Inflammatory and functional effects of increasing asthma treatment with formoterol or double dose budesonide

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KEYWORDS
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Peak expiratory flow rate;
Eosinophils;
Cell cultures

Summary
Adding a long-acting β2-agonist to inhaled corticosteroids (ICS) for asthma treatment is better than increasing ICS dose in improving clinical status, although there is no consensus about the impact of this regimen on inflammation. In this double-blind, randomized, parallel group study, asthmatics with moderate to severe disease used budesonide (400 mcg/day) for 5 weeks (run-in period); then they were randomized to use budesonide (800 mcg/day — BUD group) or budesonide plus formoterol (400 mcg and 24 mcg/day, respectively — FORMO group) for 9 weeks (treatment period). Home PEF measurements, symptom daily reporting, spirometry, sputum induction (for differential cell counts and sputum cell cultures), and hypertonic saline bronchial challenge test were performed before and after treatments. TNF-α, IL-4 and eotaxin-2 levels in the sputum and cell culture supernatants were determined. Morning and night PEF values increased in the FORMO group during the treatment period (p < 0.01), from 435 ± 162 to 489 ± 169 and 428 ± 160 to 496 ± 173 L/min, respectively. The rate of exacerbations in the FORMO group was lower than in the BUD group (p < 0.05). Neutrophil counts in sputum increased in both groups (p < 0.05) and leukocyte viability after 48 h-culture increased in the FORMO group (p < 0.05). No other parameter changed significantly in either group. This study showed that adding formoterol to budesonide improved home PEF and provided protection from exacerbations, although increase of leukocyte viability in cell culture may be a matter of concern and needs further investigation.

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Introduction
Asthma is a chronic inflammatory airway disorder characterized by reversible airflow obstruction and bronchial hyperresponsiveness to a variety of stimuli; several types of cells and mediators are involved in its pathophysiology. The mainstay of persistent asthma management is the regular use of inhaled corticosteroids (ICSs); they modulate many inflammatory processes by reducing the number of inflammatory cells in the airways of asthmatic subjects and by inhibiting the release of inflammatory mediators, contributing to the achievement of clinical control. Nonetheless, there is concern about possible systemic side effects of these drugs, so it is advised that the minimum dose needed to control symptoms should be used. Other largely used controller drugs are the long-acting β2-agonists (LABAs), which are considered to be smooth muscle relaxants, while their action upon inflammation is still a matter of debate. Although LABAs must not be used as monotherapy in asthma treatment, it has been shown that subjects still symptomatic while taking only ICSs achieve greater clinical control and better lung function with addition of LABA rather than with increasing the dose of ICS. Moreover, the association of these drugs exposes the patients to less risk of ICS systemic side effects. In addition, some authors have shown (both in vitro and in vivo) that the association of the drugs may have beneficial effects even on inflammatory parameters.

The safety of LABAs has been questioned since their introduction as controller therapy, and a recent multicenter study showed greater mortality in the group of patients who used this class of drug, strengthening the controversy. Furthermore, some studies have shown that LABAs may have a pro-inflammatory effect, and their addition to ICSs may mask ongoing inflammation while offering satisfactory symptomatic relief.

The aim of the present study was to observe clinical and inflammatory outcomes of two 9-week treatment regimens: budesonide, 800 μg daily and budesonide, 400 μg daily plus formoterol, 24 μg daily in patients who were previously taking budesonide 400 μg daily.

Materials and methods

Subjects
Male and female asthmatic patients aged 18–60 years were recruited from the asthma clinic of the University Hospital (University of São Paulo, Ribeirão Preto, Brazil). All of them were considered to suffer from asthma with positive bronchial challenge test and forced expiratory volume in 1 s (FEV₁) ≥60% of the predicted value at baseline evaluation. Exclusion criteria included current smoking or a history of more than 10 pack-years smoking, respiratory disorders other than asthma, exacerbation, airway infections and/or use of systemic corticosteroids during the previous 4 weeks.

Study design
This was a double-blind, randomized, parallel group study consisting of a run-in period and a treatment period. During the open-label run-in period, all patients received inhaled budesonide 200-μg bid as control medication for 5 weeks and were then randomized to a 9-week treatment with twice daily inhalations of either budesonide 400 μg (BUD group) or budesonide 200 μg plus formoterol 12 mcg (FORMO group).

Budesonide was administered in 200 μg doses, thus BUD group was treated with two 200 μg-doses bid (i.e., 800 μg/day) and FORMO group also received two doses (one 200 μg budesonide dose and one 12 μg formoterol dose) bid. Dry powder inhaler (aerolizer) system was used for both budesonide and formoterol. Inhaled albuterol was used as the only reliever drug throughout the study. Treatment with other anti-asthma drugs was not allowed.

