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PARTICLE SIZE MATTERS: DIAGNOSTICS AND TREATMENT OF SMALL AIRWAYS INVOLVEMENT IN ASTHMA

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ABSTRACT

Aim

Small airways are an important site of inflammation and obstruction in asthma, which contributes to the severity of airway hyperresponsiveness (AHR) that is usually measured by nebulisation of large-particle stimuli. We investigated whether small and large particle sizes of aerosolized adenosine-‘5-monophospate (AMP) provide similar severity of AHR. Additionally, effects of small-particle ICS ciclesonide and large-particle ICS fluticasone on AHR to large- and small-particle size AMP were assessed.

Methods

After a 4-week run-in period using open-label fluticasone 100µg b.i.d., 37 mild-to-moderate asthmatics underwent provocations with standard size (3.7 micron), large-particle (9.9 micron) and small-particle (1.06 micron) AMP. Subjects received 4-week ciclesonide 160µg s.i.d. or fluticasone 100µg b.i.d. (double-blind, double-dummy) followed by large- and small-particle AMP provocation.

Results

Small-particle AMP induced a 20% fall in FEV_1 (PC_{20}) at a significantly higher dose than large-particle AMP. Ciclesonide and fluticasone had comparable effects on AMP provocations. Not all subjects reached a PC_{20} at the highest AMP dose. In those who did, ciclesonide improved small-particle PC_{20}AMP by 1.74 doubling doses (DD) (p=0.03), whereas fluticasone did not. Conversely, fluticasone improved large-particle PC_{20}AMP significantly (1.32DD, p=0.03), whereas ciclesonide did not.

Conclusions

Small-particle AMP provocation appears a promising tool to assess changes in small airways inflammation. Future adjustments are necessary taking into account the very small-particle size used, with large exhaled fractions. In asthmatics reaching a PC_{20} with small- and large-particle AMP provocations, ciclesonide improves hyperresponsiveness with small-particle size AMP, and fluticasone with large-particle size. This warrants further research to target provocations and treatment to specific airway sizes.

250 words

INTRODUCTION

Asthma is an inflammatory disease affecting large, intermediate and small airways [1,2]. In the past, involvement of the small airways, i.e. airways with an internal diameter < 2 mm, could poorly be assessed due to limitations of *in vivo* sampling techniques and lack of specificity of physiologic measurements to assess peripheral airway obstruction. However, analyses of post-mortem and resected lung tissue samples have demonstrated that considerable small airway disease exists in asthma [3,4]. More recently, advances in less invasive techniques such as measurement of alveolar and bronchial exhaled nitric oxide (eNO), multibreath washout technique [5] and volumetric measurement of air trapping have confirmed the important role of small airway inflammation in mild-to-moderate asthma [6,7].

Since small airways are an important therapeutic target, novel formulations of inhaled corticosteroids (ICS) have been developed. These newer formulations use hydrofluoroalkane (HFA) propellants, and some yield smaller particle sizes that allow better access to the distal airways. Imaging studies suggest that these new formulations produce beneficial changes in the distal airways as well [6,8]. For example, the HFA formulation of BDP enabled effective asthma control at much lower doses than CFC-BDP [9]. Ciclesonide is a novel small-particle inhaled corticosteroid (ICS), which has shown clinical efficacy in asthma in low doses [10-13].

In both clinical work-up and research settings, methacholine challenge tests are being used to quantify airway hyperresponsiveness, a core feature of asthma. Another provocative stimulus, adenosine-‘5-monophosphate (AMP), better reflects eosinophilic airway inflammation than methacholine [14]. Research groups have managed to assess small airway changes by means of pulmonary function tests and high-resolution computed tomography after methacholine bronchoprovocation [6,7,15]. However, it remains unclear whether methacholine has actually reached the small airways to cause these effects. Deposition in the small airways of inhaled particles is dependent on the inhalation maneuver and particle size which is a.o. determined by the type and output rate of the nebulizer. For methacholine provocation tests, the DeVilbiss 646 is the nebulizer of recommendation [16]. This nebulizer can yield variable particle sizes depending mainly on its output flow rate [17] and at a flow rate of 12L/min, methacholine is aerosolized to particles of 3.7 microns [18]. Particles of 5-7 microns in mass mean aerodynamic diameter (MMAD) as produced by an ultrasonic nebulizer do not reach the lower airways in contrast to particles of 2-3 microns in MMAD produced by a jet nebulizer [19]. Theoretically, nebulizers producing particles that may reach smaller airways can be used in bronchoprovocation tests, which then may reflect the hyperresponsiveness of the small airways in asthma.

