

C-reactive protein levels in the serum of asthmatic patients

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Background: Asthma is a chronic airway inflammatory disease caused by immune cells such as T lymphocytes and eosinophils. Recently, highly sensitive C-reactive protein (hs-CRP) assays have become available for detecting small changes in CRP levels within the reference range, allowing for the evaluation of clinical inflammation.

Objective: To investigate the relationship between hs-CRP levels and bronchial asthma.

Methods: We collected blood samples from 109 patients with bronchial asthma, with or without attacks, and measured serum eosinophil cationic protein levels, pulmonary function, and serum CRP levels using an hs-CRP assay.

Results: Mean serum hs-CRP levels were significantly higher in patients without attacks (0.473 mg/L) and with attacks (0.908 mg/L) ($P < .001$ for both) than in controls (0.262 mg/L). Serum hs-CRP levels were inversely correlated with forced expiratory volume in 1 second/forced vital capacity in asthmatic patients ($r = -0.4915$; $P < .01$).

Conclusion: Serum hs-CRP levels may be related to the state of asthma exacerbation and allergic inflammation.

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INTRODUCTION

Asthma is a syndrome associated with allergen-induced chronic airway inflammation and airway hyperresponsiveness, which are caused or aggravated by immune cells such as T lymphocytes and eosinophils. Eosinophils release cytotoxic products at the site of inflammation that are associated with epithelial damage and long-lasting structural changes in the bronchial wall, referred to as airway remodeling.^{1,2} In this regard, the control and monitoring of inflammatory changes in the airway are considered critical in the management of bronchial asthma.

Several clinical markers of allergic airway inflammation have been evaluated, including eosinophil cationic protein (ECP) in sputum or blood and eosinophil protein X/eosinophil-derived neurotoxin in urine.³ However, these eosinophilic inflammatory markers are problematic in terms of sample handling, accuracy, and variance,^{3–5} and they are not routinely used in clinical practice. Kuwasaki et al⁶ previously showed that well-controlled asthmatic patients had significantly more EG2 (monoclonal antibody to ECP)-positive eosinophils compared with controls. Although a treatment strategy directed at normalization of the induced sputum eosinophil count reduces asthma exacerbation and hospital admissions without the need for additional anti-inflammatory

treatment,⁷ it is not easy to obtain suitable sputum samples. Thus, it is necessary to find new, noninvasive surrogate or reinforcing measures of inflammation in asthma.

C-reactive protein (CRP) is an acute-phase reactant secreted by hepatocytes in response to circulating inflammatory cytokines. C-reactive protein has long been used clinically to evaluate the presence and degree of inflammation because CRP blood levels increase as much as 1,000-fold within 24 hours after the onset of inflammation.⁸ Recently, based on the recognition that atherosclerosis is an inflammatory process,^{9,10} a highly sensitive CRP (hs-CRP) assay has been evaluated as a predictor of the risk of a coronary event. We evaluated the relationships among hs-CRP, asthma, pulmonary function, IgE, and ECP to investigate the clinical significance of hs-CRP level as an indicator of allergic inflammation in asthma.

MATERIALS AND METHODS

Study Participants

We collected blood samples from 109 patients with bronchial asthma (Table 1). Asthma was diagnosed according to the criteria of the American Thoracic Society.¹¹ None of the patients with asthma had complications of other lung diseases or a history suggesting systemic infection, tumors, or autoimmune diseases. Patients with sinus disease were excluded by means of interview. The severity of asthma attacks was classified according to the National Heart, Lung, and Blood Institute/World Health Organization Workshop on the Global Strategy for Asthma Management and Prevention.¹² All the patients were treated with an inhaled β_2 -agonist when required. Furthermore, we collected serial samples in identical patients during the symptomatic and asymptomatic periods. Samples collected during symptomatic periods were selected

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from patients whose serum samples were collected previously during asymptomatic periods. Samples from patients after treatment with systemic corticosteroids were excluded. Pulmonary function was monitored in only 60 patients, who agreed to the evaluation of lung function, by measuring the expiratory peak flow rate and spirometry. The controls (32 men and 38 women; median age, 51.7 years) were healthy volunteers who visited Akita University Hospital for a routine check-up during the same period. They were matched in terms of age and sex, and they had no history of allergic disease, infection, or systemic disease for at least 2 weeks before the blood was collected. Individuals with elevated specific IgE levels in serum were excluded from the control group. All samples from controls and asthmatic patients without attacks were collected between 10 and 11 AM in consideration of the daily variation of the hs-CRP level in 1 patient. Informed consent was obtained from each participant, and the clinical research ethics committee of Akita University approved this study.

