Effects of 24-week add-on treatment with ciclesonide and montelukast on small airways inflammation in asthma

*Hitoshi Nakaji[1,2], *Guergana Petrova[1], Hisako Matsumoto[1], Toshiyuki Iwata[1], Isao Ito[1], Tsuyoshi Oguma[1], Hideki Inoue[1], Tomoko Tajiri[1], Tadao Nagasaki[1], Yoshihiro Kanemitsu[1], Akio Niimi[1,3], Michiaki Mishima[1]

[1]Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[2]Department of Respiratory Medicine, Wakayama Red Cross Hospital, Wakayama, Japan

[3]Division of Respiratory Medicine, Department of Medical Oncology and Immunology, Nagoya City University School of Medical Sciences, Nagoya, Japan

* HN and GP equally contributed to this study

Trial registration; Registry ID UMIN000001083

Corresponding author: Hisako Matsumoto, MD, PhD

Department of Respiratory Medicine

Postgraduate School of Medicine, Kyoto University

54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

Telephone: +81-75-751-3830; Fax: +81-75-751-4643

E-mail: hmatsumo@kuhp.kyoto-u.ac.jp

Authors' contributions

HN recruited and managed the patients, collected, analyzed and interpreted the data, and prepared the manuscript. GP collected, analyzed and interpreted the data, and wrote the draft. HM conceived the study, recruited and managed the patients, collected, analyzed and interpreted the data, and revised the manuscript. TI collected and analyzed data and prepared the part of the draft. AN and II recruited the patients, collected the data, and contributed to the edition of the manuscript. TO and HI performed IOS measurements and collected the data and prepared the part of the draft. TT, TN, and YK measured exhaled nitric oxide and analyzed and interpreted the data, and prepared the part of the draft. MM contributed to the discussion of the data and critical revision of the manuscript.

Abstract

Background: Eosinophilic inflammation of the small airways is a key process in asthma that often smolders in treated patients. The long-term effects of add-on therapy on the persistent inflammation in the small airways remain unknown.

Objective: To examine the effects of add-on therapy with either ciclesonide, an inhaled corticosteroid with extrafine particles, or montelukast on small airway inflammation.

Methods: Sixty patients with stable asthma receiving inhaled corticosteroid treatment were enrolled in a randomized, open-label, parallel comparison study of 24-week add-on treatment with ciclesonide or montelukast. Patients were randomly assigned to 3 groups: ciclesonide (n = 19), montelukast (n = 22) and no add-on as controls (n = 19). At baseline and at weeks 4, 12 and 24, extended nitric oxide analysis; pulmonary function tests, including impulse oscillometry; blood eosinophil counts; and asthma control tests (ACTs) were performed.

Results: A total of 18 patients in the ciclesonide group, 19 in the montelukast group and 15 in the control group completed the study and were analysed. With repeated-measures analysis of variance, ciclesonide produced a significant decrease in alveolar nitric oxide and a significant improvement in ACT scores over time. Montelukast produced significant decreases in alveolar nitric oxide concentrations and blood eosinophil counts over time and slightly improved ACT scores, whereas no such changes were observed in the control group. Alveolar nitric oxide concentrations with ciclesonide and reactance area at low frequencies with montelukast produced greater improvements over time compared with control.

Conclusions: Ciclesonide add-on therapy and montelukast add-on therapy may act differently, but both separately can improve small airway abnormalities and provide better asthma control.

Funding; funded by none

Key words: add-on treatment, alveolar nitric oxide, asthma control, ciclesonide, montelukast, small airways

Introduction

Asthma is a chronic inflammatory disease of the airways characterized by variable, recurring symptoms and reversible airflow obstruction. The immunohistopathologic features include infiltration of eosinophils and lymphocytes, mast cell activation and epithelial cell injury. To date, pathological [1, 2], physiologic [3] and radiologic findings [4] have provided sufficient evidence to support not only large but also small airways involvement in inflammation and airflow obstruction, particularly in patients with severe asthma[5, 6].

Recently, it was found that eosinophilic inflammation of the small airways could be assessed by determining alveolar nitric oxide concentrations[7, 8]. Small airway inflammation as assessed by alveolar nitric oxide concentrations is increased in patients with refractory asthma[8] and those with nocturnal asthma [9] and is associated with disease severity [10, 11] and small airways dysfunction [11]. Of note, 20% of asthmatic patients have increased alveolar nitric oxide concentrations despite treatments with inhaled corticosteroids (ICSs) and long-acting β_2 agonists [12]. Alveolar nitric oxide concentrations can also predict a future risk of disease exacerbation [13]. These findings suggest that, even in apparently stable patients taking ICSs, additional treatment targeting the small airways may lead to reaching total asthma control.

Few studies have evaluated the changes in alveolar nitric oxide concentrations based on either an uncorrected [7] or corrected [14] model of add-on medication for persistent inflammation of the small airways. Previous studies found that oral prednisolone [10], but not double doses of ICSs,[8] could decrease alveolar nitric oxide concentrations. These results suggest that alveolar nitric oxide concentrations may be resistant to a simple ICS dose elevation. In steroid-naive patients, however, extrafine particle hydrofluoroalkane–ciclesonide resulted in decreased alveolar nitric oxide concentrations [15] and hydrofluoroalkane–beclomethasone propionate

improved peripheral airway dysfunction [16]. Collectively, an extrafine particle ICS is expected to decrease alveolar nitric oxide concentrations when they are used as an add-on medication. Leukotriene receptor antagonists (LTRAs) that are administered systemically are another medication that are supposed to decrease alveolar nitric oxide concentrations. Treatment with montelukast for 4 weeks improved small airway obstruction in steroid-naive patients, which resulted in a decrease in regional air trapping [17]. So far published study data of an add-on LTRA to ICS therapy for 3 to 8 weeks with regard to alveolar nitric oxide concentrations have been conflicting[18,19]. These effects require confirmation with a longer-term study.

For this study, we hypothesized that prolonged add-on therapy to the ICS treatment with either ciclesonide or montelukast would have beneficial effects on the persistent inflammation of the small airways and would improve pulmonary function. To test this hypothesis, our primary objectives were to examine the effects of this add-on therapy on alveolar nitric oxide concentrations and to compare its effects on small airways in patients with stable asthma who had not been previously treated with extrafine particle ICSs or LTRAs.

Methods

The full details of the study methods are given in the eMethods. In brief, adult patients with stable asthma who regularly visited our outpatient asthma clinic were enrolled from April 2008 to August 2011. Asthma was diagnosed according to American Thoracic Society criteria [20]. Patients were included if they were classified as being in treatment steps 2 to 5 of ICS treatment according to the Global Initiative for Asthma guidelines [21]. These patients had no exacerbations 3 months before enrollment, had alveolar nitric oxide concentrations of 5.0 ppb or higher, and were either never-smokers or ex-smokers who had smoked fewer than 5 pack-years and had stopped more than 1 year before. The threshold level for uncorrected alveolar nitric oxide concentrations was set at 5.0 ppb; this value was the average minus 1 SD of uncorrected alveolar nitric oxide concentrations of 70 patients with asthma taking ICSs in our previous study [22].

Exclusion criteria were current or previous use of extrafine particle ICSs or LTRAs. Patients were also excluded if, during the study period, any adverse effects of the add-on therapy or asthma exacerbations, including mild exacerbations, defined as an increased need for rescue use of short-acting β_2- agonists, were noted.

This study was approved by the ethics committees of our institute and was registered in UMIN Clinical Trials Registry (Registry Identified UMIN000001083). Written informed consent was obtained from all participants.

Design and Measurements

This was a randomized, open-label, parallel comparison study of 24-week add-on treatment with either inhaled ciclesonide or montelukast. Patients were randomly assigned to 3 treatment groups: inhaled ciclesonide, 400 µg once daily add-on (ciclesonide group);

montelukast, 10 mg once daily add-on (montelukast group); and control group, who were taking current medication only. At weeks 0 (baseline), 4, 12, and 24 (end of study period) the patients underwent extended nitric oxide analysis and pulmonary function tests, including tests with an impulse oscillometry system (IOS), spirometry, and a nitrogen single-breath wash out test. At the same time points, patients completed an asthma control test (ACT) questionnaire comprising 5 questions with a best possible score of 25 [23] and were given a rhinitis symptom score (RSS), a self-assessment questionnaire comprising 4 questions, the responses to which were ranked on a Likert-type scale with a maximum of 5 points per answer. The RSS was determined based on the Japanese Guideline for Allergic Rhinitis (best score, 20) [24] (eTable 1).

