Allergology International. 2013;62:343-349
DOI: 10.2332/allergolint.13-OA-0543

ORIGINAL ARTICLE

Stratifying a Risk for an Increased Variation of Airway Caliber among the Clinically Stable Asthma

Atsushi Hayata[1,2], Kazuto Matsunaga[1], Tsunahiko Hirano[1], Keiichiro Akamatsu[1], Tomohiro Ichikawa[1], Yoshiaki Minakata[1] and Masakazu Ichinose[3]

ABSTRACT

Background: Recently, correlations of peak expiratory flow (PEF) variation have been shown to facilitate the prediction of later asthma symptoms and exacerbations. However, it has not been fully examined whether or not any patient characteristics are associated with the residual airway lability in treated asthmatics. The objective of this study is to examine a predictive marker for increased variation of PEF in patients with clinically stable asthma.

Methods: We studied 297 asthmatic patients who were monitored for PEF twice a day. Asthma Control Questionnaire (ACQ), spirometry, and exhaled nitric oxide fraction (FE_{NO}) were measured. After the assessment of baseline values, PEF measuring was continued and associations between these clinical markers and later variation of PEF over a week (Min%Max) were investigated.

Results: 17.5% of the subjects showed increased PEF variability (Min%Max < 80%). ACQ, forced expiratory volume in 1 s % of predicted (%FEV_1), and FE_{NO} were identified as independent predictors of Min%Max < 80%. An ACQ ≥ 0.4 yielded 96% sensitivity and 59% specificity, a %FEV_1 ≤ 85% yielded 62% sensitivity and 89% specificity, and a FE_{NO} ≥ 40 ppb yielded 75% sensitivity and 90% specificity for identifying the subjects with high variability in PEF. When we combine %FEV_1 ≤ 85% and FE_{NO} ≥ 40 ppb, this index showed the highest specificity (98%) for increased PEF variability.

Conclusions: These results indicate that ACQ, %FEV_1 and FE_{NO} can stratify the risk for increased variation in airway caliber among patients with stable asthma. This may help identify subjects in whom further monitoring of lung function fluctuations is indicated.

KEY WORDS

airflow limitation, airway hyperresponsiveness, airway inflammation, airway lability, peak expiratory flow rate

ABBREVIATIONS

ACQ, Asthma Control Questionnaire; AHR, Airway hyperresponsiveness; FE_{NO}, Exhaled nitric oxide fraction; ICS, Inhaled corticosteroids; Min%Max, The lowest PEF over a week, expressed as a percentage of the highest PEF; PEF, Peak expiratory flow.

INTRODUCTION

Airway hyperresponsiveness (AHR) is the susceptibility of the airways to narrow excessively in response to various stimuli and is an important physiological property of asthma.[1-3] The clinical consequences of AHR are an exaggerated fluctuation in the airway caliber known as airway lability.[1] Indeed, it has been reported that AHR correlates well with the daily/weekly variation in lung function assessed by peak expiratory flow (PEF) measurements.[4-6] Among the PEF indices, the lowest PEF over a week expressed as a percentage of the highest PEF (Min% Max) has been shown to be the best index of airway

[1]Third Department of Internal Medicine, Wakayama Medical University, School of Medicine, [2]Division of Respiratory Disease, Naga Hospital, Wakayama and [3]Department of Respiratory Disease, Tohoku University Graduate School of Medicine, Miyagi, Japan.
Conflict of interest: No potential conflict of interest was disclosed.
Correspondence: Kazuto Matsunaga, MD, PhD, Third Department of Internal Medicine, Wakayama Medical University, School of Medicine, 811–1 Kimiidera, Wakayama 641–8509, Japan.
Email: kazmatsu@wakayama–med.ac.jp
Received 28 January 2013. Accepted for publication 12 March 2013.

