

Abbreviations used

AUC:	Area under the curve
FVC:	Forced vital capacity
ICS:	Inhaled corticosteroid
LTD ₄ :	Leukotriene D ₄
LTD ₄ -BPT:	LTD ₄ bronchial provocation test
LTRA:	Leukotriene receptor antagonist
MCh-BPT:	Methacholine bronchial provocation test
PD ₂₀ FEV ₁ -LTD ₄ :	Cumulative dose of LTD ₄ causing a 20% decrease in FEV ₁
PD ₂₀ FEV ₁ -MCh:	Cumulative dose of methacholine causing a 20% decrease in FEV ₁

asthmatic patients and 21 healthy control subjects were enrolled. Asthma and control status (uncontrolled, partly controlled, and controlled) were diagnosed based on the Global Initiative for Asthma 2006 guidelines (see Table E1 in this article's Online Repository at www.jacionline.org for details regarding the definition of asthma control).¹² Eligible asthmatic patients were between 18 and 65 years of age and had no acute upper respiratory tract infections in the previous 2 weeks, normal chest radiographic results, baseline FEV₁ of greater than 60% of predicted value, and no treatment with LTRAs for 5 days, oral corticosteroids or antihistamines for 3 days, oral xanthenes or long-acting bronchodilators for 2 days, inhaled corticosteroids (ICSs) or long-acting bronchodilators for 24 hours, or short-acting bronchodilators for at least 4 hours before measurement. Excluded were asthmatic patients who had an FEV₁ decrease of 20% or greater after ethanol dilution challenge, other confirmed chronic respiratory diseases, or severe systemic diseases (eg, myocardial infarction or malignant tumor) or had poor compliance.

Healthy subjects were between 18 and 65 years of age, had no upper respiratory tract infections for the previous 2 weeks or allergic or systemic diseases, and had normal chest radiographic and lung function results. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Guangzhou Medical College. All subjects provided written informed consent before the study.

Study protocol

This was a single-center cross-over study. LTD₄-BPTs and MCh-BPTs were performed according to a randomized number sequence. The between-test interval ranged from 2 to 14 days. Neither severe asthma attack nor change in medication could be recorded during the interval. Airway responsiveness was assessed based on the cumulative dosage causing a 20% decrease in FEV₁.

Spirometry

Spirometry was performed with spirometers (LTD₄-BPTs: QUARK PFT, COSMED, Milan Italy; MCh-BPTs: JAEGER Masterscope, CareFusion Co Ltd, Hoechstberg, Germany). All procedures met the joint recommendations of the American Thoracic Society and the European Respiratory Society.¹⁴ At least 3 (but not more than 8) maneuvers were performed, with the variation between the best 2 maneuvers of less than 5% or 150 mL in forced vital capacity (FVC) and FEV₁. The maximal values of FVC, FEV₁, and peak expiratory flow were reported. Predicted values were calculated based on the model recommended by Zheng and Zhong.¹⁵

Preparations of dilutions

The methods for preparing LTD₄ dilutions have been described previously.¹⁶ Briefly, LTD₄ (200 nmol/mL in ethanol; Cayman Chemical Co, Ann Arbor, Mich) was stored in a -80°C freezer. The 0.2-mL LTD₄ aliquot was extracted with a micropipette, and the 3.2×10^4 nmol/L LTD₄ dilution (solution A) was prepared by adding 1.050 mL of normal saline. The aliquot of solution A was then pipetted into 0.3 mL of normal saline, forming a 1.6×10^4 nmol/L LTD₄ dilution (solution B). The ethanol dilution control (16%

ethanol) was prepared similarly, except that 0.20 mL of absolute ethanol was adopted.

Methacholine (Sigma-Aldrich Co, St Louis, Mo) was stored in a -20°C refrigerator. A gram of methacholine was dissolved with 10 mL of normal saline, the dilution control, for preparation of a 5.0×10^8 nmol/L dilution (solution C). The 2.5×10^8 nmol/L dilution (solution D) was prepared by adding an aliquot of normal saline into 3 mL of solution C.

