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

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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.

R1. Abstract

Background: Eosinophilic inflammation of the small airways is a key process in asthma and often smoulders in treated patients. The long-term effects of add-on therapy on the persistent inflammation in the small airways remain unknown. We examined the effects of add-on therapy with either ciclesonide, an inhaled corticosteroid (ICS) with extra fine particles, or montelukast on small airway inflammation.

Methods: Sixty patients with stable asthma on ICS 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 three groups: ciclesonide (CicG; n = 19), montelukast (MG; n = 22) and no add-on as controls (CG; n = 19). At baseline and at weeks 4, 12 and 24, extended NO analysis, pulmonary function tests including impulse oscillometry, blood eosinophil counts and asthma control tests (ACT's) were performed.

Results: A total of 18 patients in CicG, 19 in MG and 15 in CG completed the study and were analysed ~~thereafter~~. Using repeated measures analysis of variance, CicG showed significant decrease in alveolar nitric oxide (CA_{NO}), significant improvement in ACT over time. MG showed significant decreases in CA_{NO} and blood eosinophil counts over time and a trend for improved ACT, whereas no such changes were observed in CG during the time course. CA_{NO} with CicG and reactance area at low frequencies with MG showed greater improvements over time compared with CG.

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.

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

R1 manuscript with highlight

Introduction

Asthma is a chronic inflammatory disease of the airways characterized by variable, recurring symptoms and reversible airflow obstruction. The immunohistopathological features include infiltration of eosinophils and lymphocytes, mast cell activation and epithelial cell injury. To date, pathological [1 2], physiological [3] and radiological 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 (CANO) [7, 8]. Small airway inflammation as assessed by CANO levels is increased in patients with refractory asthma [8], in those with nocturnal asthma [9] and is associated with disease severity [10, 11] and small airways dysfunction [11]. Of note, 20% of asthmatics show increased CANO levels despite treatments with inhaled corticosteroids (ICS) and long-acting β_2 agonists [12]. CANO levels can also predict a future risk of disease exacerbation [13]. These findings suggest that, even in apparently stable patients on ICS, additional treatment targeting the small airways may lead to reaching total asthma control.

Few studies have evaluated the changes in CANO levels 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 ICS [8] could decrease CANO levels. These results suggest that CANO may be resistant to a simple ICS dose-elevation. In steroid-naive patients, however, extra-fine particle hydrofluoroalkane–ciclesonide resulted in decreased CANO levels [15] and hydrofluoroalkane–beclomethasone propionate improved

peripheral airway dysfunction [16]. Collectively, extra-fine particle ICS is expected to decrease C_{ANO} levels when they are used as an add-on medication. Leukotriene receptor antagonists (LTRA) that is administered systemically is another medication that is supposed to decrease C_{ANO} levels. 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 add-on LTRA to ICS therapy for 3 [18] to 8 [19] weeks with regard to C_{ANO} levels were conflicting. 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 objective was to examine the effects of this add-on therapy on C_{ANO} levels and to compare its effects on small airways in stable asthmatics who had not been previously treated with extra-fine particle ICSs or LTRAs.

Methods

For full details see the E-Supplement material.

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 2-5 on ICS treatment according to the Global Initiative for Asthma guidelines [21]. These patients had no exacerbations 3 months prior to enrolment, had C_{ANO} levels $\geqq$ 5.0 ppb and were either never-smokers or ex-smokers who had smoked less than 5 pack-years and had stopped more than 1 year before. The threshold level for uncorrected C_{ANO} was set at 5.0 ppb; this value was the average minus 1 SD of uncorrected C_{ANO} levels of 70 asthmatics on ICS in our previous study [22].

Exclusion criteria were current or previous use of extra-fine particle ICSs or LTRAs. Patients were also excluded if, during the study period, any side 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 ID 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

three treatment groups: inhaled ciclesonide 400 µg once daily add-on (CicG), montelukast 10 mg once daily add-on (MG) and control group who were on current medication only (CG). At week 0 (baseline), and at weeks 4, 12 and 24 (end of study period) the patients underwent extended NO 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. RSS was determined based on the Japanese Guideline for Allergic Rhinitis (Best score: 20) [24] (eTable 1).

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

NO levels were determined with a chemiluminescence analyzer (NOA 280; Sievers, Boulder, Colo., USA) according to current guidelines and as previously described C_{ANO} levels are provided as non-corrected one [7] and $C_{ANO_{corrected}}$ - corrected value using a trumpet shaped model with axial back diffusion [14].

After NO measurements, subjects underwent pre- and post-bronchodilator (i.e. inhalation of 200 µg of salbutamol) PFT's.

