



Plasma connective tissue growth factor levels as potential biomarkers of airway obstruction in patients with asthma

Masato Kato, MD, PhD ^{*,†}; Tomoyuki Fujisawa, MD, PhD ^{*}; Dai Hashimoto, MD, PhD ^{*}; Masato Kono, MD, PhD ^{*,‡}; Noriyuki Enomoto, MD, PhD ^{*}; Yutaro Nakamura, MD, PhD ^{*}; Naoki Inui, MD, PhD [§]; Etsuko Hamada, PhD [‡]; Osamu Miyazaki, PhD [¶]; Syunsuke Kurashita, BS ^{||}; Masato Maekawa, MD, PhD [‡]; and Takafumi Suda, MD, PhD ^{*}

^{*} Second Division, Department of Internal Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan

[†] Department of Respiratory Medicine, Seirei Mikatahara General Hospital, Hamamatsu, Japan

[‡] Department of Laboratory Medicine, Hamamatsu University School of Medicine, Hamamatsu, Japan

[§] Department of Clinical Pharmacology and Therapeutics, Hamamatsu University School of Medicine, Hamamatsu, Japan

[¶] Tsukuba Research Institute, Research & Development Division, Sekisui Medical Company Ltd, Ryugasaki, Japan

^{||} Diagnostic Products Development Department, Research & Development Division, Sekisui Medical Company Ltd, Tokyo, Japan

ARTICLE INFO

Article history:

Received for publication March 26, 2014.

Received in revised form May 12, 2014.

Accepted for publication May 29, 2014.

ABSTRACT

Background: Bronchial asthma is a chronic inflammatory disorder characterized by airway hyper-responsiveness and airflow limitation. Connective tissue growth factor (CTGF), one of the key profibrotic factors associated with transforming growth factor β , may be related to airway remodeling in asthma. However, no data are available on the association between plasma CTGF levels and clinical and physiologic parameters in patients with asthma. Recently, we developed a novel subtraction method for determination of plasma CTGF levels.

Objective: To investigate the utility of plasma CTGF level as a surrogate biomarker in asthma.

Methods: Plasma CTGF levels were measured in 67 patients with stable asthma and 81 healthy volunteers, using the subtraction method. We evaluated correlations between plasma CTGF levels and clinical and physiologic parameters in patients with asthma.

Results: Plasma CTGF levels were higher in patients with asthma than in healthy volunteers. Asthmatic patients with a percentage of predicted forced expiratory volume in 1 second (FEV₁) less than 80% had significantly higher levels of plasma CTGF than those with a percentage of predicted FEV₁ of 80% or more. In patients with asthma, plasma CTGF levels had significantly negative correlations with forced vital capacity (FVC), FEV₁, percentage of predicted FEV₁, FEV₁/FVC ratio, forced expiratory flow at 50% of the FVC (FEF_{50%}), percentage of predicted FEF_{50%}, forced expiratory flow at 75% of the FVC (FEF_{75%}), and percentage of predicted FEF_{75%}, parameters that reflect the degree of airway obstruction. Plasma CTGF levels were negatively correlated with Asthma Control Test scores, a patient-based index of clinical control of asthma.

Conclusion: Plasma CTGF may be a potential biomarker for stable asthma when evaluating the degree of persistent airway obstruction.

Trial Registration: umin.ac.jp/ctr Identifier: UMIN000013081

© 2014 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

Introduction

Bronchial asthma is a chronic inflammatory disorder characterized by airway hyperresponsiveness and reversible airflow obstruction. Current asthma practice guidelines emphasize the importance of inhaled corticosteroids (ICSs) as an anti-inflammatory therapy, which substantially contributes to improving the disease

control and the quality of life in patients with asthma.¹ However, even if inhaled corticosteroids are administered, persistent airway inflammation cannot always be completely controlled, leading to irreversible airway obstruction due to airway structural changes, referred to as airway remodeling.² These changes include epithelial detachment, goblet cell hyperplasia, increased smooth muscle mass, and subepithelial fibrosis.³ To assess the degree of airway obstruction and evaluate the clinical control of asthma, noninvasive physiologic studies, such as pulmonary function tests, are usually performed; however, the results have test-retest variability because of their dependence on the patient's condition. Therefore, surrogate

Reprints: Tomoyuki Fujisawa, MD, PhD, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan; E-mail: fujisawa@hama-med.ac.jp.

Disclosures: Authors have nothing to disclose.

<http://dx.doi.org/10.1016/j.anai.2014.05.026>

1081-1206/© 2014 American College of Allergy, Asthma & Immunology. Published by Elsevier Inc. All rights reserved.

biomarkers are being sought to further evaluate airway obstruction and disease control to provide appropriate treatments.