Measurement of Serum hs-CRP, ECP, and IgE Levels

Plasma samples from patients and controls, which were frozen at -20°C immediately after collection, were thawed, and CRP levels were measured using hs-CRP latex-enhanced immunonephelometric assays on a BN-II analyzer (Dade Behring Inc, Newark, Delaware), as previously described.¹³ After blood sampling using specially designed tubes for ECP measurements (Vacutainer tubes with SST gel; Becton Dickinson, San Jose, California), samples were left at room temperature for 1 to 2 hours. Serum was obtained by means of centrifugation to measure ECP using an ECP radioimmunoassay kit (Pharmacia, Uppsala, Sweden), as previously described.¹⁴ The number of serum ECP samples for analysis was 26.

All patients with atopic asthma had a positive radioallergen sorbent test result (specific IgE $\geq$ class 3) or a positive skin prick test reaction to 1 or more common inhalant allergen(s). Specific serum IgE levels were determined using an in vitro assay and skin prick testing. The in vitro assay was the Fluorescence-Enzyme Immunoassay kit (Pharmacia & Upjohn Diagnostics AB, Uppsala), as previously described.¹³ Five allergens were tested. Skin prick testing was performed using a metal lancet to prick the skin through a drop of allergen solution (*Depmatophagoides pteronyssinus*, cat, *Cryptomeria*, ragweed, or *Aspergillus*). Skin tests were read 15 minutes after pricking, with a positive reaction defined as erythema surrounding a wheal 3 to 5 mm greater than the negative saline control. Each participant had a positive control test result using histamine solution (10 mg/mL). The same allergens were tested using both methods.

Evaluation of Pulmonary Function

Immediately after blood sampling, forced expiratory volume in 1 second (FEV₁) and forced vital capacity (FVC) were evaluated using CHESTAC-65V (CHEST Inc, Tokyo, Japan). The FEV₁/FVC is expressed as the percentage of FVC.

Table 1. Clinical Characteristics of Controls and Patients With Bronchial Asthma^a

Characteristics	Controls (n = 70)	Patients (n = 109)
Age, mean $\pm$ SD, y	51.7 $\pm$ 7.1	52.2 $\pm$ 16.0
Male sex, No. (%)	32 (45.7)	52 (47.7)
BMI, mean $\pm$ SD	22.3 $\pm$ 3.3	22.0 $\pm$ 3.0
Smoker, No. (%)	10 (14.3)	17 (15.6)
Atopy, No. (%)	0	45 (41.3) ^b
Inhaled corticosteroid use, No. (%)	0	59 (54.1) ^b

Abbreviation: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared).

^a Differences in patient characteristics between groups were evaluated using the χ^2 test or 1-way analysis of variance.

^b $P < .05$ vs controls.

Statistical Analysis

Normally distributed data are expressed as mean $\pm$ SD. Nonnormally distributed data are presented as median (interquartile range [IQR]). Differences in patient characteristics between groups were evaluated using the χ^2 test or 1-way analysis of variance. Comparisons of serum hs-CRP levels in subgroups were tested using analysis of variance for normally distributed data and the Mann-Whitney U test, Kruskal-Wallis test, or Wilcoxon signed rank test for data not normally distributed. The relationships between normally distributed variables were examined using the Pearson correlation coefficient. Correlations between serum hs-CRP levels and other variables were determined using the Spearman rank correlation coefficient when the data were not normally distributed. Statistical significance was defined as $P < .05$.