The aim of the present study was twofold. First, we evaluated the use of small-particle and large-particle AMP bronchoprovocation tests as markers of small and large airways involvement in asthma. Second, we assessed the differential effects of small-particle versus large-particle ICS on the outcome of these bronchoprovocation tests. For small-particle ICS we used ciclesonide and for large-particle ICS we used fluticasone.

MATERIALS AND METHODS

Study design

This study was performed in a double-blind, double-dummy, randomized parallel-group design, and was registered in a public database prior to execution (clinicaltrials.gov identifier NCT00306163). Subjects were randomized for treatment with large particles (fluticasone) or small particles inhaled corticosteroids (ciclesonide). The flow chart of the study is presented in Figure 1.

Subjects

Subjects were recruited from the outpatient clinics of the Departments of Pulmonology of hospitals in the Northern provinces in the Netherlands, and with advertisements in local papers. The local Medical Ethics Committee approved the study protocol, and all subjects gave written informed consent. Subjects between 18 and 60 years of age were included with a history of asthma according to GINA criteria [20], either steroid-naïve or using ICS ≤ 500 µg fluticasone propionate or equivalent, a prebronchodilator FEV_1 > 1.20 L, and atopy, i.e. at least 1 positive skin prick test to 18 common aero-allergens.

Current or ex-smokers who quit smoking < 1 year prior to the study and those with ≥10 packyears were excluded, as were those with a history of COPD or other diseases likely interfering with the study, unstable asthma (>3 exacerbations in the past year or 1 exacerbation in the past 2 months), concomitant medication that was not allowed (e.g. oral corticosteroids < 4 wks prior to participation), intolerance for short-acting ß$_2$-agonists (SABA), and females being pregnant or lactating or lacking adequate methods of contraception. Subjects were excluded during study participation if their asthma became unstable.

Bronchoprovocation tests with aerosolized AMP

Standard AMP test

The standard AMP test was performed conform the ATS protocol (2-minute tidal breathing protocol), using a DeVilbiss 646 nebulizer with a calibrated output rate of 0.13mL/min [16]. The MMAD (range) with this method is: 3.9 (3.1-5.5) microns at an inspiratory flow rate of 10 l/min [21]. Spirometry was measured with a daily calibrated dry wedge spirometer (Jaeger Masterscope, Hoechberg, Germany). Baseline FEV_1, FVC and SVC were measured. Thereafter, 3 ml of saline, followed by doubling doses of aerosolized AMP (0.04-320 mg/mL) were administered until FEV_1 dropped ≥ 20% from baseline. If FEV_1 did not drop ≥ 20% from baseline the test was terminated after administration of the maximum dose of 320 mg/ml. PC_{20} was calculated according to standardized procedures [16]. At any given dose of AMP causing a ≥ 20% fall in FEV_1 from baseline, or after administration of the maximum AMP dose for the standard size AMP test (160 mg/mL), all subjects were given 400µg of salbutamol per spacer.

Large-particle AMP test
The large-particle AMP test was modeled on the design of a large-particle cat allergen challenge test by Lieutier-Colas and colleagues [22]. The test was performed with a Pari TIA nebulizer (Pari, Starberg, Germany) attached to a pressure reducer set at 1.2 bar. As the generated output was far larger than with standard AMP protocol, nebulization time was set to 30 seconds tidal breathing, in order to achieve administration of similar dose of AMP as with the standard AMP set-up. Particle size distributions (psd's) in the aerosols from the Pari TIA were measured 10 s from the start of nebulization and lasted 10 s, after it was checked that no changes occured within the first 30 s of nebulisation. For the measurements at room temperature and moderate relative air humidity with laser diffraction technique (Sympatec HELOS BF MAGIC) in combination with an INHALER 2000 adapter, a 100 mm lens was used (measuring range: 0.9-175 µm). Calculations were made with the Fraunhofer theory after it was checked that the Mie theory with the correct optical parameters yielded the same result. For the Pari TIA a MMAD (range) of 9.88 (9.84-9.91) micron was obtained (mean and spread of 3 measurements at 20 l/min suction rate). The droplet size distribution ranged from 3.37 (3.04-3.60 for X_{10}) to 19.03 (18.21-19.61 for X_{90}) micron. Administration of saline, followed by increasing doubling doses of AMP and measurement of spirometry was similar to the standard AMP protocol. A PC_{20} of 640 mg/mL was assigned to those subjects in whom FEV_1 did not fall ≥20% with the maximum dose of AMP (320mg/mL).