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 [16, 22]

Statistical analysis

For sample size determinations, we originally sought to enrol 90 patients based on previous findings [15, 17, 19]. However, as described in Results, we decided to stop patient enrolment at 60 due to the more frequent occurrence of exacerbations in CG, although these were mild. Statistical analysis used JMP 6.00 (SAS Institute Inc., Cary, N.C., USA) on a "per-protocol" basis. For non-normally distributed results, comparisons were made by Kruskal–Wallis test, Fisher's exact test or Wilcoxon signed-rank test as appropriate. For normally distributed results, comparisons were made by analysis of variance (ANOVA) and paired t-test. Two-way repeated-measures ANOVA (two-way ANOVA) was used to assess the variations among the three treatment modalities and at different time points. For cases with unequal variations in the treatment modalities, only one-way repeated measures ANOVA (one-way ANOVA) within one treatment group was used. For correlation analysis, the Spearman's rank-correlation test was used. Data are expressed as mean ± SD. P-values of ≤ 0.05 were considered statistically significant.

Results

Enrolment, drop out and exacerbation rates and baseline characteristics

Sixty asthmatic patients were enrolled in this study and randomly assigned to the groups: 19 in CicG, 22 in MG and 19 in CG (Fig 1).The reasons for patient drop-out were as follows: in CicG, one patient had a possible side effect (urticaria); in MG, three patients had possible side effects (two experienced mild gastro-intestinal discomfort and they preferred to discontinue the medication and one patient had mildly elevated transaminase levels); in CG, three had mild asthma exacerbations and they preferred to intensify medications and one patient discontinued ICS treatment following a general practitioner's advice. As a result 18 patients in CicG, 19 in MG and 15 in CG 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 HN and HM on each visit by checking the residual number of medications.

When the exacerbation frequencies were compared between the 19 patients in CG 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, CG had a significantly higher rate of exacerbation ($p = 0.028$; by Fisher's exact test). The baseline patient characteristics, ICS doses and biomarkers, including FeNO and CANO, were not significantly different between the 3 patients who later experienced mild exacerbations and the other 57 patients.

Asthma control test (ACT) scores and rhinitis symptom scores (RSS)

By one-way ANOVA, there was a significant improvement in ACT scores during the treatment period within CicG ($p = 0.024$; Fig 2) and there was a trend for improvement within MG ($p =$

0.076). When sub-scores for the ACT components were separately analysed in CicG, sub-scores 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 two-way ANOVA for the ACT results. 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 RSS differed. However, there was a significant difference in the time trends for RSS among the three treatment modalities ($p = 0.004$; eFig 1); in particular, using two-way ANOVA, there were significant differences for the symptom of nasal obstruction ($p = 0.046$). When comparing two different treatment modalities in a post-hoc analysis, MG exhibited a significantly better time trend for RSS than CG ($p = 0.0008$) and a trend for better scores than CicG ($p = 0.07$; eFig 1). A significant increase in RSS over time was found only in the MG ($p = 0.0001$, by one-way ANOVA).

There were no associations between changes in ACT or RSS from baseline to the end of the treatment period and changes in CANO or $CANO_{corrected}$ levels in either treatment group.

Nitric oxide results

There were no significant differences in the time trends for fractional exhaled nitric oxide (FeNO) at an expiratory flow rate of 50 ml/s among the three treatment modalities or within each of the groups (results not shown).

The time trends for uncorrected CANO levels were significantly different among the three treatment groups ($p = 0.048$, by two-way ANOVA). When comparing two different treatment

modalities in a post-hoc analysis, CicG showed a greater decrease in C_{ANO} levels over time than CG ($p = 0.027$, by two-way ANOVA). By one-way ANOVA, C_{ANO} levels in CG did not change during the time course, whereas in both of the add-on treatment groups, C_{ANO} levels significantly decreased over time ($p = 0.014$ for CicG and $p = 0.012$ for MG groups; Fig 3).

For $C_{ANO_{corrected}}$ levels, one-way ANOVA showed that there was an insignificant decrease over time in CicG ($p = 0.06$).

Pulmonary function tests

None of the spirometry indices, ΔN_2, or IOS indices of Rrs_5, Rrs_{20} or Xrs_5, showed any difference among the three treatment modalities during the treatment period regardless of pre- or post-bronchodilator conditions. No significant changes were observed within any of the three groups (data not shown).

There was a significant difference in the time trends for the reactance area (AX) among the three treatment modalities ($p = 0.038$, by two-way ANOVA). AX levels in MG were improved over time when compared with CG ($p = 0.05$, by two-way ANOVA; Fig 4). For Rrs_5–Rrs_{20}, two-way ANOVA was not used because of the unequal variations among the three treatment modalities; however, one-way ANOVA showed that there was a trend for a change over time in CicG ($p = 0.09$).

Although there were associations between $C_{ANO_{corrected}}$ levels and IOS indices of AX or Rrs_5-Rrs_{20} at baseline ($r = 0.30$, $p < 0.05$ for both, $n = 52$), there were no associations between changes in pulmonary function data from baseline to the end of the treatment period and changes in C_{ANO} or $C_{ANO_{corrected}}$ levels 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 ECP, hsCRP and YKL-40. There were no significant changes in these tests results between the beginning and the end of the treatment period, except for MG in which the eosinophil counts significantly declined after treatment (2.9 ± 2.2% at 24 weeks)($p = 0.016$, 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 extra-fine particle ICS or montelukast in steroid-treated stable asthma patients. Ciclesonide may have attenuated smouldering 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 smouldering inflammation of the small airways.