LTD₄-BPT

The methodology of the LTD₄-BPT, using a dosimeter approach, was introduced previously.¹⁶ Briefly, all dilutions were delivered through a Devilbiss nebulizer (Type 646, output: 900 μ L/min) by using compressed oxygen (pressure, 37 psi; mean particle diameter, 1-6 μ m), with the provocative concentration and cumulative dose increasing in a stepwise manner. Inhalation challenge with a 16% ethanol dilution, the concentration of which corresponded to solution A, was undertaken for exclusion of subjects hypersensitive to ethanol. LTD₄ challenge could be initiated provided that FEV₁ decrease was less than 20% and was restored to less than 10% within 1 minute. The cumulative dose of LTD₄ causing a 20% decrease in FEV₁ (PD₂₀FEV₁-LTD₄) ranged from 0.075 to 4.800 nmol in a 4-fold increment fashion. Repetitive inhalation challenges were performed at an interval of 5 minutes (Table I). These procedures should be terminated in case of a 20% or greater FEV₁ decrease, and 200 to 400 μ g of salbutamol was administered through a spacer (Volumatic; Allen & Hanbury's, London, United Kingdom) to relieve discomfort. Spirometric results were reassessed at 3, 5, and 10 minutes and thereafter, if necessary, depending on FEV₁ improvement. In this study time of recovery was defined as the end of the test to the time point at which the posttest FEV₁ value was 80% or greater of the baseline FEV₁ value. Additionally, subjects with negative test results were artificially assigned a figure of 5.00 nmol for PD₂₀FEV₁.

MCh-BPT

Spirometric tests were performed with the Masterscreen (output, 160 μ L/min) and automated APS pro system (Jaeger, Germany) based on the dosimeter method. The provocation procedure was similar to that of the LTD₄-BPT, except for initial and ultimate cumulative doses (0.390 and 12.520 μ mol, respectively)¹⁷ and the increment in cumulative dose (Table I). Likewise, subjects with negative test results were artificially assigned a figure of 12.50 μ mol for PD₂₀FEV₁.

Criteria for identification of leukotriene-responsiveness subtypes

Leukotriene-responsiveness subtypes were classified by using the combination of PD₂₀FEV₁-LTD₄ and LTD₄/methacholine potency ratio, which was defined as the cumulative provocative dose of methacholine (transformed into nanomoles accordingly) divided by that of LTD₄ (in nanomoles). Asthmatic patients having PD₂₀FEV₁-LTD₄ values of the geometric mean (in nanomoles) or less and a potency ratio of greater than the geometric mean were regarded as leukotriene responsive. Those with PD₂₀FEV₁-LTD₄ values of greater than the geometric mean (in nanomoles) and a potency ratio of less than the geometric mean among all asthmatic patients were considered leukotriene unresponsive, with the remaining subjects being categorized into the unclassified group.

Statistical analysis

Statistical analysis was performed with the SPSS 16.0 version package (SPSS, Chicago, Ill). Dot plots were depicted with Graphpad Prism Version 5.01 software (GraphPad Software, San Diego, Calif). The test of normality was conducted, and the mean $\pm$ SD ($\bar{x} \pm s$) was adopted for data with normal distribution; otherwise the geometric mean (interquartile range; [G{Q_R}]) was used. One-way ANOVA was applied for comparing baseline levels assuming normal distribution and equal variance, whereas the Kruskal-Wallis test was otherwise applied. The PD₂₀FEV₁-LTD₄ values and cumulative dose of

TABLE I. LTD₄-BPT and MCh-BPT procedures

Stimulus	Step	Concentration (nmol/L)	Inhalation times	Inhalation duration (s)	Dosage (nmol)	Cumulative dosage (nmol)
LTD ₄	0	0	5	0.375	0.000	0.000
	1	1.6×10^4	1	0.315	0.075	0.075
	2	1.6×10^4	3	0.315	0.225	0.300
	3	3.2×10^4	5	0.375	0.900	1.200
	4	3.2×10^4	10	0.750	3.600	4.800
Methacholine	0	0	5	0.600	0.000	0.000
	1	2.5×10^8	1	0.585	3.90×10^2	0.39×10^2
	2	2.5×10^8	3	0.585	1.23×10^3	1.56×10^3
	3	5.0×10^8	7	0.521	4.69×10^3	6.25×10^3
	4	5.0×10^8	9	0.504	6.25×10^3	1.25×10^4

Note: for LTD₄, 1 nmol equals 0.5 μg; for MCh, 1 μmol is equivalent to 200 μg.

TABLE II. Comparison of baseline levels

Parameter	Uncontrolled (n = 20)	Partly controlled (n = 22)	Controlled (n = 20)	Healthy subject (n = 21)	F value	P value
Anthropometry						
Age	37.5 ± 11.4	39.05 ± 8.81	40.05 ± 12.29	38.00 ± 10.78	0.221	.881
Height (cm)	160.55 ± 6.78	162.39 ± 6.80	159.28 ± 7.35	164.48 ± 9.87	4.156	.245
Weight (kg)	58.95 ± 10.91	60.25 ± 10.97	57.60 ± 6.72	60.10 ± 9.89	0.323	.809
Spirometry						
FVC (L)	3.60 ± 0.80	3.60 ± 0.82	3.36 ± 0.82	3.61 ± 0.76	0.467	.706
FVC predicted (%)	112.19 ± 15.36	110.15 ± 15.86	108.69 ± 16.49	107.03 ± 12.97	0.425	.736
FEV ₁ (L)	2.46 ± 0.56	2.58 ± 0.64	2.55 ± 0.66	3.03 ± 0.61	3.542	.018
FEV ₁ predicted (%)	91.13 ± 12.30	94.10 ± 15.85	98.52 ± 16.91	107.30 ± 11.54	5.021	.003
PEF (L/s)	6.09 ± 1.72	6.26 ± 1.66	6.27 ± 1.32	8.01 ± 1.75	6.183	.001
PEF predicted (%)	93.91 ± 25.45	96.11 ± 23.65	98.69 ± 19.62	123.05 ± 25.58	6.610	.000

All data were presented as means ± SDs unless otherwise stated.

