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Effects of beclomethason/formoterol and budesonide/formoterol fixed combinations on lung function and airway inflammation in patients with mild to moderate asthma – An exploratory study

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ABSTRACT

Rationale: Asthma is a chronic inflammatory airway disease of the whole bronchial tree. In this exploratory study we investigated the effects of beclomethasone/formoterol (becl/form) and budesonide/formoterol (bud/form) fixed combinations on lung function and airway inflammation in patients with mild to moderate asthma.

Methods: 22 adult patients with asthma (mean FEV1 91.6% pred.) were recruited to this prospective phase IV, double-blind, double-dummy, two-way cross-over, single-centre, randomised study. After a 7 days run-in period with bud 200 μg bid patients were randomised to receive 4 weeks of becl/form (100/6 μg) bid in a pressurised metered dose inhaler or bud/form (160/4.5 μg) bid administered via dry powder inhaler. We measured spirometry, bodyplethysmography, impulse oscillometry, nitric oxide (NO) and its alveolar fraction (C_{Alv}), and assessed sputum cellularity.

Results: C_{Alv} significantly decreased after 4 weeks of treatment in each treatment period. The adjusted geometric mean (log transformed data, end of treatment vs. baseline) was 0.942 ppb (95% CI: 0.778 −1.141 ppb) for becl/form and 0.903 ppb (95% CI: 0.741−1.099 ppb) for bud/form. Impulse oscillometry revealed a significant decrease in mean Delta R5−R20 of −0.033 kPa * L^{-1} * sec^{-1} for becl/form (95% CI: −0.064 to −0.002) and of −0.048 033 kPa * L^{-1} * sec^{-1} for bud/form (95% CI: −0.079 to −0.017). Other parameters of lung function and NO showed numerically small and in most cases statistically non-significant changes.

Conclusions: In patients with mild to moderate asthma pre-treated with inhaled corticosteroids, the use of ICS/LABA formulations led to improvements of C_{Alv} and Delta R5−R20 indicating that these parameters might be helpful to further assess the effects of inhaled ICS/LABA combinations on lung function and airway inflammation.

1. Introduction

Asthma is a heterogeneous disease leading to chronic inflammation of the whole bronchial tree from central to peripheral airways [1]. The importance of the small airways for the clinical feature [2] and therapy of patients with asthma [3] is increasingly recognized. Despite their importance, small airways have been proven difficult to study [4,5]. Among the current available techniques the measurement of the alveolar fraction of exhaled nitric oxide (C_{Alv}) might be useful to assess peripheral airway inflammation in asthma, which can not be assessed by the whole fraction of exhaled nitric oxide (FENO) [6,7]. Furthermore, the forced oscillation technique with measurements of delta R5−R20 might provide insights into peripheral airway function that can not be assessed by conventional lung function techniques like spirometry [8]. Of interest, the fixed combination of budesonide/formoterol (bud/form) delivered by dry powder inhalation has recently demonstrated an improvement of several parameters of impulse oscillometry like delta R5−R20 without changing the forced expiratory volume in 1 s (FEV1) in patients with controlled asthma significantly. This study indicates that the technique of

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impulse oscillometry is helpful to assess changes in lung function not captured by conventional spirometry [9].

Recently, a fixed combination of beclomethasone dipropionate and formoterol (becl/form) delivered via a hydrofluoroalkane (HFA) pressurized metered dose inhaler (pMDI) became available. It is characterised by an extrafine particle formulation. This extrafine particle formulation ensures uniform delivery of the two active drugs to large and small airways, therefore treatment of inflammation and bronchoconstriction is expected throughout the whole bronchial tree [10]. While several studies evaluated the effects of becl/form on airway inflammation assessed by the whole fraction of exhaled NO (FENO) [11] and small airway dysfunction assessed by the nitrogen wash-out technique [12,13] no study evaluated the effects of becl/form on parameters of impulse oscillometry or C_{Alv} so far.