Small-particle AMP test
The small-particle AMP test was modeled on the design of a small-particle cat allergen challenge test by Lieutier-Colas and colleagues [22]. The test was performed with a Microcirrus nebulizer (Intersurgical BV, Uden, the Netherlands) attached to a pressure reducer set at 2.5 bar. The generated output was similar to the output of the standard AMP protocol; therefore the 2-minute tidal breathing method was adhered to. Particle size distributions from the Microcirrus were measured using the same equipment and conditions as described for the Pari TIA. Because AMP administration with the Microcirrus was over a longer period (2 min) than with the Pari TIA (30 s), measurements of 10 s with the Microcurrus were repeated 4 times during nebulization of the same volume of 3 ml with interval times of 20 s. The first measurement was started after a lag time of 10 s. Only measurements with sufficient optical concentration were used for computation of the mean for a total nebulization time of 2 min. No changes in psd as function of the nebulisation time were observed. Mean MMAD (range) of four nebulizations with the Microcirrus is 1.06 (1.04-1.08) micron. The droplet size distribution ranges from 0.60 (0.60-0.61 for X_{10}) to 2.18 (2.17-2.19 for X_{90}) µm. Administration of saline, followed by increasing doubling doses of AMP and measurement of spirometry was similar to the standard AMP protocol. A PC_{20} of 640 mg/mL was assigned to those subjects in whom FEV_1 did not fall ≥20% with the maximum dose of AMP (320mg/mL).

Bronchial and alveolar eNO
Bronchial and alveolar eNO were calculated after exhaled NO measurement with a NIOX (Aerocrine, Stockholm, Sweden) at multiple flow rates (30, 50,

100, and 200 mL/s). The mean of three technically acceptable attempts per flow rate was used for analysis. Bronchial and alveolar fractions were calculated with a modification of the mathematical model of Tsoukias and George [23].

Borg dyspnea score
Dyspnea was scored according to the modified Borg scale, range 0 (no dyspnea) to 10 (maximal breathlessness) [24] before and after each administration of either saline, AMP or salbutamol at the end of each AMP test.

Airway resistance
Hypothetically, if only small airways become obstructed, FEV_1 may not be reduced to a large extent, Therefore we additionally measured airway resistance three time points: before each AMP test, at the moment of bronchoconstriction (i.e. immediately after PC_{20} was reached, or in the absence of a PC_{20} after administration of the maximum dose), and 10 minutes after administration of 400µg salbutamol. Airway resistance was measured with a shutter-interrupter technique with the measurement programme ROcclusion attached to the spirometer (Jaeger Masterscope, Würzburg, Germany).

Randomization
Subjects were randomized to treatment with either small corticosteroid particles: ciclesonide 160µg s.i.d., or large corticosteroid particles: fluticasone 100µg b.i.d. Both investigators and patients were blinded to treatment. A double-dummy procedure was adhered to in order to garantuee blinding, as ciclesonide was administered as an MDI and fluticasone as a DPI. Randomization codes were broken after the last patient had completed the study.

Statistical Analysis
Data were entered by double-entry protocol into a database. A power calculation was performed prior to study execution. It was assumed that the treatment effect on the difference between PC_{20} large-particle and PC_{20} small-particle would amount to approximately 1 to 1.5 doubling doses (DD) of AMP. Based on experience with trials using AMP bronchoprovocations the standard deviation of PC_{20}AMP was assumed to amount to 1 doubling dose. Using a subject size of 40, a power calculation yielded a minimal detectable difference of 0.44 DD with a power of 80%, and an alpha of 5%

Data were expressed as medians (interquartile ranges). A p-value <0.05 was considered to indicate statistical significance. Differences between PC_{20} large particles and small particles were tested with Wilcoxon signed rank tests, and differences between groups with Mann Whitney U tests. Within and between treatment differences in lung function were tested by means of paired and independent t-test. Spearman's nonparametric rank correlation was calculated to assess the relationship between airway resistance and PC_{20} with the three particle sizes.

RESULTS

Study population

A total of 101 subjects gave informed consent, 64 did not enter or discontinued the study due to various reasons and 37 asthmatics completed the study protocol (Figure 2). Characteristics of the latter subjects are shown in Table 1. Nineteen subjects were randomized to ciclesonide treatment, and 18 to fluticasone. After a 4-week run-in period with fluticasone there were no statistically significant differences in clinical characteristics between the treatment groups (Table 1). Side effects were comparable between Fluticasone and ciclesonide (individual data not shown).