RESULTS

Clinical Characteristics of the Study Groups

The clinical characteristics of each group are listed in Table 1. No significant differences in age, sex, body mass index (BMI), or current smoking status were observed between groups.

Serum Levels of hs-CRP

In this study hs-CRP values ranged from less than 0.01 to 5.385 mg/L. To study the clinical significance of CRP in asthma, we first measured the serum levels of hs-CRP in asthmatic patients with and without attacks. Serum samples from patients with asthma attacks were collected before treatment. It has been reported that CRP is related to obesity¹⁵ and aging.¹⁶ In agreement with the present study, there was a significant correlation between serum hs-CRP levels and BMI or age in the control group. However, there was no significant correlation between serum hs-CRP levels and BMI or age in asthmatic patients (Table 2). In addition, there was no significant difference between male and female hs-CRP levels in this study. Furthermore, no significant differences in serum hs-CRP levels were found in patients with asthma in terms of smoking history, state of atopy, or inhaled corticosteroid therapy (data not shown).

Table 2. Relationship Between Serum hs-CRP Levels and Age or BMI in Controls and Patients With Asthma^a

	Controls (n = 70)		Patients (n = 109)	
	r	P value	r	P value
Age	0.357	.049	0.027	.87
BMI	0.414	.04	0.076	.47

Abbreviations: BMI, body mass index; hs-CRP, highly sensitive C-reactive protein.

^a Because all data were normally distributed, correlations were tested using the Pearson correlation coefficient.

As shown in Figure 1A, hs-CRP levels were significantly elevated in patients without attacks (median, 0.473 mg/L; IQR, 0.174–1.630 mg/L) and with attacks (median, 0.908 mg/L; IQR, 0.130–5.385 mg/L) ($P < .001$ for both) compared with controls (median, 0.262 mg/L; IQR, 0.005–0.879 mg/L; Kruskal-Wallis test, $P < .001$). We further examined serum hs-CRP levels in 31 identical patients during attacks and asymptomatic periods. The hs-CRP levels were significantly higher during attacks than during remission ($P < .001$) (Fig 1B).

Relationship Between Serum hs-CRP Levels and Pulmonary Function Test Results

Pulmonary function tests were performed immediately after blood sampling. The median FEV₁/FVC in 26 asthmatic patients with attacks was 59.77% (IQR, 37.1%–70.4%) vs 74.01% (IQR, 44.1%–92.3%) in 34 patients without attacks. In 61 controls, the median value was 85.45% (IQR, 79.5%–98.1%). Serum hs-CRP levels were inversely correlated with FEV₁/FVC in all 60 asthmatic patients ($r = -0.4915$; $P < .01$) (Fig 2A). Furthermore, in the 34 asthmatic patients without attacks, there was a significant correlation between

hs-CRP levels and FEV₁/FVC ($r = -0.5352$; $P < .001$) (Fig 2B). However, in the 26 asthmatic patients with attacks, there was no significant correlation between hs-CRP levels and FEV₁/FVC ($r = 0.3253$; $P = .11$) (Fig 2C).

Relationship Between Serum hs-CRP Levels and Variables of Eosinophil Activation

Eosinophils are the primary effector cells in the etiology of asthma. Serum ECP level is known as a marker for eosinophils and is elevated in accordance with asthma exacerbation.³ Peripheral eosinophil counts and serum ECP levels were compared with hs-CRP levels. Although there was no correlation between hs-CRP levels and peripheral eosinophil counts ($n = 84$; $r_s = 0.071$; $P = .71$), a significant correlation was found between serum hs-CRP levels and ECP levels in the 26 asthmatic patients during attacks ($r_s = 0.415$; $P = .04$) (Fig 3A).

Relationship Between Serum hs-CRP Levels and Atopy

Atopic and nonatopic asthmatic patients had significantly higher levels of serum hs-CRP than controls during attacks and during stable periods (Fig 3B). Furthermore, there is no significant difference between atopic and nonatopic asthmatic patients during attacks and remission.

