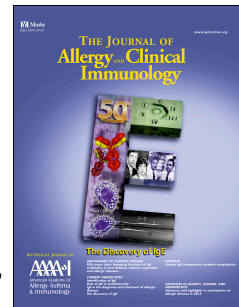


Accepted Manuscript

CCSP Serum Level is A Surrogate Marker of Small Airway Involvement in Asthma

Sebastien Bommart, MD-PhD, Grégory Marin, PhD, Nicolas Molinari, PhD, Lucie Knabe, PhD, Aurélie Petit, PhD, Pascal Chanez, MD-PhD, Anne Sophie Gamez, MD, Catherine Devautour, RN, Isabelle Vachier, PhD, Arnaud Bourdin, MD-PhD



PII: S0091-6749(17)30039-8

DOI: [10.1016/j.jaci.2016.09.063](https://doi.org/10.1016/j.jaci.2016.09.063)

Reference: YMAI 12581

To appear in: *Journal of Allergy and Clinical Immunology*

Received Date: 28 January 2016

Revised Date: 8 September 2016

Accepted Date: 27 September 2016

Please cite this article as: Bommart S, Marin G, Molinari N, Knabe L, Petit A, Chanez P, Gamez AS, Devautour C, Vachier I, Bourdin A, CCSP Serum Level is A Surrogate Marker of Small Airway Involvement in Asthma, *Journal of Allergy and Clinical Immunology* (2017), doi: 10.1016/j.jaci.2016.09.063.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

CCSP SERUM LEVEL IS A SURROGATE MARKER OF SMALL AIRWAY INVOLVEMENT IN ASTHMA

Sebastien Bommart MD-PhD^{3,4} and Grégory Marin PhD^{1,2}, Nicolas Molinari PhD^{1,4,5}, Lucie Knabe PhD^{2,4}, Aurélie Petit PhD², Pascal Chanez MD-PhD⁶, Anne Sophie Gamez MD², Catherine Devautour RN², Isabelle Vachier PhD² and Arnaud Bourdin MD-PhD^{2,4,5}

§: the two first co-authors equally contributed to this work

1. Department of Medical Information. Montpellier University Hospital, France
2. Department of Respiratory Diseases, Montpellier University Hospital, France
3. Department of Radiology, Montpellier University Hospital, France
4. PhyMedExp, University of Montpellier, INSERM U1046, CNRS UMR 9214, Montpellier University Hospital, France
5. APARD: Association pour l'Assistance et la Réhabilitation à Domicile, Montpellier, France
6. Department of Respiratory Diseases APhM, INSERM Laboratory U1067, CNRS UMR 7333, Aix-Marseille University Marseille France

Correspondence to:

Pr Arnaud Bourdin

Department of Respiratory Diseases

Hôpital Arnaud de Villeneuve

CHU Montpellier

371 Av. Doyen G.Giraud

34295 MONTPELLIER Cedex 5, France

Tel: + 33 4 67 33 61 26

Fax: +33 4 67 63 36 45

a-bourdin@chu-montpellier.fr

Author contributions

SB developed the protocol, performed all the quantitative CT analysis, performed statistical analysis, interpreted data, and wrote the manuscript; **BM** developed the protocol, performed all the quantitative CT analysis, interpreted data, and wrote the manuscript; **ASG, CD and AB** undertook patient recruitment, performed patient recruitment and data collection; **LK and AP** performed serum analysis; **NM** supervised statistical analyses and acted as scientific advisor; **PC and IV** codeveloped the protocol and acted as scientific advisors; **AB** developed the protocol, supervised the study, obtained funding for the study, acted as scientific advisor, and cowrote the manuscript. All authors critically reviewed and approved the manuscript.

Capsule summary

Serum CCSP level was independently related to small airway hyperresponsiveness, measured by the slope of air-trapping progression measured using CT. In asthmatic patients, this blood biomarker may identify small airways involvement.