At the start and end of the study period, blood samples were obtained for blood eosinophil counts and serum high sensitivity C-reactive protein [25], serum eosinophil cationic protein,[26] and serum YKL-40, a chitinase like protein [27]. Blood samples for eosinophil cationic protein determinations were collected in SST tubes (Becton Dickinson, Mountain View, California) and were processed as previously described [26]. YKL-40 levels were determined using an enzyme-linked immunosorbent assay kit (Quidel, San Diego, California) following the manufacturer's instructions [27].

Nitric oxide levels were determined with a chemiluminescence analyzer (NOA 280; Sievers, Boulder, Colorado) according to current guidelines, and as previously described alveolar nitric oxide concentrations are provided as noncorrected [7] and corrected values using a trumpet-shaped model with axial back diffusion (eMethods) [14].

After nitric oxide measurements, patients underwent prebronchodilator and postbronchodilator (ie, inhalation of 200 μg of salbutamol) pulmonary function tests.

Spirograms were obtained as recommended by the American Thoracic Society/European Respiratory Society [28]. A nitrogen single-breath washout test was performed only before the inhalation of salbutamol to assess ventilation inhomogeneity by measuring the slope of phase 3 of the nitrogen washout curve (ΔN_2).

Respiratory impedance was determined by IOS using a Jaeger MasterScreen, IOS^{TM} (Erich Jaeger, Hoechberg Germany) that met standard recommendations (eMethods). [16, 22]

Statistical analysis

For sample size determinations, we originally sought to enroll 90 patients based on previous findings [15, 17, 19]. However, as described in the "Results" section, we decided to stop patient enrollment at 60 because of the more frequent occurrence of exacerbations in the control group, although these were mild.

Statistical analysis used JMP 6.00 (SAS Institute Inc., Cary, North Carolina) on a per-protocol basis. For non-normally distributed results, comparisons were made by the Kruskal–Wallis test, Fisher exact test or Wilcoxon signed-rank test as appropriate. For normally distributed results, comparisons were made by analysis of variance (ANOVA) and the paired *t*-test. Two-way repeated-measures ANOVA was used to assess the variations among the 3 treatment modalities and at different time points. For cases with unequal variations in the treatment modalities, only 1-way repeated-measures ANOVA within 1 treatment group was used. For correlation analysis, the Spearman rank-correlation test was used. Data are expressed as mean ± SD. $P \leq 0.05$ were considered statistically significant.

Results

Enrollment, Dropout, and Exacerbation Rates and Baseline Characteristics

Sixty asthmatic patients were enrolled in this study and randomly assigned to the groups: 19 in the ciclesonide group, 22 in the montelukast group, and 19 in the control group (Fig 1).The reasons for patient dropout were as follows: in the ciclesonide group, 1 patient had a possible adverse effect (urticaria); in the montelukast group, 3 patients had possible adverse effects (2 experienced mild gastrointestinal discomfort and they preferred to discontinue use of the medication and 1 patient had mildly elevated transaminase levels); and in the control group, 3 had mild asthma exacerbations and they preferred to intensify medications and 1 patient discontinued ICS treatment following a general practitioner's advice. As a result 18 patients in the ciclesonide group, 19 in the montelukast group, and 15 in the control group completed the study and were analyzed thereafter (Table 1). For these patients, adherence to the add-on and current medications was satisfactory, which was confirmed by 2 of the authors (H.N. and H.M.) on each visit by checking the residual number of medications.

When the exacerbation frequencies were compared between the 19 patients in the control group and the 41 patients in the add-on therapy groups and assuming that the 5 patients who dropped out for reasons other than exacerbation would complete the protocol without exacerbation, the control group had a significantly higher rate of exacerbation ($p = 0.03$; by Fisher exact test). The baseline patient characteristics, ICS doses, and biomarkers, including fractional exhaled nitric oxide (FeNO) and alveolar nitric oxide concentrations, were not significantly different among the 3 patients who later experienced mild exacerbations and the other 57 patients.

ACT scores and RSSs

By 1-way ANOVA, there was a significant improvement in ACT scores during the treatment period within the ciclesonide group ($p = 0.02$; Fig 2), and there was a trend for improvement within the montelukast group ($p = 0.08$). When subscores for the ACT components were separately analyzed in the ciclesonide group, subscores for ACT question 3 concerning nocturnal symptoms and question 5 for self-rating were marginally and insignificantly improved over time ($p = 0.05$ and $p = 0.06$, respectively). Because of the unequal variations among the 3 treatment modalities, we did not conduct 2-way ANOVA for the ACT scores. Details on ACT scores across the treatment steps are presented in eTable 2.

Among the 3 groups, neither the proportions of patients with allergic rhinitis nor their baseline RSSs differed. However, a significant difference was seen in the time trends for RSS among the 3 treatment modalities ($p = 0.004$; eFig 1); in particular, using 2-way ANOVA, significant differences were seen for the symptom of nasal obstruction ($p = 0.046$). When comparing 2 different treatment modalities in a post hoc analysis, the montelukast group exhibited a significantly better time trend for the RSS than the control group ($p < 0.001$) and a trend for better scores than the ciclesonide group ($p = 0.07$; eFig 1). A significant increase in RSS over time was found only in the montelukast group ($p < 0.001$, by 1-way ANOVA).

There were no associations between changes in ACT or RSS from baseline to the end of the treatment period and changes in alveolar nitric oxide concentrations or corrected alveolar nitric oxide concentrations in either treatment group.

Nitric Oxide Results

No significant differences were found in the time trends for FeNO at an expiratory flow rate of 50 mL/s among the 3 treatment modalities or within each of the groups (results not shown).

The time trends for uncorrected alveolar nitric oxide concentrations were significantly different among the 3 treatment groups ($p = 0.048$, by 2-way ANOVA). When comparing 2 different treatment modalities in a post hoc analysis, the ciclesonide group had a greater decrease in alveolar nitric oxide concentrations over time than the control group ($p = 0.03$, by 2-way ANOVA). By 1-way ANOVA, alveolar nitric oxide concentrations in the control group did not change during the study period, whereas in both of the add-on treatment groups, alveolar nitric oxide concentrations significantly decreased over time ($p = 0.01$ for the ciclesonide and montelukast groups; Fig 3).

For corrected alveolar nitric oxide concentrations, 1-way ANOVA showed that there was an insignificant decrease over time in the ciclesonide group ($p = 0.06$).

Pulmonary Function Tests

None of the spirometry indices, ΔN_2, or IOS indices of respiratory resistance at 5 Hz (Rrs_5), respiratory resistance at 20 Hz (Rrs_{20}), or respiratory reactance at 5 Hz (Xrs_5) revealed any difference among the 3 treatment modalities during the treatment period regardless of prebronchodilator or postbronchodilator conditions. No significant changes were observed within any of the 3 groups (data not shown).

A significant difference was found in the time trends for the reactance area (AX) among the 3 treatment modalities ($p = 0.04$, by 2-way ANOVA). The AX levels in the montelukast group improved over time when compared with the control group ($p = 0.05$, by 2-way ANOVA; Fig 4). For Rrs_5–Rrs_{20}, 2-way ANOVA was not used because of the unequal variations among

the 3 treatment modalities; however, 1-way ANOVA revealed that there was a trend for a change over time in the ciclesonide group ($p = 0.09$).

Although there were associations between corrected alveolar nitric oxide concentrations and IOS indices of AX or Rrs_5-Rrs_{20} at baseline ($r = 0.30$, $p < 0.05$ for both, $n = 52$), no associations were found between changes in pulmonary function data from baseline to the end of the treatment period and changes in alveolar nitric oxide concentrations or corrected alveolar nitric oxide concentrations in either treatment group.

