

Changes in High-Sensitivity C-Reactive Protein in Serum and Exhaled Breath Condensate after Intensive Exercise in Patients with Allergic Asthma

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Key Words

Asthma • Exercise-induced bronchoconstriction • High-sensitivity C-reactive protein • Airway inflammation • Exhaled breath condensate

Abstract

Background: Exercise-induced bronchoconstriction (EIB) in asthmatics depends on the presence of allergic inflammation. This study was performed to assess the possible association of EIB with low-grade systemic inflammation, whose presence was revealed in asthmatic patients. **Methods:** The study was conducted in a group of 24 asthmatics (14 with EIB, 10 without EIB) and 8 healthy volunteers. Changes in serum and exhaled breath condensate (EBC) high-sensitivity C-reactive protein (hs-CRP) levels induced by intensive exercise were determined. Moreover, the possible correlation of these measurements with the results of other tests used in the diagnosis of asthma as well as laboratory tests commonly associated with asthma were investigated. **Results:** In asthmatic patients with EIB, a statistically significant increase in hs-CRP levels both in serum and EBC after an exercise test was observed. Twenty-four hours after the exercise test in the group of asthmatics with EIB, a statistically significant increase in exhaled nitric oxide (F_{ENO}), serum eosinophil cationic protein (ECP) concentrations and bronchial hyperreac-

tivity to histamine was revealed. A statistically significant correlation between the maximum increase in hs-CRP levels both in serum and EBC after exercise and either baseline F_{ENO} and an increase in serum ECP or F_{ENO} 24 h after exercise in the group of asthmatics with EIB was revealed. **Conclusions:** We show that, as a result of intensive exercise leading to bronchoconstriction, an increase in serum and EBC hs-CRP occurs. Our observations could suggest that in asthmatic patients, as a consequence of exercise-induced bronchoconstriction, an intensification of low-grade systemic inflammation can be observed.

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Introduction

Asthma is characterized by chronic inflammation of the airways [1]. The increased levels of plasma fibrinogen and serum amyloid A observed in patients with asthma suggest that in these patients not only local but also systemic inflammation is present [2].

Yoshikawa et al. [3] have revealed that there is a relationship between the severity of exercise-induced bronchoconstriction (EIB) and the eosinophil count in induced sputum as well as in serum eosinophil cationic protein (ECP) levels in adult asthmatic patients. This sug-

Table 1. Characteristics of study subjects and healthy volunteers

Characteristics	Patients with EIB	Patients without EIB	Differences between groups of asthma patients, p	Healthy volunteers
Number of patients	14	10		8
Sex, F/M	8/6	6/4		4/4
Age, years	31.7 ± 6.9	32.3 ± 6.4	0.92	29.9 ± 7.1
Duration of symptoms, years	4.0 ± 3.2	3.3 ± 2.5	0.62	
Baseline FEV ₁ , % predicted	86.29 ± 8.17	92.10 ± 5.58	0.29	98.37 ± 4.45*, +
PC ₂₀ histamine FEV ₁ , mg/ml	1.36 ± 0.93	1.57 ± 1.07	0.66	
ECP (serum), µg/l	13.65 ± 2.91	11.49 ± 2.08	0.045	2.88 ± 0.84*, +
Total IgE (serum), kU/l	258 ± 115	194 ± 66	0.31	39 ± 12*, +
Blood eosinophil count, cells/mm ³	252 ± 131	183 ± 107	0.21	68 ± 37*, +
Baseline F _{ENO} , ppB	75 ± 46	59 ± 35	0.59	15 ± 5*, +
Hs-CRP (EBC), mg/l	0.20 ± 0.03	0.18 ± 0.03	0.2	0.07 ± 0.02*, +
Hs-CRP (serum), mg/l	1.35 ± 0.63	1.11 ± 0.57	0.56	0.29 ± 0.08*, +
BMI	23.5 ± 3.6	22.4 ± 3.1	0.87	21.9 ± 2.7
Maximum decrease of FEV ₁ after exercise, %	22.8 ± 11.8	3.4 ± 2.1	0.0003	0.91 ± 3.7*, +

Data are presented as medians. PC₂₀ histamine FEV₁ = Provocative concentration of histamine that caused a 20% fall in FEV₁.
 * Values significantly different from patients with EIB, p < 0.05. + Values significantly different from patients without EIB, p < 0.05.

gests that the presence of sputum eosinophilia predicts the airway responsiveness induced by an exercise challenge. Eosinophilic inflammation is also augmented by postexercise bronchoconstriction, which in turn is stimulated by many mediators released mainly by mast cells [4]. We observed an upregulation of airway inflammation, expressed as an increase in exhaled nitric oxide (F_{ENO}) levels and in bronchial hyperreactivity (BHR) to histamine after postexercise bronchoconstriction in allergic asthma patients [5].

There are no studies confirming the connection between postexercise bronchoconstriction and systemic inflammation in asthmatic patients. C-reactive protein (CRP) is one of the most characteristic markers of the inflammatory process. Therefore, the use of high-sensitivity assays for CRP determination (hs-CRP) could be a helpful tool for assessment of low-grade systemic inflammation [6, 7].

In the present study we have decided to investigate the effect of postexercise bronchoconstriction on low-grade systemic inflammation in asthmatic patients. We have determined changes in serum and exhaled breath condensate (EBC) hs-CRP levels following intensive exercise in order to establish the possible correlation of these measurements with the parameters of airway inflammation and their changes after exercise.

Methods

Subjects

The study involved a group of 24 patients with mild allergic asthma. Asthma was diagnosed according to the criteria recommended by the GINA [8]. The presence of an allergy to common allergens (house dust mite, trees, weeds, grasses, cat, *Alternaria*, or *Cladosporium*) was demonstrated in all patients by positive skin prick tests. The characteristics of the studied patients are presented in table 1. All patients had been in a stable condition, free from respiratory tract infections and acute exacerbations during the past 4 weeks. Patients with other factors which could influence F_{ENO} levels (except for asthma, features of atopy or allergic rhinitis) were excluded from the study. Before the beginning of this study, patients were allowed to take short-acting β₂-agonists. Asthmatic patients who had been treated with inhaled steroids in the past 3 months were excluded from the study. 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. Subjects with heart disease, diabetes mellitus, cancer status, obesity (body mass index >30) and systemic inflammatory disorders (such as collagen vascular diseases) were excluded from the study.