DISCUSSION

In this study, we investigated the clinical relevance of serum CRP levels in bronchial asthma. Buyukozturk et al¹⁷ found no difference in CRP levels between controls and asthmatic patients. However, by using highly sensitive measurements, we found that serum hs-CRP levels were elevated in asthmatic patients with and without attacks, and serum hs-CRP levels were higher during an attack compared with when stable. Evidence of the difference in methods between the 2 studies is demonstrated by the CRP measurements being 50

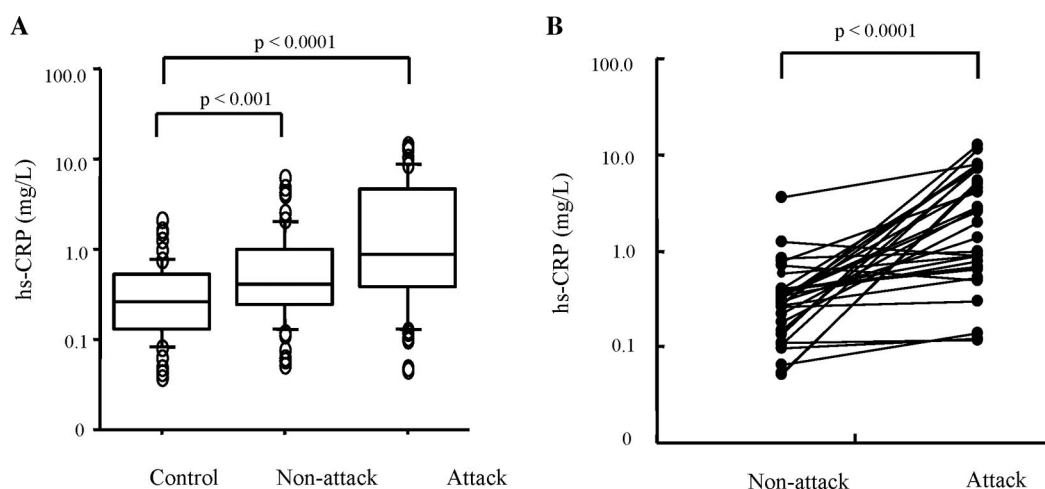


Figure 1. Serum levels of highly sensitive C-reactive protein (hs-CRP) in controls and patients with bronchial asthma without attacks and with attacks. A, Serum levels of hs-CRP were significantly higher in patients with bronchial asthma without attacks ($n = 70$) and with attacks ($n = 70$) than in controls ($n = 70$) (Kruskal-Wallis test, $P < .001$). B, Increase in serum hs-CRP levels in 31 patients with asthma before and after attacks (Wilcoxon rank sum test, $P < .001$). Central horizontal bars represent medians; boxes, 25th to 75th percentiles; whiskers, 10th to 90th percentiles; and circles, outliers.