The aim of the present exploratory study was to evaluate changes in lung function and airway inflammation following treatment with becl/form and bud/form fixed combinations applying a comprehensive panel of lung function techniques and assessments of airway inflammation in patients with mild to moderate asthma.

2. Material and methods

2.1. Study design

This was a prospective phase IV, double-blind, double-dummy, two-way cross-over, single-centre, randomised study (Fig. 1). After a screening visit, the patients entered a 7 day run-in period and inhaled budesonide 200 μg bid. Patients then were randomly assigned to received the two study mediations, i.e. bud/form combination (160/4.5) μg bid and becl/form combination (100/6 μg) bid for 28 days each in a crossover setting, separated by a seven days wash-out period with inhalation of budesonide 200 μg bid.

2.2. Eligibility criteria for participants

The study was conducted between March 2009 and May 2010 at the Pulmonary Research Institute at LungClinic Grosshansdorf, Germany. To be eligible, patients had to fulfil the following main inclusion criteria: adult male or female aged 18–65 years; clinical diagnosis of mild to moderate asthma; treatment with inhaled corticosteroids (ICS) equivalent to budesonide 200 μg bid (with or without long-acting beta agonists (LABA), including fixed ICS/LABA combinations); pre-bronchodilator FEV1 ≥65% pred.; an increase of FEV1 of ≥12% and 200 ml after inhaled salbutamol 400 μg, and/or a hyper-responsiveness to methacholine (PC_{20} < 8 mg/ml); and FENO >30 ppb. Main exclusion criteria included current smokers or ex-smokers ≥10 pack-years and a medical history of COPD. In addition, patients had to be withdrawn from the study in case they took more than 8 puffs of salbutamol per day.

Prohibited concomitant medications at study entry were extrafine formulations of ICS, fixed combinations of extrafine ICS and LABA, leukotriene modifiers, inhaled long-acting and short-acting anticholinergics, monoamine oxidase inhibitors, beta-blockers, tricyclic antidepressants, and selective serotonin re-uptake inhibitors (unless taken at stable doses at the screening visit).

All subjects gave their written informed consent. The study was conducted under the provisions of the Declaration of Helsinki, and in accordance with the International Conference on Harmonisation (ICH) Consolidated Guideline on Good Clinical Practice (GCP). Ethical approval was obtained.

2.3. Study medication

Extrafine becl/form fixed combination (100/6 μg) bid was delivered via an HFA pressurized MDI. Bud/form fixed combination (160/4.5 μg bid corresponding to an emitted dose of 200/6 μg) was inhaled via the Turbuhaler dry-powder inhaler (DPI).

Budesonide was provided via Turbuhaler DPI, and patients inhaled 200 μg bid during run-in and wash-out periods. Salbutamol 100 μg pMDI as reliever medication was allowed throughout the study.

2.4. Pulmonary function tests

2.4.1. Spirometry

Spirometry was performed with a self-calibrated computer-operated pneumotachographic spirometer (Masterscope, CareFusion, Hoechberg, Germany) according to the ATS/ERS recommendations [14].

2.4.2. Bodyplethysmography

A constant volume plethysmograph (MasterScreenBody, CareFusion, Hoechberg, Germany) was used to measure specific airway conductance (sGaw), airway resistance (Raw), functional residual capacity (FRC) total lung capacity (TLC), residual volume (RV) [15].

2.4.3. Exhaled nitric oxide

Exhaled nitric oxide measurements were performed with the NIOX FLEX- (Aerocrine, Solna, Sweden). FENO measurements were performed at standard flow rate (50 mL/s) and at additional flow rates of 10, 100, 200, and 300 mL/s. Bronchial flux of NO ($J_{NO, Br}$; nL/min) and C_{Alv} (ppb) were calculated [6,16]. All data were mean values derived from two technically satisfactory measurements.