Blood Test Results

Blood samples were obtained at baseline and at the end of the treatment period to determine blood eosinophil counts and serum levels of eosinophil cationic protein, high sensitivity C-reactive protein, and YKL-40. No significant changes were found in these tests results between the beginning and the end of the treatment period, except for the montelukast group in which the eosinophil counts significantly declined after treatment ($2.9 \pm 2.2\%$ at 24 weeks)($p = 0.02$, paired *t* test).

Discussion

To the best of our knowledge, this is the first long-term study that clarified the benefits and potential role of add-on therapy with either ciclesonide of extrafine particle ICS or montelukast in steroid-treated patients with stable asthma. Ciclesonide may have attenuated smoldering inflammation of the small airways and significantly improved asthma control over time. Montelukast ameliorated the remnant dysfunction of the small airways and reduced nasal symptoms and blood eosinophil counts. To a lesser extent than ciclesonide, montelukast also improved smoldering inflammation of the small airways.

Alveolar nitric oxide concentration is an established marker of small airway inflammation and is correlated with eosinophil counts in bronchoalveolar lavage fluid [8]. In the ciclesonide group, alveolar nitric oxide concentrations significantly decreased over time when compared with the control group and the ciclesonide intragroup analysis. Our data confirmed earlier findings of the effects of 5-week treatment with ciclesonide on alveolar nitric oxide concentrations in steroid-naïve patients [15] and reinforced the advantage of extrafine particle ICSs to treat smoldering inflammation of the small airways, even in patients already taking ICSs. There remains the possibility that the addition of ciclesonide to the patients' current medication may have exerted anti-inflammatory effects *via* the increase in the total amount of ICS, which may have suppressed the remnant inflammation throughout the airways. However, this is unlikely because FeNO at 50 mL/s did not change over time. Taken the results of the previous short-term study and current study together, ciclesonide would be capable of treating the small airways potentially because of its particles size, which was sufficiently small to reach the peripheral airways.

In contrast to uncorrected alveolar nitric oxide concentrations, corrected alveolar nitric oxide concentrations only showed a trend toward being decreased in the ciclesonide group ($p = 0.06$, 1-way ANOVA). Although corrected alveolar nitric oxide concentrations reflect airway dysfunction [22, 29], as do alveolar nitric oxide concentrations, corrected alveolar nitric oxide concentrations do not reflect disease severity [14, 22] or asthma control status [29]. It is also not increased during asthma exacerbations in adults [30], a finding that is in contrast to several lines of evidence for alveolar nitric oxide concentrations. Although alveolar nitric oxide concentrations are contaminated with bronchial nitric oxide, potentially from small conducting airways where diffusion begins to replace bulk flow, our findings on alveolar nitric oxide concentrations imply that relatively small airways, albeit not actual peripheral airways, are still important in the management of asthma.

Studies of add-on medication using LTRAs that have evaluated changes in alveolar nitric oxide concentrations in persistent inflammation of the small airways reported inconsistent findings. Previous add-on studies of montelukast to fluticasone [18] or fluticasone and salmeterol treatment [12] did not find any significant benefits for montelukast with regard to decreases in alveolar nitric oxide concentrations after montelukast add-on therapy. However, these earlier studies were relatively short-term, with treatment periods of only 3 to 4 weeks. Yasui et al. investigated pranlukast use in patients with stable asthma and found significant decreases in both corrected and uncorrected alveolar nitric oxide concentrations after 8-week crossover of add-on therapy with pranlukast [19]. In agreement with that study, we found that alveolar nitric oxide concentrations in the montelukast group decreased during the 24-week add-on period, although these levels were not significantly different from the control group. As with the ciclesonide group, FeNO at 50 mL/s did not change over time. These findings indicate that add-on treatment

297 with LTRAs for longer than 8 weeks suppresses the remnant inflammation in the small airways.
298 In addition, our intervention study that covered the 2 seasons for allergic rhinitis (spring and
299 autumn) provided additional evidence of the established benefit of montelukast on allergic
300 rhinitis [31] and justified a role for LTRA in the therapy for patients with stable asthma with
301 concomitant allergic rhinitis, even those with minimal symptoms.
302 Symptoms and airway obstruction are integral to the definition of asthma, and represent
303 important components for assessing asthma control in both clinical practice and clinical trials.
304 Therefore, one of the end points in our study was ACT scores. Despite the disadvantage in
305 adherence to inhalation use, as reported previously on adherence to LTRA of 67.7% vs. 33.8%
306 for ICS [32], ACT scores significantly improved over time in the ciclesonide group. In addition,
307 there was a marginal improvement in the subscore of ACT question 3 concerning nocturnal
308 symptoms in the ciclesonide group. To date, a number of studies have confirmed that
309 eosinophilic inflammation worsens in patients with nocturnal asthma, particularly in the
310 peripheral airways [33]. Lehtimaki et al[9] reported that nocturnal symptoms in asthmatic patients
311 were related to higher alveolar nitric oxide concentrations. These results are in accordance with
312 our results showing that ciclesonide add-on treatment reduced inflammation in the small airways,
313 as assessed by alveolar nitric oxide concentrations, and improved nocturnal symptoms, as
314 assessed by ACT subscores. Care must be taken when interpreting these findings, however,
315 because the minimally important difference in ACT scores that reflects a clinically meaningful
316 change is considered to be 3 points [34], and the increase in ACT composite scores in our
317 ciclesonide group did not achieve this. Despite this minimal change, these statistically significant
318 changes would still favour add-on therapy for patients with seemingly stable asthma.

We did not find any significant changes in spirometry function results or ΔN_2 between the control and therapy add-on groups, which may seem unexpected. Spirometry is not sensitive enough to detect early small airway involvement because the small airways are pathways of very low resistance and only contribute to approximately 10% of the total airway resistance [35]. Instead of using ΔN_2, ventilation heterogeneity within conductive and acinar airways could have been separately assessed using a nitrogen multiple-washout test [36]. Another possible reason could be that our patients had already good pulmonary function, so that changes in alveolar nitric oxide concentrations were not reflected in the airway function. However, in the montelukast group, the $AX^{16, 22}$ significantly decreased over time when compared with the control group, as was found in our previous intervention study in steroid-naïve patients [16]. Montelukast may have reversed remodeling in the airway walls by reducing airway smooth muscle layer thickening and subepithelial fibrosis in long-term treatment, as has been shown in an animal model [37]. More significant findings might be expected in extended studies in a larger number of patients.

A limitation of our study was that it was a parallel, open-label, and unblinded study, which might have influenced subjective measures, such as asthma symptoms and rescue use of short-acting β_2 agonists. Another issue is the use of 2 different inhalers for corticosteroids, although we achieved good adherence in the ciclesonide group. In future studies with more patients and longer treatment periods, this issue could be resolved.

In addition, we may have missed some patients with occult inflammation in the small airways by excluding those with alveolar nitric oxide concentrations less than 5 ppb, given that some patients who have high FeNO and low alveolar nitric oxide concentrations exhibit paradoxical increases in alveolar nitric oxide concentrations after treatment [38], possibly because of dilatation of constricted small airways from terminal to respiratory bronchioles. However, by

setting this threshold for alveolar nitric oxide concentrations during patient enrollment, the changes of alveolar nitric oxide concentrations in this study could be simply interpreted.

Finally, from the ethical standpoint, we stopped enrollment at 60 patients because of a higher, albeit mild, exacerbation rate in the control group, which was consistent with the finding that elevated alveolar nitric oxide concentration was associated with risk of asthma exacerbation[13]. Thus, some of the insignificant findings, particularly of the pulmonary function data in this study, may be due to lesser statistical power. Lack of associations between the changes in alveolar nitric oxide concentrations and changes in pulmonary function data or ACT scores might be another issue. However, we did not set the sample size to seek significant associations between changes in alveolar nitric oxide concentrations and any other clinical indices because of their potentially large variations during the treatment period, although alveolar nitric oxide concentrations, pulmonary function, and ACT were intuitively thought to behave in parallel. Despite these limitations, the current findings of a decrease in alveolar nitric oxide concentrations with add-on treatment are sufficient to be used as a future reference when intensifying treatment with extrafine particle ICS or LTRA add-on therapy, even in patients with seemingly stable asthma who are receiving ICS treatment but still have evidence of small airways inflammation as assessed by alveolar nitric oxide concentrations.