C_{ANO} is an established marker of small airway inflammation and is correlated with eosinophil counts in bronchoalveolar lavage fluid [8]. In CicG, C_{ANO} levels significantly decreased over time when compared with CG as well as in CicG intragroup analysis. Our data confirmed earlier findings for the effects of 5-weeks treatment with ciclesonide on C_{ANO} in steroid naïve patients [15] and reinforced the advantage of extra-fine particle ICS to treat smouldering inflammation of the small airways, even in patients already on ICS. 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 levels did not change over time. Taken the results of previous short term study and current study together, ciclesonide would be capable of treating the small airways potentially due to its particles size that was sufficiently small to reach the peripheral airways.

In contrast to uncorrected C_{ANO} levels, $C_{ANO\ corrected}$ levels only showed a trend toward being decreased in CicG ($p = 0.06$, one-way ANOVA). Although $C_{ANO_{corrected}}$ levels reflect airway dysfunction [22, 29], as does C_{ANO}, $C_{ANO_{corrected}}$ does not reflect disease severity [14, 22] or

asthma control status [29]. It is also not increased during asthma exacerbations in adults [30], which is in contrast to several lines of evidence for CANO. Although CANO is contaminated with bronchial NO, potentially from small conducting airways where diffusion begins to replace bulk flow, our findings on CANO imply that relatively small airways, albeit not actual peripheral airways, are still important in the management of asthma.

Studies of add-on medication using LTRA that have evaluated changes in CANO levels 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 CANO levels after montelukast add-on. However, these earlier studies were relatively short-term with treatment periods of only 3–4 weeks. Yasui et al. investigated pranlukast use in stable asthmatics and found significant decreases in both corrected and uncorrected CANO levels after 8 weeks cross-over of add-on therapy with pranlukast [19]. In agreement with that study, we found that CANO levels in MG decreased during the 24 week add-on period, although these levels were not significantly different from CG. As with CicG, FeNO at 50 ml/s levels did not change over time. These findings indicate that add-on treatment with LTRA for longer than 8 weeks suppresses the remnant inflammation in the small airways. In addition, our intervention study that covered the two seasons for allergic rhinitis (spring and autumn) provided additional evidence for the established benefit of montelukast on allergic rhinitis [31] and justified a role for LTRA in the therapy for stable asthmatics with concomitant allergic rhinitis, even those with minimal symptoms.

Symptoms and airway obstruction are integral to the definition of asthma, and represent important components for assessing asthma control both in clinical practice and clinical trials.

Therefore, one of the end-points in our study was ACT scores. Despite the disadvantage in compliance for inhalation use, as reported previously on adherence to LTRA of 67.7% vs. 33.8% for ICS [32], ACT scores significantly improved over time in CicG. In addition, there was a marginal improvement in the sub-score of ACT question 3 concerning nocturnal symptoms in CicG. To date, a number of studies have confirmed that eosinophilic inflammation worsens in patients with nocturnal asthma, particularly in the peripheral airways [33]. Lehtimaki et al. showed that nocturnal symptoms in asthmatic patients were related to higher CANO levels [9]. These results are in accordance with our results showing that ciclesonide add-on treatment reduced inflammation in the small airways, as assessed by CANO levels and improved nocturnal symptoms, as assessed by ACT sub-scores. Care must be taken when interpreting these findings, however, because the minimally important difference in ACT that reflects a clinically meaningful change is considered to be 3 points [34], and the increase in ACT composite scores in our CicG did not achieve this. Despite this minimal change, these statistically significant changes would still favour add-on therapy for seemingly stable asthma patients.

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 since the small airways are pathways of very low resistance and only contribute to about 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 CANO were not reflected in the airway function. However, in MG, AX, the reactance area at low frequencies that is sensitive and sufficiently accurate to determine small airway dysfunction [16, 22], did significantly decrease over

time when compared with CG, as was found in our previous intervention study in steroid naïve patients [16]. Montelukast may have reversed remodelling 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 two different inhalers for corticosteroids, although we achieved good adherence in CicG. 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 CANO levels < 5 ppb, given that some patients who have high FeNO and low CANO levels exhibit paradoxical increases in CANO levels after treatment [38], possibly due to dilatation of constricted small airways from terminal to respiratory bronchioles. However, by setting this threshold for CANO levels during patient enrolment, the changes of CANO in this study could be simply interpreted.

Finally, from the ethical standpoint, we stopped enrolment at 60 patients due to a higher, exacerbation rate in the control group, albeit they were mild, which was consistent with the finding that elevated CANO 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 CANO 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 CANO and any other clinical

indices because of their potentially large variations during the treatment period albeit C_{ANO}, pulmonary function, and ACT were intuitively thought to behave in parallel. Despite these limitations, the current findings of decrease in C_{ANO} with add-on treatment are sufficient to be used as a future reference when intensifying treatment with extra-fine particle ICS or LTRA add-on, even in seemingly stable asthma patients on ICS treatment who still have evidence of small airways inflammation as assessed by C_{ANO} levels.