Connective tissue growth factor (CTGF) is a 38-kDa, cysteine-rich secreted peptide that was originally isolated from human umbilical vein endothelial cells.⁴ It is a member of the CCN family of immediate early proteins and comprises 4 modules: an insulin-like growth factor-binding protein domain (module 1), a von Willebrand factor domain (module 2), a thrombospondin-homology domain (module 3), and a cysteine knot, heparin-binding domain (module 4).^{5–10} The biological functions of CTGF include angiogenesis and chondrogenesis, as well as cell adhesion, migration, proliferation, and differentiation.^{5–10} In the clinical setting, overexpression of CTGF has received attention as a biological marker for some fibrotic diseases.^{11–16} Plasma CTGF exists as an N-terminal CTGF fragment, which is considered to be an important factor in fibrosis because CTGF binds to transforming growth factor β (TGF- β) as an essential cofactor through the N-terminal von Willebrand factors domain (module 3) to augment TGF- β activity. Platelet-derived CTGF, which exists as full-length CTGF and is released into the plasma by platelet activation during or after blood collection, may interfere with determination of plasma CTGF levels existing as N-terminal fragments; thus, accurate measurement of plasma N-terminal CTGF levels requires subtraction of the platelet-derived full-length CTGF level from the total CTGF level. We have recently developed a novel subtraction method for determination of N-terminal CTGF levels.¹⁷ Using this method, we found that plasma N-terminal CTGF levels were increased in idiopathic pulmonary fibrosis, a major fibrotic lung disease, and negatively correlated with 6-month changes in forced vital capacity (FVC) in patients with idiopathic pulmonary fibrosis, which indicated that plasma CTGF is a potential biomarker for idiopathic pulmonary fibrosis.¹⁸

Previous studies found that CTGF was up-regulated in smooth muscle cells and fibroblasts isolated from patients with asthma,^{19,20} which induced cell proliferation and extracellular matrix synthesis. These findings suggest that CTGF plays a role in the development of airway remodeling and that CTGF levels could be a clinical biomarker to evaluate the impairment of pulmonary functions and disease severity in patients with asthma. However, no reports are available on the association between plasma CTGF levels and clinical and physiologic parameters in patients with asthma, perhaps because of the lack of an accurate method for measuring plasma N-terminal CTGF levels. In the present study, we measured plasma CTGF levels using the novel subtraction method¹⁷ and evaluated correlations between plasma CTGF levels and clinical physiologic parameters in patients with stable asthma.

Methods

Patients

A total of 67 patients with asthma, whose disease was classified as “controlled” under adequate treatment according to the Global Initiative for Asthma criteria for asthma control,¹ were included in the study (30 men and 37 women). Briefly, the patients were free of asthma symptoms, activity limitations, and exacerbation and had no more than 2 rescue treatments a week, if any. Each patient had received ICSs, the mainstay of anti-inflammatory therapy in asthma, or ICSs plus other antiasthmatic drugs for 1 year or more, with no change in dose within the previous 8 weeks. Patients who met the following criteria were excluded from this study: (1) treatment with oral or intravenous corticosteroids in the previous 4 weeks, (2) a smoking history of more than 10 pack-years, or (3) other pulmonary diseases, including chronic obstructive pulmonary disease. The study also included 81 healthy volunteers (37 men and 44 women with a mean [SD] age of 48.7 [14.4] years) as a control group. No patients had any other fibrotic diseases, such as

skin, pancreas, heart, liver, or kidney fibrosis, that might cause elevated N-terminal CTGF levels.

The study protocol was approved by the Ethical Committee of the Hamamatsu University School of Medicine (approval 19–88), and written informed consent was obtained from each patient. The study was registered in the UMIN Clinical Trials Registry system (Identifier: UMIN000013081).

Data Collection

Clinical data were collected from medical records. Laboratory data, pulmonary function tests, and Asthma Control Test (ACT) scores were obtained at the time of measurement of plasma N-terminal CTGF levels. Pulmonary function tests were performed using an Auto-Spirometer System 7 (Minato Medical Science Co, Osaka, Japan), according to the recommendations of the American Thoracic Society guidelines. FVC, forced expiratory volume in 1 second (FEV₁), forced expiratory flow at 50% of the FVC (FEF_{50%}), and forced expiratory flow at 75% of the FVC (FEF_{75%}) were assessed.

Assay for N-terminal CTGF Levels in Plasma

We obtained whole blood samples from peripheral venous blood with sodium EDTA. The plasma samples were separated by centrifugation at 2,080g for 15 minutes at 4°C, stored frozen at –80°C, and then thawed by incubating at 25°C for 5 minutes in a water bath just before the CTGF assay.