Key words

Asthma; small airways; CT; methacholine; E/I MLD; CCSP; SCGB1A1; CC10; CC16; biomarker

Abbreviations (in order of appearance):

CCSP: Club Cell Secretory Protein

FVC: Forced Vital Capacity

PC20: Provocative Concentration causing a 20% fall in FEV1 (see below)

CT: Computed Tomodensitometry

ICS: Inhaled Corticosteroids

MLD: Mean Lung Density

E/I MLD: Expiratory to Inspiratory ratio of Mean Lung Density

FE_{NO}: Fractional Exhaled Nitric Oxide concentration

Calv_{NO}: Alveolar Nitric Oxide concentration

GINA: Global Initiative for Asthma

LABA: Long-Acting Beta-Agonist

ACQ-6: Asthma Control Questionnaire

FEV1: Forced Expiratory Volume in One Second

Pi10: Square root of wall area of a 10-mm luminal perimeter

DRS: Dose-Response Slope

BMI: Body Mass Index

IRB approval: "CPP Sud-Méditerranée III": ref 2011.12.05; approval date Jan 16, 2012;

amendment ref 2011.12.05ter; approval date Feb 8, 2012

Funding: This is an academic study funded by “Direction Inter-régionale de la Recherche Clinique – Sud Méditerranée”. (Appel d’Offre Interne du GCS MERRI 2011). Montpellier. France (ID RCB: 2011-A01396-35)

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 asthma, especially in the serum, since compartmentalization may occur between blood and airways. Blood eosinophil counts do not represent an overall view of airway inflammation and exhaled nitric oxide measurements at different flow rates (Fractionated Exhaled Nitric Oxide (FE_{NO}) and Alveolar NO (Calv_{NO})) have been developed and validated to reflect more accurately proximal and distal airway inflammation (2). CCSP serum level has been shown to be associated with Chronic Obstructive Pulmonary Disease, Bronchiolitis Obliterans Syndrome and sarcoidosis, which are all predominantly diseases involving small airways. The Ranges of CCSP concentrations in healthy subjects, reproducibility, and the relationships between serum and airway levels are known and can be used as a potential surrogate marker. Our aim was to assess small airway disease in asthma and to find a related biomarker. We used a dynamic assessment of gas trapping employing CT imaging of the chest during methacholine challenge as a marker of small airway disease.

Thirty-two non-smoking asthmatic women treated with ICS (GINA step 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. Prior to the bronchial provocation test coupled with thoracic CT scan, FE_{NO} and Calv_{NO} concentrations were measured (HypairFeNO, Medisoft, Sorinnes, Belgium). CCSP concentrations were measured in serum using an ELISA test according to manufacturer's instructions (validated range of 80–4000 pg/ml, intra-assay coefficient of variation 3.4%, inter-assay coefficient of variation 4.7%; Biovendor, Brno, Czech Republic).

Methacholine challenges were performed according to the American Thoracic Society guidelines with the five-breath dosimeter method (ADD Aerodoser with Atomisor NL11AD nebulizer, ©Atomisor, CE 0459, Dir. Eur. 93/42/CEE) in the CT room, the patient lying on the table in the supine position. For each methacholine dose from baseline to PC20 (provocative concentration causing a 20% fall in FEV₁), expiratory slices were acquired using a 64-detector row CT scanner (LightSpeed VCT; GE Healthcare, Waukesha, USA). Expiratory acquisition of two small volumes of ten consecutive slices of

1 mm each at standardized sites (first volume between the apices and the carina and second between the carina and the diaphragm) were acquired to limit irradiation (3). Each chest CT examination was reconstructed using a standard filter and images were acquired using spirometric gating (WinspiroPRO; Medical International Research Waukesha, USA) to monitor lung volumes and determine respiratory endpoints. CT air trapping was quantified using expiratory to 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 Pi10 index (4). In order 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 (last MLD value – baseline MLD value) divided by methacholine dose. The evolution of E/I MLD throughout the bronchial provocation tests was analyzed using a mixed model with an autoregressive covariance matrix in order to study the possible impact of the methacholine dose on E/I MLD.