We conclude that ciclesonide and montelukast may act differently, but that both separately can improve small airway abnormalities (eTable 3). By co-administration 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 in order to reach the ultimate asthma treatment goal: ideal control.

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Figure legends

Figure 1. Registration and randomization

Figure 2. Asthma control test (ACT) scores in the three groups. *Significant changes in ACT scores within the ciclesonide add-on group ($p = 0.024$, by one-way ANOVA).

Figure 3. CANO levels in the three groups. *Significant difference in the time trends for CANO levels among the three treatment modalities ($p = 0.048$, by two-way ANOVA). †Significant changes in CANO levels in ciclesonide add-on group ($p = 0.027$ vs. control group, by two-way ANOVA) ($p = 0.014$, by one-way ANOVA). ‡Significant changes within montelukast add-on group ($p = 0.012$, by one-way ANOVA).

Figure 4. AX levels in the three groups. *Significant difference in the time trends for AX levels among the three treatment modalities ($p = 0.038$, by two-way ANOVA), †post-hoc analysis between montelukast add-on and control groups ($p = 0.05$, by two-way ANOVA).

Table 1. Patients' Characteristics

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

Data are presented in mean ± SD, except for IgE, median (range); by analysis of variance, the Kruskal-Wallis test or Fisher's exact test

1) According to GINA2006, 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

ICS; inhaled corticosteroids, LABA; long-acting β_2 agonist, hsCRP; high sensitivity CRP

E-Supplement material

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

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Trial registration; Registry ID UMIN000001083

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eMethods

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].

NO levels were determined with a chemiluminescence analyzer (NOA 280; Sievers, Boulder, Colo., USA) according to current guidelines and as previously described [e2]. The analyzer was daily calibrated with gas without NO and a standard concentration of 640 ppb NO. Lower detection limit for NO was 2 ppb. The concentrations were determined using a data analysis program (NOA Analysis™ Software; Sievers). Seated subjects 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 NO plateau was taken as the FeNO value. By varying expiratory resistances, we measured FeNO levels at three expiratory flows of 50, 100 and 200 ml/s in that order. C_{ANO} levels are provided as non-corrected one[e3], and $C_{ANO_{corrected}}$ - corrected value using trumpet shaped model and axial back diffusion [e2, e4].

After NO measurements, subjects underwent pre- and post-bronchodilator (i.e., inhalation of 200 μg salbutamol) PFT's. Respiratory impedance was determined by IOS followed by spirometric test and a nitrogen single-breath washout test. FVC, FEV_1 and forced mid-expiratory flow ($FEF_{25-75\%}$) 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 (ΔN2).

Respiratory impedance was determined using a Jaeger MasterScreen, IOS™ (Erich Jaeger, Hoechberg Germany) that met standard recommendations [e6]. In brief, rectangular mechanical impulses containing a continuous power spectrum ranging from 0 to 100 Hz, generated by a loudspeaker at intervals of 0.2 s, were applied to the respiratory system through a

mouthpiece during tidal breathing. The resulting pressure and flow signals were measured next to the mouthpiece, and were analyzed for amplitude and phase differences using a fast Fourier transform to determine resistance (Rrs) and reactance (Xrs) of the total respiratory system. To reduce loss of energy in the upper airways, the chin and cheeks were supported by the subjects' hands. As proxies for peripheral airway function, we used the negative frequency dependence of Rrs between 5 and 20 Hz (Rrs5-Rrs20), Xrs at 5 Hz (Xrs5), and reactance area (AX) that is the integral of Xrs from 5 Hz to the resonant frequency at which Xrs crosses zero [e2,e7] .

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eFigure legends

eFigure 1. Rhinitis symptom scores (RSS) in the three groups. *Significant difference in the time trends for RSS among the three treatment modalities (p = 0.004, by two-way ANOVA). †Significant changes in RSS in montelukast add-on group (p = 0.0008 vs. control group, by two-way ANOVA) (p = 0.0001, by one-way ANOVA).

eTable 1.

Rhinitis symptom score [e8] (originally in Japanese)

A. Average 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. Average 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. 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 (n = 18)	ACT scores	23.1 ± 1.9	16	NS
	total/good/no control (n)	7/10/0	0/0/1	<0.01
Montelukast (n = 19)	ACT scores	23.3 ± 2.4	22.3 ± 2.3	NS
	total/ good/no control (n)	7/7/2	1/2/0	NS
Control (n = 15)	ACT scores	23.7 ± 1.7	21.3 ± 5.5	NS
	total/good/no control (n)	5/7/0	1/1/1	NS

Data are presented in mean ± SD.