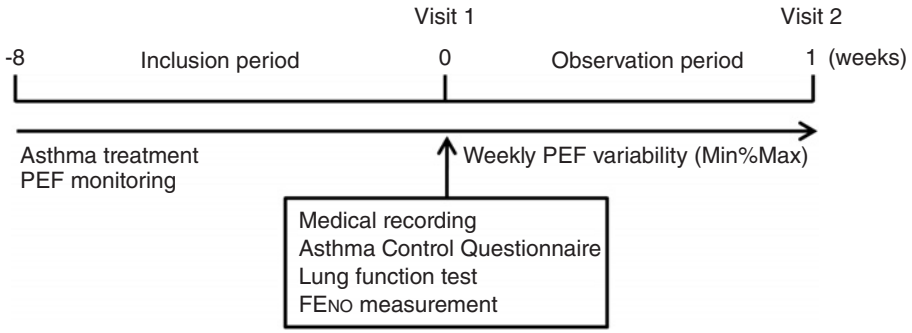

Fig. 1 Study design.

lability because it more strongly correlates with AHR than any other pulmonary physiological parameters.[1,5,6]

Importantly, it is widely accepted that AHR remains in patients with stable asthma and it is associated with future risk of adverse outcomes.[7-11] Actually, asthma management plans that include AHR measurements showed greater efficacy in reducing the exacerbation rate compared to plans without AHR measurements.[7,8] More recently, correlations of PEF data have been demonstrated to facilitate the prediction of later asthma symptoms and exacerbations.[12-15] Thus, predicting increased fluctuation in airway caliber might be useful to detect the highly reactive asthma phenotype. However, it has not been fully examined whether or not any patient characteristics are associated with the residual airway lability in stable asthma.

In this prospective observational study, we simultaneously examined the predictive value of clinical markers for assessing variations in airway caliber. We studied 297 stable asthma patients who were monitored for PEF twice a day. Asthma Control Questionnaire (ACQ), spirometry, and exhaled nitric oxide fraction (FE_{NO}) were measured. After the assessment of the baseline values, PEF measuring was continued and associations between these clinical markers and later variations in PEF over a week (Min%Max) were investigated.

METHODS

STUDY SUBJECTS

Subjects over 20 years old were eligible if they satisfied the standard criteria for asthma.[1] We included non-smoking asthmatic patients who were clinically stable (no history of exacerbation of asthma) following the treatment of inhaled corticosteroids (ICS) with or without inhaled long-acting β_2-agonist, leukotriene receptor antagonist, or theophylline during and/or 8 weeks prior to the study. Asthma exacerbations were defined as events that required urgent action for worsening asthma including unscheduled office visits, emergency department visits, or hospitalization requiring systemic steroids therapy.[16] Subjects were excluded if they had a history of pulmonary diseases except for asthma such as chronic obstructive pulmonary disease and bronchiectasis, or were poor adherence to asthma treatment (defined <80% adherence based on prescription refill data). Specific IgE for common inhaled allergens was examined. Positive specific IgE to at least one allergen was assumed to confirm the presence of atopy. This study was approved by the local ethics committee, (IRB #526) and informed consent was obtained from each participant.

STUDY DESIGN

This was a prospective observational study (Fig. 1). We consecutively studied non-smoking asthmatic patients who were clinically stable and monitored PEF for more than 8 weeks (inclusion period). Patient medical records were obtained and ACQ, spirometry, and FE_{NO} were examined on one occasion. After the assessment of baseline values, PEF measuring was continued for one week, and associations between these clinical markers and later variations of PEF over a week (Min%Max) were investigated (observation period).

ASTHMA CONTROL VARIABLES

The pre-bronchodilator PEF was measured twice a day using an Assess® PEF meter. The Min%Max was assumed to represent weekly PEF variability and increased PEF variability was defined by Min%Max < 80%.[1] The average of weekly PEF variability during the inclusion period (8 weeks) was also obtained. The forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) were measured as previously described.[6] The predictive values were estimated by the prediction formula of the Japanese Respiratory Society. The ACQ-5 is a questionnaire that assesses asthma condition according to five items, each of which can be rated on a seven point scale.[17] 0 represents excellent asthma control and 6 represents extremely poor control. The overall score was the mean of the five responses. The FE_{NO} level was measured according to the standard procedures using an online electrochemical nitric oxide analyzer (NIOX MINO; Aerocrine AB, Solna, Sweden).[18,19] Exhalations were

Table 1 Demographic and baseline data for the study population stratified by PEF variability

