

The Fractional Exhaled Nitric Oxide and Serum High Sensitivity C-Reactive Protein Levels in Cough Variant Asthma and Typical Bronchial Asthma

Terufumi Shimoda¹, Yasushi Obase², Reiko Kishikawa¹,
Tomoaki Iwanaga¹, Akihiko Miyatake³ and Soji Kasayama⁴

ABSTRACT

Background: Fractional exhaled nitric oxide (FeNO) is known to be a good marker of airway eosinophilic inflammation in bronchial asthma. Recently, serum high sensitivity C-reactive protein (hs-CRP) has been shown to be also useful to detect the airway inflammation.

Methods: Newly diagnosed 90 cough variant asthma and 92 bronchial asthma patients were enrolled. FeNO, serum hs-CRP, pulmonary function tests, bronchial hyperresponsiveness, IgE and sputum eosinophils ratio were compared. Ninety healthy control subjects were set for FeNO and serum hs-CRP normal range reference. We have compared the clinical utilities of FeNO and serum hs-CRP to differentiate bronchial asthma and cough variant asthma.

Results: FeNO was significantly higher in bronchial asthma (92.6 ± 85.5 ppb) than in cough variant asthma (35.6 ± 43.3 ; $p < 0.001$) and both were significantly higher than normal range (18.0 ± 6.4 , $p < 0.001$, respectively), and in differentiating between the two groups showed a sensitivity of 0.69 and a specificity of 0.73 at the cutoff value of 28 ppb. Serum hs-CRP did not differ significantly between bronchial asthma (723 ± 1162 ng/ml) and cough variant asthma (558 ± 758) even if both were significantly higher than normal range (345 ± 401 , $p < 0.01$ and $p < 0.05$ respectively).

Conclusions: FeNO is more useful than serum hs-CRP in differentiating patients with bronchial asthma from those with cough variant asthma, and healthy persons.

KEY WORDS

airway inflammation, bronchial asthma, cough variant asthma, fractional exhaled nitric oxide, high sensitivity CRP

INTRODUCTION

Fractional exhaled nitric oxide (FeNO) is known to be a convenient, sensitive marker that can be measured noninvasively to monitor eosinophilic lower airway inflammation.^{1,2} It was reported that the levels of FeNO rise in patients with bronchial asthma or cough variant asthma³ but do not rise in nonallergic cough.⁴ Sato *S et al.* suggested the possibility of that FeNO is

able to distinguish bronchial asthma and cough variant asthma from other cough by the cutoff 38.8 ppb.

High sensitivity C-reactive protein (hs-CRP) is highly sensitive (said to have 100-fold higher sensitivity than the conventional method) and quantifiable at extremely low concentrations (0.01 to 0.02 mg/dl).^{5,6} Serum hs-CRP was recently reported as a useful marker of inflammation in bronchial asthma⁷.

Cough variant asthma seems to be a precursor of

¹Clinical Research Center, Fukuoka National Hospital, Fukuoka,

²Department of Respiratory Medicine, Kawasaki Medical School, Okayama, ³Miyatake Asthma Clinic and ⁴Department of Internal Medicine, Nissay Hospital, Osaka, Japan.

Authors' contributions: YO analyzed the data. RK assisted in the design of the study. TI coordinated patient recruitment. AM assisted in study design. SK assisted in the data interpretation. TS wrote the manuscript as the first author.

Conflict of interest: No potential conflict of interest was disclosed.

Correspondence: Terufumi Shimoda, MD, Clinical Research Center, Fukuoka National Hospital, 4-39-1 Yakatabaru, Minami-ku, Fukuoka 811-1394, Japan.

Email: t-shimoda@mfukuoka2.hosp.go.jp

Received 5 November 2012. Accepted for publication 15 January 2013.

©0000 Japanese Society of Allergology

asthma, the beginning of asthma pathogenesis.⁸ Differentiation of cough variant asthma from typical asthma may lead to understanding incipency of the disease. There is no report about the cutoff of FeNO or hs-CRP for the differentiation between bronchial asthma and cough variant asthma.⁹ The present study aimed to compare the clinical utility of FeNO and serum hs-CRP as a marker of differentiation between bronchial asthma and cough variant asthma.