The control group was 8 healthy volunteers. F_{ENO}, flow/volume spirometry and prick tests with common inhaled allergens were performed in all healthy persons. All of them were free of chronic or recurrent respiratory symptoms with no past history of significant pulmonary disease. They were nonatopic and had forced expiratory volume in 1 s (FEV₁) >80% predicted. They were free of respiratory tract infection within 2 months prior to the study as well as free from other significant illnesses known to

affect the measurement of F_{ENO} or hs-CRP levels (which have been described previously).

All studied asthmatic patients and healthy volunteers were nonsmokers and during the last year had not been passive smokers.

The Schema of the Protocol

Bronchial provocation test (BPT) with histamine was performed 24 h before and 24 h after an exercise test. EBC was collected before and 30 min after BPT with histamine as well as before, 30 min and 24 h after the exercise test. F_{ENO} was measured before BPT with histamine, before the exercise test and 24 h after the exercise test. Before and 24 h after the exercise test, serum ECP and peripheral blood eosinophilia were measured.

The study protocol was approved by the Ethics of Research Committee of the Medical University of Bialystok, number of agreement: R-I-003/80/2006. Informed consent was obtained from every patient entered into the study.

Measurements

The baseline spirometry was performed using a MasterScreen Pneumo PC spirometer (Jaeger, Hoechberg, Germany). Spirometry was performed according to ATS standards [9]. FEV_1 was evaluated. Before the examination, the patients did not take any medications that could change spirometry results. The highest value from 3 technically satisfactory attempts was attached.

A nonspecific BPT with histamine was carried out according to the method described by Ryan et al. [10]. Provocation was performed using a De Villbiss nebuliser 646 (Viasys Healthcare GmbH, Hoechberg, Germany) at an air pressure of 0.15 MPa linked to a Rosenthal-French dosimeter (Applied Immunology, Baltimore, Md., USA). The results were presented as PC_{20} FEV_1 – the concentration of histamine which causes a decrease in FEV_1 of exactly 20% in comparison to initial values.

An exercise test was performed on a bicycle ergometer for 9 min with a fixed work load adjusted to increase the heart rate to 85% of the maximum predicted for the age of each patient [11]. Serial FEV_1 measurements were made 5 min before exercise and at 5, 10, 15, 20, 25, 30 and 60 min as well as 24 h after exercise. Those patients whose maximum decrease in FEV_1 was greater than 15% from baseline were considered to have EIB. The EBC and blood samples were collected before, 30 min after and 24 h after the exercise test.

F_{ENO} was measured by the chemiluminescence technique, using a Sievers 280i NO Analyzer (Boulder, Colorado, USA). The measurements were performed at an expiratory flow of 50 ml/s according to ATS recommendations for on-line measurement of F_{ENO} in adults [12].

EBC was collected by using a commercially available condenser (EcoScreen; Jaeger) according to the current ATS/ERS guidelines [13]. All measurements were performed at the same time (between 8.00–10.00 a.m.) to avoid possible circadian rhythm of mediator concentrations in EBC. All patients were asked to refrain from eating and drinking before collecting EBC. Exhaled air entered and left the chamber through one-way valves and an inlet and outlet, thus keeping the chamber closed. A low temperature inside the condensing chamber throughout the collection time produced a cooling-down sample. The temperature of collection was around 0°C [13, 14]. Patients were instructed to breathe tidally for 10 min with a nose clip. The respiratory rate

ranged from 15 to 20 breaths/min. Patients were asked to swallow their saliva periodically and to temporarily discontinue collection if they needed to cough. At the end of collection, 1.5- to 3.5-ml aliquots of condensate were transferred to Eppendorf tubes and immediately frozen. Samples were stored at –80°C [15].

Serum total IgE concentrations and serum ECP were measured using ImmunoCAP™ Technology (Pharmacia Diagnostics, Uppsala, Sweden). The minimum detectable level of ECP was 2.0 µg/l. Blood eosinophil count was measured using a hematology analyzer (Coulter Electronics GmbH, Miami, Fla., USA). The concentrations of hs-CRP in EBC and serum were determined using a highly sensitive CRP assay (Konelab, Waltham, Mass., USA). The method is based on measurement of immunoprecipitation at 540 nm. Microparticles coated with anti-human CRP are added to buffered samples. The increase in absorbance caused by immunoprecipitation is recorded when the reaction has reached its endpoint. The change in absorbance is proportional to the amount of antigen (CRP) in solution. The minimum detectable level of hs-CRP was 0.05 mg/l. All measurements were performed in a blind fashion. All samples were run in duplicate.

Analysis

Statistical significance was analyzed by using analysis of variance followed by Bonferroni's *t* test post hoc to determine statistical differences. All values were expressed as means $\pm$ SD; *p* values <0.05 were considered significant. The relationship between studied parameters was assayed by correlation. Pearson's linear correlation coefficient was used.

Results

Characteristics of asthmatic patients and healthy volunteers are presented in table 1. In the studied group of asthmatics, 14 patients had a positive and 10 had a negative exercise test. In none of the healthy volunteers were spirometric indices worse after exercise.

In the group of asthmatics with EIB compared with asthmatic patients without EIB, slightly higher levels of markers of allergic inflammation were revealed; however, statistical significance was observed only in ECP levels. In asthmatic patients (both with or without EIB) compared with healthy volunteers, in serum as well as in EBC statistically significantly higher hs-CRP levels were revealed (serum hs-CRP of asthmatic patients: 1.25 ± 0.61 mg/l vs. healthy volunteers: 0.29 ± 0.08 , $p < 0.0001$; EBC hs-CRP of asthmatic patients: 0.19 ± 0.04 mg/l vs. healthy volunteers: 0.07 ± 0.02 , $p < 0.0001$).