PEF, Peak expiratory flow.

methacholine causing a 20% decrease in FEV₁ (PD₂₀FEV₁-MCh) were further processed with logarithmic transformation. This was followed by comparison of their distribution pattern by using a dot plot. Scatter dots were applied for comparing the correlation between the logarithm of PD₂₀FEV₁-LTD₄ (LogPD₂₀FEV₁-LTD₄) and the logarithm of PD₂₀FEV₁-MCh (LogPD₂₀FEV₁-MCh) and between LogPD₂₀FEV₁-LTD₄ and the potency ratio logarithm in asthmatic patients with different responsiveness to inhaled LTD₄. The row-column table was analyzed by using the χ^2 test. Diagnostic value was assessed by using the receiver operating characteristic curve for computation of the area under the curve (AUC) and the 95% CI. A *P* value of less than .05 was taken as statistically significant.

RESULTS

Of 104 subjects who underwent screening procedures, 99 subjects, including 74 asthmatic patients and 25 healthy control subjects, met the inclusion criteria. A total of 20 patients with uncontrolled, 22 with partly controlled, and 20 with controlled asthma, as well as 21 healthy control subjects, who finished both LTD-BPTs and MCh-BPTs were included in the final analysis (Fig 1).

No significant differences were found in anthropometric parameters (all *P* > .05). Major spirometric parameters in asthmatic patients differed considerably (*P* < .05) compared with those in healthy control subjects, with the exception of FVC (*F* = 0.467, *P* = .706) and FVC percent predicted (*F* = 0.425, *P* = .736; Table II).

Neither BPT yielded a marked distinction (*P* > .05) in spirometric parameter decreases, except for the FEV₁ percentage decrease (*t* = -2.459, *P* = .023) and peak expiratory flow percentage

decrease (*t* = -3.37, *P* = .003) in healthy control subjects. A larger decrease was associated with asthmatic patients with worse symptom control, particularly the uncontrolled group. Methacholine resulted in a greater reduction among asthmatic patients. By contrast, a greater decrease was produced by inhaled LTD₄ among healthy control subjects (see Fig E1 in this article's Online Repository at www.jacionline.org). Baseline levels were not correlated with the decrease in spirometric parameters after inhalation challenge (all *P* > .05, data not shown).

Patients with uncontrolled asthma were noted as having the lowest geometric mean of PD₂₀FEV₁-LTD₄ and PD₂₀FEV₁-MCh (0.272 nmol vs 0.945 μmol), followed by patients with partly controlled (0.387 nmol vs 1.933 μmol) and controlled (1.484 nmol vs 3.946 μmol) asthma. A similar trend was shown regarding the interquartile ranges. Data were logarithmically transformed in Fig 2.

The ratio of PD₂₀FEV₁-MCh to PD₂₀FEV₁-LTD₄, referred to as the potency ratio, was highest in patients with partly controlled asthma (see Fig E2 in this article's Online Repository at www.jacionline.org). The logarithms of cumulative dosage of LTD₄ and methacholine were positively correlated in all asthmatic subgroups, with the highest slope in patients with controlled asthma (*r* = 0.965, *P* = .000), followed by patients with partly controlled (*r* = 0.661, *P* = .001) and uncontrolled (*r* = 0.504, *P* = .023) asthma (scatter plots are not shown).

Given the geometric mean of 0.533 nmol and 3647 for PD₂₀FEV₁-LTD₄ and the potency ratio, respectively, 18 asthmatic patients were categorized into the leukotriene-responsive group, 19 into the leukotriene-unresponsive group, and 25 into