METHODS

SUBJECTS

The subject population consisted of 90 patients with cough variant asthma, 92 patients with bronchial asthma, and 90 healthy control persons. Both patients with cough variant asthma and bronchial asthma were to be free of attacks and newly diagnosed. None of the patients received any type of steroid, and all patients were untreated with any anti-allergy medication within 24 hours before enrollment. Patients with concurrent hypertension, diabetes mellitus, or hyperlipidemia were excluded from the study. The patients with too severe cough to measure bronchial hyperresponsiveness were also excluded from the study. All measurements were performed within a week from the first visit. The diagnoses of cough variant asthma and bronchial asthma were based on the method reported by Corrao *et al.*^{8,10,11} and the Global Initiative for Asthma (GINA) guidelines,¹² respectively. The patients with cough variant asthma were referred to our clinic for chronic cough persisting for longer than 8 weeks but without wheezing or dyspnea. They had no past history of asthma or other respiratory diseases. Wheeze or rhonchi were not audible on chest auscultation even at forced expiration. The subjects all had bronchial hyperresponsiveness (BHR) to inhaled acetylcholine (see below for method). Bronchodilators (inhaled beta2-agonists) were effective against their coughs. No other apparent causes of cough were present. They did not have any signs or symptoms of postnasal drip or gastro-oesophageal reflux, and had not been taking angiotensin-converting enzyme inhibitors. They had normal chest radiograph results. The patients with bronchial asthma had a history of episodic dyspnoea, wheezing and cough. They had at least 15% reversibility of forced expiratory volume in one second (FEV₁) after inhalation of 200 µg of salbutamol and/or BHR to acetylcholine. The healthy subjects had no past history of asthma, atopic diseases, or other respiratory diseases and had no current respiratory symptoms.

This study was approved by the Institutional Review Board of Fukuoka National Hospital. The project approval number is 20-12. The purpose of the research and experimental protocols was explained to all participants, and their prior written informed consent was obtained.

BHR MEASUREMENT

The challenge test was performed using a standardized method.¹³ All antiasthma medications were withheld for at least 24 hrs before the BHR measurement. Subjects inhaled acetylcholine aerosol from a hand-held nebulizer (PARI BOY 038, PARI GmbH, Starnberg, Germany) with tidal breathing for 2 min. The operating airflow rate was 5 L/min. Isotonic saline was inhaled first as a control. This was followed by progressive doubling of concentrations of acetylcholine from 0.039 to 20 mg/ml. The FEV₁ was measured after each inhalation with a spirometer (Chest Graph HI-701, Chest M.I., Tokyo, Japan). The percentage fall in FEV₁ was calculated from the post-saline FEV₁. The test was continued until the FEV₁ had fallen >20% or until the maximal concentration of acetylcholine had been administered. The BHR was expressed as PC₂₀. Subjects with a PC₂₀ of less than 8000 µg/ml were considered to have BHR according to the criteria of the American Thoracic Society¹³.

hs-CRP MEASUREMENT

Levels of hs-CRP were measured by latex nephelometry. Blood drawn from the cubital vein was centrifuged to obtain serum, which was frozen at -80°C until testing. According to the United States Food and Drug Administration requirements for hs-CRP assay reagents, the sensitivity of measurement should be 0.02 mg/dl or less, and the coefficient of variation at a CRP concentration of 0.1 mg/dl should not exceed 3%.¹⁴

FeNO MEASUREMENT

FeNO were measured according to the guidelines of the American Thoracic Society (ATS) by the single-breath method (on-line measurement) using a fast response (0.02 s) chemiluminescence analyzer (NOA 280; Sievers Instruments Inc., Boulder, CO, USA).¹⁵ All measurements were made using a mouth pressure of 16 cmH₂O corresponding to an expiratory flow of 50 mL/s. Repeated exhalations were performed to achieve three NO values that agreed at the 5% level. NO concentrations were recorded as the average of these three values. The stability of NO measurement among other NO analyzers is almost confirmed.¹⁶