Control status is defined as total when ACT = 25 points, good when ACT ≥ 20, no control when ACT <20

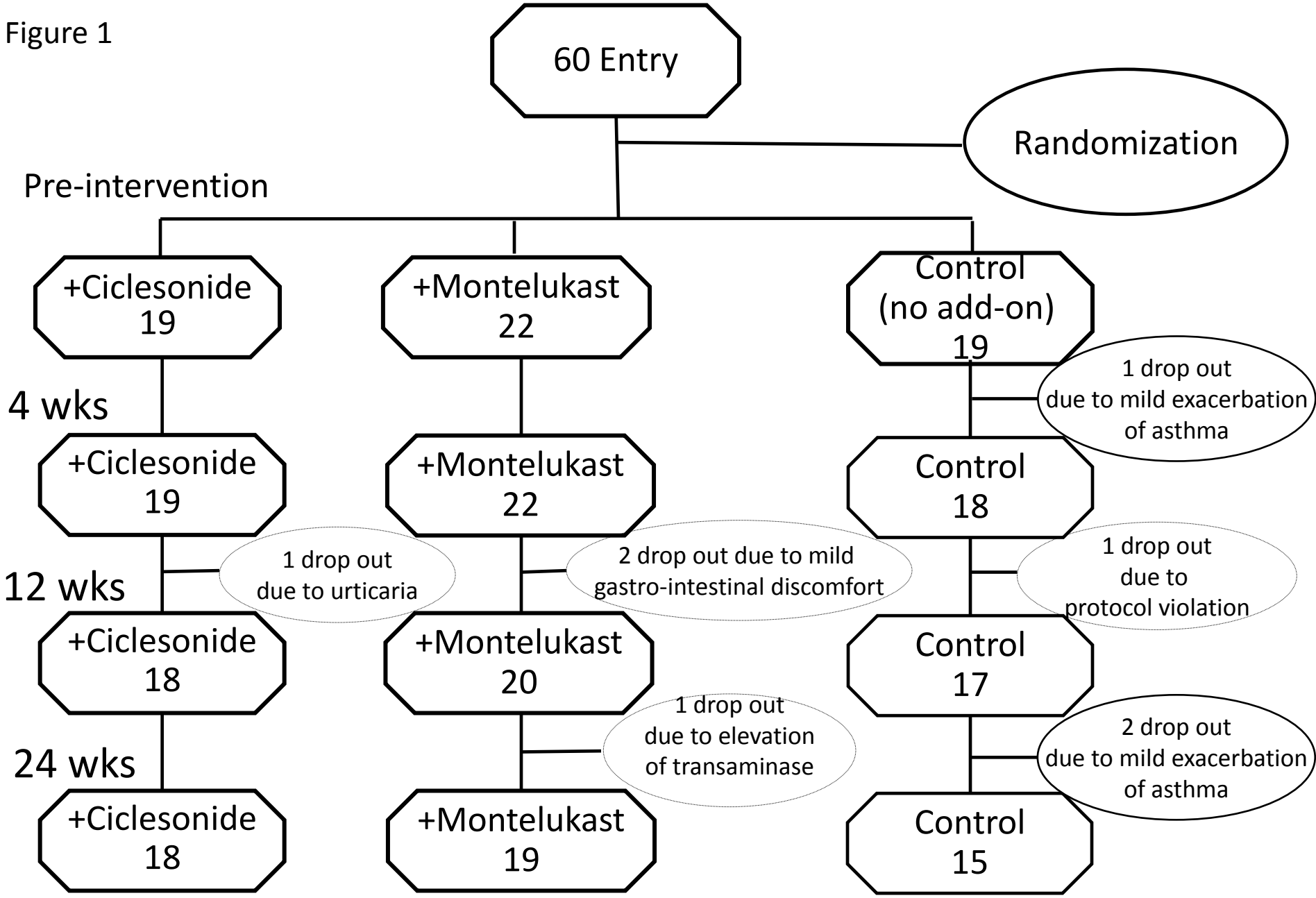
NS; no significant difference by Wilcoxon rank-sum test or χ^2 test.

eTable 3. Summary of the results

		Ciclesonide add-on	Montelukast add-on	CG
FeNO		NS	NS	NS
CANO	*vs* other groups	Significant decrease *vs* CG	NS	-
	within the treatment modality	Decreased	Decreased	NS
$CANO_{corrected}$	within the treatment modality	Insignificantly decreased	NS	NS
AX	*vs* other groups	NS	Significant decrease *vs* CG	-
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
CG: control group (no add-on)
NS; no significant difference or no significant changes