Anti-CTGF antibodies were prepared, and each sandwich enzyme-linked immunosorbent assay (ELISA) was performed as described previously.^{17,18} The full-length CTGF levels were determined by a sandwich ELISA using 2 monoclonal antibodies against modules 1 and 4. Total CTGF levels were determined by a sandwich ELISA using 2 monoclonal antibodies against modules 1 and 2. N-terminal CTGF levels were calculated using a subtraction method, as follows: (N-terminal CTGF Level) = (Total CTGF Level) – (Full-Length CTGF Level). All assays were performed in duplicate, and the mean value is reported for each.

Statistical Analysis

Statistical analysis was performed using JMP Start Statistics (SAS Institute Inc, Cary, North Carolina). Normally distributed variables were expressed as means and SDs. Nonnormally distributed variables were expressed as medians with interquartile ranges. Differences among the groups were assessed using an unpaired *t* test. The continuous data from the 3 groups were compared using a 1-way analysis of variance test. When the result was significant, each pairing was examined using an unpaired *t* test. Correlations among different parameters were evaluated with the Spearman correlation test. For all analyses, *P* < .05 was considered significant.

Results

Baseline Characteristics

Baseline characteristics of patients with asthma are summarized in Table 1. Briefly, 30 men and 37 women with a mean (SD) age of 64.9 (12.8) years were enrolled. The median duration of asthma was 15 years. Twenty-seven patients were atopic, as judged by clinical manifestations and positive specific IgE antibodies to at least 1 common inhalant allergen. Modest decreases were observed in the percentage of predicted FEV₁, FEV₁/FVC ratio, percentage of predicted FEF_{50%}, and percentage of predicted FEF_{75%}, which suggests the presence of a certain level of obstructive impairment in the central and peripheral airways. Sixty of the 67 patients had ACT scores higher than 19, which indicates that the asthma of most of the patients in the present study was well controlled.²¹ All patients were treated with inhaled corticosteroids (ICSs), which are the mainstay drug of anti-inflammatory therapy for asthma, and the

Table 1
Baseline characteristics

Characteristic	Asthmatic patients (N = 67)
Age, mean (SD), y	64.9 (12.8)
Female sex, No.	37
Former smokers, No.	18
Patients with atopy, No.	27
Duration of asthma, median (IQR), y	15 (10–22)
FVC, mean (SD), L	2.69 (0.83)
FVC, mean (SD), % predicted	94.0 (16.0)
FEV ₁ , mean (SD), L	1.78 (0.72)
FEV ₁ , mean (SD), % predicted	76.5 (20.6)
FEV ₁ /FVC ratio, mean (SD), %	65.8 (13.9)
FEF _{50%} , mean (SD), L/s	1.56 (1.12)
FEF _{50%} , mean (SD), % predicted	49.5 (32.1)
FEF _{75%} , mean (SD), L/s	0.46 (0.49)
FEF _{75%} , mean (SD), % predicted	38.2 (24.9)
ACT score, median (IQR)	23 (21–24)
Patients with ACT scores > 19, No.	60
Total IgE level, median (IQR), IU/mL	258 (75–531)
Patients taking ICSs, No.	67
ICS dose, median (IQR), $\mu\text{g}/\text{d}^a$	400 (200–800)
Patients taking long-acting β_2 -agonists, No.	44
Leukotriene receptor antagonists, No.	27

Abbreviations: ACT, Asthma Control Test; FEF_{50%}, forced expiratory flow at 50% of the FVC; FEF_{75%}, forced expiratory flow at 75% of the FVC; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; ICS, inhaled corticosteroid; IQR, interquartile range.

^aFluticasone equivalent.

median dose of ICS was 400 μg (fluticasone propionate conversion). Forty-four patients were treated with inhaled long-acting β_2 -agonist add-on therapy in addition to ICSs. Leukotriene receptor antagonists were used in 27 patients.

Plasma N-terminal CTGF Levels in Bronchial Asthma and Healthy Controls

Patients with asthma had significantly higher levels of plasma N-terminal CTGF than healthy controls (1,389 [454] pmol/L vs 1,135 [287] pmol/L, $P < .001$). We divided the patients with asthma into 2 groups, percentage of predicted FEV₁ of 80% or greater ($n = 33$) and percentage of predicted FEV₁ less than 80% ($n = 34$), and compared the plasma N-terminal CTGF level among healthy controls, asthmatic patients with percentage of predicted FEV₁ of 80% or greater, and asthmatic patients with percentage of predicted FEV₁ less than 80%. As shown in Figure 1, asthmatic patients with percentage of predicted FEV₁ less than 80% had significantly higher level of plasma N-terminal CTGF (1,578 [471] pmol/L) than not only healthy volunteers (1,135 [287] pmol/L, $P < .001$) but also asthmatic patients with percentage of predicted FEV₁ of 80% or greater (1,198 [340] pmol/L, $P < .01$). There was no statistical difference in plasma N-terminal CTGF levels between asthmatic patients with percentage of predicted FEV₁ of 80% or greater and healthy volunteers.