Variables	Low PEF variability (Min%Max ≥ 80%) *n* = 245	High PEF variability (Min%Max < 80%) *n* = 52	*p* value
Mean age (years)	47.7 ± 15.1	51.7 ± 13.5	0.08
Gender (male), *n* (%)	102 (41.6)	27 (51.9)	0.18
Body mass index (kg/mm^2)	22.4 ± 3.7	23.4 ± 4.1	0.10
Ex-Smokers, *n* (%)	77 (31.4)	25 (48.1)	<0.05
Atopy, *n* (%)	185 (75.5)	43 (82.7)	0.27
Asthma duration (years)	14.4 ± 8.4	13.6 ± 7.8	0.53
Dose of inhaled steroid (μg/day)†	356 ± 133	433 ± 225	<0.005
LABA use, *n* (%)	104 (42.4)	35 (67.3)	<0.005
LTRA use, *n* (%)	47 (19.2)	19 (36.5)	<0.005
Theophylline use, *n* (%)	22 (9.0)	7 (13.5)	0.32
FVC (L)	3.57 ± 0.84	3.15 ± 0.86	<0.001
FVC % of predicted (%)	105.8 ± 12.4	95.9 ± 12.3	<0.001
FEV_1 (L)	2.78 ± 0.70	2.21 ± 0.72	<0.001
FEV_1/FVC ratio (%)	78.1 ± 9.1	70.3 ± 10.6	<0.0001
FEV_1 % of predicted (%)	100.4 ± 12.8	82.8 ± 12.3	<0.001
The average of weekly PEF variability during the inclusion period (%)	88.7 ± 4.8	73.2 ± 3.9	<0.0001
Min%Max (%)	89.4 ± 4.2	76.4 ± 3.0	<0.0001
FE_{NO} (ppb)	25.3 ± 12.8	51.8 ± 22.1	<0.001
Mean ACQ (points)	0.4 ± 0.4	0.9 ± 0.5	<0.001

†Dose of inhaled steroid, expressed as fluticasone propionate equivalents. Abbreviations: Min%Max, the lowest PEF over a week, expressed as the percentage of the highest PEF; LABA, Inhaled long-acting β_2-agonist; LTRA, Leukotriene receptor antagonist; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; FE_{NO}, exhaled nitric oxide fraction; ACQ, Asthma Control Questionnaire. Mean (SD) values are provided unless otherwise indicated.

repeated to obtain two acceptable measurements within 10% deviation, and the average of these two values was registered.[18,19]

STATISTICAL ANALYSIS

All data were expressed as mean values ± SD. For categorical variables, the numbers of observations and percentages were given in each category. Comparisons between groups were performed by Fisher's exact test and Kruskal-Wallis test. Multivariate logistic regression analysis was used to assess the association between the binary outcome (Min%Max < 80%) and the set of clinical covariates. The variables with *p*-values < 0.20 in the univariate analysis were included in the multivariate model. Using a receiver operating curve, we determined the cutoff points of the predictors for identifying the subjects with Min%Max < 80%. A positive likelihood ratio [LR (+)] was calculated as true-positive rate/false-positive rate. An LR (+) reflects increased the odds of having a Min%Max < 80% after a positive test result. A negative likelihood ratio [LR (-)] is true-negative rate/false-negative rate and reflects reduced the odds of having a Min%Max < 80% after a negative test result. A *p*-value of <0.05 was considered significant.

Table 2 Predictive value of variables in assessing the high PEF variability according to the multivariate analysis

Variables	Odds ratio (95% CI)	*p* value
Age	1.04 (0.99-1.08)	0.13
Male	1.30 (0.43-3.92)	0.65
Body mass index	1.09 (0.96-1.24)	0.19
EX-Smokers	2.22 (0.65-7.59)	0.20
Dose of inhaled steroid†	1.00 (1.00-1.01)	0.09
LABA use	1.80 (0.58-5.60)	0.31
LTRA use	1.73 (0.55-5.47)	0.35
FVC % of predicted (%)	1.01 (0.95-1.07)	0.69
FEV_1/FVC ratio (%)	1.03 (0.95-1.12)	0.45
FEV_1 % of predicted (%)	1.14 (1.05-1.24)	<0.005
FE_{NO}	1.08 (1.05-1.11)	<0.0001
ACQ	11.86 (3.55-39.61)	<0.005