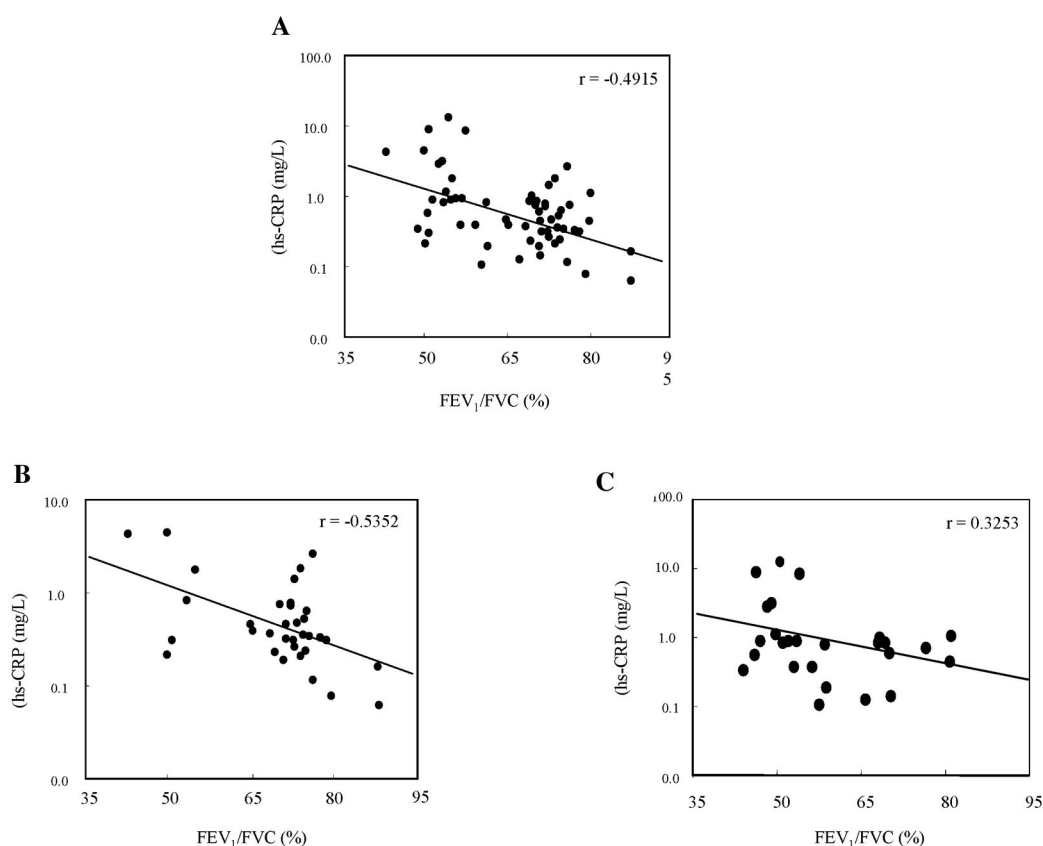


Figure 2. Relationship between serum highly sensitive C-reactive protein (hs-CRP) levels and pulmonary function in patients with asthma. There were significant negative correlations between serum hs-CRP levels and forced expiratory volume in 1 second/forced vital capacity (FEV_1/FVC) in all 60 asthmatic patients (Pearson correlation coefficient, $r = -0.4915$; $P < .01$) (A) and in the 34 patients without attacks (Pearson correlation coefficient, $r = -0.5352$; $P < .001$) (B). There was no correlation between serum hs-CRP levels and FEV_1/FVC in the 26 patients with attacks (Pearson correlation coefficient, $r = 0.3253$; $P = .11$) (C).

to 100 times greater in this study compared with that by Buyukozturk et al. To our knowledge, this is the first study to report the relationships among hs-CRP, ECP, and lung function in bronchial asthma.

The hs-CRP assay detects small changes in CRP levels within the reference range, allowing for the evaluation of subclinical inflammation as a potential new marker of vascular risk.¹⁸ The increased production of CRP that predicts atherothrombotic events may reflect subclinical infections such as *Chlamydia pneumoniae* and *Helicobacter pylori*.¹⁹ Consequently, elevated hs-CRP levels in asthmatic patients may also reflect respiratory tract or other local site infections. Therefore, in this study, we selected asthmatic patients without objective evidence of infection based on interview information and either the absence of fever, sore throat, and sputum or abnormal laboratory values.

It was recently reported that raised levels of hs-CRP are associated with respiratory symptoms in asthmatic patients.¹³ In agreement with this study, the present study shows that the increase in serum hs-CRP levels correlate with the degree of

airway obstruction as evaluated by FEV_1/FVC . These findings suggest that hs-CRP levels may be related to the state of asthma control and chronic allergic inflammation and may be helpful in the management of asthmatic patients. However, hs-CRP levels of some asthmatic patients with attacks were unchanged or reduced compared with results during an asymptomatic period. Furthermore, in asthmatic patients with attacks, there was no significant correlation between hs-CRP levels and FEV_1/FVC . One reason for this finding may be that the interval between the beginning of the attack and sample collection for the attack was variable.