Exercise-Induced Changes in Baseline Levels of hs-CRP

A comparison of the baseline concentrations of serum hs-CRP between both groups of asthma patients and healthy volunteers demonstrated statistically significant-

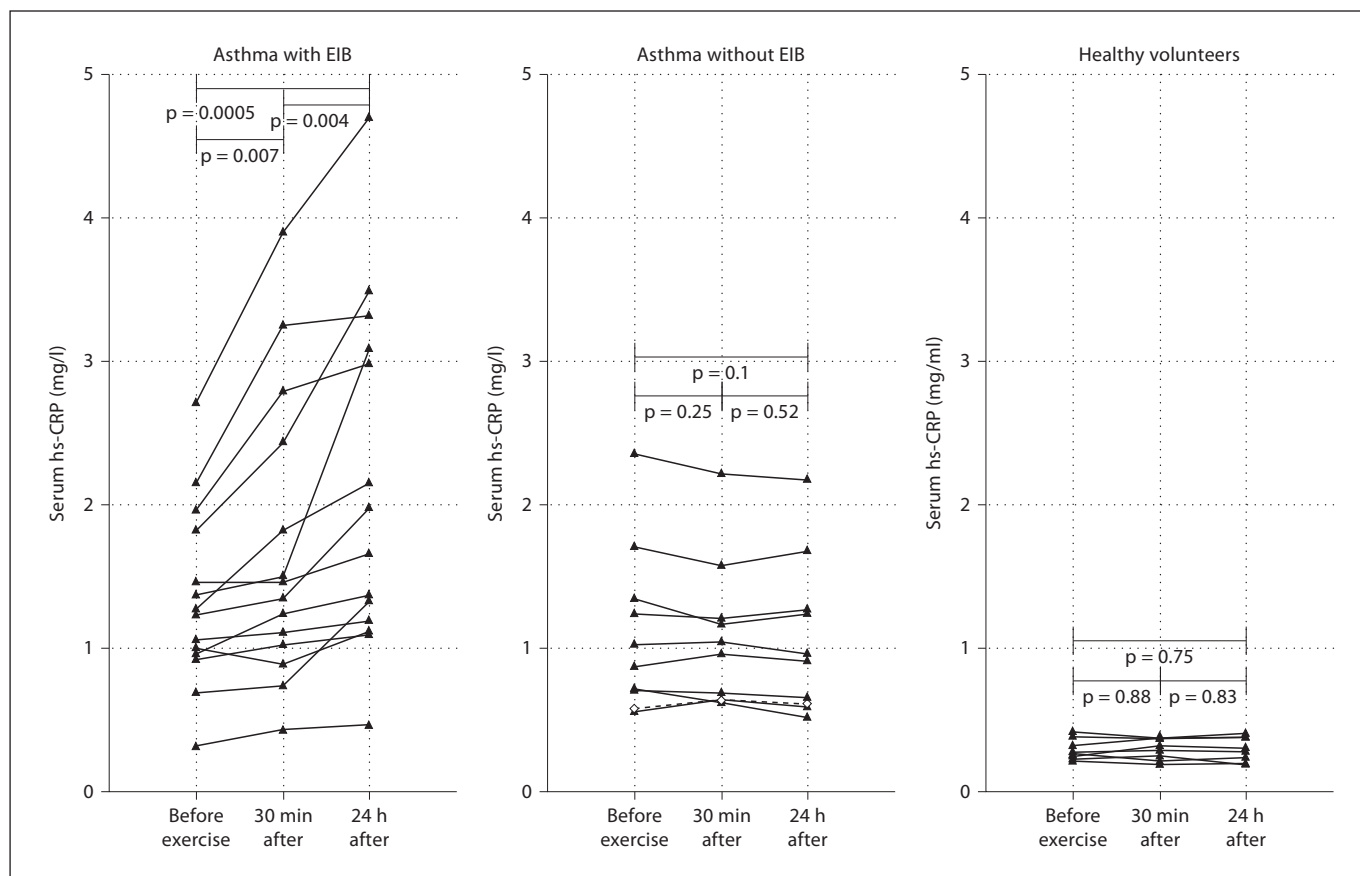


Fig. 1. Changes in the serum hs-CRP induced by intensive exercise in the studied groups of asthmatics and healthy volunteers.

ly higher levels in asthmatics, both with or without EIB (patients with EIB: $1.35 \text{ mg/l} \pm 0.63$ vs. patients without EIB: 1.11 ± 0.57 , $p = 0.56$; vs. healthy volunteers: 0.29 ± 0.08 , $p = 0.0005$; asthma without EIB vs. healthy volunteers: $p = 0.01$). Thirty minutes after exercise, in the group of asthmatic patients with EIB, a statistically significant elevation in the concentration of serum hs-CRP was revealed ($1.71 \pm 1.01 \text{ mg/l}$). Twenty-four hours after exercise, a further statistically significant increase in hs-CRP was observed (2.13 ± 1.20). There were no significant changes in serum hs-CRP in the group of asthma patients without EIB and healthy volunteers after exercise (fig. 1).

The concentrations of hs-CRP in EBC before exercise in both groups of asthma patients were statistically significantly higher in comparison to the group of healthy volunteers (patients with EIB: $0.20 \text{ mg/l} \pm 0.03$ vs. patients without EIB: 0.18 ± 0.03 , $p = 0.2$; vs. healthy volunteers: 0.07 ± 0.02 , $p < 0.0001$; asthma without EIB vs. healthy volunteers: $p < 0.0001$). In the group of patients

with EIB, a statistically significant increase in hs-CRP in EBC was demonstrated 30 min after exercise ($0.23 \text{ mg/l} \pm 0.05$). Twenty-four hours after exercise, a further, marked increase in the concentration of hs-CRP in EBC was still observed (0.27 ± 0.07). There were no significant changes in EBC hs-CRP in the group of asthma patients without EIB and healthy volunteers after exercise (fig. 2).

Correlations between hs-CRP Levels and Other Studied Parameters

A statistically significant correlation between hs-CRP levels in serum and EBC in both studied groups of asthmatics was revealed (asthma with EIB: $r = 0.96$, $p < 0.0001$; asthma without EIB: $r = 0.88$, $p = 0.0006$). Such correlation was not observed in healthy volunteers.