†Dose of inhaled steroid, expressed as fluticasone propionate equivalents. Abbreviations: LABA, Inhaled long-acting β_2-agonist; LTRA, Leukotriene receptor antagonist; FVC, forced vital capacity; FEV_1, forced expiratory volume in one second; FE_{NO}, exhaled nitric oxide fraction; ACQ, Asthma Control Questionnaire. Multivariate logistic regression analysis *p* value.

Table 3 Sensitivity and specificity of ACQ, %FEV_1 and FENO for identifying the subjects with high PEF variability

Variables	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR (+)	LR (-)
ACQ	96.2	58.8	33.1	98.6	2.33	15.3
%FEV_1	61.5	89.0	54.2	91.6	5.58	2.31
FE_{NO}	75.0	89.8	60.9	94.4	7.35	3.59

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR (+), positive likelihood ratio (true-positive ratio/false-positive ratio); LR (-), negative likelihood ratio (true-negative ratio/false-negative ratio); ACQ, Asthma Control Questionnaire; %FEV_1, forced expiratory volume in one second % of predicted; FE_{NO}, exhaled nitric oxide fraction.

RESULTS

The study subjects were recruited from June 2011 to August 2011 to avoid the influence of the cedar pollen season in Japan. All variables were obtained from 297 patients, and 52 subjects (17.5%) showed increased variability of PEF (Min%Max < 80%). There was no significant difference in the PEF variability between the inclusion period and the observation period (p = 0.32). The study subjects were divided into a low (Min%Max ≥ 80%) and high PEF variability group (Table 1). Compared to the low PEF variability group, the subjects with high PEF variability were more likely to be ex-smokers ($p < 0.05$) and were receiving more intensive asthma therapy (dose of ICS, inhaled long-acting β_2-agonist use, leukotriene receptor antagonist use; all $p < 0.005$). The FVC, FVC % of predicted, FEV_1, FEV_1/FVC ratio, and FEV_1 % of predicted (%FEV_1) were significantly lower in the high PEF variability group (all $p < 0.001$) and this group showed more evidence of airway inflammation detected by FE_{NO} measurement and more severe asthma symptoms (all $p < 0.001$).

Among the variables with p-values < 0.20 in the univariate analysis, ACQ, %FEV_1, and FE_{NO} were identified to be independent predictors of Min%Max < 80% according to the multivariate analysis (OR 11.86, $p < 0.005$, OR 1.14, $p < 0.005$, and OR 1.08, $p < 0.0001$, respectively) (Table 2). Using a receiver operating curve for identifying the subjects with Min%Max < 80%, an ACQ ≥ 0.4 yielded 96.2% sensitivity and 58.8% specificity, a %FEV_1 ≤ 85% yielded 61.5% sensitivity and 89.0% specificity, and a FE_{NO} ≥ 40 ppb yielded 75.0% sensitivity and 89.8% specificity (Table 3, Fig. 2). The area under the curve (AUC) of each variable was 0.84, 0.84, and 0.87, respectively. To examine these findings, we also retrospectively evaluated the predictive values of ACQ, %FEV_1, and FE_{NO} for assessing increased weekly PEF variability during the inclusion period. An ACQ ≥ 0.4 yielded 88.9% sensitivity and 59.7% specificity, a %FEV_1 ≤ 86% yielded 57.1% sensitivity and 88.0% specificity, and a FE_{NO} ≥ 40 ppb yielded 65.1% sensitivity and 90.1% specificity. The AUC of each variable was 0.80, 0.82, and 0.84, respectively.

When we combine these predictors, %FEV_1 ≤ 85% and FE_{NO} ≥ 40 ppb showed the highest specificity (98.4%) for increased PEF variability (Table 4). The distribution of the 52 subjects with high PEF variability stratified by baseline %FEV_1 and FE_{NO} is shown in Figure 3. Twenty-six subjects with combined %FEV_1 ≤ 85% and FE_{NO} ≥ 40 ppb had LR (+) of 26.4 and a positive predictive value of high PEF variability of 84.6%. Alternatively, 200 subjects with combined %FEV_1 > 85% and FE_{NO} < 40 ppb had LR (-) of 13.9 and negative predictive value of high PEF variability of 98.5%.