Olafsdottir and coworkers¹³ also suggested that hs-CRP levels were increased in patients without atopy but not in those with atopy; however, the present results differ in that we show that atopic and nonatopic asthmatic patients during attacks had significantly higher levels of serum hs-CRP than controls. Therefore, in the present results, hs-CRP levels may be a marker of atopic and nonatopic asthma. The different results between the 2 studies may be related to the different range of hs-CRP used. The present data suggest that elevated

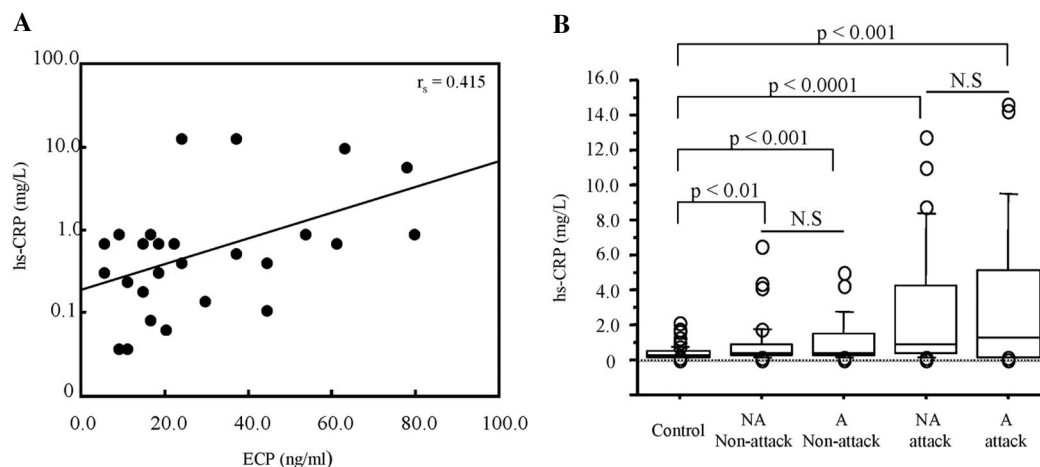


Figure 3. A, Relationship between serum eosinophil cationic protein (ECP) and serum highly sensitive C-reactive protein (hs-CRP) levels in 26 patients with asthma during attacks. There was a significant correlation between ECP and hs-CRP levels in the serum of asthmatic patients during attacks (Spearman rank correlation test, $r_s = 0.415$; $P = .04$). B, Serum levels of hs-CRP in patients with atopic asthma without attacks ($n = 30$), atopic asthma with attacks ($n = 28$), nonatopic asthma without attacks ($n = 40$), nonatopic asthma with attacks ($n = 42$), and controls ($n = 70$). Serum hs-CRP levels in all 4 patient groups were significantly higher than those in controls (Kruskal-Wallis test, $P < .001$). A indicates atopy; NA, nonatopy; NS, not significant. Central horizontal bars represent medians; boxes, 25th to 75th percentiles; whiskers, 5th to 95th percentiles; and circles, outliers.

hs-CRP levels in serum do not indicate IgE-dependent inflammation but rather eosinophilic inflammation independent of stimuli. Indeed, in this study, there was a significant correlation between serum hs-CRP and ECP levels, which is one of the eosinophilic activity markers. This finding possibly suggests that serum hs-CRP level reflects eosinophilic inflammation in asthma and other eosinophilic airway diseases.

In conclusion, increased hs-CRP levels may be associated with allergic inflammation, particularly eosinophilic inflammation, and the degree of airway obstruction in asthmatic patients. Although several clinical markers of asthma, including ECP, have been evaluated in many previous studies, the ideal measure of asthma activity has not been determined. It is important to explore a new marker that can help physicians care for asthmatic patients. In asthma, serum hs-CRP measurement is noninvasive and easier than measurement in induced sputum or bronchoalveolar lavage fluid. Therefore, hs-CRP might be a useful clinical marker of eosinophilic airway inflammation in asthma and might assist in the clinical management of the disease. However, there was some overlap among controls and asthmatic patients with and without exacerbation. Thus, future studies are necessary to clarify the role of hs-CRP in the management of asthma.

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