All patients were evaluated in a first visit (visit 1), in which the investigators assessed the fulfillment of inclusion criteria, provided the run-in period medication, symptom diary cards and peak expiratory flow (PEF) meters. Patients were instructed to perform PEF measurements and symptom diary recordings daily during weeks 3 and 4. By the end of week 4, the patients were evaluated in visit 2, in which they underwent spirometry and induction of sputum. During week 5, they used the same run-in medication and by the end of that week they underwent a hypertonic saline (HS) challenge test and were randomized to one of the treatment groups in visit 3. By the end of week 9, in visit 4, they underwent spirometry, received symptom diary cards, and were instructed to record symptoms and PEF measurements daily throughout weeks 12 and 13. By the end of week 13, another spirometry and sputum induction were performed in visit 5; all patients kept using the same treatment during week 14, by the end of which a HS challenge test was performed, in visit 6. The randomized code was withheld from the investigators until the completion of the study. The study was approved by the Ethics Committee of the University of São Paulo Medical School at Ribeirão Preto, and all subjects gave written informed consent.

Peak expiratory flow measurements and symptom diary cards recordings
The patients were carefully instructed about the use of the Mini-Wright PEF meters (Clement Clarke, Essex, UK). The measurements were performed prior to the inhalation of any medication, and the highest value of three consecutive measurements was recorded both in the morning and at night. The variability of PEF measurements was calculated using the formula:

$$\Delta\text{PEF} = \frac{\text{night PEF} - \text{morning PEF}}{\text{night PEF}} \times 100$$

Patients also recorded symptoms of asthma on symptom diary cards.

Spirometry
All spirometry measurements were performed by the same investigator, in the morning, using a Koko pneumotachograph and software (PDS Instrumentation, Inc., Louisville, Colorado, USA). Post-bronchodilator measurements were performed 15 min after the inhalation of 200 μg of
albuterol. The patients were instructed not to use asthma medications during the 12 h that preceded the procedure.

**Sputum induction and processing**

The guidelines of the Task Force on Induced Sputum of the European Respiratory Society were applied. All patients had an FEV₁ ≥ 60% immediately before the procedure. After pre-medication with 200 μg of inhaled albuterol and gargling of nistatin (to minimize fungus contamination), sputum induction was performed by inhalation of a hypertonic saline (4.5% NaCl) aerosol delivered by an ultrasonic nebulizer (Ultra-Neb; DeVilbiss, Somerset, PA, USA). The inhalation duration was 20 min, divided into four 5-min periods. For safety reasons, a PEF measurement was performed every 5 min and the procedure was stopped if the PEF fell to the critical value (a 10% fall from the basal value) or if there were bothersome symptoms. If a subject tolerated less than 20 min of induction in visit 1, then the duration of induction in visit 2 would be the same as in visit 3. During the induction, the subjects were encouraged to spit saliva into a plastic container and sputum into another pre-weighed sterile one anytime they wished. The saliva was discarded and the sputum was weighed; 1 mL of 0.1% dithiothreitol (DTT; GIBCO BRL, Grand Island, NY, USA) per gram of sputum was added and the suspension was shaken in a vortex mixer for a few seconds and incubated in a shaking water bath (Dubnoff TE-053; TECNAL, Piracicaba, SP, Brazil) at 37 °C (150 cycles/min) for 15 min, with periodic brief aspirations. The sample was centrifuged at 750×g for 10 min (Allegra™ 21R Centrifuge; Beckman, Palo Alto, CA, USA) and the supernatant was aspirated and stored at −85 °C. The cell pellet was re-suspended in 1 mL of culture medium containing 300 mg/L glutamine, 100 U/mL penicillin G, 100 μg/mL streptomycin sulphate (RPMI-1640; GIBCO BRL, Grand Island, NY, USA) and 10% calf serum. Total cell number and cell viability were determined by the Trypan blue exclusion method in a Neubauer chamber (Fein BRL, Grand Island, NY, USA) and the supernatant was aspirated and stored at −85 °C until the dosages of mediators were performed.

**Hypertonic saline challenge test**

The HS challenge tests were carried out according to the protocol developed by Anderson and Brannan. The ultrasonic nebulizer (De Vilbiss Ultra-neb 2000; DeVilbiss, Somerset, PA, USA) was connected to a two-way non-rebreathing valve (Hans Rudolph 2700 series; Kansas City, MO, USA) via 100 cm of corrugated aerosol tubing (internal diameter = 2.2 cm, smooth internal surface) with a rubber mouthpiece and a nose clip. The nebulizer canister was filled with 200 mL of 4.5% NaCl, and the output was set to maximum. Previous studies have shown that the output of this nebulizer system, at tidal volumes of 300–500 mL and respiratory rates of 12–20/min, ranges from 1.9 to 2.5 mL/min, with a particle size distribution of mass median aerodynamic diameter between 2.33 and 2.87 μm. After three reproducible measurements of baseline FEV₁, subjects breathed through the valve with the nebulizer switched off for 2 min and then started breathing the HS aerosol with an initial exposure period of 30 s. After this first inhalation of 30 s, subjects breathed increasing doses of HS by doubling the duration of nebulization (1, 2, 4, and 8 min). FEV₁ was measured in duplicate, 90 s after breathing through the valve and after each inhalation. If FEV₁ fell less than 10% of baseline, the exposure time was doubled. If the reduction of FEV₁ was more than 10% and less than 20%, the exposure time was repeated rather than doubled. The test was stopped when a fall of 20% or more was obtained or with a total exposure time of 15.5 min. Then, 200 μg albuterol was administered. The nebulizing canister and tubing were weighed before the challenge and after the final inhalation step using an electronic balance (AS 2000; Marte, São Paulo, SP, Brazil) to assess the total amount of aerosol nebulized and the nebulizer output. Patients were questioned about the intensity of dyspnea at every inhalation. The tests were performed with a Koko pneumotachograph and software. All patients were asked to withhold all inhaled drugs 12 h before the tests.