DISCUSSION

The main objective of this study was to examine the potential of clinical markers for predicting variations in airway caliber in patients with stable asthma. 17.5% of the study subjects showed increased variability of PEF and they were characterized by lower FVC and FEV_1 values, more airway inflammation, and more severe asthma symptoms. Using multivariate analysis, ACQ, %FEV_1 and FE_{NO} were identified as independent predictors of increased PEF variability. When the baseline %FEV_1 ≤ 85% and FE_{NO} ≥ 40 ppb were combined, this index showed the highest specificity for high PEF variability.

Current asthma guidelines have highlighted the importance in considering the future risk of adverse outcomes.[1,20] Interestingly, several recent studies have shown that fluctuation analysis of lung function is useful in the assessment of the risk for future loss of asthma control.[12-15] Frey *et al*. found that the time series of PEF showed long-range correlations that changed significantly with disease severity, approaching a random process with increased variability in the most severe cases.[12] Moreover, in two populations with differing asthma severity, airway caliber fluctuations were associated with asthma control and exacerbations.[14] There is also evidence that variability of airway caliber predicts the loss of asthma control following withdrawal of ICS treatment.[13] These results suggest that the characterization of fluctuations in lung function might provide a quantitative basis for objective risk prediction of asthma episodes. This study firstly demonstrated that ACQ, %FEV_1 and FE_{NO} were independent predictors of later increased variation in airway caliber. The similar predictive properties of ACQ, %FEV_1, and FE_{NO} for assessing increased weekly PEF variability during the inclusion period were consistent with these findings. In our

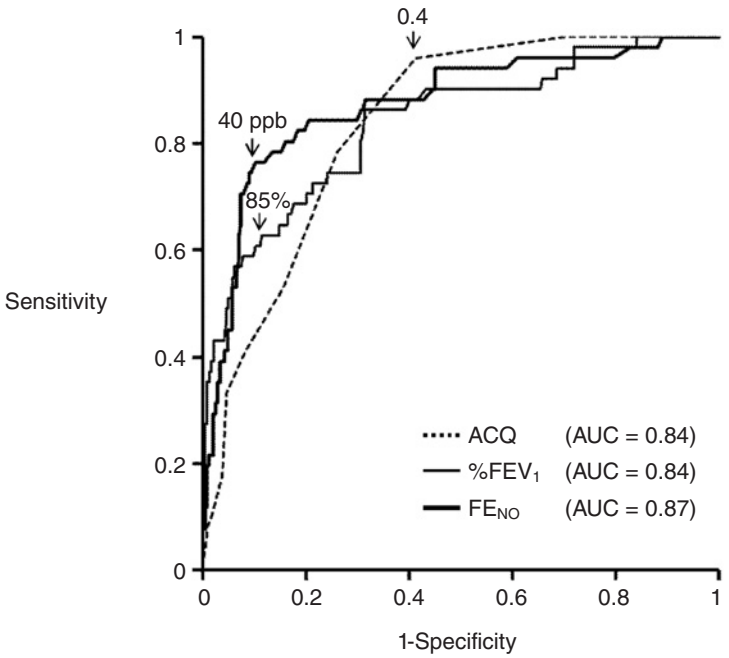

Fig. 2 Receiver operating curve for ACQ, $\%FEV_1$ and FE_{NO}. The dotted line represents ACQ, the solid thin line represents $\%FEV_1$ and the solid thick line represents FE_{NO}. AUC, area under the curve.