Association Between N-terminal CTGF Levels and Clinical Parameters in Bronchial Asthma

We next examined the association between plasma N-terminal CTGF levels and various clinical and physiologic parameters in patients with asthma (Table 2). Plasma N-terminal CTGF levels were significantly correlated with percentage of predicted FVC ($r = -0.44$, $P < .001$), FEV₁ ($r = -0.49$, $P < .001$), percentage of predicted FEV₁ ($r = -0.44$, $P < .001$), FEV₁/FVC ($r = -0.49$, $P < .001$), FEF_{50%} ($r = -0.56$, $P < .001$), percentage of predicted FEF_{50%} ($r = -0.47$, $P < .001$), FEF_{75%} ($r = -0.49$, $P < .001$), and percentage of predicted FEF_{75%} ($r = -0.36$, $P = .004$) (Fig 2). In addition, ACT scores were negatively correlated with plasma CTGF levels ($r = -0.44$, $P < .001$) (Fig 3). A weak correlation was found between duration of asthma and plasma CTGF level ($r = -0.30$, $P = .02$). No

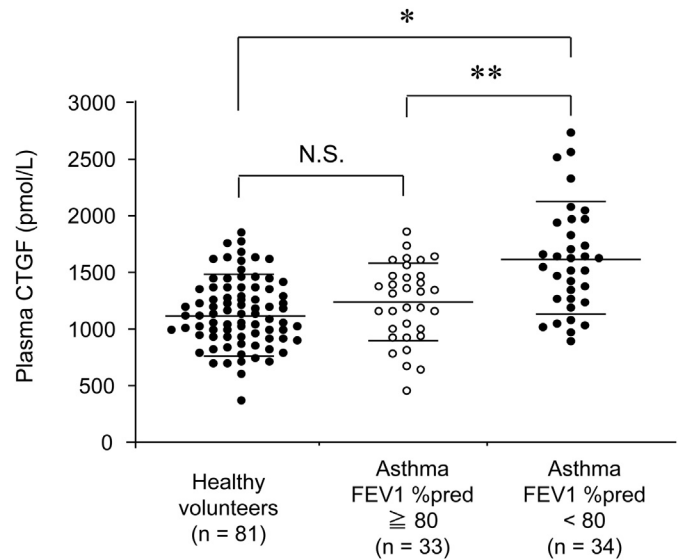


Figure 1. Plasma N-terminal connective tissue growth factor (CTGF) levels in patients with asthma and healthy volunteers. The patients with asthma were divided into 2 groups, percentage of predicted forced expiratory volume in 1 second (FEV₁) of 80% or greater ($n = 33$) and percentage of predicted FEV₁ less than 80% ($n = 34$), and the plasma N-terminal CTGF levels were compared among healthy volunteers, asthmatic patients with percentage of predicted FEV₁ of 80% or greater and those with percentage of predicted FEV₁ less than 80%. Asthmatic patients with percentage of predicted FEV₁ less than 80% had significantly higher levels of plasma N-terminal CTGF (mean [SD], 1,578 [471] pmol/L) than not only healthy volunteers (1,135 [287] pmol/L, $P < .001$) but also asthmatic patients with percentage of predicted FEV₁ of 80% or greater (1,198 [340] pmol/L, $P < .01$). Each dot represents an individual patient, and horizontal lines indicate means and SDs. * $P < .001$, ** $P < .01$. NS indicates not significant.

correlation was observed between plasma CTGF levels and serum IgE levels.

Discussion

In the present study, we measured plasma N-terminal CTGF levels, using a novel subtraction method, in patients with stable asthma and investigated the correlations between plasma CTGF levels and clinical and physiologic parameters. To our knowledge, this is the first time that plasma CTGF levels in asthmatic patients with percentage of predicted FEV₁ less than 80% were found to be significantly higher than those in asthmatic patients with percentage of predicted FEV₁ of 80% or greater and healthy volunteers.