PC_{20} AMP

PC_{20} AMP tests according to the standard-, large-, and small-particle protocol did not differ significantly between treatment groups at randomization or after the treatment period (Table 2, Figure 3).

In the total study population, PC_{20} measured with standard-particle AMP did not differ significantly from PC_{20} large-particle AMP, but both were significantly smaller than the PC_{20} value obtained with small-particle AMP, median (interquartile range) 12.0 (6.6-59.4), 9.6 (4.5-109.7) and 225.8 (32.1-640.0) mg/mL respectively, $p<0.001$.

There were no significant differences in treatment effect on ΔFVC% at PC_{20}, ΔSVC% at PC_{20} or ΔFVC/SVC% at PC_{20} measured with either small or large particle sizes of AMP.

Not all subjects reached a PC_{20} with the small- and large-particle AMP protocol at baseline. The 23 subjects with a PC_{20} small-particle AMP<640 mg/mL had significantly lower $FEF_{25-75\%}$% predicted than the 14 subjects with PC_{20}>640 mg/mL, median (IQR) 56.3% (36.1-72.7) versus 67.6% (58.2-98.0) respectively ($p=0.04$). In agreement with this, $FEF_{50\%}$% predicted was significantly smaller in those with PC_{20} small-particle AMP<640 mg/mL, 55.4% (38.9-70.6) versus 69.1% (59.3- 102.1) respectively ($p=0.012$).

The following sub-analyses on treatment effects were performed on all subjects with PC_{20}<640 mg/mL (n=23).

Large-particle protocol: fifteen out of 19 subjects in the ciclesonide group and 16 out of 18 subjects in the fluticasone group had a baseline PC_{20} <640 mg/mL. Within the fluticasone-group there was a significant treatment effect on improvement of ^{2}log PC_{20} of 1.32 DD ($p=0.03$). In the ciclesonide group there was an improvement of ^{2}log PC_{20} of 0.74 DD ($p=0.17$) (Table 2, Figure 3).

Small-particle protocol: eleven out of 19 subjects in the ciclesonide group and 12 out of 18 subjects in the fluticasone group had a baseline PC_{20} <640 mg/mL. Within the ciclesonide-group there was a significant treatment effect on improvement of ^{2}log PC_{20} of 1.74 DD ($p=0.03$). In the fluticasone group there was an improvement of ^{2}log PC_{20} of 0.8 DD ($p=0.17$) (Table 2, Figure 3).

Exhaled NO
A small number of subjects could not perform eNO tests in the study due to technical problems with the NIOX equipment. Fluticasone changed alveolar eNO values significantly more than ciclesonide, by -1.1 (-2.1-0.1) versus 0.4 (-0.8-3.9), p=0.03, respectively (Table 3), whereas changes in bronchial eNO values were comparable.

Borg dyspnea score
The change in Borg dyspnea score from before to after the last dose of AMP test was significantly higher with the standard-particle AMP test than with small-particle AMP, median (IQR) 3 steps (2-5) versus 2 steps (1-4), p=0.043, respectively.
There were no significant differences in change in Borg dyspnea score between standard-particle and large-particle AMP, or between large-particle and small-particle AMP. There were no significant between-treatment differences in change in dyspnea score.

Airway resistance
Airway resistance was significantly higher immediately after than before each bronchoprovocation test, p<0.001 (Table 4). There were no significant differences between changes in airway resistance with AMP provocation tests with the three different particle sizes. Also, there were no significant differences in treatment effects on the change in airway resistance after AMP provocation tests.
There was a significant association between the severity of hyperresponsiveness to small-particle AMP and the change in airway resistance after provocation with small-particle AMP: a higher PC_{20} (thus less hyperreponsiveness) was significantly correlated with a smaller change in airway resistance after the small-particle AMP test, r_s=-0.59, p<0.001. There were no significant associations between PC_{20} values obtained with large-or standard-particle AMP and changes in airway resistance.

Spirometry
Fluticasone improved mean (SD) FEV_1 %predicted significantly more than ciclesonide, by 0.5% (4.3) versus -3.0% (4.6) respectively, p=0.021. There were no significant differences in treatment effects of ciclesonide and fluticasone on FVC, SVC, FVC/SVC, or $FEF_{50\%}$. $FEF_{25-75\%}$ however, improved more with fluticasone than with ciclesonide, by 0.6% (5.6) predicted versus -3.6% (6.0), p=0.034.