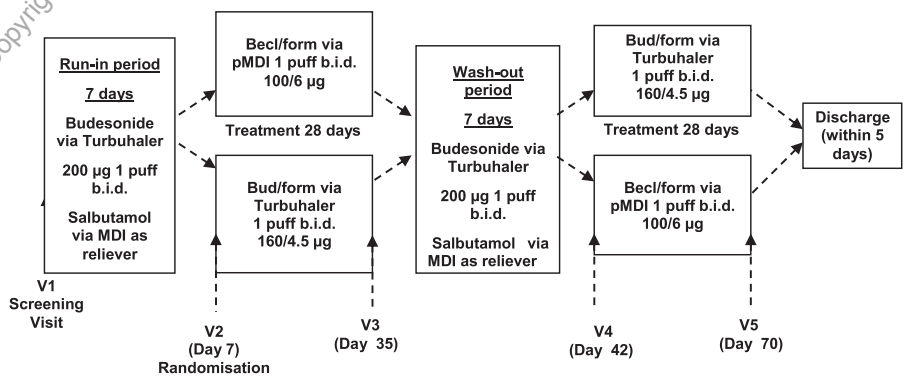

Fig. 1. Flowchart of the study.

2.4.4. Impulse oscillometry

Impulse oscillometry was performed using a MasterScreen IOS, CareFusion, Hoechberg, Germany. Total resistance (Rrs) and reactance (Xrs) of the respiratory system were derived from measuring the impedance (Zrs), the total mechanical load of the respiratory system. Large and small airway mechanics were inferred from responses at both high (20 Hz) and low frequencies (5 Hz). The following parameters were analysed: R5 (representing total airway resistance), R20 (representing central airway resistance), Delta R5–20 (representing peripheral airway resistance), X5 (representing distal reactance), and resonant frequency (RF) [8,17]. For each subject, the mean of three technically satisfactory measurements of every single parameter was used for analysis.

2.5. Induced sputum

Induction of sputum was performed by inhalation of hypertonic saline with increasing concentrations of 3%, 4%, and 5% sodium chloride, each applied for 10 min using an ultrasonic nebulizer. Processing of sputum samples was performed as previously described [18,19].

2.6. Treatment allocation and randomisation

Allocation of each subject to a given treatment sequence was described in a randomization list prepared by the Statistics and Data Management Department of Chiesi Farmaceutici S.p.A. using (ClinPro/LBL™ v.8 software) (Clinical System Inc., USA). The randomization was done considering a 2*2 cross-over design with balanced blocks of 4. Treatment allocation at the study site started from the lowest number available. Both, patients and study personnel were blinded to treatment.

2.7. Statistical methods

Data analyses were performed in three different patient groups. The Intention-to-Treat population (ITT) comprised all randomised subjects who received at least one administration of study medication and who had at least one efficacy evaluation after baseline. The Per-Protocol population (PP) included all subjects from the ITT population without any major protocol deviations (i.e., wrong inclusions, poor compliance, absence of baseline measurement of an outcome variable, prohibited concomitant medications), as assessed during a blinded review of the data. The Safety population was defined as all randomised subjects who took at least one dose of study medication. Outcome variables were analysed both, in the ITT and in the PP populations. Safety analysis was performed in the Safety population.

2.8. Outcome variables

The variables of interest were assessed with analysis of covariance (ANCOVA) for cross-over designs, using the baseline values of each period as covariates and sequence, period, and treatment as factors. ANCOVA provided the estimation of the least square means (LSMEANS) and the 95% confidence intervals.

C_{Alv} and FENO did not follow a normal distribution and were log transformed before analysis. Descriptive statistics were provided for all outcome variables. No formal sample size calculation was performed, because this was an exploratory study. Twenty completed patients were thought to be adequate to provide sufficient information.

3. Results

3.1. Patients

Out of 33 patients with asthma screened for enrolment, 22 subjects fulfilled inclusion and exclusion criteria and entered the study. Since one patient erroneously received becl/form in both treatment periods and one patient had a major protocol violation (randomization despite the use of more than 8 puffs of salbutamol per day before randomization) the PP population consisted of $n = 20$ patients for either drug. There were no premature withdrawals from the study.