Table 2

Association between plasma n-terminal connective tissue growth factor levels and clinical and physiologic parameters in patients with asthma

Parameter	Correlation coefficient	P value
Duration of asthma	0.30	.02
Pulmonary function tests		
FVC	-0.24	.051
FVC, % predicted	-0.44	<.001
FEV ₁	-0.49	<.001
FEV ₁ , % predicted	-0.53	<.001
FEV ₁ /FVC ratio	-0.44	<.001
FEF _{50%}	-0.56	<.001
FEF _{50%} , % predicted	-0.47	<.001
FEF _{75%}	-0.49	<.001
FEF _{75%} , % predicted	-0.36	.004
ACT scores	-0.44	<.001
Total IgE level	0.09	.56

Abbreviations: ACT, asthma control test; FEF_{50%}, forced expiratory flow at 50% of the FVC; FEF_{75%}, forced expiratory flow at 75% of the FVC; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity.

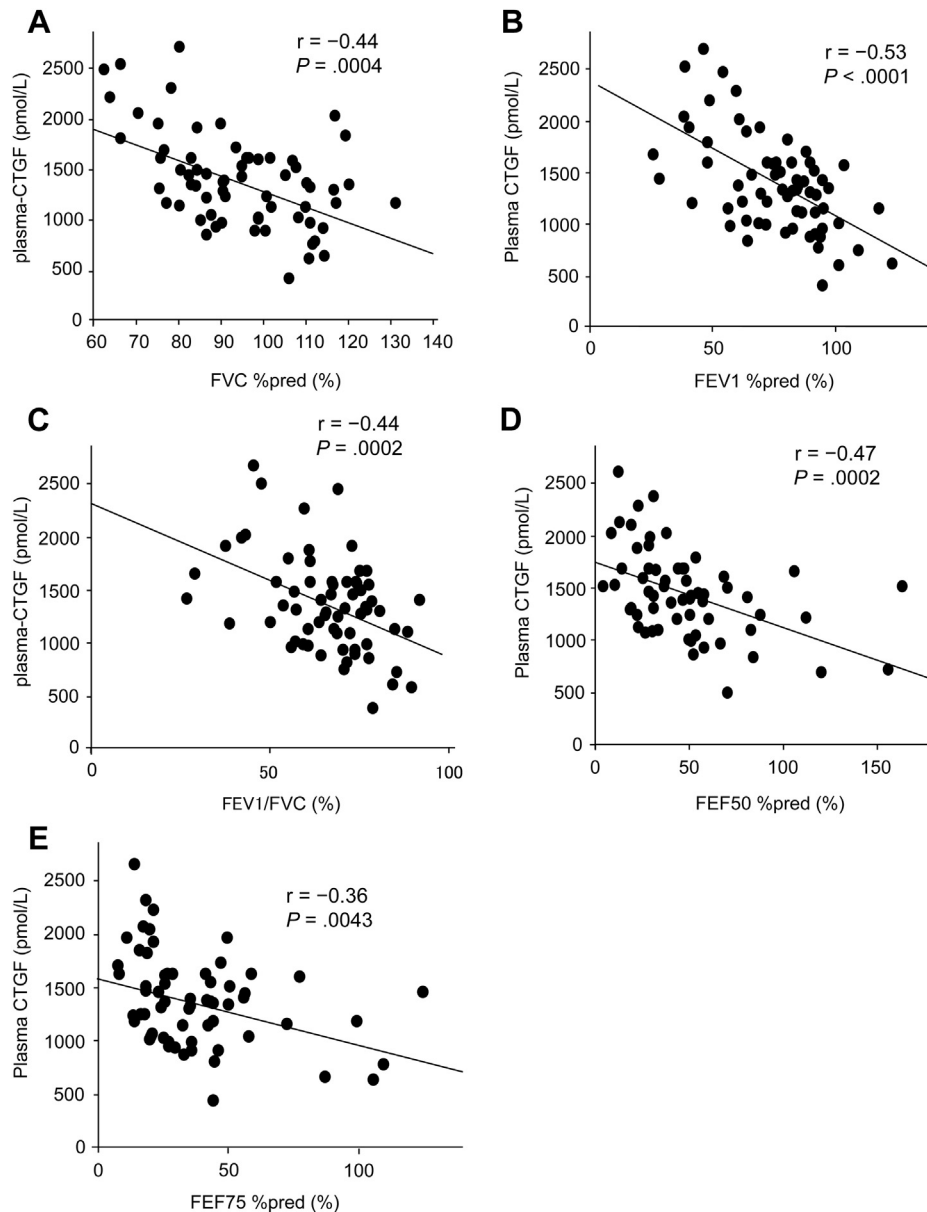


Figure 2. Correlation between plasma N-terminal connective tissue growth factor (CTGF) levels and parameters of pulmonary function tests in patients with asthma. Significant negative correlations were found between plasma N-terminal CTGF levels and percentage of predicted forced vital capacity (FVC) ($r = -0.44$, $P < .001$) (A), percentage of predicted forced expiratory volume in 1 second (FEV₁) ($r = -0.53$, $P < .001$) (B), FEV₁/FVC ratio ($r = -0.44$, $P < .001$) (C), percentage of predicted forced expiratory flow at 50% of the FVC (FEF_{50%}) ($r = -0.47$, $P < .001$) (D), and percentage of predicted forced expiratory flow at 75% of the FVC (FEF_{75%}) ($r = -0.36$, $P = .004$) in patients with asthma.