We revealed statistically significant positive correlations between levels of hs-CRP in either serum or EBC (before exercise) and either F_{ENO} or serum ECP in both groups of asthma patients before exercise. There was no

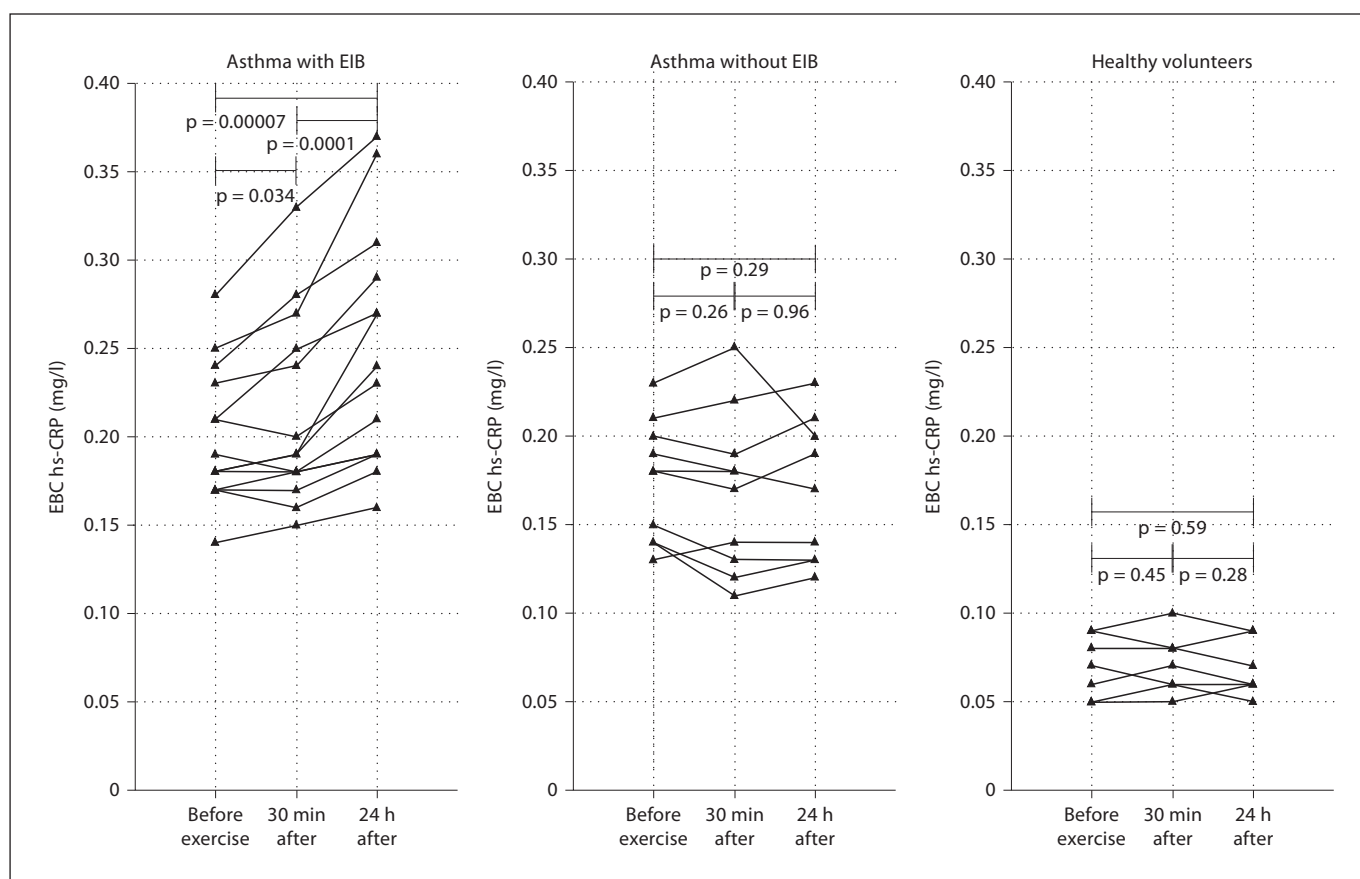


Fig. 2. Concentrations of hs-CRP in EBC at rest, and subsequent changes during the exercise test in groups of patients with asthma and healthy volunteers.

statistically significant correlation between hs-CRP in serum or EBC (before exercise) and other studied parameters in any of the studied groups of asthmatics or healthy volunteers. These results are presented in tables 2 and 3.

A statistically significant correlation between the increase in hs-CRP concentration both in serum ($r = 0.63$, $p = 0.01$) or EBC ($r = 0.68$, $p = 0.006$) observed 24 h after exercise and F_{ENO} before exercise was revealed. There was no correlation between changes in hs-CRP in serum and EBC after exercise and other studied parameters before exercise in all studied groups.

There were no significant correlations between the severity of EIB and hs-CRP both in serum and EBC before the exercise (serum: $r = 0.48$, $p = 0.08$; EBC: $r = 0.53$, $p = 0.053$) or hs-CRP changes 24 h after the exercise (serum: $r = 0.47$, $p = 0.08$; EBC: $r = 0.48$, $p = 0.079$) (fig. 5). We did not reveal significant correlations between the severity of EIB and measurements of other studied parameters assessed before and 24 h after the exercise.

Correlations between the Changes in F_{ENO} , BHR and Serum ECP 24 h after Exercise and Other Studied Parameters

Changes in F_{ENO} , BHR and serum ECP 24 h after exercise are presented in table 4. Twenty-four hours after the exercise test in the group of asthmatics with EIB, a statistically significant increase in F_{ENO} , serum ECP concentrations and BHR to histamine was revealed. Such changes were not observed in the group of asthmatic patients without EIB and in healthy volunteers (table 4).