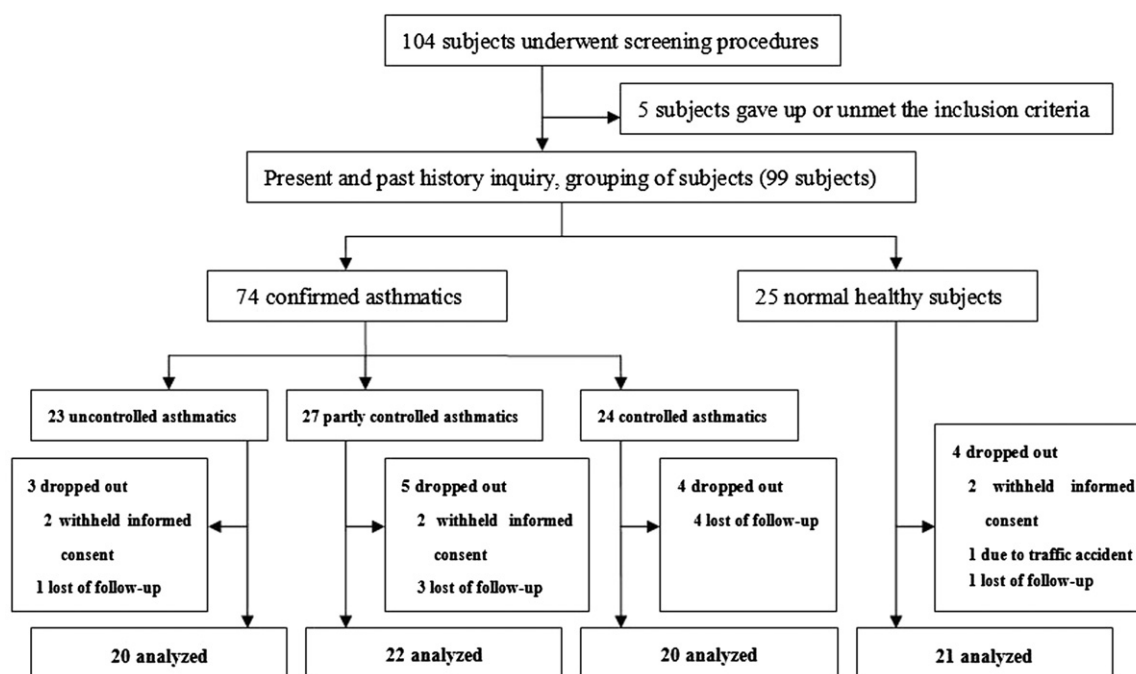


FIG 1. Patients' profiles.

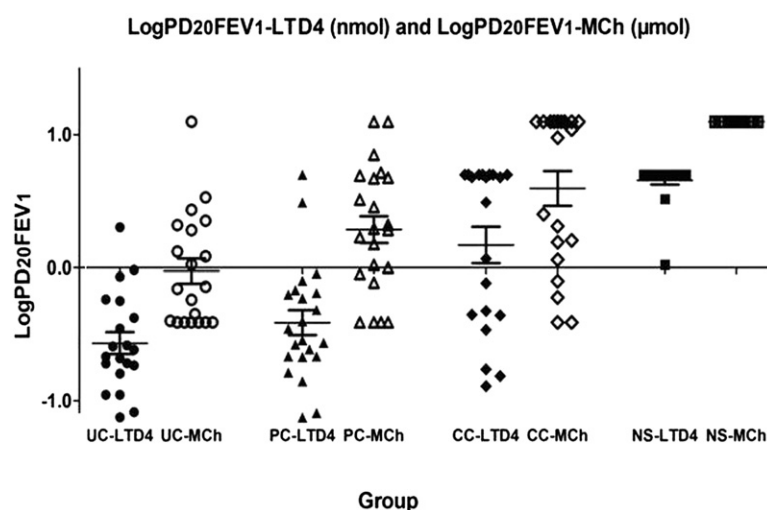


FIG 2. Distribution characters of Log₁₀PD₂₀FEV₁-LTD₄ and Log₁₀PD₂₀FEV₁-MCh values. LTD₄-BPT: Between-group comparison, $P < .01$, except for UC compared with PC, $P > .05$. MCh-BPT: Between-group comparison, $P < .01$, except for UC compared with PC, $P < .05$, and CC compared with NS, $P > .05$. CC, Controlled asthma group; NS, healthy subject group; PC, partly controlled asthma group; UC, uncontrolled asthma group.

the unclassified group. The correlation between logarithm of potency ratio and Log₁₀PD₂₀FEV₁-LTD₄ values (in nanomoles) was shown in Fig 3, A. Leukotriene-unresponsive asthmatic patients exhibited the highest within-group variation in PD₂₀FEV₁-LTD₄ values (data not shown). Additionally, patients with uncontrolled (6/18 [33.3%]) and partly controlled (10/18 [55.6%]) asthma accounted for the majority in the leukotriene-responsive group, whereas patients with controlled asthma predominated in the leukotriene-unresponsive group (13/19 [68.4%]). The logarithm of PD₂₀FEV₁-LTD₄ values (in nanomoles) correlated significantly with that of PD₂₀FEV₁-MCh (in micromoles) in asthmatic patients with various responsiveness

to inhaled LTD₄ ($r = 0.810$, $P = .000$). Data of leukotriene-responsive asthmatic patients were plotted below the regression line and were well discriminated from those of leukotriene-unresponsive subjects (Fig 3, B).