The baseline characteristics are presented in Table 1. All patients were Caucasians. The mean baseline values of all outcome variables were comparable in the two treatment sequence groups.

Because the results did not differ between the ITT and the PP populations, only data from the PP population are presented.

3.2. Outcome variables of small airway dysfunction

Patients had small improvements in nitric oxide measurements after four weeks of treatment with both ICS/LABA fixed combinations (Fig. 2; Table 2). Mean C_{Alv} decreased after 4 weeks of treatment in each treatment period. The adjusted geometric mean (log transformed data, end of treatment vs. baseline) was 0.942 ppb (95% CI: 0.778–1.141 ppb) with becl/form and 0.903 ppb (95% CI: 0.741–1.099 ppb) with bud/form.

Table 1
Patient characteristics.

	Sequence becl/form-bud/form ($N = 11$) (means ± SD)	Sequence bud/form-becl/form ($N = 11$) (means ± SD)	All patients ($N = 22$) (means ± SD)
Demographics			
Males (N, %)	4 (36.4%)	5 (45.5%)	9 (40.9%)
Females (N, %)	7 (63.6%)	6 (54.5%)	13 (59.1%)
Age, years	38.55 ± 8.79	36.18 ± 9.46	37.36 ± 8.99
Weight, kg	80.45 ± 14.8	79.18 ± 12.2	79.82 ± 13.3
Height, cm	179.2 ± 10.4	176.1 ± 7.45	177.6 ± 8.98
BMI, kg/m^2	25.21 ± 5.56	25.49 ± 3.23	25.35 ± 4.44
Spirometry			
FEV1 (L)	3.63 ± 0.92	3.44 ± 0.81	3.54 ± 0.85
FEV1 (% predicted)	92.93 ± 15.1	90.35 ± 11.2	91.64 ± 13.0
FEV1/FVC (%)	69.73 ± 6.00	68.34 ± 6.47	69.03 ± 6.13
FVC (L)	5.23 ± 1.27	5.02 ± 1.03	5.13 ± 1.13
FVC (% predicted)	110.8 ± 12.4	111.7 ± 7.89	111.3 ± 10.1
Bodyplethysmography			
sGaw (kPa^{-1} * sec^{-1})	1.04 ± 0.49	1.06 ± 0.52	1.05 ± 0.49
Raw (kPa * sec * L^{-1})	0.33 ± 0.31	0.29 ± 0.20	0.31 ± 0.26
FRC (L)	4.38 ± 1.11	4.12 ± 0.69	4.25 ± 0.91
TLC (L)	7.58 ± 1.64	7.28 ± 1.25	7.43 ± 1.43
RV	2.45 ± 0.60	2.37 ± 0.62	2.41 ± 0.60
RV/TLC (%)	32.62 ± 5.93	32.54 ± 6.32	32.58 ± 5.98
Impulse oscillometry			
R5 (kPa * L^{-1} * sec^{-1})	0.48 ± 0.25	0.47 ± 0.21	0.47 ± 0.23
R20 (kPa * L^{-1} * sec^{-1})	0.38 ± 0.13	0.37 ± 0.10	0.37 ± 0.11
Delta R5–R20 (kPa * L^{-1} * sec^{-1})	0.10 ± 0.16	0.10 ± 0.13	0.10 ± 0.14
X5 (kPa/L/sec)	−0.17 ± 0.21	−0.17 ± 0.11	−0.17 ± 0.16
RF (Hz)	16.00 ± 8.07	16.62 ± 7.61	16.31 ± 7.66
Exhaled NO			
FENO at 50 mL/s (ppb)	39.22 ± 21.6	33.42 ± 8.65	36.32 ± 16.3
$J_{NO, Br}$ (nL/min)	88.25 ± 36.8	97.45 ± 58.8	92.85 ± 48.1
C_{Alv} (ppb)	5.70 ± 4.23	4.27 ± 2.75	4.99 ± 3.56
Induced sputum (% of non-squamous cells)			
Eosinophils (%)	2.80 ± 3.53	1.51 ± 1.22	2.12 ± 2.61
Neutrophils (%)	55.81 ± 19.9	34.27 ± 18.9	44.53 ± 21.9
Macrophages (%)	37.66 ± 22.2	57.19 ± 21.8	47.89 ± 23.7
Lymphocytes (%)	1.91 ± 1.56	1.97 ± 1.02	1.94 ± 1.27