In the group of asthmatic patients with EIB a statistically significant correlation between the increase in serum hs-CRP concentrations 24 h after exercise and an increase in F_{ENO} ($r = 0.54$, $p = 0.04$) or an increase in ECP ($r = 0.57$, $p = 0.03$) 24 h after exercise was revealed (fig. 3). Such correlations between the increase in hs-CRP concentrations in EBC 24 h after exercise and an increase in F_{ENO} ($r = 0.54$, $p = 0.04$) or an increase in ECP ($r = 0.86$, $p = 0.0006$) 24 h after exercise were also observed (fig. 4).

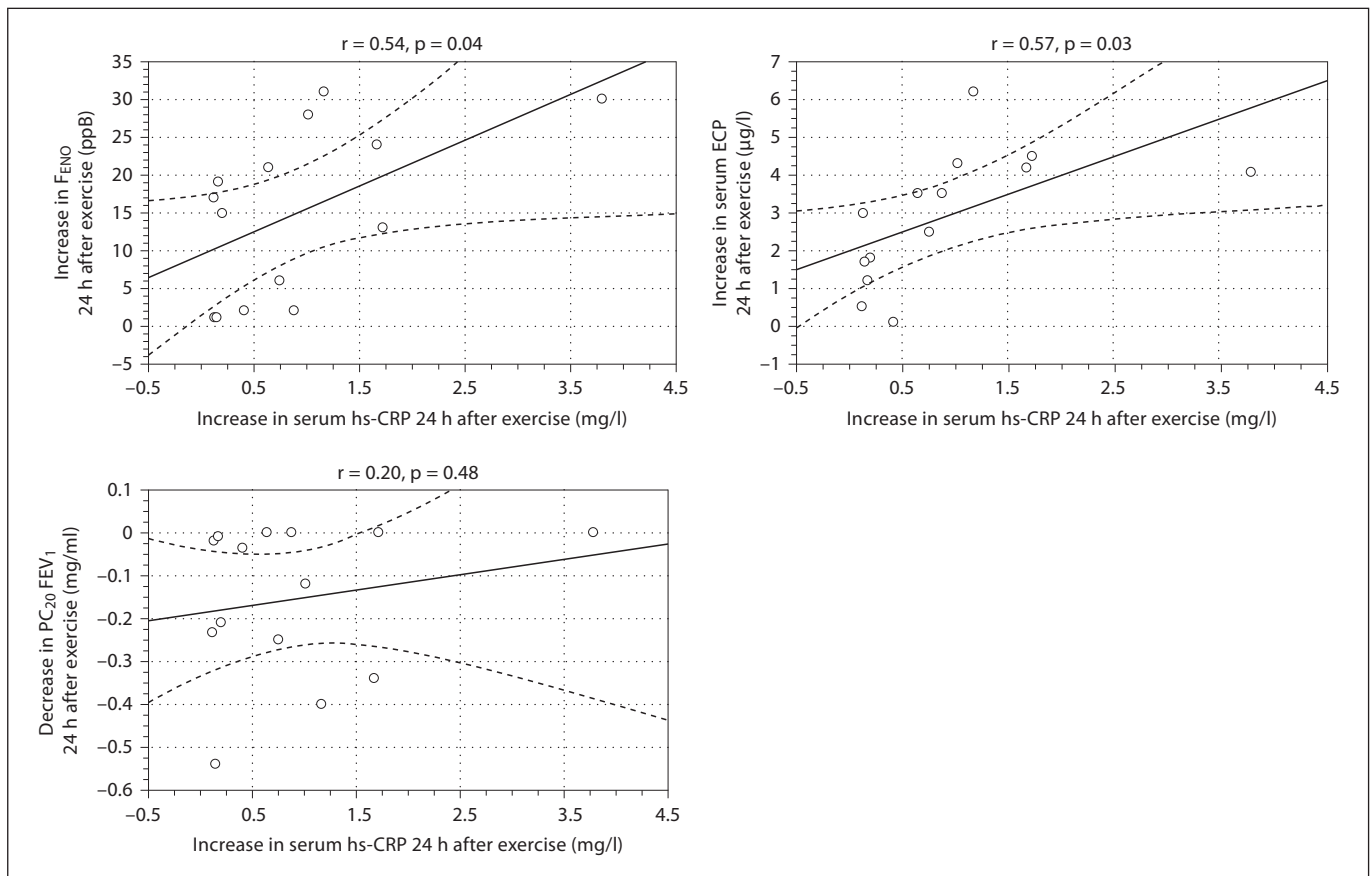


Fig. 3. Correlations between the increase in serum hs-CRP 24 h after exercise and increase in F_{ENO} , ECP or BHR to histamine 24 h after exercise.

Table 2. Correlations between baseline levels of hs-CRP in serum and other studied parameters in the groups of asthma patients and healthy volunteers before exercise

Studied groups	F_{ENO}	Serum ECP	Blood eosinophil count	Serum total IgE	Baseline FEV_1	BHR to histamine
Asthma with EIB						
r	0.85	0.60	0.42	0.27	0.13	0.15
p	0.00009	0.02	0.12	0.34	0.65	0.62
Asthma without EIB						
r	0.91	0.68	0.41	0.54	0.51	-0.46
p	0.0002	0.03	0.09	0.10	0.13	0.18
Healthy volunteers						
r	-0.04	0.41	0.36	-0.40	0.17	
p	0.92	0.30	0.16	0.32	0.68	

Table 3. Correlations between baseline levels of hs-CRP in EBC and other studied parameters in the groups of asthma patients and healthy volunteers before exercise

Studied groups	F_{ENO}	Serum ECP	Blood eosinophil count	Serum total IgE	Baseline FEV_1	BHR to histamine
Asthma with EIB						
r	0.88	0.71	0.32	0.32	0.13	0.13
p	0.00002	0.003	0.53	0.24	0.64	0.66
Asthma without EIB						
r	0.84	0.68	0.17	0.60	0.35	-0.23
p	0.002	0.02	0.52	0.06	0.32	0.24
Healthy volunteers						
r	0.03	0.15	0.38	-0.37	0.04	
p	0.93	0.71	0.12	0.35	0.91	