DISCUSSION

This study renews the discussion on the role of particle size in the deposition of inhaled substances. Particle size appears to be important both with respect to diagnostic tools such as bronchoprovocation tests and the inhaled therapeutics used in asthma. First, we found that small-particle size AMP induced a 20% fall in FEV_1 after a significantly higher dose than a larger-particle size AMP provocation. Second, our double-blind study with large- and small-particle inhaled corticosteroids in mild-to-moderate asthma showed that there were no significant differences in clinical effects of inhaled corticosteroids with a small and a large particle size, i.e. ciclesonide and fluticasone respectively. Not all subjects reached a 20% fall in FEV_1 at the highest applied AMP dose (320mg/mL). In the group of asthmatics who did achieve this, ciclesonide improved $^2\log PC_{20}$ AMP with small-particle size by 1.74 DD (p=0.03), whereas fluticasone did not. Conversely, fluticasone improved $^2\log PC_{20}$ with large-particle size AMP significantly, i.e. by 1.32 DD (p=0.03), whereas ciclesonide did not.

We demonstrated that PC_{20} values differ significantly when they are calculated from tests with standard-particle, small-particle or large-particle sizes. Surprisingly, PC_{20} large-particle values were smaller than those of PC_{20} standard-particle, which in turn was smaller than PC_{20} small-particle. When designing this study, we hypothesized that the opposite would occur, i.e. the lowest PC_{20} was to be expected with small-particle nebulisation as the small airways account for >50% of total airway surface [25], and a larger response at already a lower dose of AMP was expected. How can we explain this intriguing finding? First, it may be that due to the very small size of the small-particle AMP (MMAD 1.04 micron) a considerable fraction of particles may not have been deposited onto the airway surface and was exhaled subsequently. This then will cause a smaller bronchoconstrictive response than with larger particles that tend to deposit onto the airway surface far more easily. An alternative explanation may be the properties of the airway structure of small and large airways. Although small airways are involved in the inflammatory process of mild-to-moderate asthma, this does not automatically imply that small airways also show a similar severity of hyperresponsiveness as compared to intermediate or large airways, as there are also marked structural differences between airways of different size and generation [26]. Furthermore, PC_{20} is defined by the 20% fall in FEV_1, which may also explain why PC_{20} small-particle is larger than with larger particle sizes. FEV_1 is thought to be generated to a large extent by the airway diameter of the first four generations of the bronchial tree [27], and therefore may not be much reduced in case small airways are even extensively obstructed. All of the above-mentioned explanations may be applicable and are in accordance with findings in an earlier study by Casset *et al* on the effects of particle sizes of inhaled allergens, which demonstrated lower PD_{20} values with larger particle sizes as well [28]. It would have been an advantage if we had had access to more adequate tools to evaluate small airways bronchoconstriction, besides the interrupter resistance technique used in this study. Forced oscillation or impulse oscillometry possibly allows for a much better analysis of AMP-induced changes in the small airways. In addition the multibreath washout test with determination of Scond and Sacin is a promising and highly sensitive

tool, as confirmed by a recent paper by Verbanck et al [29]. Unfortunately, at the time of the study, these techniques were not available to the study site.

A small number of subjects did not have a larger than 20% decrease in FEV_1 after the highest administered concentration of AMP with either small-particle and/or large-particle protocol. This may be the reason why there were no significant treatment effects on PC_{20} when analyzed in the full group of participants. Sub-analyses within individuals who demonstrated a PC_{20}<640 mg/mL revealed that ciclesonide, the small-particle ICS, significantly improved small airways obstruction, as reflected by PC_{20} small-particle. Furthermore, fluticasone, the large-particle ICS, improved large airways obstruction, which was also reflected by improvements in PC_{20} large-particle. These results do meet the beforehand-defined hypothesis that small-particle treatment has a larger effect on small airways obstruction than large-particle treatment. Of interest, subjects who did respond to the small-particle AMP challenge tests with PC_{20}<640 mg/mL differed from subjects without such a response by demonstrating more small airways obstruction, as reflected by $FEF_{25-75\%}$ and $FEF_{50\%}$. This finding is in support of the hypothesis that in patients with more pronounced small airways involvement in asthma, there is more small airways hyperresponsiveness as demonstrated by PC_{20} small-particle AMP, and it is this specific subgroup that appears to benefit most from small-particle treatment with ciclesonide. Nevertheless, ciclesonide is known to improve both large and small airway parameters [30, 31] and therefore the surprising finding in this study that fluticasone improves PC_{20} large-particle more than ciclesonide is subject to speculation, more so as ciclesonide was equivalent to fluticasone in improving PC_{20} AMP in a previous study [32]. One may argue whether the duration of the treatment period in our study was sufficiently long to detect the hypothesized treatment effects. Longer treatment periods may offer larger effect sizes of PC_{20} values by increasing power. Nevertheless, both ciclesonide and fluticasone have been demonstrated to improve BHR to either methacholine or AMP significantly after a period of 4 weeks [30-32]. Moreover, a shorter duration of the treatment period has a lower chance for intercurrent spontaneous changes in the clinical expression of asthma and dropping out of patients. Therefore, we believe that the investigated study periods were sufficiently long to enable adequate detection of improvement in BHR.