The thirty-two patients were 42 ± 17 years old, with a mean BMI of 28 ± 9 kg/m² (48% obese, 72% atopic). Mean and standard deviation baseline FEV1 were $81.34\% \pm 11.70\%$ of predicted values. All patients were treated with ICS (daily dose 500 ± 250 µg) and 72% were receiving a LABA through a fixed combination device. All patients reached a 20% fall in FEV1, with a mean and standard deviation PC20 of 615.79 ng/ml ± 551.02 . E/I MLD significantly increased during bronchial provocation. Univariate analyses highlighted a statistically significant relationship between E/I MLD DRS and age ($p=0.007$) as well as CCSP serum concentration ($p<0.001$) and FVC DRS (after log transformation, $p<0.001$). The multivariate analysis showed a negative relationship between log (E/I MLD DRS) and serum CCSP concentration (β coefficient: -0.45 ; $p<0.001$), and the FVC DRS (β coefficient 1.18 ; $p<0.001$) (Table 1). FVC changes with methacholine have been associated with small airway disease in the literature (5). Noteworthy, exhaled biomarkers including CalvNO were unrelated to E/I MLD DRS. We established a threshold using ROC curve analyses and verified diagnostic performance of this biomarker in this context (figure 1a). Boxplot representations of E/I MLD values at baseline and PC20 for patients with low and high blood CCSP levels are displayed in figure 1b, the cut-off value of CCSP levels having been statistically defined as the median of the distribution (4.3 ng/ml). Representative images of expiratory CT-scan slices acquired at baseline and PC20 in patients

with low (a) and high (b) serum CCSP levels are shown figure 1c and full acquisition can be seen **online**.

This study was designed to improve dynamic assessment of small airways in asthma. We tried to better understanding the dynamics of small airway closure during bronchoconstriction, since 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 asthma and in other conditions were related to aging, obesity, inflammation, mucus plugging and peribronchiolar fibrosis. Other teams have identified the role of pseudo-emphysema and loss of elastic recoil due to 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 may have produced different results. Surprisingly, and contrary to previous studies the intensity of small airway dysfunction was unrelated to airway remodeling assessed by Pi10, airway inflammation (assessed by 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 levels are associated with incomplete lung growth and a reduced small airway numbers, as indicated by previous publications where CCSP is called CC16 (6). Lower CCSP levels may 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 (7). Noteworthy, reference values for CCSP concentrations in healthy controls (8) (mean and SD of 6.4 ± 4.1 ng/ml) are higher than in our subjects (mean and SD of 5.1 ± 2.2 ng/ml for the whole population, 6.7 ± 2.2 for high CCSP group, 3.5 ± 0.5 for low CCSP group). Identifying a biomarker related to small airways in asthma may have relevance from several perspectives. We chose CCSP as a potential biomarker since 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 asthma. <Further studies of sensitivity and specificity are needed using a longitudinal cohort to confirm clinical utility.

Sebastien Bommart and Grégory Marin, Nicolas Molinari, Lucie Knabe, Aurélie Petit, Pascal Chanez, Anne Sophie Gamez, Catherine Devautour, Isabelle Vachier and Arnaud Bourdin.

REFERENCES

1. Thompson BR, Douglass JA, Ellis MJ, Kelly VJ, O’Hehir RE, King GG, et al. Peripheral lung function in patients with stable and unstable asthma. *J Allergy Clin Immunol.* 2013 May;131(5):1322–8.
2. Szeffler SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, Hamilton RG, et al. Asthma outcomes: biomarkers. *J Allergy Clin Immunol.* 2012 Mar;129(3 Suppl):S9–23.
3. McCollough CH, Bushberg JT, Fletcher JG, Eckel LJ. Answers to Common Questions About the Use and Safety of CT Scans. *Mayo Clin Proc.* 2015 Oct;90(10):1380–92.
4. Bommart S, Marin G, Bourdin A, Revel MP, Klein F, Hayot M, et al. Computed tomography quantification of airway remodelling in normal ageing subjects: a cross-sectional study. *Eur Respir J.* 2015 Apr;45(4):1167-70.
5. Alfieri V, Aiello M, Pisi R, Tzani P, Mariani E, Marangio E. Small airway dysfunction is associated to excessive bronchoconstriction in asthmatic patients. *Respir Res.* 2014 Aug 27;15:86.
6. Guerra S, Halonen M, Vasquez MM, Spangenberg A, Stern DA, Morgan WJ, et al. Relation between circulating CC16 concentrations, lung function, and development of chronic obstructive pulmonary disease across the lifespan: a prospective study. *Lancet Respir Med.* 2015 Jul 6;
7. Guerra S, Vasquez M, Spangenberg A, Halonen M, Martin R. Club cell secretory protein in serum and bronchoalveolar lavage of patients with asthma. *J Allergy Clin Immunol.* 2016 May 11. pii: S0091-6749(16)30274-3. doi: 10.1016/j.jaci.2016.03.047
8. Lomas DA, Silverman EK, Edwards LD, Miller BE, Coxson HO, Tal-Singer R. Evaluation of serum CC-16 as a biomarker for COPD in the ECLIPSE cohort. *Thorax.* 2008 Dec;63(12):1058-63