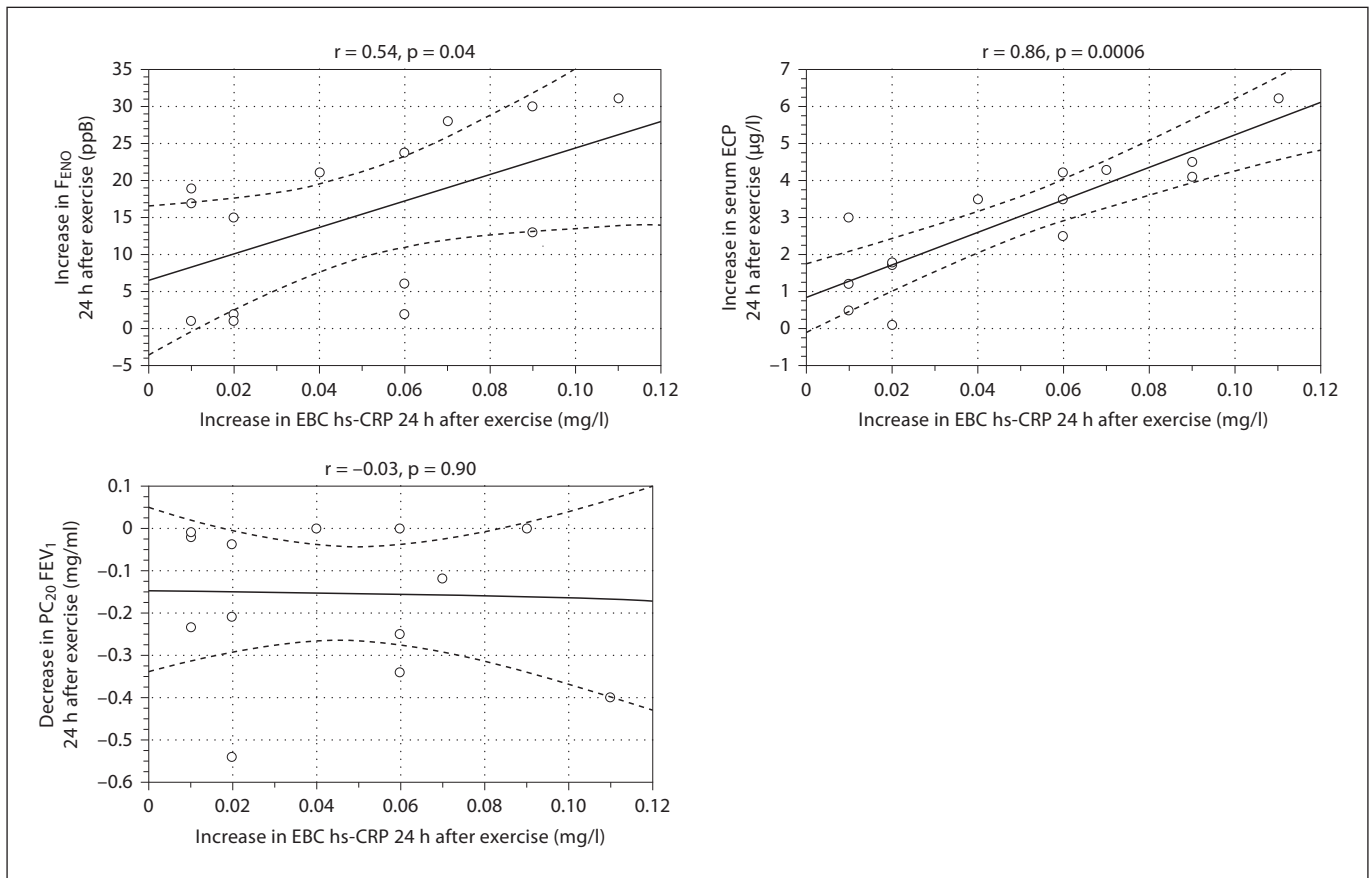


Fig. 4. Correlations between the increase the levels of hs-CRP in EBC 24 h after exercise and increase in F_{ENO}, ECP or BHR to histamine 24 h after exercise.

Table 4. Changes in F_{ENO}, BHR to histamine and serum ECP 24 h after exercise in the studied groups of asthmatics and healthy volunteers

Studied groups	F _{ENO} , ppB		BHR to histamine, PC ₂₀ FEV ₁		ECP, μg/l	
	before exercise	24 h after exercise	before exercise	24 h after exercise	before exercise	24 h after exercise
Patients with EIB	75.00 ± 46.82	90.00 ± 55.64	1.36 ± 0.93	1.16 ± 0.91	13.65 ± 2.91	16.59 ± 4.23
p	0.0002		0.009		0.0002	
Patients without EIB	59.30 ± 35.40	61.60 ± 36.95	1.57 ± 1.07	1.54 ± 1.05	11.49 ± 2.08	11.70 ± 2.40
p	0.15		0.34		0.54	
Healthy volunteers	15.02 ± 5.10	14.41 ± 3.80			2.88 ± 0.84	2.61 ± 0.75
p	0.51				0.53	

PC₂₀ histamine FEV₁ = Provocative concentration of histamine that caused a 20% fall in FEV₁.