Unexpectedly, fluticasone improved FEV_1, $FEF_{25-75\%}$ and alveolar eNO better than ciclesonide did. In other trials ciclesonide has been proven as equivalent to fluticasone in increasing FEV_1 [33]. The smaller effect of ciclesonide on small airway parameters such as $FEF_{25-75\%}$ and alveolar eNO compared to fluticasone is even more surprising. Whether these results are true or rather reflect noise remains unclear: it is possible that pre-treatment differences have influenced the results by regression to the mean. However, if these results are true, a few factors may be recognized to have influenced these effects. First of all, the difference in dosing regimen may account for the difference in treatment effect, as fluticasone was dosed twice-daily as opposed to the once-daily dosing of ciclesonide. Furthermore, in sharp contrast to our study, earlier studies demonstrating equivalent efficacy of ciclesonide to fluticasone, were performed without run-in periods in which ICS

were being used. The use of fluticasone in the run-in period may have provided add-on effects to fluticasone that was used during the treatment period, which is when effects on lung function were evaluated. In contrast: the switch from fluticasone in the run-in period to ciclesonide during the treatment period may have caused a smaller beneficial effect on conventional lung function parameters.

In summary, the currently investigated small-particle and large-particle AMP challenge tests appear to be promising tools for differentiating which patient will benefit most from either large- or small-particle ICS. Some adjustments may improve this. Possibly, the use of a different parameter than FEV_1 to establish small airways obstruction will aid in improving the small-airways bronchoprovocation test. Furthermore, a slightly larger small-particle of AMP (e.g. between 1.4-2.0 microns) will possibly prove to be a better indicator of small airways involvement in asthma than the extremely small particles that were applied in this study. The use of slightly larger small-particle size AMP may then overcome the issue of large exhaled fractions of AMP due to insufficient deposition in peripheral airways of the extreme small particles. When slightly increasing the size of the particles, this may result in a larger number of respondents to the small-particle bronchoprovocation test, which in turn could lead to better identification of subjects with more outspoken small airways involvement in asthma. This study has demonstrated that within the group of responders to small- and large-particle AMP bronchoprovocation tests, ciclesonide is more effective than fluticasone in treating hyperresponsiveness as tested with small-particle size. More investigations with even larger study populations are necessary in order to establish a reliable test reflecting small and large airways inflammation respectively. Hopefully this will enable us to create patient-adjusted treatment regimens, with treatment specifically targeted to the most prevalent site of inflammation.

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Table 1 Baseline characteristics

N=37	Ciclesonide (n=19)	Fluticasone (n=18)	
	median (IQR) or N (%)	median (IQR) or N (%)	p-value
Male. n (%)	6 (31.6%)	10 (55.6%)	.14
Ex-smoker n (%)	2 (10.5%)	5 (27.8%)	.23
Age, years	39.6 (32.5-46.6)	46.0 (37.9-55.5)	.10
Lung function after run-in with 4-week fluticasone 100µg bid (day 33)			
FEV_1 % predicted	91.3 (76.7-109.3)	86.8 (81.6-100.1)	.69
FVC % predicted	110.2 (92.9-119.7)	104.2 (90.5-118.9)	.72
SVC % predicted	104.9 (94.6-122.0)	106.1 (91.7-118.3)	.83
FVC%SVC	97.9 (95.3-100.5)	95.5 (92.3-100.3)	.20
FEV_1%FVC %predicted	92.9 (85.0-102.0)	90.1 (83.6-99.4)	.67
$FEF_{50\%}$ % predicted	68.1 (48.6-83.2)	53.6 (46.8-70.9)	.52
$FEF_{25-75\%}$ % predicted	65.3 (45.7-86.2)	58.4 (47.5-76.9)	.58