SPUTUM INDUCTION AND PROCESSING

Sputum was induced by inhalation of 5 ml of 3% NaCl solution, using an ultrasonic nebulizer (Nescosonic nebulizer, UN-511, Nesco, USA). The output was about 5 L/min. Before coughing up sputum, subjects were asked to rinse their mouths and blow their noses to minimize contamination with saliva and postnasal drip. Subjects were asked to cough during and after inhalation, and to expectorate into empty containers. We modified the methods described by Gershman *et al.* to collect both central and peripheral

Table 1 Characteristics of the subjects

	Healthy control (n = 90)	Cough variant asthma (n = 90)	Bronchial asthma (n = 92)	P value*
Male/Female	47/43	32/58	44/48	0.09
Age, years	37.4 ± 11.5	44.7 ± 14.7	38.6 ± 13.8	0.004
Body mass index	22.3 ± 2.8	21.8 ± 3.1	22.4 ± 3.1	0.19
Symptom duration, years	-	2.5 ± 4.4	6.0 ± 8.8	0.001
Never/current or ex-smoker	75/15	80/10	68/24	0.009
Non atopic/atopic	90/0	42/48	12/80	0.0001

*p-value is for between cough variant asthma and bronchial asthma.

sputum.¹⁷ Sputum was induced over 20 minutes. The portion induced for the first 10 minutes was defined as 'central sputum', and that induced for the second 10 minutes as 'peripheral sputum'. Sputum was processed within 30 minutes. The samples were processed by the method described by Metso *et al.*, to collect cells for cytospin and cell-free supernatant.¹⁸ Cytospins from sputum were allowed to air-dry for 30 minutes and were then stained using the Giemsa stain method. At least 400 non-squamous cells, including eosinophils, neutrophils, lymphocytes, macrophages and ciliated epithelial cells were counted differentially. Results were expressed as percentages of total non-squamous counts. When examination of slides revealed macrophages and ciliated epithelial cells, the sample was considered to be of bronchial origin and was included in the study.

STATISTICAL ANALYSIS

Differences were analyzed using the Wilcoxon signed rank test with the level of significance (*p*-value) set at 0.05. Receiver operating characteristic (ROC) analysis was performed by means of the statistical analysis software StatMate IV (ATMS Co., Ltd., Tokyo, Japan). Multiple regression analysis was performed by least squares linear regression using JMP8 statistical analysis software (Statistical Discovery™, SAS Institute, Cary, NC, USA).

RESULTS

Characteristics of the subjects are shown in Table 1. The each number of bronchial asthma patients for severity was Step 1, mild intermittent, 43; Step 2, mild persistent, 39; Step 3, moderate persistent, 10. In comparison the cough variant asthma and bronchial asthma groups, the mean age were higher in the cough variant asthma group (*p* = 0.004), whereas the duration of symptoms, the proportion of smokers, and the proportion of atopic patients were higher in the bronchial asthma group (*p* < 0.01 each).

All respiratory function parameters were significantly lower (*p* < 0.001) and eosinophil counts in both central and peripheral sputum samples and serum IgE levels were significantly higher in the bronchial asthma group than cough variant asthma group (*p* =

0.03 and *p* < 0.001, respectively, Table 2). The log PC₂₀ was significantly lower in the bronchial asthma group than in the cough variant asthma group (*p* < 0.001).

FeNO levels were significantly higher in patients with cough variant asthma (35.6 ± 43.3 ppb) and in those with bronchial asthma (92.6 ± 85.5) than in healthy controls (18.0 ± 6.4; *p* < 0.001 each). The FeNO values were significantly higher in patients with bronchial asthma than in those with cough variant asthma (*p* < 0.001). The FeNO values were significantly lower in mild bronchial asthma patients (mild intermittent, 57.8 ± 60.2) than in moderate patients (mild persistent or more; 128.2 ± 93.4; *p* < 0.001).