and that plasma CTGF level was negatively correlated with parameters of the pulmonary function test (percentage of predicted FVC, FEV₁, percentage of predicted FEV₁, FEV₁/FVC ratio, FEF_{50%}, percentage of predicted FEF_{50%}, FEF_{75%}, and percentage of predicted FEF_{75%}) and ACT scores, a self-assessment tool for asthma control, in patients with asthma. These findings demonstrate that plasma N-terminal CTGF levels reflect the degree of airway obstruction and asthma control and that they may be useful as surrogate biomarkers in patients with asthma.

We found that asthmatic patients with percentage of predicted FEV₁ less than 80% had significantly higher levels of plasma CTGF compared with those with percentage of predicted FEV₁ of 80% or higher and healthy volunteers and that plasma CTGF levels were negatively correlated with FEV₁, FEV₁/FVC ratio, FEF_{50%}, and FEF_{75%}, indicating that plasma CTGF levels reflect the degree of airway obstruction in patients with stable asthma. CTGF is an angiogenetic and profibrotic factor that is linked to many fibrotic

diseases.^{12–14,18,22} Several studies have found that CTGF may participate in the development of airway remodeling and pathogenesis of asthma.^{19,20,23,24} Burgess et al²⁴ reported that the expression of CTGF in asthmatic human airway smooth muscle cells was greater than that in nonasthmatic smooth muscle cells. CTGF expression, induced by TGF- β in smooth muscle cells and fibroblasts isolated from asthmatic patients, promoted accumulation of extracellular matrix, such as fibronectin and collagen I.^{19,20} Deposition of extracellular matrix and connective tissue components onto airway walls is clinically known as subepithelial fibrosis,^{25,26} which is one of the major findings of airway remodeling in asthma and leads to loss of lung function and the development of progressive airflow obstruction.^{27,28} These findings suggest that CTGF plays a role in the development of airway remodeling and airflow limitation in the asthmatic airway. However, little is known about the clinical implications of plasma CTGF levels in patients with asthma. To our knowledge, the present

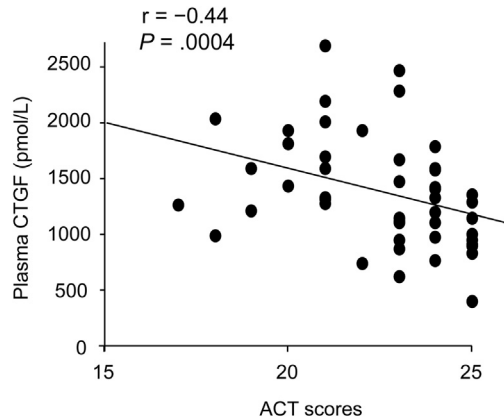


Figure 3. Correlation between plasma N-terminal connective tissue growth factor (CTGF) levels and Asthma Control Test (ACT) scores in patients with asthma. Plasma N-terminal CTGF levels were negatively correlated with ACT scores in patients with asthma ($r = -0.44$, $P < .001$).

study is the first to find that an increase in plasma CTGF level was significantly correlated with decreases in FEV₁, FEV₁/FVC ratio, FEF_{50%}, and FEF_{75%} levels in patients with stable asthma, indicating that plasma CTGF level could be a surrogate biomarker for assessing airflow limitation in patients with asthma. Recently, Weng and colleagues²⁹ compared the expression of CTGF in circulating fibrocytes from healthy people, patients with asthma with no obstruction (FEV₁ >80% predicted), and patients with chronic obstructive asthma (postbronchodilator FEV₁ <60% predicted). They reported that the percentage of CTGF expression in fibrocytes after 14 days in culture was significantly higher in patients with chronic obstructive asthma than in healthy people and in those with asthma with no obstruction, which suggests that CTGF contributes to the pathogenesis of airway obstruction in patients with chronic obstructive asthma. The present findings of negative correlations between plasma CTGF levels and FEV₁, FEV₁/FVC ratio, FEF_{50%}, and FEF_{75%} may support a role for CTGF in the development of airflow limitation and airway remodeling in asthma.