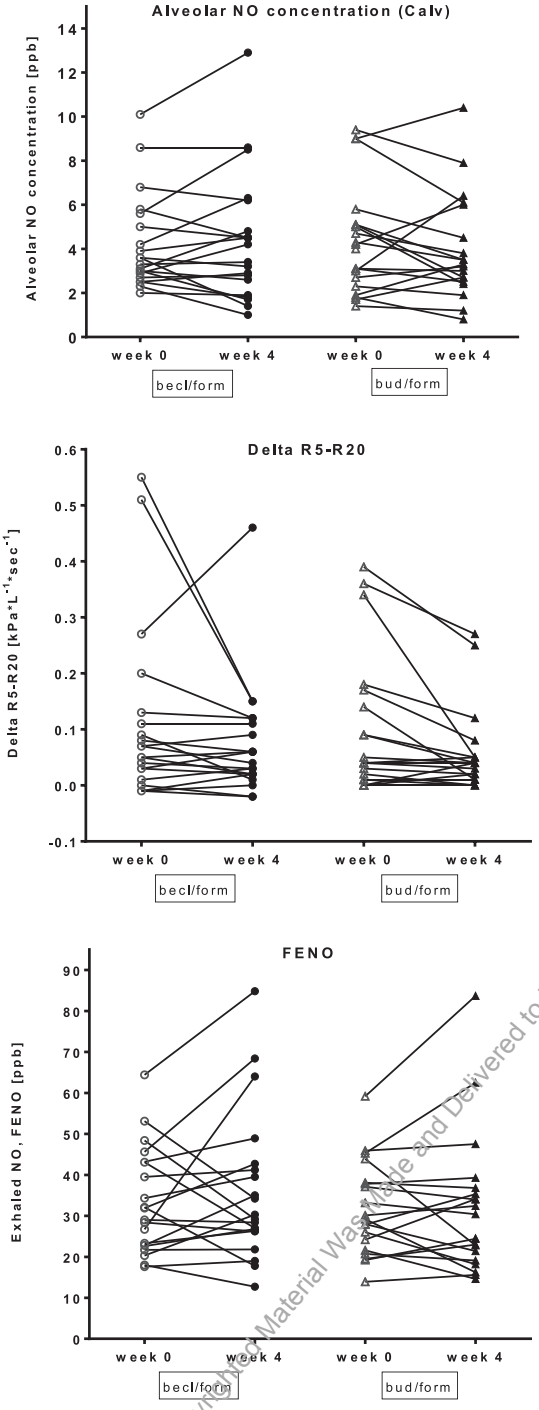

Fig. 2. Alveoloar nitric oxide concentrations (upper graph), Delta R5–R20 (middle graph), and exhaled nitric oxide (lower graph) in individual patients before (week 0) and after four weeks of treatment (week 4) with extrafine becl/form or bud/form.

Impulse oscillometry revealed a significant decrease in mean Delta R5–R20 after each treatment period (Fig. 2; Table 2).

3.3. Other outcome variables of lung function and airway inflammation

No substantial changes of FENO compared to baseline were observed (Fig. 2, Table 3). Table 3 summarises the outcome variables of lung function. After either study drug numerically small and in most cases statistically non-significant changes in further parameters of impulse oscillometry, forced spirometry, and bodyplethysmography were observed. Cellular composition of inflammatory sputum cells remained unaffected by either treatment (data not shown).