Serum hs-CRP levels were significantly higher in patients with cough variant asthma (558 ± 758 ng/ml) and in those with bronchial asthma (723 ± 1162) than in healthy controls (345 ± 401; *p* < 0.05 and *p* < 0.01, respectively). However, no significant difference was detected between cough variant asthma and bronchial asthma groups. In addition, hs-CRP levels were not different between bronchial asthma subgroups divided by severity (data not shown).

ROC curves were constructed to establish the cut-off points of hs-CRP and FeNO to discriminate healthy controls, cough variant asthma, and bronchial asthma each other (Fig. 1). FeNO had significant cut-off levels to differentiate the groups (20 ppb for healthy control vs. bronchial asthma, sensitivity, 0.72 and specificity, 0.83; 28 ppb for bronchial asthma vs. cough variant asthma, 0.69 and 0.73). On the other hand, though the serum hs-CRP had only a cutoff level 170 ng/ml to discriminate bronchial asthma and healthy persons (0.63 and 0.64), but not for cough variant asthma discrimination from healthy controls or bronchial asthma group.

Single correlations and multiple regression analysis of FeNO or serum hs-CRP and the characteristics factors (sex, age, etc), respiratory function (FEV1% predicted), BHR (logPC₂₀), sputum eosinophils rate, etc are shown in Table 3a (FeNO) and 3b (serum hs-CRP). Multiple regression analysis clarified the independent significant correlations of FeNO with severity, atopy and sputum eosinophil rate in bronchial asthma group (*p* = 0.002, 0.03, <0.0001, respectively)

Table 2 Comparison of respiratory function, airway inflammation, serum IgE, FeNO and serum hs-CRP between cough variant asthma and bronchial asthma

		Healthy controls (n = 90)	Cough variant asthma (n = 90)	Bronchial asthma (n = 92)	P value
FEV _{1.0} /FVC, %		N.D.	80.2 ± 6.7	75.4 ± 10.2	c, <0.001
FEV _{1.0} , %predicted		N.D.	100.5 ± 13.4	89.0 ± 17.0	c, <0.001
V ₅₀ , %predicted		N.D.	82.0 ± 19.9	66.9 ± 27.2	c, <0.001
V ₂₅ , %predicted		N.D.	63.1 ± 24.3	50.2 ± 24.7	c, <0.001
Induced-sputum eosinophils (%)	Central	N.D.	2.3 ± 6.2	13.8 ± 19.9	c, <0.001
	Peripheral	N.D.	2.2 ± 5.3	11.5 ± 18.4	c, <0.001
logPC ₂₀		N.D.	3.0 ± 0.6	2.6 ± 0.6	c, <0.001
IgE, IU/ml		N.D.	249.7 ± 670.1	529.1 ± 962.1	c, <0.05
FeNO, ppb		18.0 ± 6.4	35.6 ± 43.3	92.6 ± 85.5	a, <0.001; b, <0.001; c, <0.001
hs-CRP, ng/ml		345 ± 401	558 ± 758	723 ± 1162	a, <0.05; b, <0.01; c, n.s.

a, healthy controls vs. cough variant asthma; b, healthy controls vs. bronchial asthma; c, cough variant asthma vs. bronchial asthma.