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

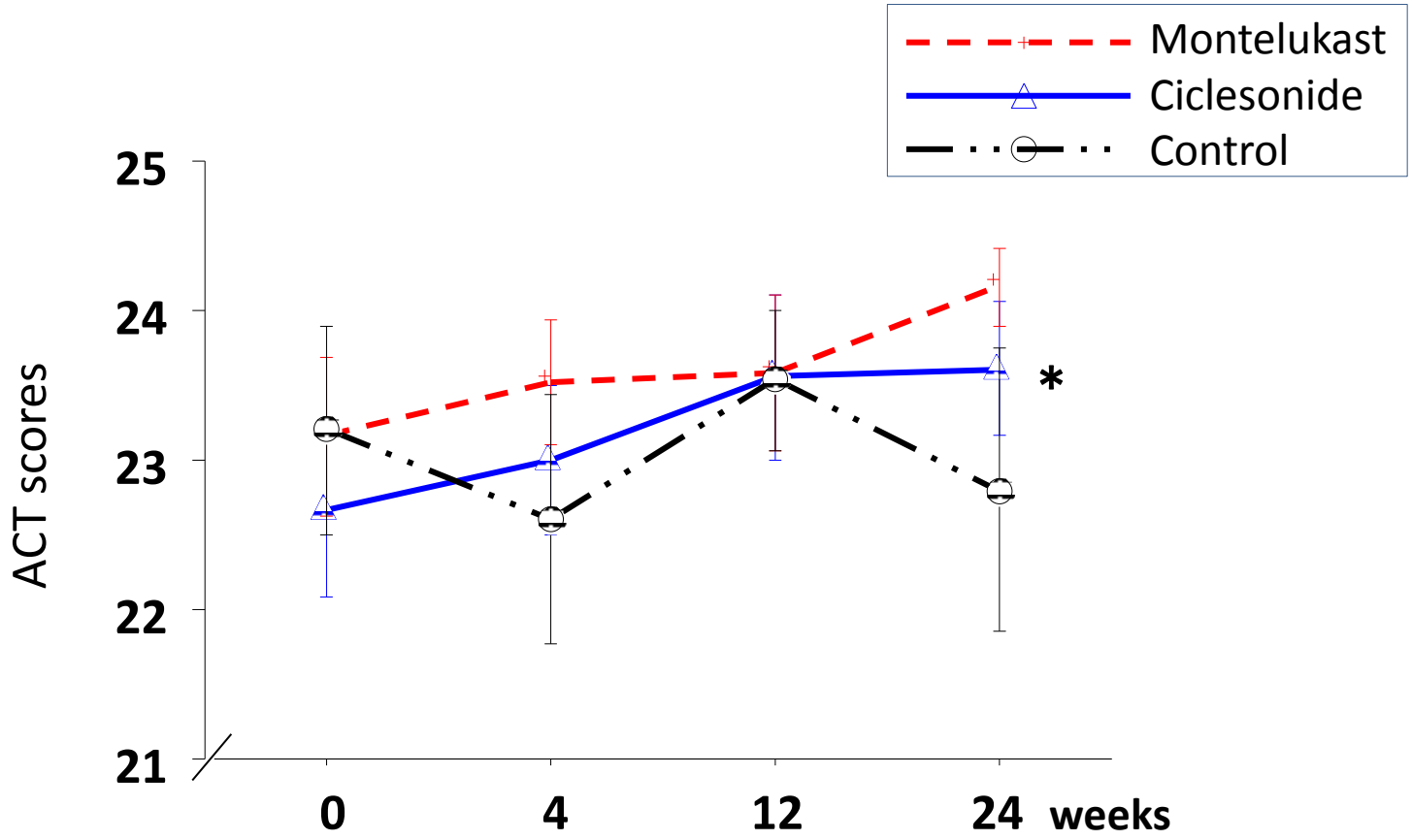

Montelukast
Ciclesonide
Control
ACT scores
25
24
23
22
21
0