Table 4 Sensitivity and specificity for identifying the subjects with high PEF variability, when combining each variables

Variables	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	LR (+)	LR (-)
ACQ and $\%FEV_1$	61.5	94.3	69.6	92.0	10.8	2.45
ACQ and/or $\%FEV_1$	98.1	53.5	30.9	99.2	2.11	28.2
$\%FEV_1$ and FE_{NO}	42.3	98.4	84.6	88.9	26.4	1.71
$\%FEV_1$ and/or FE_{NO}	94.2	80.4	50.5	98.5	4.81	13.9
FE_{NO} and ACQ	75.0	92.2	67.2	94.6	9.62	3.69
FE_{NO} and/or ACQ	96.2	56.7	32.1	98.6	2.22	14.9
ACQ and $\%FEV_1$ and FE_{NO}	42.3	98.4	91.7	89.0	26.4	1.71
ACQ and/or $\%FEV_1$ and/or FE_{NO}	98.1	51.8	30.2	99.2	2.03	27.3

Abbreviations: PPV, positive predictive value; NPV, negative predictive value; LR (+), positive likelihood ratio (true-positive ratio/false-positive ratio); LR (-), negative likelihood ratio (true-negative ratio/false-negative ratio); ACQ, Asthma Control Questionnaire; $\%FEV_1$, forced expiratory volume in one second % of predicted; FE_{NO}, exhaled nitric oxide fraction.

study, approximately 18% of the subjects showed increased variability of PEF, and they had more severe asthma symptoms, more airflow limitation, and more airway inflammation, which might reinforce this hypothesis. Indeed, poor asthma control has been suggested to be predictive of later asthma exacerbations.[20,21]

Although the precise mechanism of airway lability is uncertain, several components such as airway inflammation, neural reflexes, airway geometric factors and genetic factors have been proposed to explain the mechanism of AHR.[1] Among these components, airway inflammation has been reported to be a key factor that seems to cause AHR *via* two mechanisms.[3,22] It has been suggested that one component is variable AHR, and the other is persistent AHR.[22] The main mechanism of the former AHR is active inflammation through the release of chemical mediators from immune cells, and that of the latter AHR is modification of the airway resident cells by chronic inflammation.[3,22] In our study, FE_{NO} was identified as one of the predictors associated with high PEF variability. A possible explanation for this is that the FE_{NO} levels may reflect the variable AHR because sequential FE_{NO} measuring identified the subgroup of asthma with residual airway inflammation. Indeed, recent studies have shown that the grouping of asthma by FE_{NO} provides an independent classification of asthma severity, and the subgroups with sustained high levels of FE_{NO} are suggested to be the highly re-

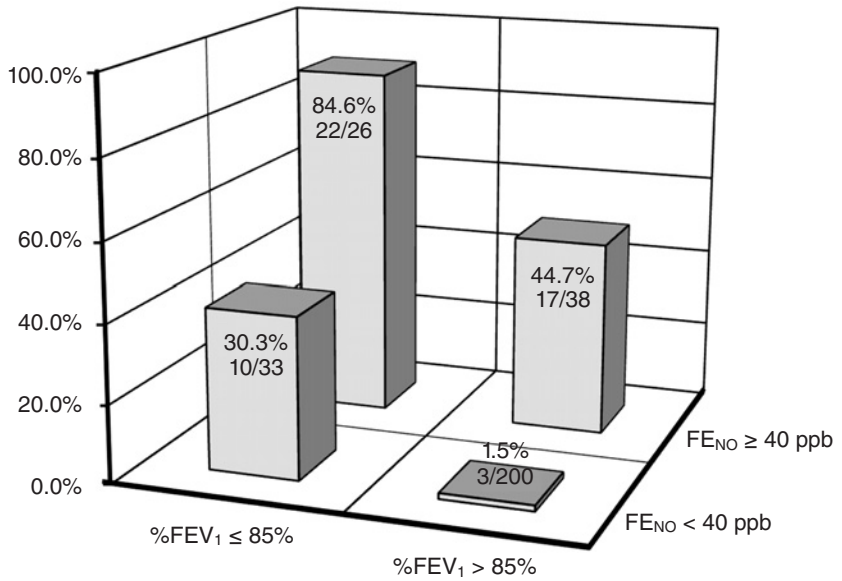

Fig. 3 Distribution of the increased PEF variability in 52 of 297 asthmatic patients stratified by baseline $\%FEV_1$ and FE_{NO} levels.

active phenotype of asthma.[23,24] We selected 40 ppb as the cutoff point, a value that was within previously published cutoff points for identifying subjects with uncontrolled asthma.[23-26] In this study, we also found that $\%FEV_1$ was an independent predictor for high PEF variability. A close association between the degree of airflow limitation and AHR[9,10,22] suggests that FEV_1 may reflect persistent AHR due to narrowing of the airway caliber. A recent study demonstrated that the majority of patients with severe asthma with low lung function do not have irreversible airflow limitation,[27] which is consistent with the results of the present study. However, FEV_1 might have low sensitivity for detecting the individuals with variable AHR.