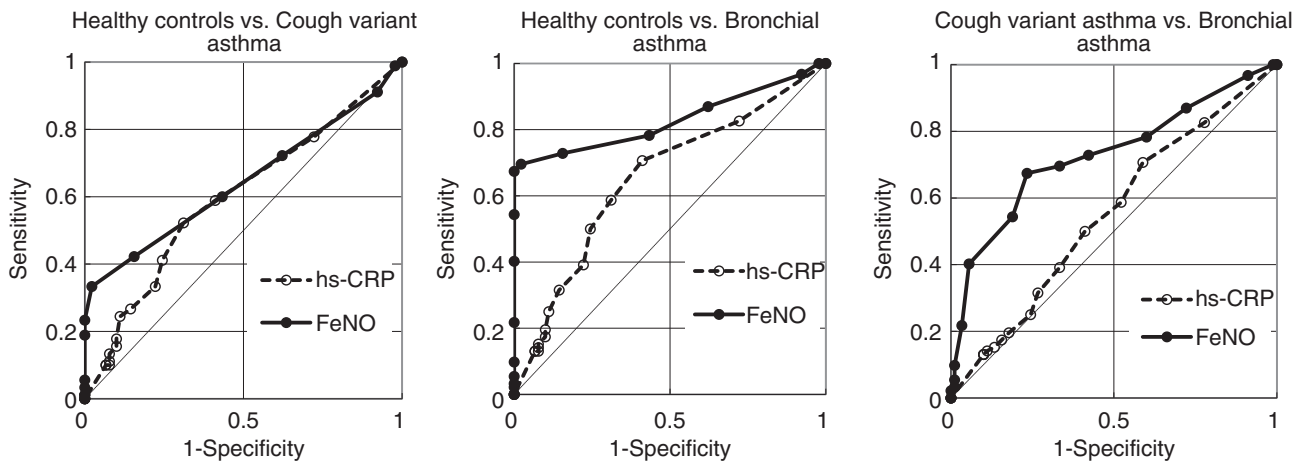


Fig. 1 Receiver operating characteristic (ROC) curves. Both FeNO (black circle and solid line) and serum hs-CRP (white circle and dash line) were barely able to differentiate between healthy controls and cough variant asthma. The ROC curve of FeNO describes an arc that approaches the upper-left corner, indicating higher differentiation ability to differentiate healthy controls and bronchial asthma. FeNO was able to differentiate between cough variant asthma and bronchial asthma but serum hs-CRP was not.

and that with sputum eosinophil rate in cough variant asthma group ($p < 0.0001$), and the significant correlation of hs-CRP with sex, age, symptom duration and PC₂₀ in bronchial asthma group ($p = 0.02, 0.001, 0.02$ and 0.026 , respectively) and with BMI in cough variant asthma group ($p = 0.004$).

DISCUSSION

In the study, we found that the FeNO may be useful to distinguish the cough variant asthma from bronchial asthma to some extent but the serum hs-CRP may not.

As reported by Corrao *et al.*,¹⁰ cough variant asthma is characterized by the sole complaint of

chronic cough, not accompanied by wheeze or dyspnea. Respiratory function in these patients is almost normal, but BHR to methacholine is increased. Cough symptoms are usually resolved rapidly after bronchodilator therapy. In most cases of cough variant asthma, parameters of central airway obstruction fall within the normal range.^{10,19} BHR levels in patients with cough variant asthma versus those with bronchial asthma is controversial; some report lower values in cough variant asthma than in bronchial asthma but other papers show comparable values.²⁰⁻²² In our study, the patients with cough variant asthma had mild BHR.

FeNO was higher in patients with cough variant

Table 3 Single correlation and multiple regression FeNO or serum hs-CRP and other factors in bronchial asthma and cough variant asthma patients**a. FeNO**

Bronchial asthma	Single correlation		Multiple regression analysis	
	r	P value	RC	P value
Male/Female	-0.003	0.98	0.888	0.379
Age	-0.151	0.15	-0.007	0.994
BMI	-0.117	0.27	-1.429	0.651
Severity	0.371	0.002	50.95	0.0021
FEV _{1.0} , %predicted	0.065	0.54	0.308	0.592
Non-atopic/atopic	0.256	0.013	70.40	0.031
Duration, years	-0.284	0.005	-0.863	0.443
log PC20	-0.207	0.054	-3.886	0.838
IgE	0.215	0.043	0.003	0.855
Central sputum eosinophils	0.616	<0.0001	2.317	<0.0001
Non-smoking/smoking	0.137	0.195	3.224	0.655
hs-CRP	0.134	0.203	6.705	0.771
Cough variant asthma	r	P value	RC	P value
Male/Female	-0.155	0.146	-2.676	0.552
Age	0.076	0.480	0.180	0.200
BMI	-0.035	0.743	0.053	0.939
Severity	-	-	-	-
FEV _{1.0} , %predicted	0.071	0.509	0.052	0.731
Non-atopic/atopic	0.242	0.022	-4.171	0.338
Duration, years	0.063	0.559	0.403	0.408
log PC20	-0.005	0.966	-1.980	0.483
IgE	0.108	0.314	0.002	0.342
Central sputum eosinophils	0.873	<0.0001	3.493	<0.0001
Non-smoking/smoking	-0.159	0.134	-3.081	0.045
hs-CRP	-0.056	0.603	-0.003	0.170