We conclude that ciclesonide and montelukast may act differently but that both separately can improve small airway abnormalities (eTable 3). By coadministration of these medications, cumulative effects on inflammation and small airways function can be expected and should be clarified in a future study. We can achieve additional benefits by treating inflammation of the small airways in patients with stable asthma to reach the ultimate asthma treatment goal: ideal control.

Acknowledgments

The authors are grateful to Ms Aya Inazumi and Ms Yuko Maeda for their technical assistance.

References

1. Hamid Q, Song Y, Kotsimbos TC, et al. Inflammation of small airways in asthma. J Allergy Clin Immunol. 1997;100: 44-51.
2. Carroll N, Cooke C, James A. The distribution of eosinophils and lymphocytes in the large and small airways of asthmatics. Eur Respir J. 1997;10: 292-300.
3. in 't Veen JC, Beekman AJ, Bel EH, Sterk PJ. Recurrent exacerbations in severe asthma are associated with enhanced airway closure during stable episodes. Am J Respir Crit Care Med. 2000;161: 1902-1906.
4. Ueda T, Niimi A, Matsumoto H, et al. Role of small airways in asthma: investigation using high-resolution computed tomography. J Allergy Clin Immunol. 2006;118: 1019-1025.
5. Johnson JR, Hamid Q. Appraising the small airways in asthma. Curr Opin Pulm Med. 2012;18: 23-28.
6. Contoli M, Kraft M, Hamid Q, et al. Do small airway abnormalities characterize asthma phenotypes? In search of proof. Clin Exp Allergy. 2012;42: 1150-1160.
7. Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. J Appl Physiol. 1998;85: 653-666.
8. Berry M, Hargadon B, Morgan A, et al. Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma. Eur Respir J. 2005;25: 986-991.
9. Lehtimaki L, Kankaanranta H, Saarelainen S, Turjanmaa V, Moilanen E. Increased alveolar nitric oxide concentration in asthmatic patients with nocturnal symptoms. Eur Respir J. 2002;20: 841-845.
10. Gelb AF, Taylor CF, Nussbaum E, et al. Alveolar and airway sites of nitric oxide inflammation in treated asthma. Am J Respir Crit Care Med. 2004;170: 737-741.
11. van Veen IH, Sterk PJ, Schot R, et al. Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma. Eur Respir J. 2006;27: 951-956.
12. Gelb AF, Taylor CF, Shinar CM, Gutierrez CA, Zamel N. Effect of fluticasone 250 microg/salmeterol 50 microg and montelukast on exhaled nitric oxide in asthmatic patients. Can Respir J. 2008;15: 193-198.

13. 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: 1492-1499.
14. Condorelli P, Shin HW, Aledia AS, Silkoff PE, George SC. A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and an axial diffusion model. J Appl Physiol. 2007;102: 417-425.
15. Cohen J, Douma WR, ten Hacken NH, et al. Ciclesonide improves measures of small airway involvement in asthma. Eur Respir J. 2008;31: 1213-1220.
16. Yamaguchi M, Niimi A, Ueda T, et al. Effect of inhaled corticosteroids on small airways in asthma: investigation using impulse oscillometry. Pulm Pharmacol Ther. 2009;22: 326-332.
17. Zeidler MR, Kleerup EC, Goldin JG, et al. Montelukast improves regional air-trapping due to small airways obstruction in asthma. Eur Respir J. 2006;27: 307-315.
18. Fritscher LG, Rodrigues MT, Zamel N, Chapman KR. The effect of montelukast on exhaled nitric oxide of alveolar and bronchial origin in inhaled corticosteroid-treated asthma. Respir Med. 2009;103: 296-300.
19. Yasui H, Fujisawa T, Inui N, et al. Impact of add-on pranlukast in stable asthma; the additive effect on peripheral airway inflammation. Respir Med. 2012;106: 508-514.
20. 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: 225-244.
21. Global Initiative for Asthma Management and Prevention: National Institutes of Health, National Heart, Lung and Blood Institute, 2006.
22. Matsumoto H, Niimi A, Jinnai M, et al. Association of alveolar nitric oxide levels with pulmonary function and its reversibility in stable asthma. Respiration. 2011;81: 311-317.
23. Nathan RA, Sorkness CA, Kosinski M, et al. Development of the asthma control test: a survey for assessing asthma control. J Allergy Clin Immunol. 2004;113: 59-65.
24. Okubo K, Kurono Y, Fujieda S, et al. Japanese guideline for allergic rhinitis. Allergol Int. 2011;60: 171-189.

25. Takemura M, Matsumoto H, Niimi A, et al. High sensitivity C-reactive protein in asthma. Eur Respir J. 2006;27: 908-912.
26. Matsumoto H, Niimi A, Minakuchi M, Izumi T. Serum eosinophil cationic protein levels measured during exacerbation of asthma: characteristics of patients with low titres. Clin Exp Allergy. 2001;31: 637-643.
27. Otsuka K, Matsumoto H, Niimi A, et al. Sputum YKL-40 Levels and Pathophysiology of Asthma and Chronic Obstructive Pulmonary Disease. Respiration. 2012;83: 507-519.
28. 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: 912-930.
29. Mahut B, Trinquart L, Le Bourgeois M, et al. Multicentre trial evaluating alveolar NO fraction as a marker of asthma control and severity. Allergy. 2010;65: 636-644.
30. Gelb AF, George SC, Silkoff PE, et al. Central and peripheral airway/alveolar sites of exhaled nitric oxide in acute asthma. Thorax. 2010;65: 619-625.
31. Lagos JA, Marshall GD. Montelukast in the management of allergic rhinitis. Ther Clin Risk Manag. 2007;3: 327-332.
32. Jones C, Santanello NC, Boccuzzi SJ, et al. Adherence to prescribed treatment for asthma: evidence from pharmacy benefits data. J Asthma. 2003;40: 93-101.
33. Kraft M, Djukanovic R, Wilson S, Holgate ST, Martin RJ. Alveolar tissue inflammation in asthma. Am J Respir Crit Care Med. 1996;154: 1505-1510.
34. Schatz M, Kosinski M, Yarlas AS, et al. The minimally important difference of the Asthma Control Test. J Allergy Clin Immunol. 2009;124: 719-723 e711.
35. Macklem PT. The physiology of small airways. Am J Respir Crit Care Med. 1998;157: S181-183.
36. Verbanck S, Schuermans D, Vincken W. Inflammation and airway function in the lung periphery of patients with stable asthma. J Allergy Clin Immunol. 2010;125: 611-616.
37. Henderson WR, Jr., Chiang GK, Tien YT, Chi EY. Reversal of allergen-induced airway remodeling by CysLT1 receptor blockade. Am J Respir Crit Care Med. 2006;173: 718-728.
38. Van Muylem A, Kerckx Y, Michils A. Acinar effect of inhaled steroids evidenced by exhaled nitric oxide. J Allergy Clin Immunol. 2010;126: 730-735 e732.