Among the examined variables, when we combined baseline $\%FEV_1 \leq 85\%$ and $FE_{NO} \geq 40$ ppb, this index showed the highest specificity for increased PEF variability. Alternatively, the subjects with combined $\%FEV_1 > 85\%$ and $FE_{NO} < 40$ ppb had a negative predictive value of high PEF variability of 98.5%. This result suggests that combined measurements of both FEV_1 and FE_{NO} may be a useful diagnostic marker for identifying the subjects who are clinically stable but have increased fluctuations of airway caliber. Interestingly, Gelb *et al.* have demonstrated that decreased FEV_1 and increased FE_{NO} are independent risk factors for exacerbations of asthma.[28]

ACQ is a well validated composite measure for asthma control[17] and it was also related to increased variation of airway caliber. ACQ-defined asthma control is identified according to the following criteria: well controlled ≤ 0.75 and inadequately controlled ≥ 1.5.[29] In our study, ACQ ≥ 0.4 yielded 96.2% of sensitivity and 58.8% of specificity for identifying the subjects with high PEF variability. ACQ was suggested to be a good screening test for exaggerated variation in lung function, but it is necessary to note that the specificity reduced when stricter cutoff point was applied.

There would be some limitations in our study. First, a selection bias is possible because it was a purely observational study. Second, although all subjects had been carefully educated on the correct PEF measurements, we could not verify their techniques objectively. Finally, the study period was too short to assess the relationship between the airway caliber fluctuations and loss of asthma control.

In conclusion, this study indicates that ACQ, $\%FEV_1$ and FE_{NO} can stratify the risk for increased variation in airway caliber among patients with stable asthma. This may help identify subjects in whom further monitoring of lung function fluctuations is indicated.

ACKNOWLEDGEMENTS

The authors thank Mr. Brent Bell for reading the manuscript. This work was supported by the Grant of 2011 General Researches on Health Damage Caused by Air Pollution from the Environmental Restoration and Conservation Agency of Japan.

REFERENCES

1. Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. Updated 2008. Available at: http://www.ginasthma.org.
2. American Thoracic Society. Guidelines for methacholine and exercise challenge testing-1999. *Am J Respir Crit Care Med* 2000;**161**:309-29.