b. hs-CRP

Bronchial asthma	Single correlation		Multiple regression analysis	
	r	P value	RC	P value
Male/Female	-0.179	0.089	-0.306	0.021
Age	0.310	0.003	0.018	0.001
BMI	0.199	0.058	0.018	0.363
Severity	-0.002	0.986	-0.128	0.243
Symptom duration	-0.225	0.031	-0.016	0.020
Non-atopic/atopic	-0.078	0.463	-0.235	0.268
FEV _{1.0} , %predicted	-0.113	0.282	-0.002	0.631
log PC20	0.041	0.710	-0.262	0.026
IgE	-0.02	0.850	-0.001	0.666
Central sputum eosinophils	0.165	0.187	0.005	0.193
Non-smoking/smoking	-0.120	0.257	0.008	0.868
FeNO	0.134	0.203	0.001	0.771
Cough variant asthma	r	P value	RC	P value
Male/Female	-0.088	0.409	-268.49	0.371
Age	0.208	0.049	-0.658	0.945
BMI	0.462	<0.0001	126.7	0.004
Severity	-	-	-	-
Symptom duration	-0.082	0.443	-15.5	0.635
Non-atopic/atopic	0.060	0.578	44.49	0.879
FEV _{1.0} , %predicted	0.148	0.163	-12.237	0.227
log PC20	0.024	0.828	-220.12	0.241
IgE	0.155	0.145	0.121	0.453
Central sputum eosinophils	0.004	0.977	52.81	0.186
Non-smoking/smoking	0.030	0.778	59.68	0.571
FeNO	-0.056	0.603	-13.93	0.170

asthma and bronchial asthma than in healthy controls, as previously reported.⁹ Kowal *et al.* reported the cutoff value to differentiate a chronic cough with and without asthma was 40 ppb (sensitivity, 0.88; specificity, 0.83).²³ The present study indicated a cutoff value of 20 ppb (sensitivity, 0.72; specificity, 0.83) for differentiation between bronchial asthma and healthy controls, and a cutoff value of 28 ppb (sensitivity, 0.69; specificity, 0.73) for differentiation between bronchial asthma and cough variant asthma. Ludviksdottir *et al.* reported that atopic patients with asthma had a significantly higher mean exhaled NO than non-atopic subjects with asthma.²⁴ In the study, the FeNO levels had a significantly positive correlation with the eosinophil ratio in induced sputum, atopy, and the severity in bronchial asthma patients and with the eosinophil ratio in induced sputum in cough variant asthma patients. These data supports that FeNO is a useful marker to monitor the airway eosinophilic inflammation in cough variant asthma and bronchial asthma patients. FeNO and sputum eosinophil relate each other significantly when the sputum eosinophil is more than 3%.²⁵ In the study, sputum eosinophil number was not so high in cough variant asthma. The discrepancy between FeNO and sputum eosinophil number may be due to the very low inflammation level. Differentiation of cough variant asthma from typical asthma might lead to understanding incipency of the disease.

Takemura *et al.* reported a negative correlation ($r = -0.63$, $p = 0.004$) between serum hs-CRP and pulmonary function (FEV1.0/FVC),²⁶ in bronchial asthma patients. Our study, however, demonstrated no correlation of serum hs-CRP with FEV1 %predicted. Olafsdottir *et al.* reported that serum hs-CRP was significantly higher in patients with non-allergic asthma than in those with allergic asthma.²⁷ Our study showed that serum hs-CRP was higher in both patients with cough variant asthma and bronchial asthma than in healthy controls and no statistically significant influence by atopic status by multiple regression analysis. Hs-CRP increase in cough variant asthma may be resulted by the several weak asthma related factors together without independency.