Figure legends

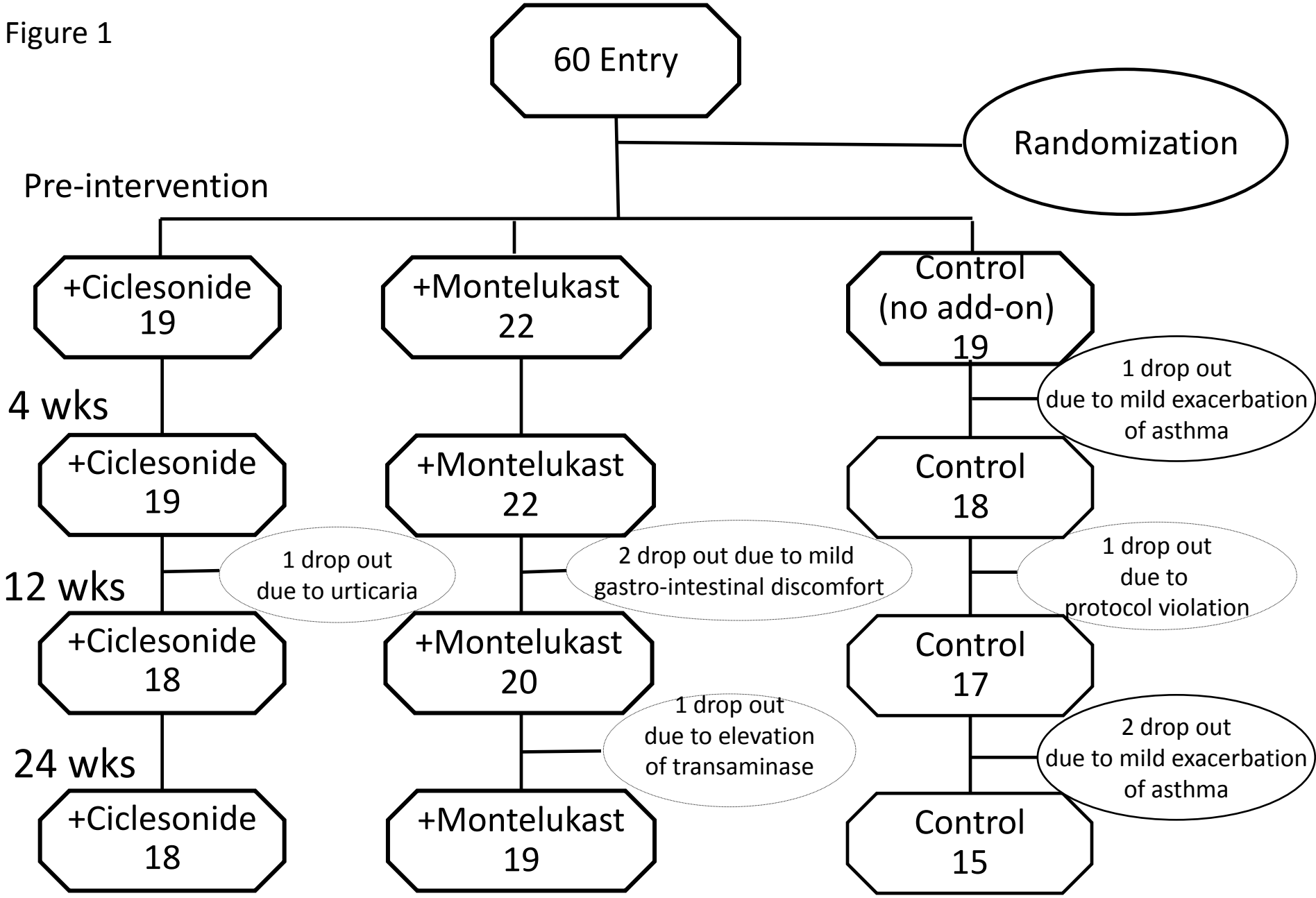
Figure 1. Registration and randomization

Figure 2. Asthma control test (ACT) scores in the 3 study groups. *Significant changes in ACT scores within the ciclesonide add-on group ($p = 0.02$, by 1-way analysis of variance).

Figure 3. Alveolar nitric oxide concentrations in the 3 study groups. *Significant difference in the time trends for alveolar nitric oxide concentrations among the 3 treatment modalities ($p = 0.048$, by 2-way analysis of variance [ANOVA]). †Significant changes in alveolar nitric oxide concentrations in the ciclesonide add-on group ($p = 0.03$ vs the control group, by 2-way ANOVA) ($p = 0.01$, by 1-way ANOVA). ‡Significant changes within montelukast add-on group ($p = 0.01$, by 1-way ANOVA).

Figure 4. Reactance area (AX) levels in the 3 study groups. *Significant difference in the time trends for AX levels among the 3 treatment modalities ($p = 0.04$, by 2-way analysis of variance [ANOVA]), †posthoc analysis between the montelukast add-on and control groups ($p = 0.05$, by 2-way ANOVA).

Figure 1


60 Entry
Randomization
Pre-intervention
+Ciclesonide 19
+Montelukast 22
Control (no add-on) 19
1 drop out due to mild exacerbation of asthma
4 wks
+Ciclesonide 19
+Montelukast 22
Control 18
1 drop out due to urticaria
2 drop out due to mild gastro-intestinal discomfort
1 drop out due to protocol violation
12 wks
+Ciclesonide 18
+Montelukast 20
Control 17
1 drop out due to elevation of transaminase
2 drop out due to mild exacerbation of asthma
24 wks
+Ciclesonide 18
+Montelukast 19
Control 15

