text{methacholine dose}}.$$

The evolution of E/I MLD throughout the bronchial provocation tests was analyzed by using a mixed model with an autoregressive covariance matrix to study the possible effect of the methacholine dose on E/I MLD.

The 32 patients were 42 ± 17 years old, with a mean body mass index of 28 ± 9 kg/m² (48% obese and 72% atopic). Mean ± SD baseline FEV₁ values were 81.34% ± 11.70% of predicted values. All patients were treated with inhaled corticosteroids (daily dose, 500 ± 250 µg), and 72% were receiving a long-acting β-agonist through a fixed-combination device. All patients reached a 20% decrease in FEV₁, with a mean ± SD PC₂₀ of 615.79 ± 551.02 ng/mL. E/I MLD significantly increased during bronchial provocation. Univariate analyses highlighted a statistically significant relationship between E/I MLD DRS and age (*P* = .007), as well as CCSP serum concentration (*P* < .001) and forced vital capacity (FVC) DRS (after log transformation, *P* < .001). The multivariate analysis showed a negative relationship between log (E/I MLD DRS) and serum CCSP concentration (β coefficient, -0.45; *P* < .001) and the FVC DRS (β coefficient, 1.18; *P* < .001; Table I). FVC changes with methacholine have been associated with small-airway disease in the literature.⁵ It

is noteworthy that exhaled biomarkers, including CalvNO, were unrelated to E/I MLD DRS. We established a threshold using receiver operating characteristic curve analyses and verified the diagnostic performance of this biomarker in this context (Fig 1, A). Box plot representations of E/I MLD values at baseline and PC₂₀ for patients with low and high blood CCSP concentrations are displayed in Fig 1, B, with the cutoff value of CCSP having been statistically defined as the median of distribution (4.3 ng/mL). Representative images of expiratory CT scan slices acquired at baseline and PC₂₀ in patients with low (a; upper row) and high (b; lower row) serum CCSP concentrations are shown Fig 1, C, and full acquisition can be seen online.

This study was designed to improve dynamic assessment of the small airways in asthmatic patients. We tried to better understand the dynamics of small-airway closure during bronchoconstriction because controversies exist about the sequence of events that leads to asthma storms. In the present study radiation doses remained far below the threshold considered at risk.³ In various studies air trapping and small-airway involvement in patients with asthma and other conditions were related to aging, obesity, inflammation, mucus plugging, and peribronchiolar fibrosis. Other teams have identified the role of pseudoemphysema and loss of elastic recoil because of decreased elastin content and performance. If we assume that methacholine-induced bronchoconstriction is due to direct smooth muscle constriction, our findings probably apply to this mechanism only, and adenosine or mannitol challenge might have produced different results. Surprisingly, and contrary to previous studies, the intensity of small-airway dysfunction was unrelated to airway remodeling assessed by using Pi10, airway inflammation (assessed based on FENO and CalvNO), or the level of control but reflected better by a specific small airway-related biomarker represented by CCSP. We acknowledge that our findings are not a definitive demonstration. Potentially, low CCSP concentration are associated with incomplete lung growth and reduced small-airway numbers, as indicated by previous publications in which CCSP is called CC16.⁶ Lower CCSP concentrations might relate to damaged (unexplored by Pi10 index) or inflamed (but unexplored by FENO and CalvNO) small airways, which in turn might be more responsive to methacholine and manifest as more rapidly progressing air trapping on CT scans.⁷ It is noteworthy that reference values for CCSP concentrations in healthy control subjects (mean ± SD, 6.4 ± 4.1 ng/mL)⁸ are higher than in our subjects (mean ± SD, 5.1 ± 2.2 ng/mL for