Hs-CRP levels are influenced by various factors including systemic inflammation, age, body mass index, smoking status, serum lipid levels, blood pressure, diabetic status, and exercise, as well as airway inflammation,²⁸ causing difficulties in obtaining stable values. Serum hs-CRP was higher in men than women, in older, and in bronchial hyperresponded bronchial asthma patients and in the cough variant asthma patients with high BMI, but not in long time suffering patients unexpectedly. The marker is also reported to have a relationship not only with cardiovascular disease and diabetes but also with obesity itself, in association with IL-6 derived from adipocytes.²⁹ Smoking is another cause of increased serum

hs-CRP, and smoking cessation is reported to reduce hs-CRP.³⁰ Serum hs-CRP is thus considered inappropriate as a marker of airway inflammation.

Thus, FeNO is deemed more useful than serum hs-CRP as a marker of airway inflammation. As shown by ROC curves, FeNO was useful to differentiate patients with bronchial asthma from those with cough variant asthma and healthy controls but was minimally capable of differentiating between those with cough variant asthma and healthy controls. Serum hs-CRP was not able to reliably differentiate between healthy controls and those with cough variant asthma or bronchial asthma and between cough variant asthma and bronchial asthma. Panaszek reports that serum hs-CRP concentration in asthma did not correlate with BHR which, in turn, indirectly suggests its poor relationship with asthmatic inflammation.³¹ Probably the serum hs-CRP is not the direct indicator for airway inflammation but for general indirect inflammatory status of the subjects.

In conclusion, FeNO is a more useful marker to differentiate between bronchial asthma and cough variant asthma than serum hs-CRP.

ACKNOWLEDGEMENTS

We thank Miss Kojima and Miss Oda for their technical assistance in doing this study.

REFERENCES

1. Barnes PJ, Liew FY. Nitric oxide and asthmatic inflammation. *Immunol Today* 1995;**16**:128-30.
2. Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airways. *Am J Respir Crit Care Med* 1994;**149**:538-51.
3. Crater SE, Peters EJ, Martin ML, Murphy AW, Platts-Mills TA. Expired nitric oxide and airway obstruction in asthma patients with an acute exacerbation. *Am J Respir Crit Care Med* 1999;**159**:806-11.
4. Ricciardolo F, Sterk P, Gaston B, Folkerts G. Nitric oxide in health and disease of the respiratory system. *Physiol Rev* 2004;**84**:731-65.
5. Kuller LH, Tracy RP, Shaten J, Meilahn EN. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. *Am J Epidemiol* 1996;**144**:537-47.
6. Clearfield DO. C-reactive protein: a new risk assessment tool for cardiovascular disease. *J Am Osteopath Assoc* 2005;**105**:409-16.
7. Jousilahti P, Salomaa V, Hakala K, Rasi V, Vahtera E, Palosuo T. The association of sensitive systemic inflammation markers with bronchial asthma. *Ann Allergy Asthma Immunol* 2002;**89**:381-5.
8. Corrao WM, Braman SS, Irwin RS. Chronic cough as the sole presenting manifestation of bronchial asthma. *N Engl J Med* 1979;**300**:633-7.
9. Sato S, Saito J, Sato Y *et al.* Clinical usefulness of fractional exhaled nitric oxide for diagnosing prolonged cough. *Respir Med* 2008;**102**:1452-9.
10. Niimi A, Amitani R, Suzuki K, Tanaka E, Murayama T, Kuze F. Eosinophilic inflammation in cough variant asthma. *Eur Respir J* 1998;**11**:1064-9.