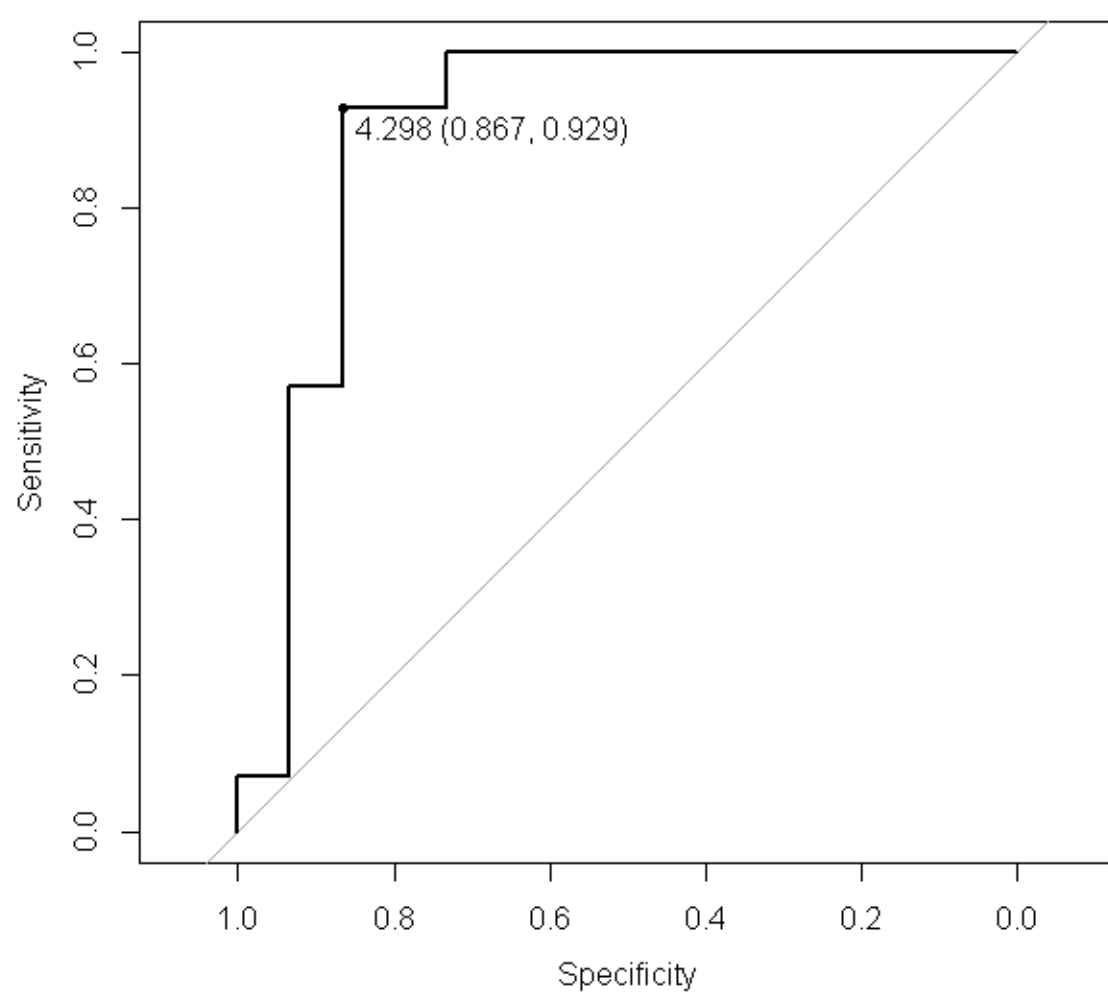
TABLE AND FIGURE LEGENDS

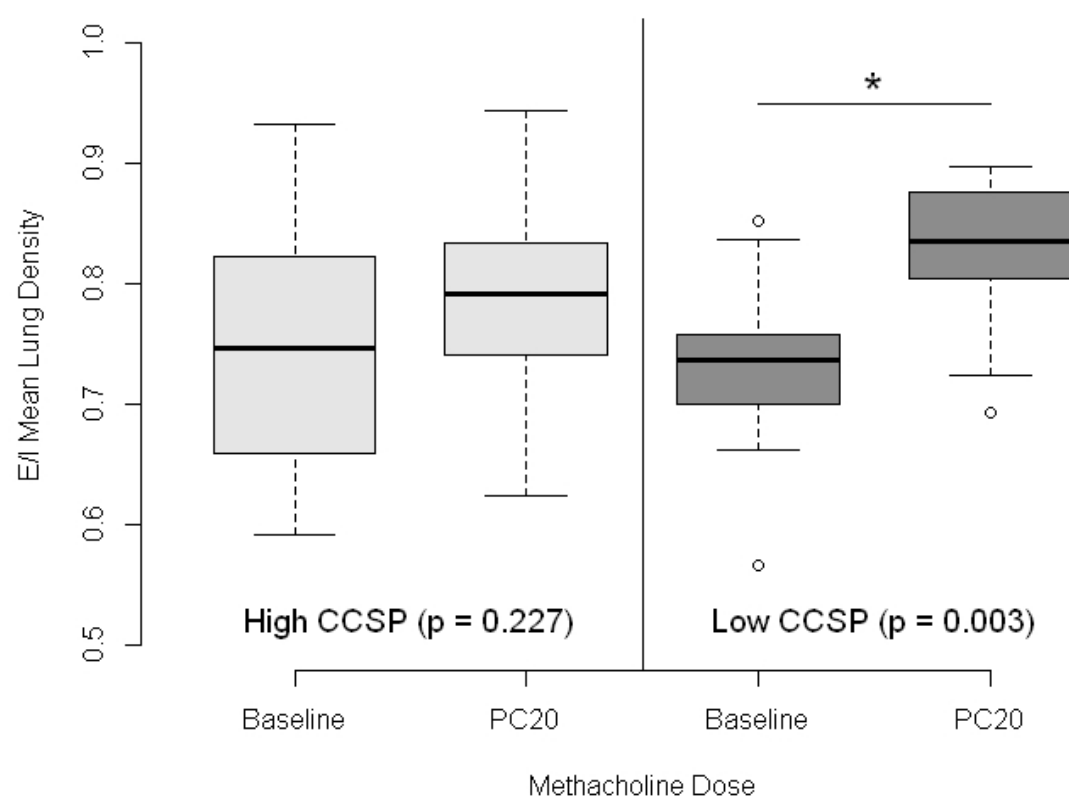
Table 1. Relationship between $\log$ (E/I MLD DRS) and clinical and respiratory parameters. Variables with p -values lower than 0.15 (in **bold**) were considered for the multivariate analysis.

Figure 1. **a)** ROC curves and diagnostic performances of blood CCSP concentrations to detect intensive small airway impairment in asthma (using the dose response slope of E/I MLD during methacholine provocation). Area Under 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 values = 0.87 [0.69-1]; negative predictive value = 