In terms of positive rate, between-group comparison yielded marked difference (LTD₄-BPT: $\chi^2 = 49.163$, $P = .000$; MCh-BPT: $\chi^2 = 50.775$, $P = .000$), except between patients with uncontrolled and partly controlled asthma (both $\chi^2 = 0.000$, $P = 1.000$). Both patients with uncontrolled and those with partly controlled asthma had higher positive rates than patients with controlled asthma. Furthermore, the LTD₄-BPT was characterized by higher sensitivity yet lower specificity. Better

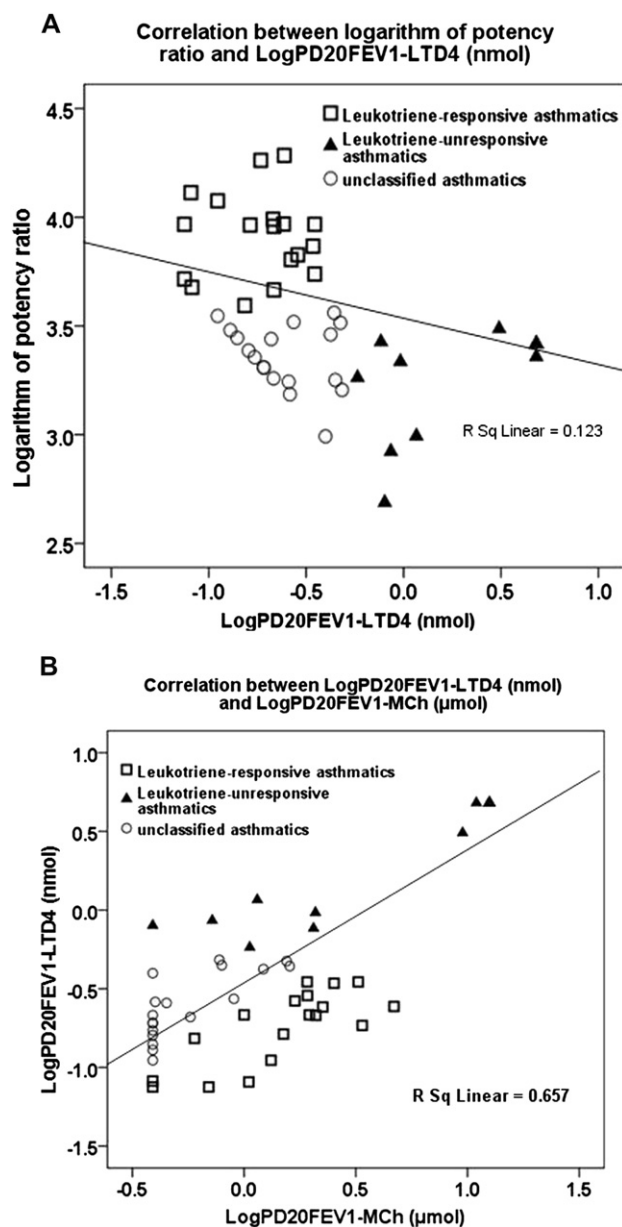


FIG 3. A, Correlation between logarithm of PD₂₀FEV₁-LTD₄ value and LTD₄/MCh potency ratio ($r = -0.351$, $P = .005$). B, Correlation between logarithm of PD₂₀FEV₁-LTD₄ and PD₂₀FEV₁-MCh values ($r = 0.810$, $P = .000$).

asthma control was associated with lower sensitivity and accuracy (see Table E2 in this article's Online Repository at www.jacionline.org).

Receiver operating characteristic curves revealed similar yet slightly superior screening capability of the LTD₄-BPT (AUC, 0.914; 95% CI, 0.855-0.974) over the MCh-BPT (AUC, 0.900; 95% CI, 0.835-0.965; Fig 4).

Adverse events were mainly found in those with positive responses. The major adverse events were tachypnea, chest tightness, wheezing, and coughing. The incidence rate of adverse events was similar in both tests in asthmatic patients with either control status. Furthermore, the incidence rate was similar between those with uncontrolled and partly controlled asthma and was higher than that of patients with controlled asthma,

suggesting that lower incidence correlated with better asthma control (Table III). Adverse events were well tolerated, and subjects recovered within 15 minutes after inhalation of approximately 200 to 400 μ g of salbutamol administered through a metered-dose inhaler, if necessary. Additionally, the mean time of recovery in asthmatic patients with positive test results to inhaled LTD₄ with a 30% or greater and less than 30% decrease in FEV₁ was 7.8 minutes (range, 3-15 minutes) and 5.2 minutes (range, 3-10 minutes), respectively. In contrast, the average time of recovery in asthmatic patients with positive test results to inhaled methacholine with a 30% or greater and less than 30% decrease in FEV₁ was 8.7 minutes (range, 3-20 minutes) and 6.1 minutes (range, 2-15 minutes), respectively. Baseline FEV₁ was not correlated with the incidence of adverse events in either measurement. No nebulization of bronchodilators or oxygen therapy was needed. No serious adverse event was found.