IQR: interquartile range. Between-group differences are tested with Mann-Whitney U test

Table 2 PC_{20} AMP with the different particle sizes, measurement at baseline and after treatment with ciclesonide or fluticasone

	Ciclesonide N=19	Fluticasone N=18		Ciclesonide N=11	Fluticasone N=12	
	Mean (SD)	Mean (SD)	p-value	Mean (SD)	Mean (SD)	p-value
	Baseline PC_{20}			**Individuals with Baseline PC_{20} <640mg/mL**		
AMP Standard	4.2 (2.8)	3.5 (2.1)	.41			
AMP Large	4.9 (3.3)	3.8 (3.2)	.30	3.7 (2.6)	3.0 (2.4)	0.44
AMP Small	6.8 (3.6)	6.6 (2.7)	.87	4.9 (3.7)	5.2 (2.1)	0.81
	Post-treatment PC_{20}					
AMP Large	4.8 (2.7)	4.5 (2.3)	.77	4.5 (2.3)§	4.3 (2.4) •	0.89
AMP Small	7.4 (2.0)	6.7 (2.8)	.42	6.6 (2.0) *	6.0 (2.7) °	0.53

Values are mean ^{2}log PC_{20}AMP values. SD: standard deviation. Between-group differences are tested with T-test, statistical significance demonstrated as p-values in columns besides the table. Within-group treatment differences: § p=0.17, • **p=0.03**, * p=0.03. ° **p=0.17**.

Table 3 Exhaled NO

		Ciclesonide N=19		Fluticasone N=18	
	N	median (IQR)	N	median (IQR)	p-value
Baseline					
Alveolar NO	18	4.78 (3.52-8.12)	17	5.52 (4.72-6.83)	.51
Bronchial NO	18	0.66 (0.49-1.39)	17	0.79 (0.67-1.12)	.41
Post-treatment					
Alveolar NO	16	4.61 (3.29-6.91)	16	4.73 (3.69-5.69)	.88
Bronchial NO	16	0.67 (0.42-1.44)	16	0.82 (0.46-1.33)	.90
Differences					
Alveolar NO	**15**	**0.40 (-0.82-3.85)**	**15**	**-1.06 (-2.41-0.07)**	**.03**
Bronchial NO	15	0.17 (-0.11-0.70)	15	0.02 (-0.15-0.09)	.22

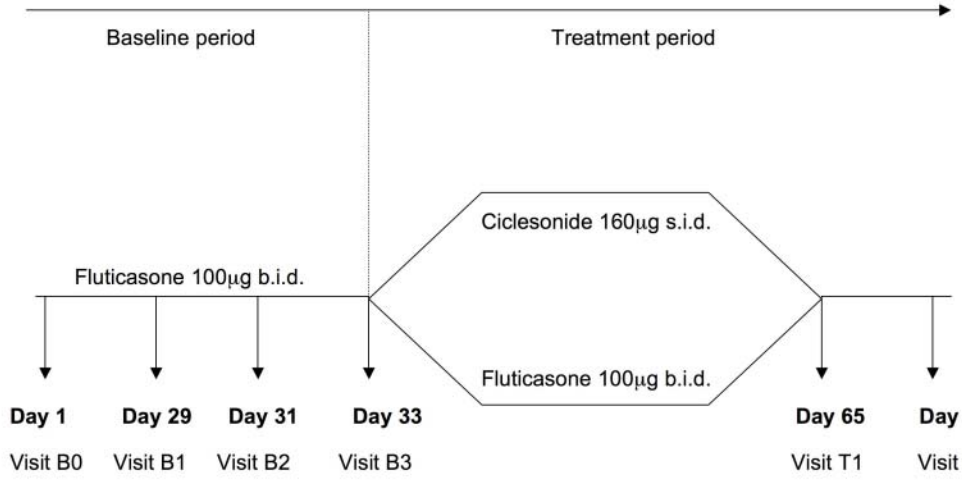
IQR: interquartile range. Between-group differences are tested with Mann-Whitney U test.