Table 2
Outcome variables of small airway dysfunction and nitric oxide.

	becl/form		bud/form	
	LS mean[a]	95% CI	LS mean[a]	95% CI
C_{Alv} (ppb)[a]	0.942	0.778, 1.141	0.903	0.741, 1.099
Delta R5–R20 (kPa * L^{-1} * sec^{-1})	−0.033	−0.064, −0.002	−0.048	−0.079, −0.017
FENO at 50 mL/s (ppb)	1.049	0.895, 1.229	0.964	0.820, 1.135
$J_{NO,\ Br}$ (nL/min)	1.028	0.871, 1.214	0.940	0.792, 1.114

[a] Ratio between end of treatment and baseline of the alveolar fraction of exhaled nitric oxide (C_{Alv}; ANCOVA Model) and change versus baseline of Delta R5–R20 derived from impulse oscillometry (ANCOVA Model).

3.4. Treatment compliance

Adherence to study medication as recorded by patients on diary cards was between 98% and 99% during becl/form, bud/form and placebo treatments, respectively. During run-in and wash-out periods, compliance rates with budesonide therapy were 96% and 100%, respectively.

3.5. Safety

Treatment-emergent adverse events were observed in 8 patients in each study group. One patient experienced an adverse drug reaction (cough) during becl/form treatment. There were no premature discontinuations of study drugs due to adverse events, no serious adverse events, and no deaths.

Adverse events that occurred in more than one patient were nasopharyngitis (4 patients during becl/form and 1 patient during bud/form), headache (1 patients during becl/form and 3 patients during bud/form), respiratory disorders (2 patients during bud/form), and skin disorders (2 patients during bud/form).

4. Discussion

This exploratory study evaluated the efficacy of two ICS/LABA combinations on a comprehensive panel of parameters of lung function and airway inflammation in patients with mild to moderate asthma. The primary variables of interest of the study were alveolar nitric oxide concentrations and peripheral lung resistance measured by forced oscillation as both assessments seem to reflect small airway dysfunction. We observed a decrease in alveolar NO (C_{Alv}) and distal airway resistance (Delta R5–R20) after four weeks of therapy with both combinations.

The C_{Alv} levels observed in our study were comparable to the C_{Alv} levels reported for patients with mild asthma and normal lung function [7]. To the best of our knowledge there are no studies available that evaluated the effects of an ICS/LABA combination on C_{Alv} levels in patients with mild asthma before. However, the extrafine beclomethasone dipropionate formulation demonstrated to lower C_{Alv} NO levels in patients with asthma before, while a non-extrafine beclomethasone dipropionate formulation failed to demonstrate significant treatment effects on C_{Alv} [20]. While it is difficult to speculate on the clinical relevance of lowering C_{Alv} in our patients in this study it should be noted that the alveolar component of exhaled NO is associated with asthma control in patients with mild untreated asthma [7]. Clearly, more studies are needed to evaluate the role of C_{Alv} as an indicator of asthma control under treatment.

Table 3
Other variables of airway inflammation and lung function.

	becl/form		bud/form	
	Mean change from baseline (SD)	95% CI on mean change	Mean change from baseline (SD)	95% CI on mean change
Impulse oscillometry				
R5 (kPa * L^{-1} * sec^{-1})	−0.057 (0.14)	−0.122, 0.008	−0.072 (0.12)	−0.127, −0.016
R20 (kPa * L^{-1} * sec^{-1})	−0.020 (0.07)	−0.051, 0.012	−0.028 (0.06)	−0.057, 0.002
X5 (kPa * L^{-1} * sec^{-1})	0.025 (0.14)	−0.042, 0.091	0.053 (0.08)	0.016, 0.089
RF (Hz)	−1.276 (3.32)	−2.831, 0.280	−4.008 (4.88)	−6.292, −1.723
Spirometry				
FEV1 (L)	0.025 (0.27)	−0.099 to 0.149	0.098 (0.36)	−0.070 to 0.266
FVC (L)	−0.044 (0.25)	−0.162 to 0.074	0.042 (0.24)	−0.068 to 0.152
FEV1/FVC (%)	1.135 (3.83)	−0.660 to 2.930	1.555 (4.64)	−0.614 to 3.724
Body plethysmography				
sGaw (kPa^{-1} * sec^{-1})	0.231 (0.39)	0.050, 0.413	0.206 (0.66)	−0.101, 0.513
Raw (kPa * sec * L^{-1})	−0.084 (0.21)	−0.184, 0.016	−0.065 (0.10)	−0.113, 0.017
FRC (L)	−0.174 (0.47)	−0.392, 0.044	−0.096 (0.35)	−0.262, 0.070
TLC (L)	−0.051 (0.38)	−0.331, 0.030	−0.127 (0.29)	−0.265, 0.012
RV (L)	−0.117 (0.49)	−0.346, 0.112	−0.186 (0.36)	−0.356, −0.016
RV/TLC	−1.206 (6.18)	−4.097, 1.686	−2.157 (4.61)	−4.313, −0.001