In the present study, we used the new subtraction method for measurement of plasma CTGF levels. The CTGF level in blood, existing as N-terminal CTGF fragments, is considered to reflect fibrosis in a variety of organs.^{11–13} CTGF is also known to be present in platelets at a very high level, and it is released into the blood plasma as a result of platelet activation during or after blood collection.^{30,31} Therefore, accurate determination of plasma CTGF levels requires inhibition of CTGF released from platelets. The subtraction method that we previously developed enabled us to measure plasma N-terminal CTGF levels precisely.¹⁷ By using this method, we found significant correlations between plasma CTGF levels and clinical and physiologic parameters (eg, pulmonary function tests and ACT scores) in patients with stable asthma, suggesting that the plasma N-terminal CTGF level has potential as a new clinical biomarker in patients with stable asthma, reflecting airway obstruction and clinical control of asthma.

The ACT is a short and simple patient-based, 5-item questionnaire to assess clinical control of asthma,³² which has been widely used in clinic practice. Although the ACT does not include a pulmonary function test, ACT scores have been reported to be correlated with percentage of predicted FEV₁, peak expiratory flow, and airway inflammation in patients with asthma,^{32–34} which indicates that persistent airflow limitation and airway inflammation impair clinical control of asthma. Interestingly, the present study found that plasma CTGF levels were negatively correlated with ACT scores, as well as parameters of pulmonary function tests. Given

that CTGF may play an important role in the development of airway remodeling and chronic airway obstruction,^{29,35} it is reasonable that CTGF levels are related not only to airflow limitation but also to the clinical control of asthma, which would ensure the usefulness of plasma CTGF level as a clinical biomarker of asthma. Further studies using large sample sizes are required to validate whether plasma CTGF levels reflect clinical control of asthma.

There were some limitations in the present study. First, this was a single-institution study, and the sample size was relatively small. Second, we did not investigate the association between plasma CTGF level and pathologic changes in airway remodeling in asthma. Bronchomucosal biopsies using bronchoscopy are required to provide pathologic evidence of airway remodeling; however, it is difficult to perform the biopsies in patients with asthma because of the invasiveness of the procedure. Future studies will be required to determine whether plasma CTGF levels are associated with pathologic changes in airway remodeling, leading to persistent airflow limitation in patients with asthma. Third, it is unclear whether the plasma CTGF levels vary in the patients with asthmatic attack and unstable asthma because we did not examine the plasma CTGF level in those patients in the present study. That will be a topic of the future studies to evaluate the plasma CTGF levels in various conditions of asthma.

To summarize, plasma N-terminal CTGF levels were elevated in patients with stable asthma and were correlated with parameters of pulmonary function tests and asthma control. Plasma N-terminal CTGF levels detected using the novel subtraction method may be a useful surrogate marker for evaluation of persistent airway obstruction and disease control in asthma.

References

- [1] Bateman ED, Hurd SS, Barnes PJ, et al. Global strategy for asthma management and prevention: GINA executive summary. *Eur Respir J*. 2008;31:143–178.
- [2] Fish JE, Peters SP. Airway remodeling and persistent airway obstruction in asthma. *J Allergy Clin Immunol*. 1999;104:509–516.
- [3] Bergeron C, Al-Ramli W, Hamid Q. Remodeling in asthma. *Proc Am Thorac Soc*. 2009;6:301–305.
- [4] Bradham DM, Igarashi A, Potter RL, Grotendorst GR. Connective tissue growth factor: a cysteine-rich mitogen secreted by human vascular endothelial cells is related to the SRC-induced immediate early gene product CEF-10. *J Cell Biol*. 1991;114:1285–1294.
- [5] Bork P. The modular architecture of a new family of growth regulators related to connective tissue growth factor. *FEBS Lett*. 1993;327:125–130.
- [6] Brigstock DR. The connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. *Endocr Rev*. 1999;20:189–206.
- [7] Moussad EE, Brigstock DR. Connective tissue growth factor: what's in a name? *Mol Genet Metab*. 2000;71:276–292.
- [8] Blom IE, Goldschmeding R, Leask A. Gene regulation of connective tissue growth factor: new targets for antifibrotic therapy? *Matrix Biol*. 2002;21:473–482.
- [9] Perbal B. CCN proteins: multifunctional signalling regulators. *Lancet*. 2004;363:62–64.
- [10] Leask A, Abraham DJ. All in the CCN family: essential matricellular signaling modulators emerge from the bunker. *J Cell Sci*. 2006;119:4803–4810.
- [11] Leask A, Parapuram SK, Shi-Wen X, Abraham DJ. Connective tissue growth factor (CTGF, CCN2) gene regulation: a potent clinical bio-marker of fibroproliferative disease? *J Cell Commun Signal*. 2009;3:89–94.
- [12] Dziadzio M, Usinger W, Leask A, et al. N-terminal connective tissue growth factor is a marker of the fibrotic phenotype in scleroderma. *QJM*. 2005;98:485–492.
- [13] Sato S, Nagaoka T, Hasegawa M, et al. Serum levels of connective tissue growth factor are elevated in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. *J Rheumatol*. 2000;27:149–154.
- [14] Gressner AM, Yagmur E, Lahme B, Gressner O, Stanzel S. Connective tissue growth factor in serum as a new candidate test for assessment of hepatic fibrosis. *Clin Chem*. 2006;52:1815–1817.
- [15] Koitabashi N, Arai M, Niwano K, et al. Plasma connective tissue growth factor is a novel potential biomarker of cardiac dysfunction in patients with chronic heart failure. *Eur J Heart Fail*. 2008;10:373–379.
- [16] Nguyen TQ, Tarnow L, Jorsal A, et al. Plasma connective tissue growth factor is an independent predictor of end-stage renal disease and mortality in type 1 diabetic nephropathy. *Diabetes Care*. 2008;31:1177–1182.
- [17] Miyazaki O, Kurashita S, Fukamachi I, Endo K, Ng PS, Takehara K. Subtraction method for determination of N-terminal connective tissue growth factor. *Ann Clin Biochem*. 2010;47:205–211.