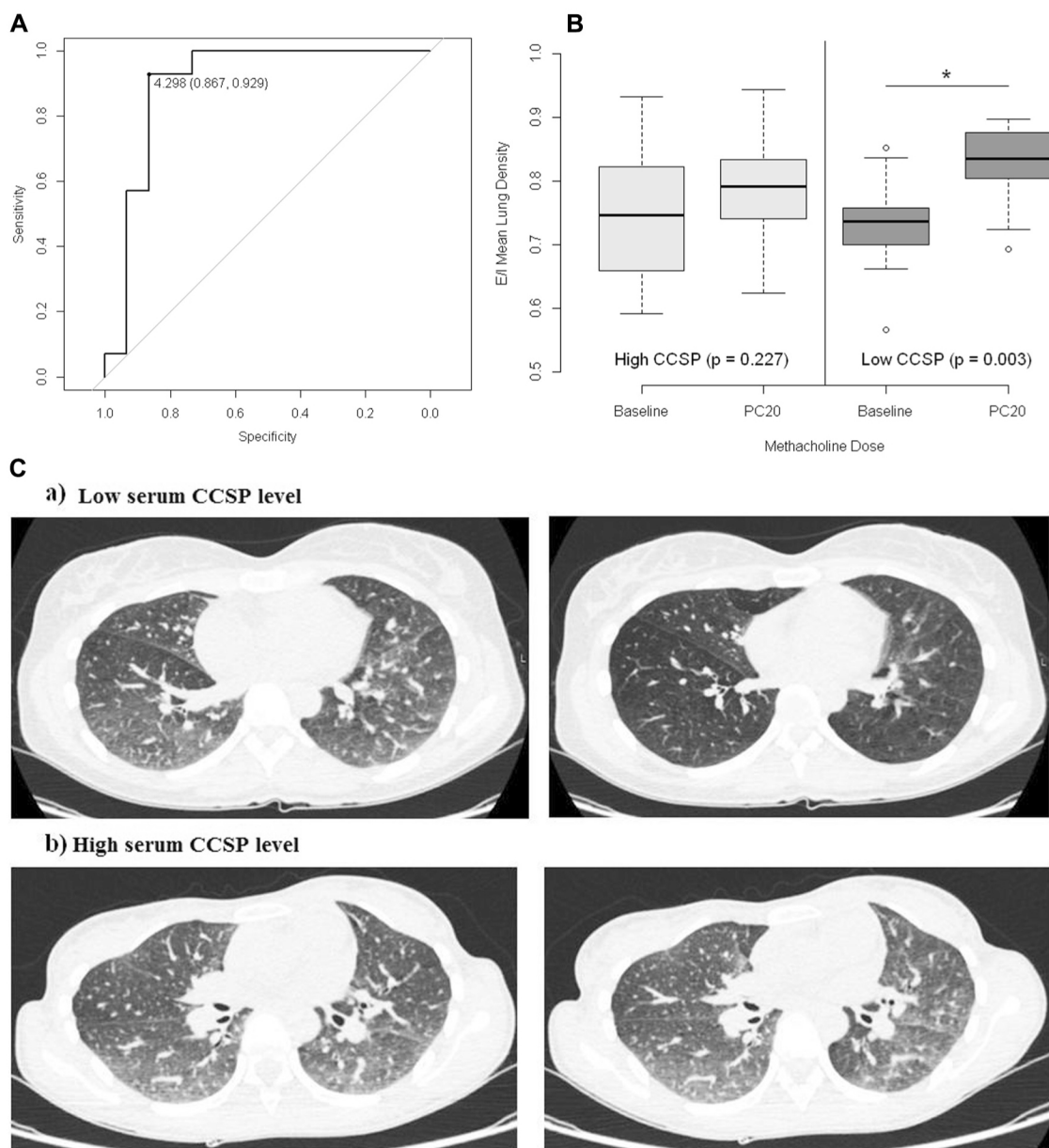


FIG 1. A, Receiver operating characteristic curves and diagnostic performances of blood CCSP concentrations to detect intensive small-airway impairment in asthmatic patients (using the DRS of E/I MLD during methacholine provocation). Area under the curve, 0.90 (0.77-1); CCSP threshold, 4.30 ng/mL; specificity, 0.87 (0.67-1); sensitivity, 0.93 (0.79-1); positive predictive value, 0.87 (0.69-1); and negative predictive value, 0.93 (0.79-1). **B**, Box plot representation of E/I MLD at baseline and at PC₂₀ in subgroups of patients with high or low CCSP concentrations (greater than or less than the threshold of 4.30 ng/mL). **C**, CT scan images at baseline (left) and PC₂₀ (right) of a patient with low CCSP concentration and a patients with a high CCSP concentration, showing variations in mean lung densities. All sequential images can be seen as videos in this article's Online Repository at www.jacionline.org. * $P = .003$.

the whole population, 6.7 ± 2.2 ng/mL for the high-CCSP group, and 3.5 ± 0.5 ng/mL for the low-CCSP group). Identifying a biomarker related to the small airways in asthmatic patients might have relevance from several perspectives. We chose CCSP as a potential biomarker because it fulfills most of the criteria required to be a candidate biomarker: easy to assess, reproducible, unaffected by steroids, and relevant to disease pathophysiology.

In conclusion, we have identified a potential blood biomarker relevant to small-airway hyperresponsiveness observed during dynamic CT acquisition in asthmatic patients. Further studies of

sensitivity and specificity are needed using a longitudinal cohort to confirm clinical utility.

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