Values are adjusted geometric mean ratios between end of treatment and baseline.

With respect to impulse oscillometry, Delta R5–R20 as a parameter of small airway function has been demonstrated to significantly correlate with health status, dyspnoea, and disease control in patients with asthma [21]. A clinical trial with ciclesonide in 30 subjects with mild asthma showed a significant improvement in resistance of small airways (Delta R5–R20) and distal reactance (X5) when compared with fluticasone propionate [22]. In the same trial, spirometry indices did not change in either group, suggesting that impulse oscillometry is more capable of detecting treatment effects in peripheral airways. This observation is in line with data from a recent study of Hozawa et al. demonstrating significant changes in small airway resistance with bud/form without significant changes of FEV_1 in patients with controlled asthma [9]. In our study we also observed significant changes of Delta R5–R20 without significant changes of FEV1, which further strengthens the mounting evidence that in the absence of FEV1 improvements significant changes of small airway resistance following intervention can be demonstrated.

Looking at the changes of lung function parameters assessed by bodyplethysmography in our study we observed numerically small changes following intervention with both ICS/LABA treatments. The characteristics of small airway obstruction include premature airway closure and air trapping, which can be assessed by an elevation of RV or RV/TLC ratio [5]. Indeed, RV and RV/TLC ratio decreased significantly after bud/form treatment indicating that this parameter might be also used in further interventional studies related to small airway disease in asthma.

Induced sputum was collected in our study in order to evaluate changes in the cellular composition of airway inflammation. No significant changes regarding the cellular distribution in sputum were observed after treatment with both ICS/LABA combinations. Furthermore, the variability in cell counts from induced sputum was rather large. As patients were already using inhaled corticosteroids at baseline it might have been not possible to demonstrate significant effects of both study treatments on sputum cellularity in this study.

Our study has several limitations. First, it lacks a formal calculation of sample size. The power of a study depends mainly on the standard deviation of the study endpoint, the effect size when two treatments are compared, and on the number of patients tested. In the present study, both the alveolar NO concentration (C_{Alv}) and Delta R5–R20 had relatively large standard deviations in comparison to the changes observed after treatment. Second, the patients were relatively well controlled with bud 200 μg bid when study medication was commenced. Therefore, the room for improvements might have been too small in our study and it is likely that greater changes could have been observed in a different study population affected by more severe asthmaThird, there is no established duration of action of LABA based on Delta R5–R20 or C_{Alv}. Therefore, we can not rule out that the wash-out period of 7 days for LABA before each treatment period was too short with regard to the variables of small airway dysfunction in this trial. Fourth, the nitrogen wash-out technique with a measurement of the lung clearance index as an indicator of small airway dysfunction was not part of our panel of assessments as this technique was not available at our center at the time the study was conducted. As previous studies conducted with becl/form have shown positive effects on closing capacity and closing volume measured by nitrogen wash-out technique [12,13] it would have been scientifically valuable to include this information in the present study.

5. Conclusion

In conclusion, the use of both ICS/LABA formulations led to improvements of C_{Alv} and Delta R5–R20 indicating that these parameters might be helpful to further assess the effects of inhaled ICS/LABA combinations on lung function and airway inflammation in patients with mild to moderate asthma.

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