- [18] Kono M, Nakamura Y, Suda T, et al. Plasma CCN2 (connective tissue growth factor; CTGF) is a potential biomarker in idiopathic pulmonary fibrosis (IPF). *Clin Chim Acta*. 2011;412:2211–2215.
- [19] Kunzmann S, Schmidt-Weber C, Zingg JM, et al. Connective tissue growth factor expression is regulated by histamine in lung fibroblasts: potential role of histamine in airway remodeling. *J Allergy Clin Immunol*. 2007;119:1398–1407.
- [20] Johnson PR, Burgess JK, Ge Q, et al. Connective tissue growth factor induces extracellular matrix in asthmatic airway smooth muscle. *Am J Respir Crit Care Med*. 2006;173:32–41.
- [21] Schatz M, Sorkness CA, Li JT, et al. Asthma Control Test: reliability, validity, and responsiveness in patients not previously followed by asthma specialists. *J Allergy Clin Immunol*. 2006;117:549–556.
- [22] Gilbert RE, Akdeniz A, Weitz S, et al. Urinary connective tissue growth factor excretion in patients with type 1 diabetes and nephropathy. *Diabetes Care*. 2003;26:2632–2636.
- [23] Laug R, Fehrholz M, Schutze N, et al. IFN-gamma and TNF-alpha synergize to inhibit CTGF expression in human lung endothelial cells. *PLoS One*. 2012;7:e45430.
- [24] Burgess JK, Johnson PR, Ge Q, et al. Expression of connective tissue growth factor in asthmatic airway smooth muscle cells. *Am J Respir Crit Care Med*. 2003;167:71–77.
- [25] Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet*. 1989;1:520–524.
- [26] Redington AE. Fibrosis and airway remodelling. *Clin Exp Allergy*. 2000;30(suppl 1):42–45.
- [27] Shiba K, Kasahara K, Nakajima H, Adachi M. Structural changes of the airway wall impair respiratory function, even in mild asthma. *Chest*. 2002;122:1622–1626.
- [28] Sumi Y, Hamid Q. Airway remodeling in asthma. *Allergol Int*. 2007;56:341–348.
- [29] Weng CM, Chen BC, Wang CH, et al. The endothelin A receptor mediates fibrocyte differentiation in chronic obstructive asthma: the involvement of connective tissue growth factor. *Am J Respir Crit Care Med*. 2013;188:298–308.
- [30] Kubota S, Kawata K, Yanagita T, Doi H, Kitoh T, Takigawa M. Abundant retention and release of connective tissue growth factor (CTGF/CCN2) by platelets. *J Biochem*. 2004;136:279–282.
- [31] Cicha I, Garlich CD, Daniel WG, Goppelt-Struebe M. Activated human platelets release connective tissue growth factor. *Thromb Haemost*. 2004;91:755–760.
- [32] Nathan RA, Sorkness CA, Kosinski M, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol*. 2004;113:59–65.
- [33] Shirai T, Furuhashi K, Suda T, Chida K. Relationship of the asthma control test with pulmonary function and exhaled nitric oxide. *Ann Allergy Asthma Immunol*. 2008;101:608–613.
- [34] Senna G, Passalacqua G, Schiappoli M, Lombardi C, Wilcock L. Correlation among FEV₁, nitric oxide and asthma control test in newly diagnosed asthma. *Allergy*. 2007;62:207–208.
- [35] Burgess JK. Connective tissue growth factor: a role in airway remodelling in asthma? *Clin Exp Pharmacol Physiol*. 2005;32:988–994.