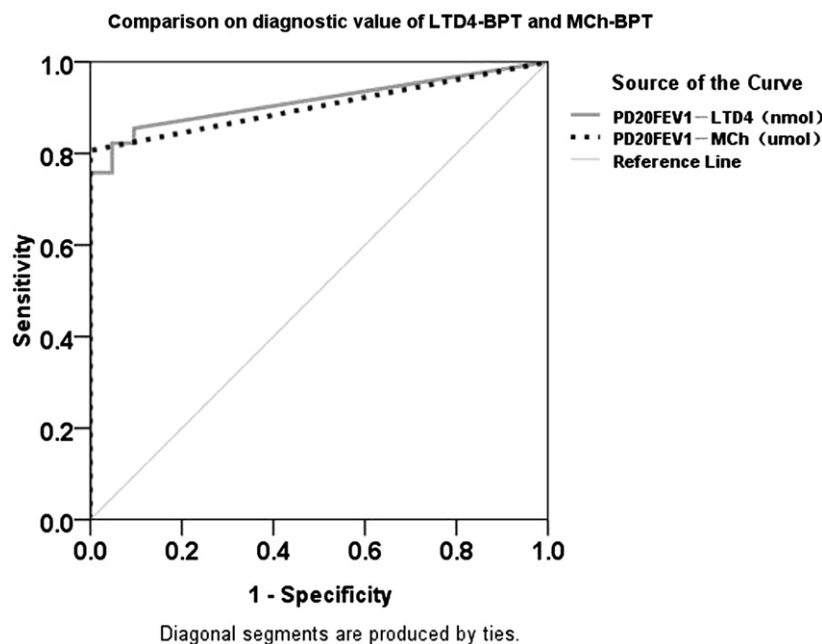
DISCUSSION

The current study showed similar diagnostic and safety profiles of LTD₄-BPTs and MCh-BPTs in asthmatic patients with different levels of control status. The fact that the potency of LTD₄ was not parallel to that of methacholine offered a rationale for classification of a leukotriene-responsiveness subtype among asthmatic patients.

Our results were consistent with previous findings. Echazarreta et al¹¹ reported a $32\% \pm 3\%$ decrease in FEV₁ after LTD₄ challenge in 10 patients with mild asthma. In another study a $32\% \pm 2\%$ reduction in FEV₁ was also found in 10 patients with stable asthma.¹⁰ Interestingly, asthmatic patients yielded larger FEV₁ decreases in MCh-BPTs but not in LTD₄-BPTs. Mulder et al⁷ documented a $39.4\% \pm 2.8\%$ and $31.4\% \pm 1.8\%$ FEV₁ decrease in 10 asthmatic patients during MCh-BPTs and LTD₄-BPTs, respectively. However, these findings might be affected by using different dosages of both bronchoconstrictors.

Airway responsiveness varied in asthmatic patients with different levels of control status. In the present study the geometric mean and interquartile range of PD₂₀FEV₁ in patients with uncontrolled and partly controlled asthma were much lower than those in patients with controlled asthma, supporting the fact that worse asthma control was associated with higher airway responsiveness and less between-individual differences. This was in tune with the discovery of Gyllfors et al,⁵ who reported lower variance in PD₂₀FEV₁-LTD₄ (approximately 0.06-6.43 nmol vs approximately 0.15-37.05 nmol) and PD₂₀FEV₁-MCh (approximately 0.089-25.457 μ mol vs approximately 0.089-37.188 μ mol) values in ICS-naïve and ICS-treated asthmatic patients. Additionally, airway responsiveness persisted despite complete control in some asthmatic patients, as reflected by their low PD₂₀FEV₁ values.