4
12
24
weeks
*

Figure 2

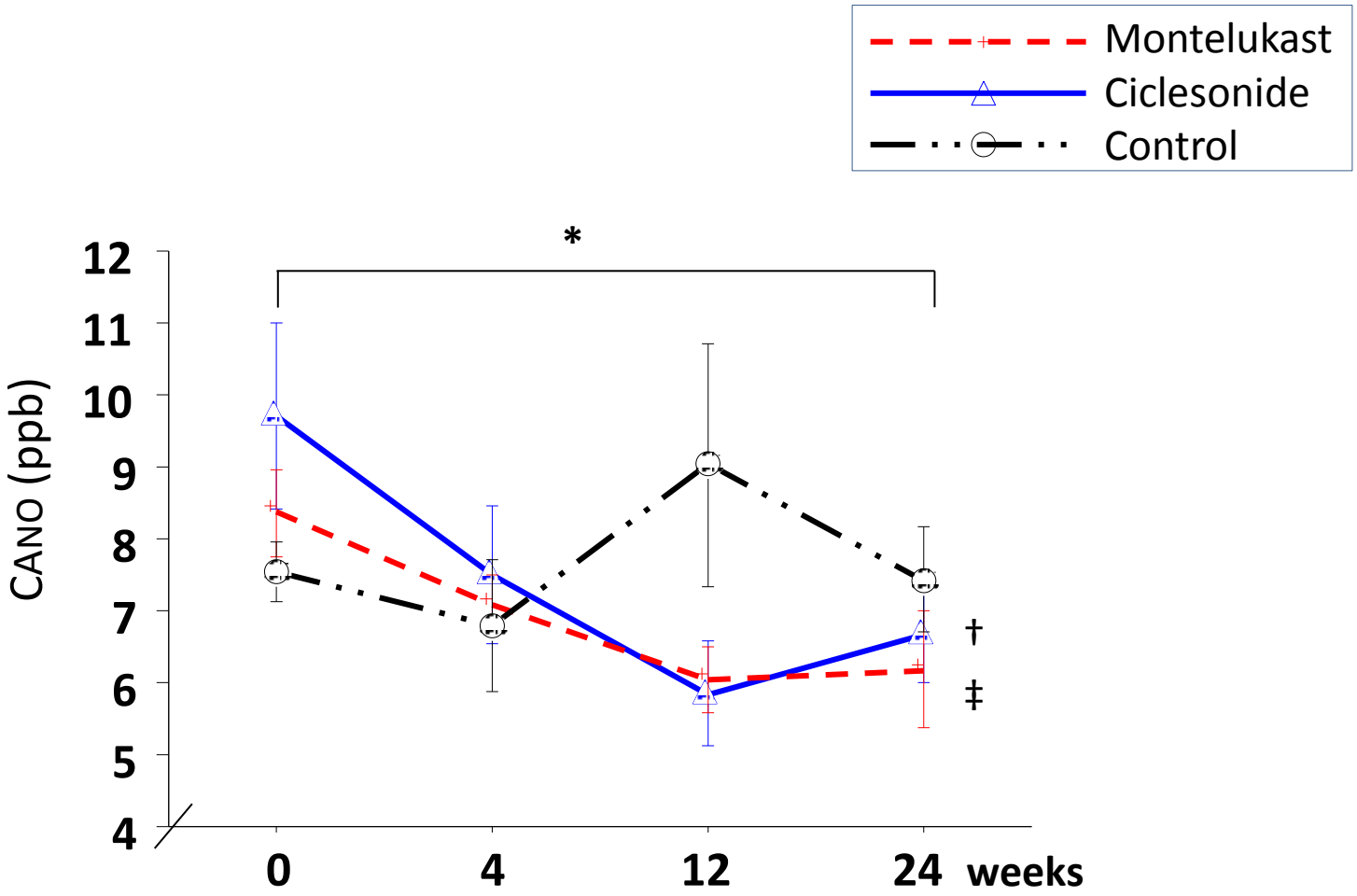

Montelukast
Ciclesonide
Control
*
12
11
10
9
8
7
6
5
4
CANO (ppb)
0
4
12
24
weeks
†
‡