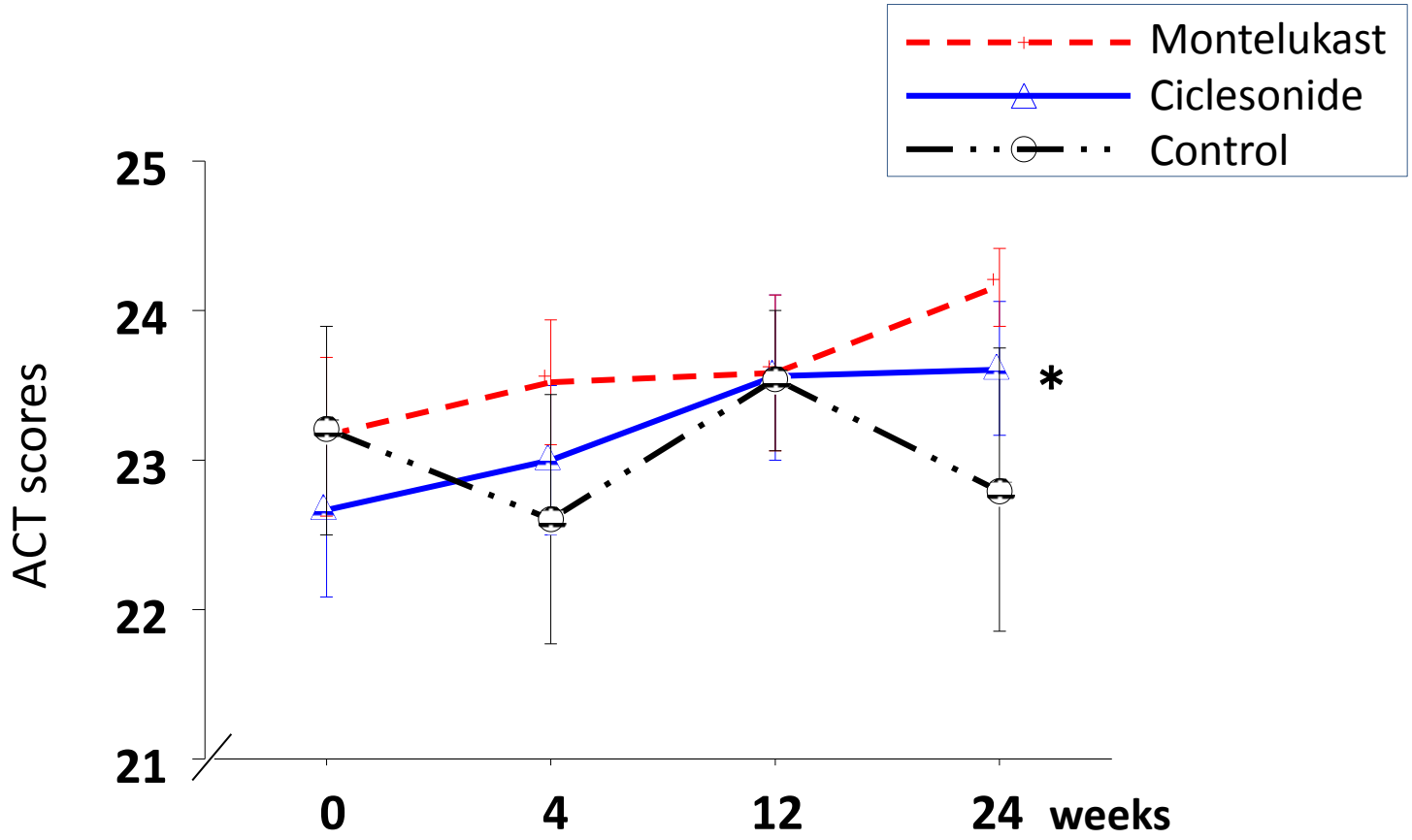

Montelukast
Ciclesonide
Control
ACT scores
25
24
23
22
21
0
4
12
24
weeks
*

Figure 2

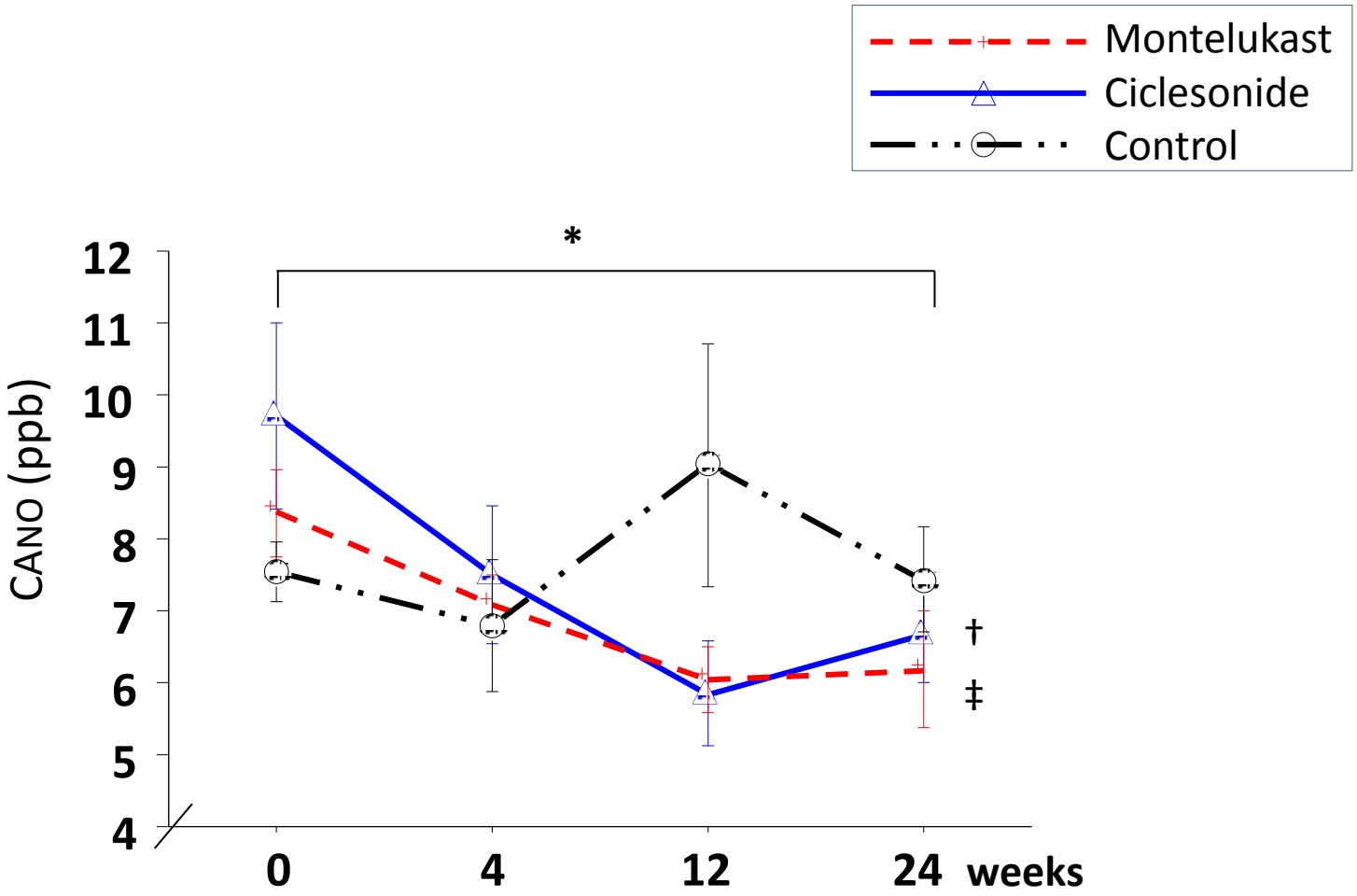

Montelukast
Ciclesonide
Control
*
12
11
10
9
8
7
6
5
4
CANO (ppb)
0
4
12
24
weeks
†
‡

Figure 3

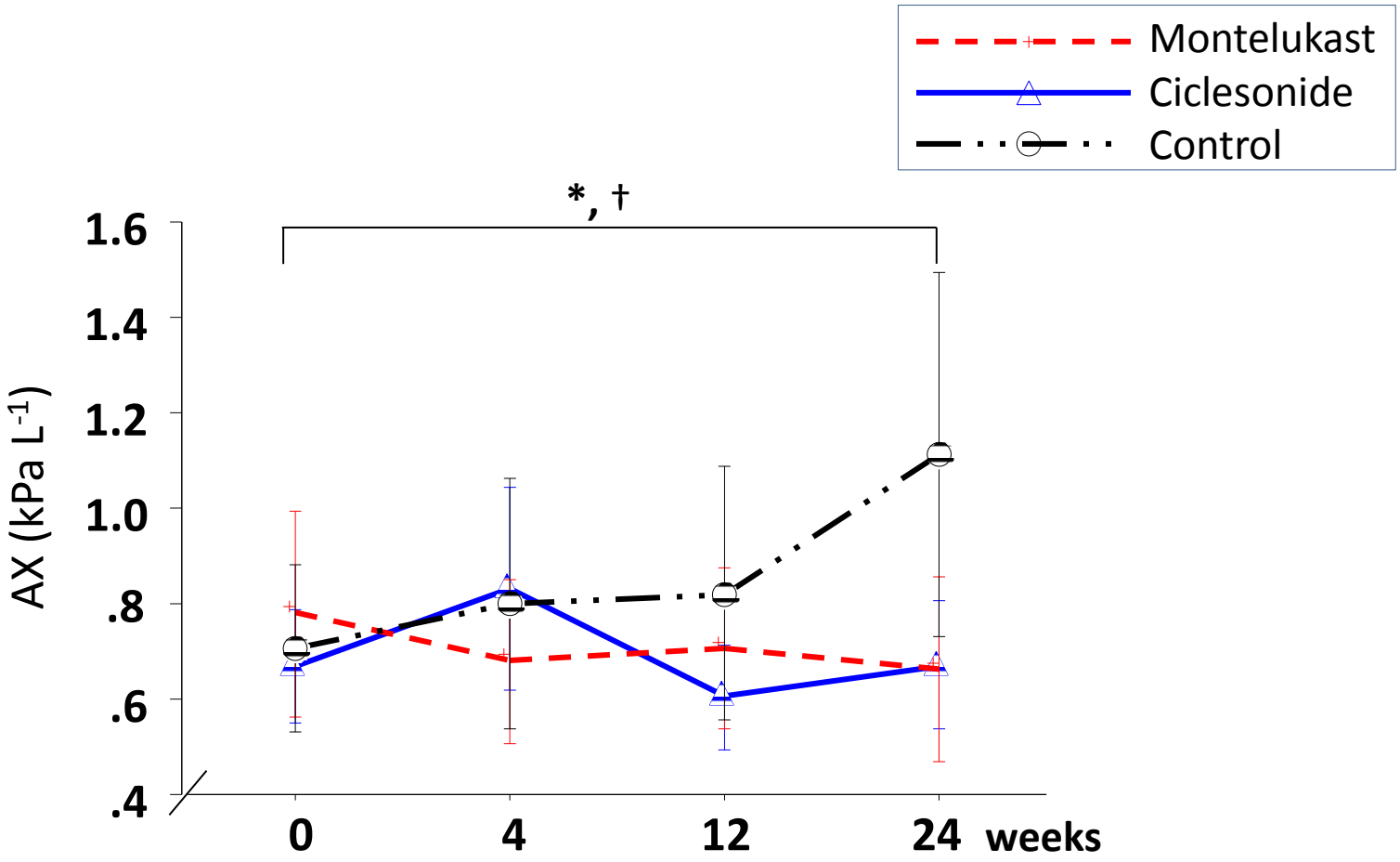

Montelukast
Ciclesonide
Control
*, †
AX (kPa L-1)
1.6
1.4
1.2
1.0
.8
.6
.4
0
4
12
24
weeks