The complexity between airway responsiveness and level of asthma control might be determined by the following factors. First, in addition to persistence of airflow limitation and hyper-responsiveness in certain asthmatic patients during remission,¹⁸ airway responsiveness was found to be not in parallel to severity of airway inflammation.¹⁹ It was therefore postulated that miscellaneous factors (airway remodeling or autonomous nerve disorder) might contribute to between-individual variance. Sont et al²⁰ reported a 1.8 times higher frequency of episodes in asthmatic patients treated with routine protocols than those who received airway responsiveness-targeted treatments as having lower thickness of reticular layer measured by using bronchial

FIG 4. Comparison of the diagnostic value of LTD₄-BPTs and MCh-BPTs.TABLE III. Adverse events in LTD₄-BPTs and MCh-BPTs (%)

	Tachypnea	Chest tightness	Wheezing	Coughing	Expectoration	Pharyngeal itching	Palpitation	Sore throat	Headache
LTD₄-BPTs									
Asthmatic patients (n = 62)	82.3	72.6	32.3	25.8	3.2	9.7	9.7	1.6	1.6
UC (n = 20)	100.0	95.0	40.0	40.0	0.0	10.0	10.0	0.0	0.0
PC (n = 22)	86.4	77.3	27.3	22.7	9.1	13.6	9.1	0.0	0.0
CC (n = 20)	60.0	45.0	30.0	15.0	0.0	5.0	10.0	5.0	5.0
NS (n = 21)	4.8	9.5	0.0	0.0	0.0	4.8	0.0	0.0	0.0
MCh-BPTs									
Asthmatic patients (n = 62)	72.6	80.6	25.8	37.1	6.5	9.7	3.2	1.6	0.0
UC (n = 20)	100.0	100.0	35.0	55.0	15.0	15.0	0.0	0.0	0.0
PC (n = 22)	72.7	90.9	27.3	45.5	0.0	13.6	9.1	4.5	0.0
CC (n = 20)	45.0	50.0	15.0	10.0	5.0	0.0	0.0	0.0	0.0
NS (n = 21)	0.0	0.0	0.0	4.8	0.0	0.0	0.0	0.0	0.0

CC, Controlled asthma group; NS, healthy subject group; PC, partly controlled asthma group; UC, uncontrolled asthma group.

biopsy, indicating persistent airway hyperresponsiveness as a result of airway remodeling.

Second, exacerbation might lead to worsened airway inflammation and remodeling, and significant improvement could consequently be observed until complete control was obtained.

Third, asthmatic patients frequently exposed to allergens or having respiratory tract infection might predispose themselves to worse asthma control. However, these subjects might not necessarily present high airway responsiveness despite their poor asthma control status.

LTD₄ and methacholine are both direct bronchoconstrictors with different mechanism of action. LTD₄ acts mainly through combining with cysteinyl leukotriene receptor 1^{2,21} and methacholine, cholinergic receptors of airway smooth muscle cells.²² This might be useful to interpret the discrepancy in their potency. Echazarreta et al¹¹ reported an average LTD₄/MCh potency ratio of 450 in 13 patients with stable asthma with intermittent episodes. Casas et al¹⁰ documented a potency ratio of 400 in 10 patients with mild and stable asthma. Gyllfors et al¹³ found a 1234 and 1326

times higher potency of LTD₄ in asthmatic patients receiving and not receiving ICS therapy, respectively. In our study, on a molar basis, LTD₄ was 3600 times more potent than methacholine among all asthmatic patients. We also found that asthmatic patients with worse control were more sensitive to LTD₄, but it was interesting that a higher potency ratio favored patients with partly controlled asthma rather than patients with uncontrolled or controlled asthma. It seemed to make sense that the level of control status, concomitant use of medications, and ethnicity might have an effect on the difference from previous reports.

LTD₄ elicited more significant bronchospasm in patients with poorly controlled asthma, suggesting that the leukotriene plays an important role in the pathogenesis of asthma. However, the results of airway responsiveness to inhaled LTD₄ alone might not accurately estimate the exact sensitivity to leukotrienes, and therefore the combination assessment of PD₂₀FEV₁-LTD₄ value and LTD₄/MCh potency ratio might be useful to distinguish leukotriene inflammation subtypes, which could offer novel perspective for optimizing antileukotriene therapy. In this study

geometric means of PD₂₀FEV₁-LTD₄ value and LTD₄/MCh potency ratio were adopted. This was based on the assumption that better efficacy of LTRAs might be associated with higher responsiveness to inhaled leukotriene and that prescription of LTRAs to leukotriene-unresponsive asthmatic patients should be discouraged. However, this deduction remained elusive, and further randomized controlled studies are warranted.

Both tests had high sensitivity and specificity. Larger AUCs in the receiver operating characteristic curve were found in asthmatic patients with worse asthma control, indicating more reliable screening capability. Noticeably, LTD₄-BPTs had higher diagnostic value in patients with partly controlled and uncontrolled asthma.

The major adverse events were tachypnea, chest tightness, wheezing, and coughing. Worse asthma control was associated with higher incidence rate and severity of adverse events. This might be partly interpreted by the correlation between asthma control and average airway responsiveness. However, the incidence rate of expectoration, palpitation, pharyngeal itching, and headache was similar in each group. Short-acting bronchodilator resulted in rapid recovery from bronchoconstriction. Both tests were well tolerated. No serious adverse events were found, even in those who had higher FEV₁ decreases or slower recovery, securing the safety of both measurements.