3. Ichinose M, Takahashi H, Sugiura N *et al*. Baseline airway hyperresponsiveness and its reversible component: role of airway inflammation and airway caliber. *Eur Respir J* 2000;**15**:248-53.
4. Ryan G, Latimar KM, Dolovich J, Hargreave FE. Bronchial responsiveness to histamine: relationship to diurnal variation of peak flow rate, improvement after bronchodilator, and airway calibre. *Thorax* 1982;**37**:423-9.
5. Reddel HK, Salome CM, Peat JK, Woolcock AJ. Which index of peak expiratory flow is most useful in the management of stable asthma? *Am J Respir Crit Care Med* 1995; **151**:1320-5.
6. Matsunaga K, Kanda M, Hayata A *et al*. Peak expiratory flow variability adjusted by forced expiratory volume in one second is a good index for airway responsiveness in asthmatics. *Intern Med* 2008;**47**:1107-12.
7. Nuijisink M, Hop WC, Sterk PJ, Duiverman EJ, de Jongste JC. Long-term asthma treatment guided by airway hyperresponsiveness in children: a randomised controlled trial. *Eur Respir J* 2007;**30**:457-66.
8. Sont JK, Willemes LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathological outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL study group. *Am J Respir Crit Care Med* 1999;**159**:1043-51.
9. Leuppi JD, Salome CM, Jenkins CR *et al*. Predictive markers of asthma exacerbation during stepwise dose reduction of inhaled corticosteroids. *Am J Respir Crit Care Med* 2001;**163**:406-12.
10. Boulet LP, Turcotte H, Brochu A. Persistence of airway obstruction and hyperresponsiveness in subjects with asthma remission. *Chest* 1994;**105**:1024-31.
11. Leuppi JD, Salome CM, Jenkins CR *et al*. Makers of airway inflammation and airway hyperresponsiveness in patients with well-controlled asthma. *Eur Respir J* 2001;**18**: 444-50.
12. Frey U, Brodbeck T, Majumdar A *et al*. Risk of severe asthma episodes predicted from fluctuation analysis of airway function. *Nature* 2005;**438**:667-70.
13. Thamrin C, Taylor DR, Jones SL, Suki B, Frey U. Variability of lung function predicts loss of asthma control following withdrawal of inhaled corticosteroid treatment. *Thorax* 2010;**65**:403-8.
14. Thamrin C, Nydegger R, Stern G *et al*. Associations between fluctuations in lung function and asthma control in two populations with differing asthma severity. *Thorax* 2011;**66**:1036-42.
15. Thamrin C, Zindel J, Nydegger R *et al*. Predicting future risk of asthma exacerbations by using individual conditional probabilities. *J Allergy Clin Immunol* 2011;**127**: 1494-502.
16. Reddel HK, Taylor DR, Bateman ED *et al*, and American Thoracic Society/European Respiratory Society Task Force on Asthma Control and Exacerbations. An official American Thoracic Society/European Respiratory Society statement. Asthma control and exacerbations. *Am J Respir Crit Care Med* 2009;**180**:59-99.
17. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. *Respir Med* 2005;**99**: 553-8.
18. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;**171**:912-30.
19. Matsunaga K, Hirano T, Akamatsu K *et al*. Exhaled nitric oxide cutoff values for asthma diagnosis according to rhinitis and smoking status in Japanese subjects. *Allergol Int* 2011;**60**:331-7.
20. Reddel HK, Taylor DR, Bateman ED *et al*, and American Thoracic Society/European Respiratory Society Task Force on Asthma Control and Exacerbations. An official ATS/ERS statement: Asthma control and exacerbations. Standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009;**180**:59-99.
21. Bateman ED, Boushey HA, Bousquet J *et al*. Can guideline-defined asthma control be achived? The gaining optimal asthma control study. *Am J Respir Crit Care Med* 2004;**170**:836-44.
22. Cockcroft DW, Davis BE. Mechanisms of airway responsiveness. *J Allergy Clin Immunol* 2006;**118**:551-9.
23. Dweik RA, Sorkness RL, Whenzel S *et al*. Use of exhaled nitric oxide measurement to identify a reactive, at-risk phenotype among patients with asthma. *Am J Respir Crit Care Med* 2010;**181**:1033-41.
24. Matsunaga K, Yanagisawa S, Hirano T *et al*. Associated demographics of persistent exhaled nitric oxide elevation in treated asthmatics. *Clin Exp Allergy* 2012;**42**:775-81.
25. Dweik RA, Boggs PB, Erzurum SC *et al*, and American Thoracic Society Committee on Interpretation of Exhaled Nitric Oxide Levels (FENO) for Clinical Applications. An official ATC clinical practice guideline: Interpretation of exhaled nitric oxide levels (FENO) for clinical applications. *Am J Respir Crit Care Med* 2011;**184**:602-15.
26. Matsunaga K, Hirano T, Kawayama T *et al*. Reference ranges for exhaled nitric oxide fraction in healthy Japanese adult population. *Allergol Int* 2010;**59**:363-7.
27. Moore WC, Bleecker ER, Curran-Everett D *et al*. Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007;**119**:405-13.
28. Gelb AF, Flynn Taylor C, Shinar CM, Gutierrez C, Zamel N. Role of spirometry and exhaled nitric oxide to predict exacerbations in treated asthmatics. *Chest* 2006;**129**: 1492-9.
29. Juniper EF, Bousquet J, Abetz L, Bateman ED. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. *Respir Med* 2006;**100**: 616-21.