Figure 4

Table 1. Characteristics of the study patients

	Ciclesonide group (n = 18)	Montelukast group (n = 19)	Control group (n = 15)
Female / male	13 / 5	13 / 6	9 / 6
Age, y	64.5 ± 9.9	61.8 ± 10.6	57.4 ± 21.1
Treatment Step 2/ 3/ 4/ 5[1)]	6 / 11 / 1 / 0	6 / 10 / 3 / 0	9 / 3 / 2 / 1
Smoking history (never / ex-smoker)	17 / 1	15 / 4	12 / 3
Atopy (yes / no) [2)]	10 / 8	12 / 7	7 / 8
Total IgE, IU/mL	120 (7-25000)	159 (8-1900)	86 (8-760)
Daily dose of ICS, μg [3)]	361 ± 263	353 ± 174	333 ± 222
Use of LABA (yes / no)	11 / 7	10 / 9	6 / 9
Use of theophylline (yes / no)	3 / 15	3 / 16	1 / 14
$FeNO_{50}$, ppb	42.4 ± 32.1	44.5 ± 36.4	37.5 ± 15.7
Alveolar nitric oxide concentrations, ppb	9.7 ± 5.6	8.4 ± 2.7	7.5 ± 1.6
Corrected alveolar nitric oxide concentrations, ppb	7.0 ± 5.5	5.7 ± 3.3	5.0 ± 2.0
FEV_1, % predicted	93.9 ± 17.5	93.7 ± 20.3	94.7 ± 23.8
FEV_1/FVC, %	74.7 ± 18.6	74.2 ± 18.2	73.6 ± 24.6
ΔN_2, %	1.8 ± 1.7	1.8 ± 1.7	2.1 ± 2.2
Rrs_5, kPa sL^{-1}	0.43 ± 0.15	0.40 ± 0.13	0.40 ± 0.14
Rrs_{20}, kPa sL^{-1}	0.35 ± 0.11	0.31 ± 0.09	0.33 ± 0.10
Rrs_5-Rrs_{20}, kPa sL^{-1}	0.08 ± 0.05	0.09 ± 0.07	0.07 ± 0.07
Xrs_5, kPa sL^{-1}	-0.14 ± 0.06	-0.14 ± 0.06	-0.14 ± 0.07
AX, kPa L^{-1}	0.67 ± 0.51	0.78 ± 0.91	0.71± 0.68
ACT score	22.7 ± 2.5	23.2 ± 2.3	23.2 ± 2.7
Rhinitis symptom score	16.9 ± 2.1	16.4 ± 2.1	17.2 ± 1.7
Blood eosinophils, %	5.3 ± 3.9	4.7 ± 2.9	4.0 ± 2.5
Serum ECP, μg/L	16.6 ± 17.6	11.4 ± 11.1	15.0 ± 15.5
Serum hsCRP, mg/dL	0.21 ± 0.37	0.10 ± 0.20	0.14 ± 0.17
Serum YKL-40, ng/dL	115.2 ± 86.0	123.2 ± 83.7	93.2 ± 116.4

Data are presented as number or mean ± SD, except for IgE, which is presented as median (range); $p>0.05$ for all characteristics according to the analysis of variance, the Kruskal-Wallis test or Fisher's exact test. 1) According to the 2006 Global Initiative for Asthma guidelines, 2) Atopy was determined based on the presence of specific serum IgE antibodies to at least 1 common inhalant allergen, including cat dander, dog dander, weed pollens, grass pollens, molds, or house dust mite, 3) equivalent to fluticasone proprionate

E-Supplement material

Effects of 24-week add-on treatment with ciclesonide and montelukast on small airways inflammation in asthma

*Hitoshi Nakaji[1,2], *Guergana Petrova[1], Hisako Matsumoto[1], Toshiyuki Iwata[1], Isao Ito[1], Tsuyoshi Oguma[1], Hideki Inoue[1], Tomoko Tajiri[1], Tadao Nagasaki[1], Yoshihiro Kanemitsu[1], Akio Niimi[1,3], Michiaki Mishima[1]

[1]Department of Respiratory Medicine, Graduate School of Medicine, Kyoto University, Kyoto, Japan

[2]Department of Respiratory Medicine, Wakayama Red Cross Hospital, Wakayama, Japan

[3]Division of Respiratory Medicine, Department of Medical Oncology and Immunology, Nagoya City University School of Medical Sciences, Nagoya, Japan

* HN and GP equally contributed to this study

Trial registration; Registry ID UMIN000001083

Corresponding author: Hisako Matsumoto, MD, PhD

Department of Respiratory Medicine

Postgraduate School of Medicine, Kyoto University

54 Kawahara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan

Telephone: +81-75-751-3830; Fax: +81-75-751-4643

E-mail: hmatsumo@kuhp.kyoto-u.ac.jp

e**Methods**

Adult patients with stable asthma who regularly visited our outpatient asthma clinic were enrolled from April 2008 to August 2011. Asthma was diagnosed according to American Thoracic Society criteria based on a history of recurrent episodes of wheezing and chest tightness, with or without cough, and documented airway reversibility with a bronchodilator or hyperresponsiveness to inhaled methacholine [e1].

Nitric oxide levels were determined with a chemiluminescence analyzer (NOA 280; Sievers, Boulder, Colorado) according to current guidelines and as previously described [e2]. The analyzer was daily calibrated with gas without nitric oxide and a standard concentration of 640 ppb nitric oxide. Lower detection limit for nitric oxide was 2 ppb. The concentrations were determined using a data analysis program (NOA Analysis™ Software; Sievers). Seated patients inserted a mouthpiece, inhaled orally to total lung capacity, exhaled immediately against a resistance and maintained mouth pressure at 20 cm H_2O, displayed on a pressure gauge. The steady-state nitric oxide plateau was taken as the fractional exhaled nitric oxide (FeNO) value. By varying expiratory resistances, we measured FeNO levels at 3 expiratory flows of 50, 100 and 200 mL/s in that order. Alveolar nitric oxide concentrations are provided as non-corrected[e3] and corrected values using trumpet-shaped model and axial back diffusion [e2, e4].

After nitric oxide measurements, patients underwent prebronchodilator and postbronchodilator (ie, inhalation of 200 μg of salbutamol) pulmonary function tests. Respiratory impedance was determined by impulse oscillometry system (IOS) followed by spirometric test and a nitrogen single-breath washout test. Forced vital capacity, forced expiratory volume in 1 second, and forced midexpiratory flow were determined using a ChestGraph HI-701 spirometer (Chest MI Corp., Tokyo, Japan). Spirograms were obtained in triplicate, and the best of 3 reproducible measurements was recorded, as recommended by the American Thoracic Society/European Respiratory Society [e5]. A nitrogen single-breath washout test was performed only before the inhalation of salbutamol to assess ventilation inhomogeneity by measuring the slope of phase 3 of the nitrogen washout curve.

570 Respiratory impedance was determined using a Jaeger MasterScreen, IOS (Erich Jaeger,
571 Hoechberg Germany), which met standard recommendations [e6]. In brief, rectangular mechanical
572 impulses containing a continuous power spectrum ranging from 0 to 100 Hz, generated by a
573 loudspeaker at intervals of 0.2 second, were applied to the respiratory system through a
574 mouthpiece during tidal breathing. The resulting pressure and flow signals were measured next
575 to the mouthpiece and were analyzed for amplitude and phase differences using a fast Fourier
576 transform to determine respiratory resistance (Rrs) and respiratory reactance (Xrs) of the total
577 respiratory system. To reduce loss of energy in the upper airways, the chin and cheeks were
578 supported by the patients' hands. As proxies for peripheral airway function, we used the negative
579 frequency dependence of Rrs between 5 and 20 Hz (Rrs5-Rrs20), Xrs at 5 Hz (Xrs5), and
580 reactance area (AX) that is the integral of Xrs from 5 Hz to the resonant frequency at which Xrs
581 crosses zero [e2,e7] .