Table 4 Airway resistance

	median (IQR)	p-value
AMP Standard		
Rocc before	0.43 (0.34-0.55)	
Rocc after	0.59 (0.45-0.89)	after vs before AMP p = .00
Rocc after salbutamol (sal)	0.33 (0.27-0.43)	after sal vs after AMP p = .00
AMP Large		
Rocc before	0.40 (0.36-0.54)	
Rocc after	0.56 (0.45-0.84)	after vs before AMP p = .00
Rocc after salbutamol (sal)	0.33 (0.28-0.42)	after sal vs after AMP p = .00
AMP Small		
Rocc before	0.41 (0.35-0.48)	
Rocc after	0.50 (0.43-0.76)	after vs before AMP p = .00
Rocc after salbutamol (sal)	0.31 (0.26-0.39)	after sal vs after AMP p = .00

IQR: interquartile range, sal: salbutamol 400µg per spacer

Figure 1



Visit B0: in/exclusion criteria, skin prick test, pulmonary function test, start fluticasone 100 b.i.d. for 4 weeks
Visit B1: exhaled NO, Standard AMP test (including airway resistance and BORG scores)
Visit B2: Large AMP test (including airway resistance and BORG scores)
Visit B3: Small AMP test (including airway resistance and BORG scores), randomization
Visit T1: exhaled NO, Large AMP test (including airway resistance and BORG scores)
Visit T2: Small AMP test (including airway resistance and BORG scores)

Figure 2

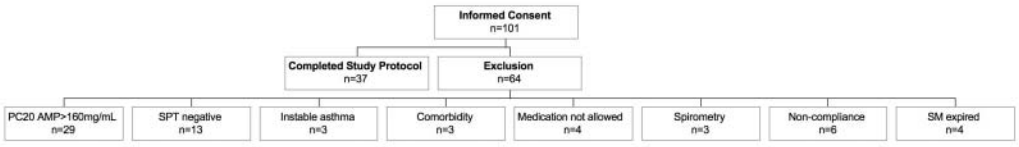


Figure 3

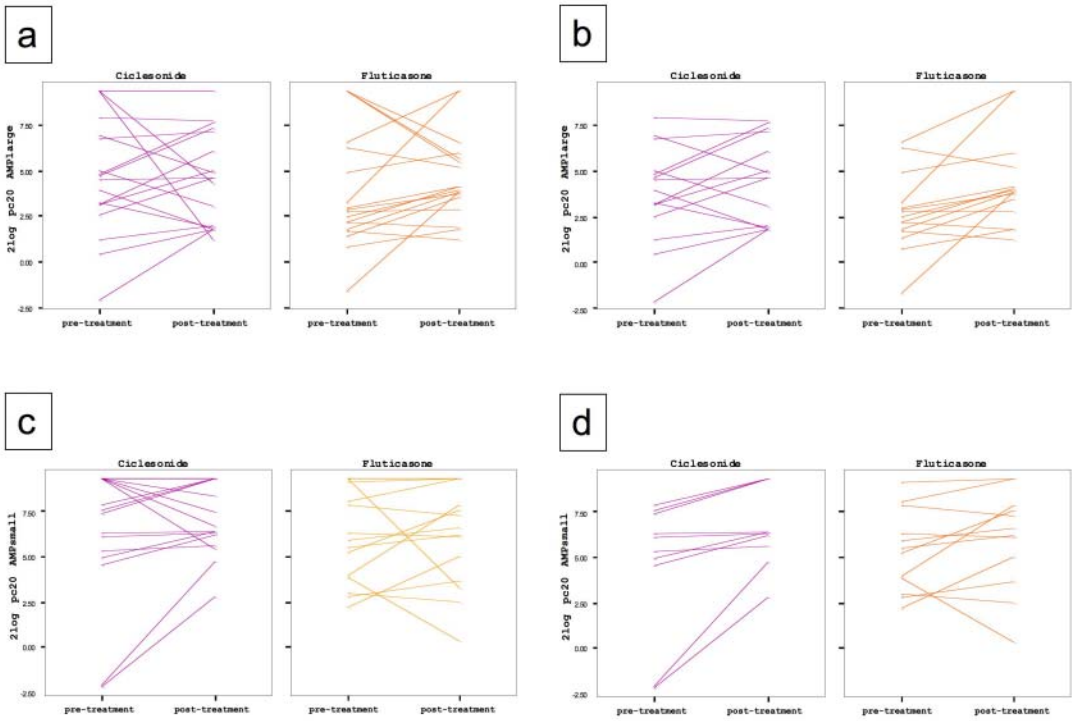


Figure 3 PC_{20} AMP large and small particle pre- and post-treatment. A) PC_{20} large particles in all subjects. B) PC_{20} large particles in only those subjects with pre-treatment PC_{20}<640 mg/mL. C) PC_{20} small particles in all subjects. D) PC_{20} small particles in only those subjects with pre-treatment PC_{20} <640 mg/mL.

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