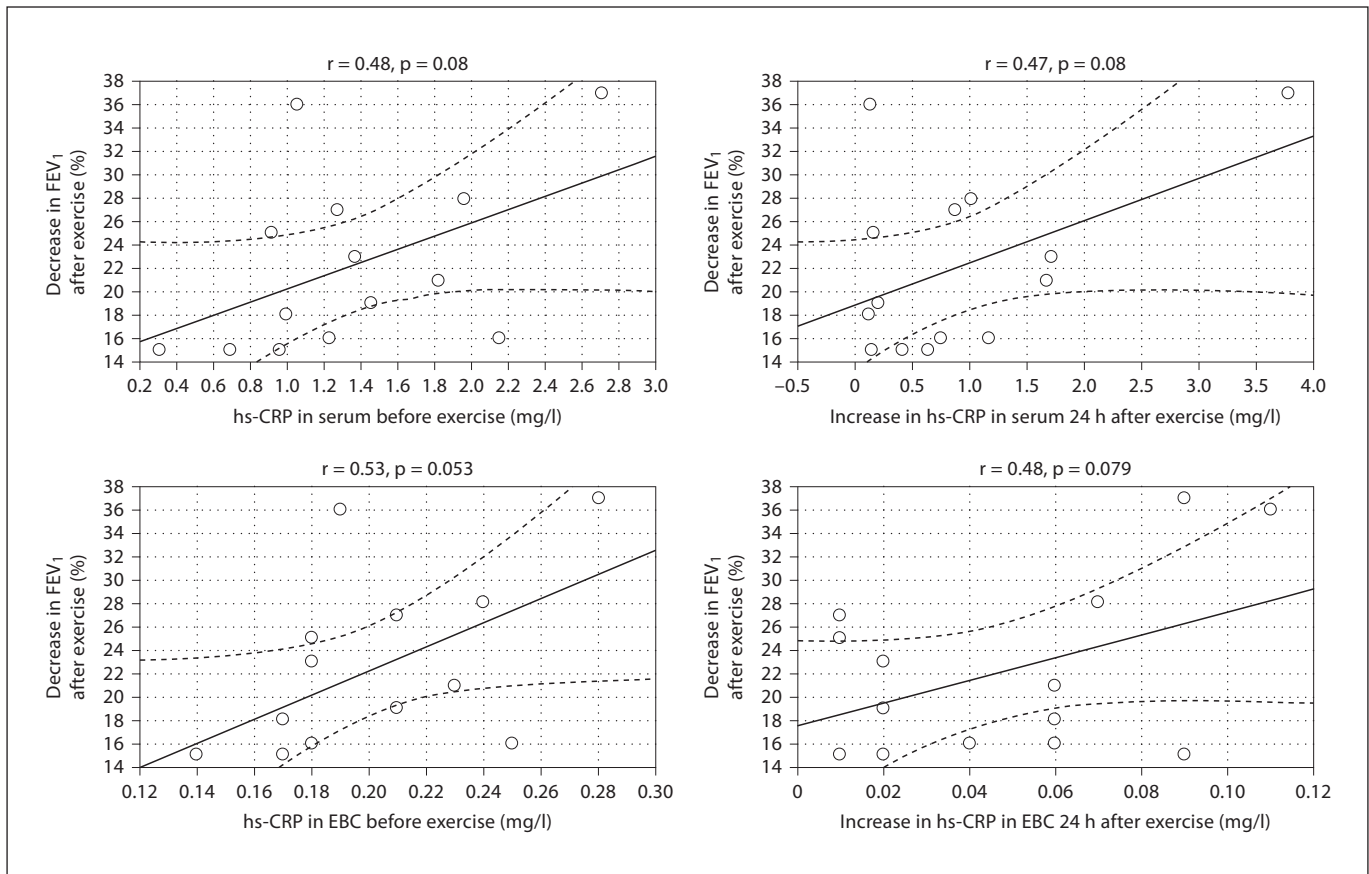


Fig. 5. Correlations between severity of EIB and hs-CRP concentrations before exercise or hs-CRP changes observed 24 h after exercise.

There was no correlation between the increase in hs-CRP concentration (both in serum or EBC) 24 h after exercise and a decrease in PC_{20} FEV_1 24 h after exercise (serum hs-CRP: $r = 0.20$, $p = 0.48$; EBC hs-CRP: $r = -0.03$, $p = 0.9$).

No changes in the mean serum and EBC concentrations of hs-CRP were found within 30 min and 24 h after a nonspecific BPT with histamine. No changes in F_{ENO} before and 24 h after BPT with histamine were observed (data not shown).

Discussion

There are many reports focused on the importance of the inflammatory process related to the activation of many cells, including eosinophils, in the pathogenesis of exercise-induced bronchoconstriction in asthma [4]. The

correlations between the severity of exercise-induced bronchoconstriction and peripheral blood eosinophilia [16] or serum ECP concentration [17] have been described. Kivity et al. [18] have demonstrated a significant increase in sputum eosinophil counts in patients with exercise-induced bronchoconstriction, but not in those with no EIB or after BPT with methacholine.

Yoshikawa et al. [3] have revealed that there is a connection between the severity of EIB and induced sputum eosinophil counts or ECP concentrations in adult asthmatics. These results suggest that eosinophilia in induced sputum predicts the occurrence of exercise-induced bronchoconstriction in asthmatic patients. On the other hand, EIB, resulting in the release of many mediators mainly from mast cells, augments the inflammatory process connected with eosinophil activation [4].

The occurrence of EIB in asthmatic patients depends on allergic inflammation. There are a few reports also

suggesting that EIB, through the release of many pro-inflammatory cytokines and activation of inflammatory cells, could augment airway inflammation and bronchial hyperreactivity [19, 20].

In our previous study we revealed that in asthmatic patients, as a consequence of intensive exercise leading to bronchoconstriction, both damage and activation of endothelial cells occur. In asthmatics, changes in endothelial cells could in turn evoke the release of inflammatory mediators and migration of inflammatory cells, and, as a result, lead to intensification of the inflammatory process in airways and bronchial hyperreactivity after exercise [5].

CRP is one of the recognized markers of the inflammatory process. CRP is produced mainly by Browicz-Kupfer cells and hepatocytes [21, 22]. The mechanism of activating CRP production is still unclear. It has been demonstrated that the main stimulators of this process are IL-1, IL-6 and TNF- α . These cytokines play an important role in the pathophysiology of asthma [22, 23]. The hs-CRP assays detect small changes in CRP levels within the reference range, and the introduction of this measurement has significantly extended the possibilities for employing CRP. Measurements of serum hs-CRP levels have suggested the presence of low-grade systemic inflammation in several disorders, such as diabetes mellitus or cardiovascular disorders [6, 7].