582

eReferences

e1. 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;136:225-244.

e2. Matsumoto H, Niimi A, Jinnai M, et al. Association of alveolar nitric oxide levels with pulmonary function and its reversibility in stable asthma. Respiration. 2011; 81: 311-317.

e3. Tsoukias NM, George SC. A two-compartment model of pulmonary nitric oxide exchange dynamics. J Appl Physiol. 1998; 85: 653-666.

e4. Condorelli P, Shin HW, Aledia AS, Silkoff PE, George SC. A simple technique to characterize proximal and peripheral nitric oxide exchange using constant flow exhalations and an axial diffusion model. J Appl Physiol. 2007; 102: 417-425.

e5. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J. 2005; 26: 319-338.

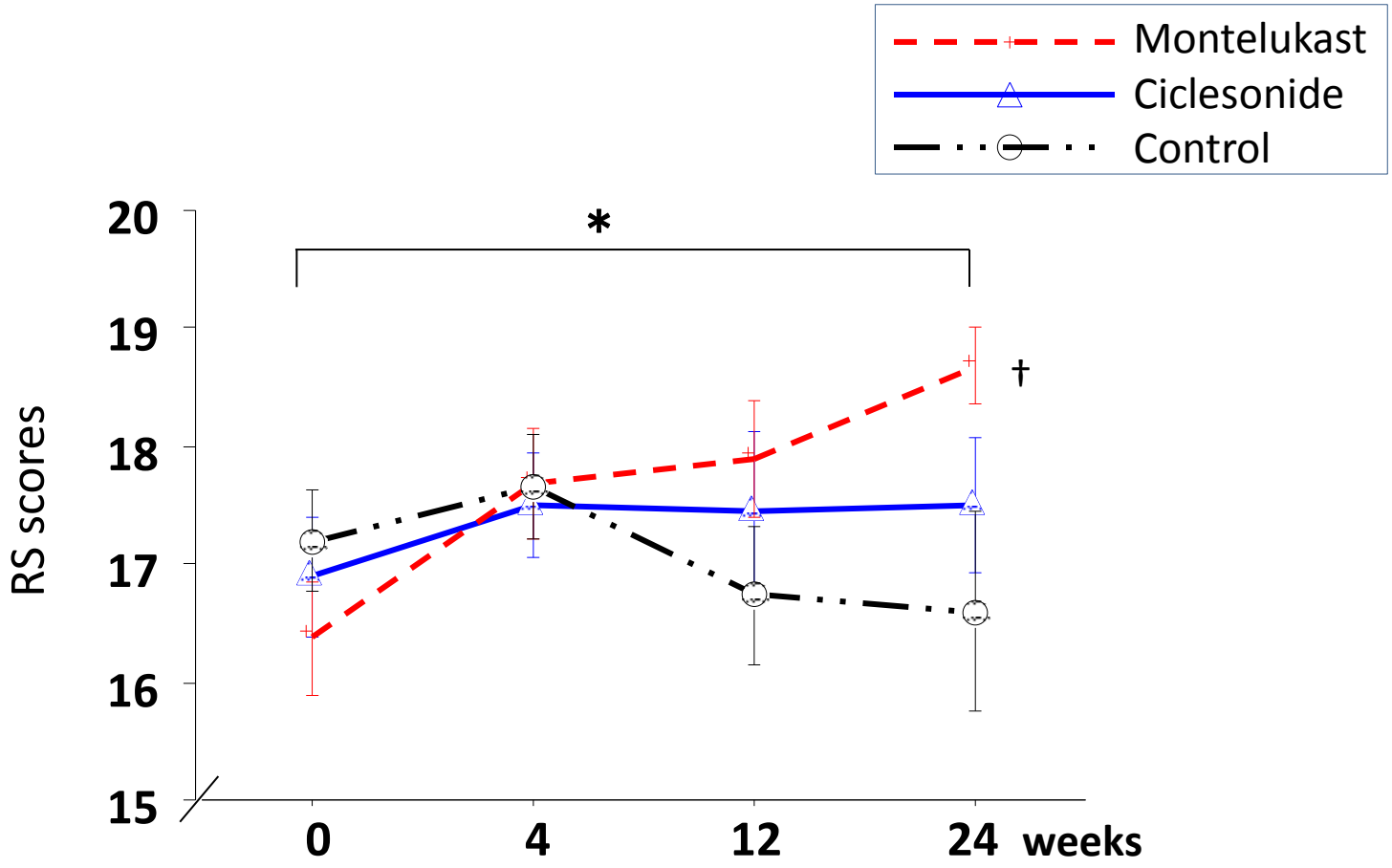
e6. Oostveen E, MacLeod D, Lorino H, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. Eur Respir J. 2003; 22: 1026-1041.

e7. Yamaguchi M, Niimi A, Ueda T, et al. Effect of inhaled corticosteroids on small airways in asthma: investigation using impulse oscillometry. Pulm Pharmacol Ther. 2009; 22: 326-332.

e8. Okubo K, Kurono Y, Fujieda S, et al. Japanese guideline for allergic rhinitis. Allergol Int. 2011;60:171-189.

eFigure legends

eFigure 1. Rhinitis symptom scores (RSS) in the 3 study groups. *Significant difference in the time trends for RSS among the 3 treatment modalities ($p = 0.004$, by 2-way analysis of variance [ANOVA]). †Significant changes in RSS in montelukast add-on group ($p < 0.001$ vs control group, by 2-way ANOVA) ($p < 0.001$, by 1-way ANOVA).


Montelukast
Ciclesonide
Control
RS scores
20
19
18
17
16
15
*
†
0
4
12
24
weeks

eFigure 1

e**Table 1.**

Rhinitis symptom scores [e8] (originally in Japanese)

A. Mean number of episodes of paroxysmal sneezing in a day

1. ≥ 21 times 2. 20-11 times 3. 10-6 times 4. 5-1 times 5. none

B. Mean number of episodes of nasal discharge a day

1. ≥ 21 times 2. 20-11 times 3. 10-6 times 4. 5-1 times 5. none

C. Nasal blockage

1. completely obstructed all day
2. severe nasal blockage causing prolonged oral breathing in a day
3. severe nasal blockage causing occasional oral breathing in a day
4. nasal blockage without oral breathing
5. not obstructed / no symptoms

D. Disturbance of daily activity (troubles with work, study, household work, sleep, going out, etc)

1. impossible
2. painful and complicating daily life
3. intermediate between 2) and 4)
4. few troubles
5. not disturbed at all

eTable 2. Asthma control test (ACT) scores and distribution of control status at baseline according to the treatment steps

		Treatment steps		
		2 and 3	4 and 5	p value
Ciclesonide	ACT scores	23.1 ± 1.9	16	NS
(n = 18)	total/good/no control (n)	7/10/0	0/0/1	<0.01
Montelukast	ACT scores	23.3 ± 2.4	22.3 ± 2.3	NS
(n = 19)	total/ good/no control (n)	7/7/2	1/2/0	NS
Control	ACT scores	23.7 ± 1.7	21.3 ± 5.5	NS
(n = 15)	total/good/no control (n)	5/7/0	1/1/1	NS

Data are presented as mean ± SD.

Control status is defined as total when ACT score was equal to 25 points, good when ACT score was 20 or higher, no control when ACT score was less than 20.

NS; no significant difference by Wilcoxon rank-sum test or χ^2 test.

eTable 3. Summary of the results

		Ciclesonide add-on	Montelukast add-on	Control
FeNO		NS	NS	NS
Alveolar nitric oxide concentrations	*vs* other groups	Significant decrease *vs* controls	NS	-
	within the treatment modality	Decreased	Decreased	NS
Corrected alveolar nitric oxide concentrations	within the treatment modality	Insignificantly decreased	NS	NS
AX	*vs* other groups	NS	Significant decrease *vs* controls	-
Blood eosinophils	within the treatment modality	NS	Decreased	NS
ACT	within the treatment modality	Improved	Insignificantly improved	NS

ACT: asthma control test

AX: reactance area at low frequencies

NS; no significant difference or no significant changes