0.93 [0.79-1]. **b)** Boxplots representation of E/I MLD at baseline and at the PC20 in the subgroups of patients with high or low CCSP levels (above or below the threshold of 4.30 ng/ml). **c)** CT-scan images at baseline (left) and at PC20 (right) of a low and a high-CCSP patient, showing variations of mean lung densities. **All sequential images can be seen as videos in the “online” repository.**

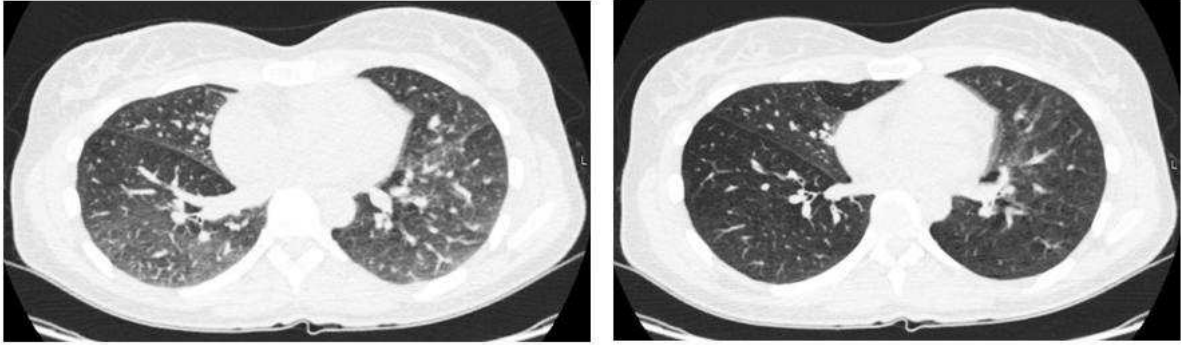
Table 1. Relationship between log (E/I MLD DRS) and clinical and respiratory parameters. Variables with p-values lower than 0.15 (in **bold**) were considered for the multivariate analysis.