Several limitations of the study were considered. First, correlation between airway responsiveness to inhaled LTD₄ and endogenous leukotriene release was not examined, which might be solved by analyzing blood and urine leukotriene levels. Second, dynamic variation in individual airway responsiveness was not monitored. This prompts further research on assessing recovery of airway responsiveness over time among asthmatic patients receiving treatments (eg, ICS and LTRA) in the future. Third, the idea that leukotriene-responsiveness subtypes might be helpful to predict the efficacy of LTRAs was based on a theoretical deduction. Randomized clinical trials are therefore warranted to determine the feasibility of these outcome measures.

In conclusion, both LTD₄-BPTs and MCh-BPTs are of high diagnostic value in asthmatic patients and are well tolerated. The combination assessment of PD₂₀FEV₁-LTD₄ value and LTD₄/MCh potency ratio is of significance to identify leukotriene-responsive or leukotriene-unresponsive asthmatic patients, which might help to identify patients with ideal responses to LTRAs and zileuton.

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Clinical implications: Both LTD₄-BPTs and MCh-BPTs are of high diagnostic value in asthmatic patients. The combination assessment of PD₂₀FEV₁-LTD₄ value and LTD₄/MCh potency ratio might be helpful to predict the response to antileukotriene therapy.

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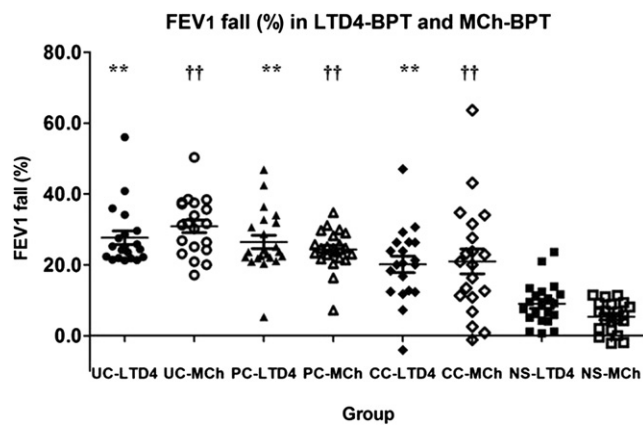


FIG E1. FEV₁ decrease after LTD₄ and methacholine challenge. ** $P < .01$ for between-group comparison with NS-LTD₄. †† $P < .01$ for between-group comparison with NS-MCh. CC, Controlled asthma group; NS, healthy subject group; PC, partly controlled asthma group; UC, uncontrolled asthma group.

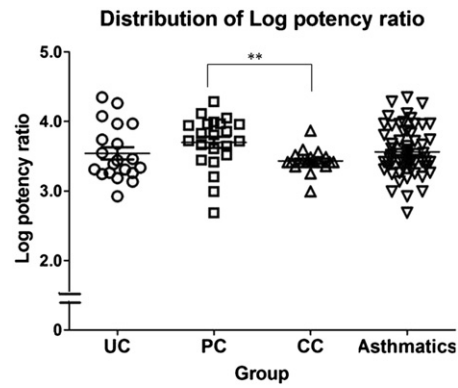


FIG E2. Distribution of logarithm of MCh/LTD₄ potency ratio. $**P < .01$. CC, Controlled asthma; PC, partly controlled asthma; UC, uncontrolled asthma.

TABLE E1. Definition of asthma control

	Controlled (all of the following)	Partly controlled (any measure present)	Uncontrolled
Daytime symptoms	None (twice or less/week)	More than twice/week	Three or more features of partly controlled
Limitation of activities	None	Any	
Nocturnal symptoms/awakening	None	Any	
Need for reliever/rescue treatment	None (twice or less/week)	More than twice/week	
Lung function (FEV ₁ or PEF)	Normal	<80% of predicted value or personal best (if known)	

Criteria were defined based on the Global Initiative for Asthma 2009 update.

PEF, Peak expiratory flow.

TABLE E2. Diagnostic parameters of LTD₄-BPTs and MCh-BPTs

	Sensitivity	Specificity	Accuracy	Positive likelihood ratio	Negative likelihood ratio
LTD ₄ -BPT					
Uncontrolled	1.000	0.905	0.905	0.909	1.000
Partly controlled	0.955	0.905	0.859	0.913	0.950
Controlled	0.600	0.905	0.505	0.857	0.704
MCh-BPT					
Uncontrolled	0.950	1.000	0.950	1.000	0.955
Partly controlled	0.909	1.000	0.909	1.000	0.913
Controlled	0.550	1.000	0.550	1.000	0.700