Jousilahti et al. [2] have demonstrated that levels of acute phase proteins (such as plasma fibrinogen and serum amyloid A) are positively associated with the prevalence of asthma. It has also been shown that in patients with asymptomatic asthma increased levels of amyloid A were present [24]. Taking these findings into account, it is very likely that systemic inflammation may exist in asthma. There are some studies concerning the possibility of using hs-CRP measurements to assess subclinical, systemic inflammation in asthma. Olafsdottir et al. [25] have revealed that increased serum levels of hs-CRP are associated with respiratory symptoms in asthmatics. The authors also demonstrate that the increase in serum hs-CRP correlates with the degree of airway obstruction. Moreover, they suggest that serum hs-CRP levels may be associated with the state of asthma control and chronic airway inflammation, and hs-CRP measurements could be a useful tool in the management of asthmatic patients [25]. Takemura et al. [26] have revealed that in patients with steroid-naïve asthma, serum levels of hs-CRP increase, compared with healthy volunteers, and correlate positively with sputum eosinophilia and negatively with indices of pulmonary function. Fujita et al. [27] suggest

that serum hs-CRP levels could be a marker of atopic and nonatopic asthma. The authors show a significant correlation between serum hs-CRP and ECP levels, and they suppose that increased hs-CRP levels may be related to allergic, particularly eosinophilic, inflammation.

There are no studies concerning the connection between postexercise bronchoconstriction and systemic inflammation in asthmatic patients. Moreover, there are no reports about the possible influence of EIB on low-grade systemic inflammation in asthma patients.

The aim of this study was to evaluate the changes in hs-CRP both in serum and exhaled breath condensate following intensive exercise in asthmatic patients, and to establish the possible correlation of these measurements with the parameters of airway inflammation, as well as their changes after exercise.

Several factors could affect serum levels of hs-CRP. The presence of diabetes mellitus, cardiovascular disease or obesity is associated with increased serum hs-CRP levels, which may be due to adipocyte-derived IL-6 [6, 28]. In smokers, elevated levels of hs-CRP have also been shown; stopping smoking leads to their reduction [29]. Aging is another factor of elevated hs-CRP levels [30]. In our study, patients and healthy volunteers were carefully selected to exclude all these confounding factors. We selected asthmatics and healthy persons without objective evidence of infection based on interview information, absence of clinical symptoms or abnormal laboratory values.

Our results confirmed the presence of low-grade systemic inflammation in asthma as shown previously [2, 24, 26, 27]. Hs-CRP levels in serum and EBC were statistically significantly higher in asthmatics than in healthy controls. We found a correlation of hs-CRP levels, measured in serum and EBC, with the results of other inflammatory markers (such as F_{ENO} and ECP).

In the study performed by Ueno et al. [31], in which CRP was measured using ELISA with a high sensitivity, the authors demonstrated raised CRP levels in the EBC in asthmatics compared with healthy volunteers. These results were confirmed by our previous report, in which the increase in hs-CRP levels both in serum and EBC and the correlation of this parameter with F_{ENO} and ECP in asthmatic patients were revealed [32]. However, Rosias et al. [33] were not able to demonstrate the presence of CRP in the EBC of asthmatic children and healthy controls. Moreover, the authors emphasized that high-sensitivity assays for measurement of inflammatory markers in EBC should be used.

In our study, hs-CRP measurements in serum were statistically significantly correlated with those in EBC. This observation suggests the possibility and usefulness of the assessment of this parameter in EBC.

In asthmatic patients, the trend to higher levels of hs-CRP in serum as well as in EBC has been observed in the group of patients with EIB; however, these differences were not statistically significant. Only in the group of patients with EIB could we observe a statistically significant increase in hs-CRP concentrations both in serum and EBC already 30 min after exercise. Twenty-four hours after exercise, a subsequent significant increase in hs-CRP concentration was still observed, which could suggest that in asthmatic patients, as a consequence of EIB, the intensification of low-grade systemic inflammation occurs. The increase in CRP levels could be, on the one side, the consequence of intensification of synthesis at the local inflammatory area (airways), which could be confirmed by the rapid increase in CRP concentration. On the other side, the increase in CRP synthesis by hepatocytes as an answer for intensification of inflammation after postexercise bronchoconstriction could also be taken into account. In airways the expression of CRP was observed in macrophages, myofibroblasts, endothelial and epithelial cells. Monocytes and lymphocytes are also capable of CRP synthesis [34]. The increase in local production of CRP could be a consequence of the intensification of airway inflammation; however, other mechanisms could also play a role, such as damage of the vascular endothelium, as well as bronchial epithelium activation, induced by postexercise bronchoconstriction.

The maximum increase in hs-CRP in serum and EBC was correlated with baseline F_{ENO} levels – which has become a more and more appreciable criterion for the evaluation of airway eosinophilic inflammation [35] – as well as with the increase in F_{ENO} or serum ECP 24 h after exercise.

In this study there were no significant correlations between the severity of EIB and the results of studied parameters (including inflammatory mediators and CRP) before exercise and their changes after exercise. The severity of EIB could be the consequence of initial intensification of eosinophilic inflammation or bronchial hyperreactivity, as well as of changes observed during and after exercise (degranulation of mast cells, activation of vascular endothelium, airway epithelium and blood platelets). Therefore, the severity of EIB could be dependent on the intensification of systemic inflammation to a lesser extent, and the lack of correlation between EIB severity and hs-CRP could confirm this observation. The

increase in hs-CRP concentration after exercise could be the consequence of the enhanced inflammatory process in which many mediators and pro-inflammatory cytokines take part. Moreover, it could not be excluded that the small group of patients, which is a possible limitation of the study, could be related to the lack of these correlations.

Hypoxia may also have an influence on changes in CRP – which was confirmed in a study performed by Tomita et al. [36] in patients with pre- and postoperative congenital heart disease. However, in our study the significant correlation between the increase in CRP, both in serum and EBC and baseline F_{ENO} or ECP, and changes in F_{ENO} 24 h after exercise could indicate that changes in CRP are closely related rather to airway inflammation and their changes after exercise.

This study was performed to assess the possible association of low-grade systemic inflammation with EIB. We show that, as a result of intensive exercise leading to bronchoconstriction, an increase in hs-CRP occurs. Based on these findings, it is considered that in asthmatic patients, as a consequence of EIB, the intensification of low-grade systemic inflammation occurs. This process is not observed in asthmatics, in whom postexercise bronchoconstriction is not found.

Proper anti-inflammatory treatment could protect asthmatic patients from postexercise bronchoconstriction and, as a consequence, from the intensification of local and systemic inflammation.

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