Variables Y = log(E/I MLD DRS)	Univariate analyses			Multivariate analysis		
	Coefficient	Std Error	P-value	Coefficient	Std Error	P-value
Age (y)	-0.054	0.018	0.007			
BMI (kg/m ²)	-0.031	0.041	0.449			
ACQ6	-0.093	0.387	0.812			
Baseline FEV ₁ (% predicted)	-2.705	2.799	0.342			
Baseline FVC (% predicted)	-1.285	2.745	0.644			
Baseline FEV ₁ /FVC	-0.081	0.045	0.086			
FVC Fall at PC20 (%)	-7.289	5.560	0.201			
Log (FVC DRS)	1.237	0.221	< 0.001	1.18409	0.15398	< 0.001
FE _{NO} (ppb)	0.017	0.013	0.197			
Corrected Calv _{NO} (ppb)	0.098	0.060	0.120			
CCSP (ng/ml)	-0.593	0.126	< 0.001	-0.45119	0.07423	< 0.001
Pi10	-0.208	0.285	0.472			

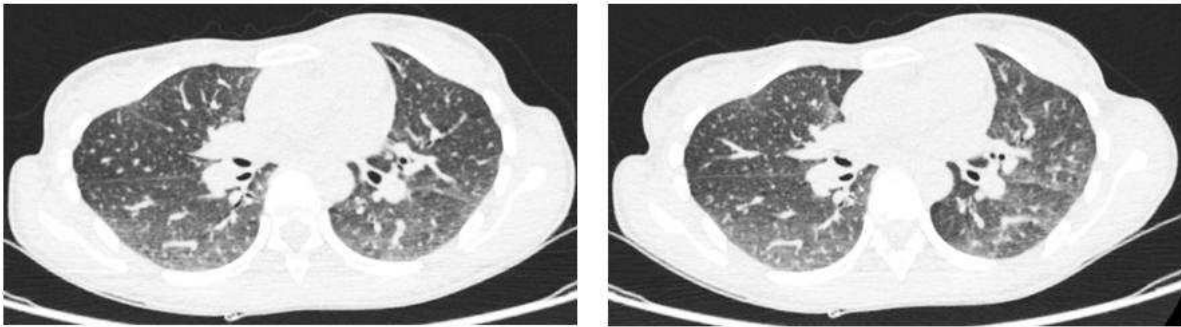




a) Low serum CCSP level

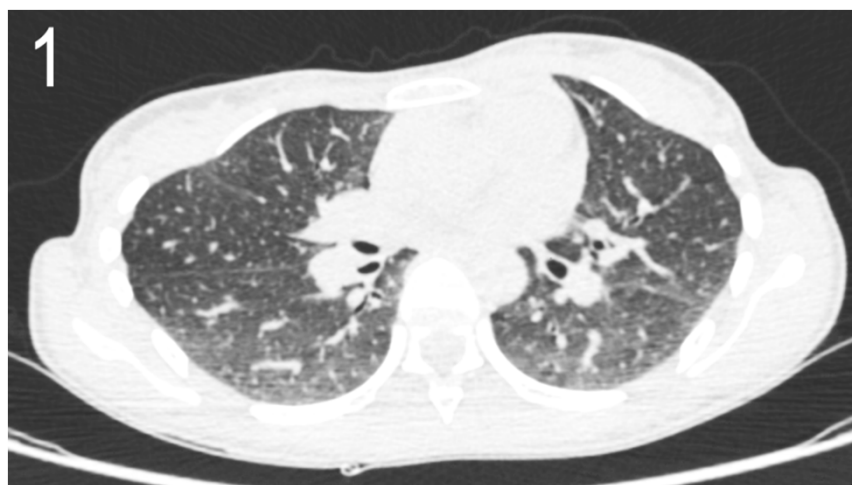


b) High serum CCSP level



Air trapping during methacholine in asthma patients

High serum CCSP level



Low serum CCSP level



Display in the slideshow mode to start animation