Figure 3

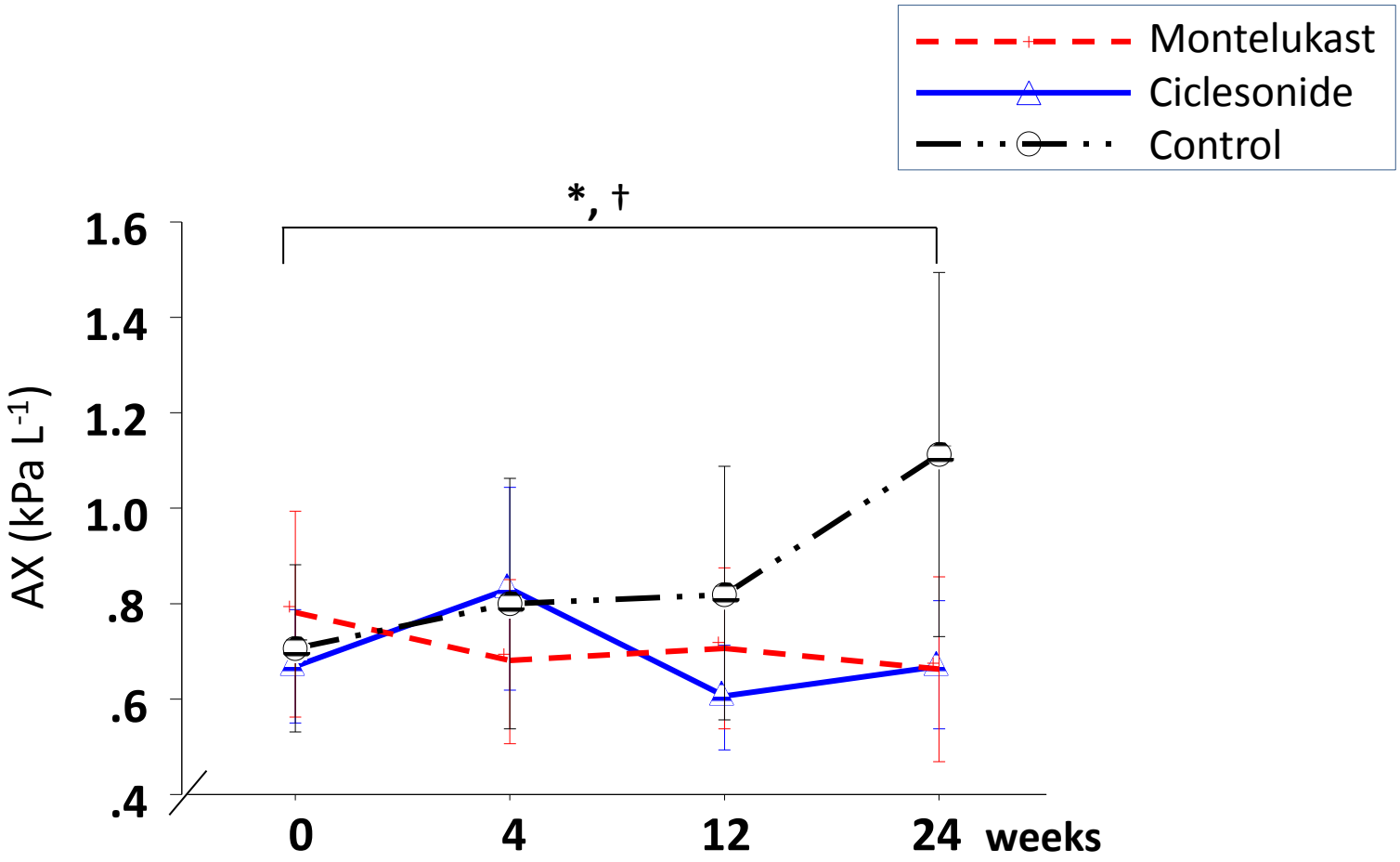
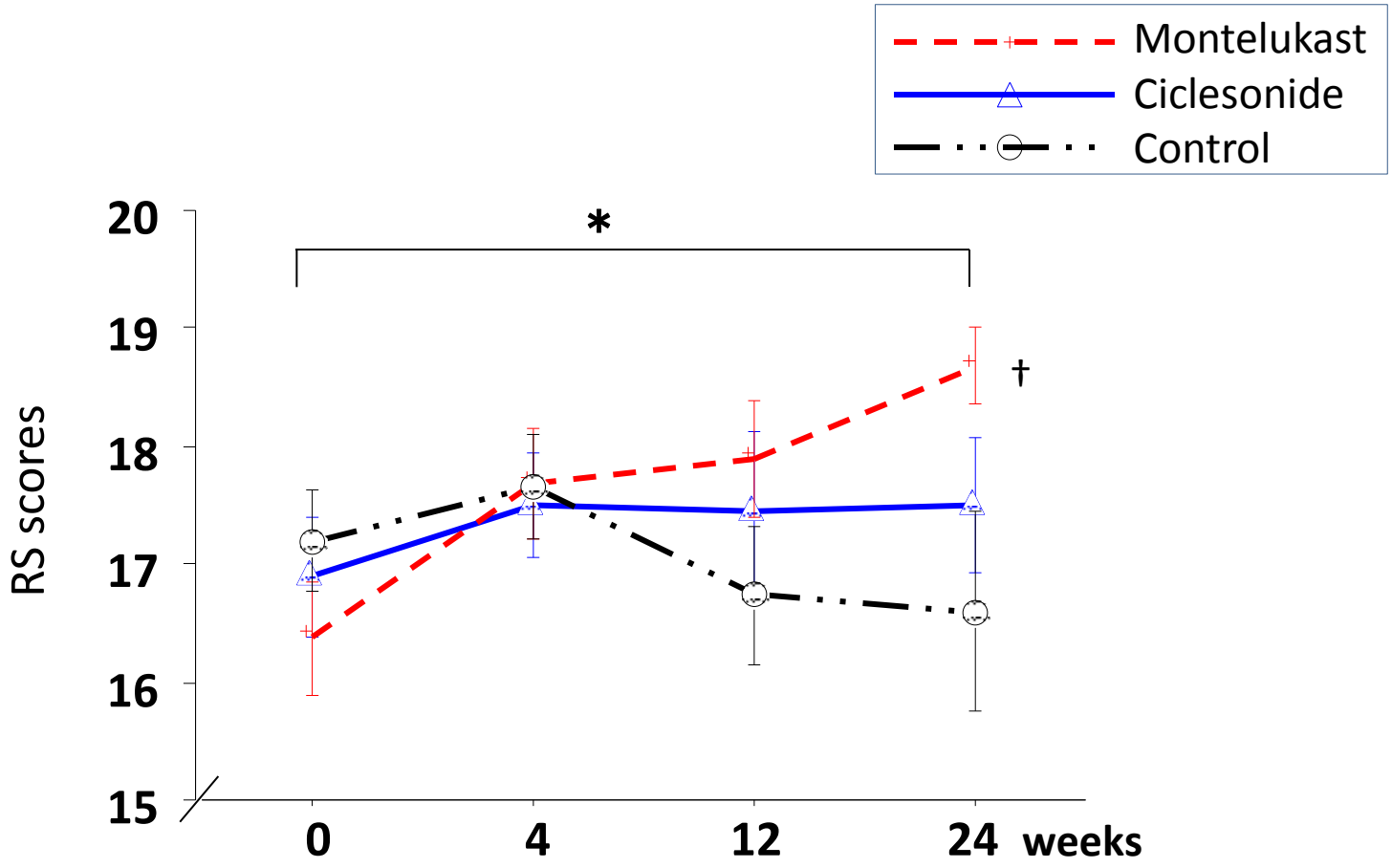

Montelukast
Ciclesonide
Control
*, †
AX (kPa L-1)
1.6
1.4
1.2
1.0
.8
.6
.4
0
4
12
24
weeks

Figure 4


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

eFigure 1