11. Committee for the Japanese Respiratory Society Guidelines for Management of Cough, Kohno S, Ishida T *et al.* The Japanese Respiratory Society guidelines for management of cough. *Respirology* 2006;**11** (Suppl 4):S135-86.
12. Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. NHLBI/WHO Report. NIH Publication: 02-3659. Updated 2006. Available at: <http://www.ginasthma.org>.
13. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease (COPD) and asthma. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, November 1986. *Am Rev Respir Dis* 1987;**136**:225-44.
14. Rifai N, Tracy RP, Ridker PM. Clinical efficacy of an automated high-sensitivity C-reactive protein assay. *Clin Chem* 1999;**45**:2136-41.
15. Smilkoff PE. Recommendation for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children-1999. *Am J Respir Crit Care Med* 1999;**160**:2104-17.
16. Dweik RA, Boggs PB, Erzurum SC *et al.* An official ATS clinical practice guideline: Interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;**184**:602-15.
17. Gershman NH, Liu H, Wong HH, Liu JT, Fahy JV. Fractional analysis of sequential induced sputum samples during sputum induction: Evidence that different lung compartments are sampled at different time points. *J Allergy Clin Immunol* 1999;**104** (Pt 1):322-8.
18. Metso T, Ryttilä P, Peterson C, Haahtela T. Granulocyte markers in induced sputum in patients with respiratory disorders and healthy persons obtained by two sputum-processing methods. *Respir Med* 2001;**95**:48-55.
19. Irwin RS, Boulet LP, Cloutier MM *et al.* Managing cough as a defense mechanism and as a symptom. A consensus Panel Report of the American College of Chest Physicians. *Chest* 1998;**114**:133S-81.
20. Matsumoto H, Niimi A, Akemura M *et al.* Features of cough variant asthma and classic asthma during methacholine-induced bronchoconstriction: a cross-sectional study. *Cough* 2009;**5**:3.
21. Koh Y, Chae SA, Min KU. Cough variant asthma is associated with a higher wheezing threshold than classic asthma. *Clin Exp Allergy* 1993;**23**:696.
22. Niimi A, Amitani R, Suzuki K, Tanaka E, Murayama T, Kuze F. Eosinophilic inflammation in cough-variant asthma. *Eur Respir J* 1998;**11**:1064-9.
23. Kowal K, Bodzenta-Lukaszyk A, Zukowski S. Exhaled nitric oxide in evaluation of young adults with chronic cough. *J Asthma* 2009;**46**:692-8.
24. Lúdvíksdóttir D, Janson C, Högman M, Hedenström H, Björnsson E, Boman G. Exhaled nitric oxide and its relationship to airway responsiveness and atopy in asthma: BHR-Study Group. *Respir Med* 1999;**93**:552-6.
25. Schleich FN, Seidel L, Sele J *et al.* Exhaled nitric oxide thresholds associated with a sputum eosinophil count $\geq 3\%$ in a cohort of unselected patients with asthma. *Thorax* 2010;**65**:1039-44.
26. Takemura M, Matsumoto H, Niimi A *et al.* High sensitivity C-reactive protein in asthma. *Eur Respir J* 2006;**27**:908-12.
27. Olafsdóttir IS, Gislason T, Thjodleifsson B *et al.* C reactive protein levels are increased in non-allergic but not allergic asthma: a multicentre epidemiological study. *Thorax* 2005;**60**:451-4.
28. Pearson TA, Mensah GA, Alexander RW *et al.* Markers of inflammation and cardiovascular disease; application to clinical and public health practice: a statement for health-care professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation* 2003;**107**:499-511.
29. Yudkin JS, Stehouwer CD, Emeis JJ, Coppack SW. C-reactive protein in healthy subjects: association with obesity, insulin resistance, and endothelial dysfunction: a potential role for cytokines originating from adipose tissue? *Arterioscler Thromb Vasc Biol* 1999;**19**:972-8.
30. Ridker PM. High-sensitivity C-reactive protein potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation* 2001;**103**:1813-8.
31. Panaszek B, Liebhart E, Liebhart J, Pawłowicz R, Fal AM. Serum concentration of C-reactive protein is not a good marker of bronchial hyperresponsiveness. *Arch Immunol Ther Exp (Warsz)* 2007;**55**:341-5.