**Measurement of inflammatory mediators**

The concentrations of interleukin-4 (IL-4), tumor necrosis factor-α (TNF-α) and eotaxin-2 in sputum fluid and cell culture medium were determined by ELISA (R&D systems, Minneapolis, MN and Pharmingen, San Diego, CA, USA).

**Statistical analysis**

Data are expressed as mean ± standard deviation, unless otherwise specified. Intra-group comparisons between run-in and treatment periods were performed by Wilcoxon...
matched-pairs test. Fisher exact test was used to compare proportions between groups. Correlations were calculated by Pearson correlation. A $p$ value of $\leq 0.05$ was considered to be statistically significant.

Results

Thirty-two patients were randomized: 19 to the BUD group and 13 to the FORMO group. In the BUD group and the FORMO group, eight and one patients, respectively, were excluded due to exacerbations, and the rates of exacerbations were significantly different between the groups ($p < 0.05$; Fisher exact test). One and two patients abandoned the BUD and the FORMO groups, respectively. Twenty patients completed both the study periods, 10 in each group. Table 1 shows the characteristics of these 20 patients at the end of the run-in period; there were no significant differences between the groups.

Clinical, functional and inflammatory parameters

In the BUD group, there were not statistically significant differences in clinical, functional or inflammatory parameters in the comparison of the run-in and the treatment periods, whereas in the FORMO group, there were increases in morning and night PEF in the treatment period (Table 2). The increase in morning PEF during the treatment period correlated with the bronchodilator response during the run-in period in the BUD group ($r = 0.78; p = 0.008$) as well as in the FORMO group ($r = 0.72; p = 0.018$).

Induced sputum

Fig. 1 shows eosinophil counts in the run-in and treatment periods in both groups; there was no significant difference between the run-in and the treatment periods in any group. Table 3 shows the differential cell counts in sputum in the run-in and treatment periods in both groups; there was an increase in neutrophil counts in both groups during the treatment period.

Cell cultures

The percentages of viable cells (vitalities) after culture were similar in the run-in and treatment periods in both groups, except for the non-stimulated non-adherent cells in the FORMO group, in which there was a raise in viability in the treatment period compared to the run-in period; run-in: 87.4 (7.2)%; treatment: 93.3 (3.9)%; $p < 0.05$.

Cytokines concentrations

There were no statistically significant differences between the periods in any group concerning the concentrations of TNF-$\alpha$, IL-4 or eosetin-2 in sputum supernatants and cell culture supernatants.

Discussion

In the present study, the addition of either 24 $\mu$g of formoterol or 400 $\mu$g of budesonide per day failed to further modify most inflammatory parameters compared to the use of budesonide 200 $\mu$g bid in patients with moderate and severe asthma, although the association of drugs improved lung function and reduced exacerbations. The superiority of the “add-on” therapy in improving symptoms and functional parameters has been previously demonstrated, and addition of a LABA to daily regimen of ICSs is the recommended step when patients do not achieve clinical control while taking medium dose of inhaled corticosteroids, according to recent protocols. Some authors believe that the add-on therapy is better than the rising of ICS doses even when patients are not clinically stable on low doses of ICSs. Our data illustrated the rising of PEF measurements, but failed to demonstrate any rise in FEV$_1$ during the use of formoterol plus budesonide. This may have happened because the use of formoterol was withheld before spirometry, but not before PEF measurements; the latter reflects more properly the actual functional status of asthmatics who take bronchodilators on a regular basis, and that is why some authors have used PEF as a primary end-point on studies assessing LABA efficacy. Doubling the dose of ICSs did not result in any improvement of lung function, perhaps because of a flat dose-response curve of ICSs (as mentioned below).

We showed significant correlation between magnitude of bronchodilator response in the run-in period and improvement of PEF during the treatment period in both groups. It seems that individuals using low dose of budesonide whose response to bronchodilator is greater may have bigger functional improvement when treatment is stepped-up.

We chose an indirect challenge test (hypertonic saline) instead of a direct challenge test because indirect stimuli act on inflammatory cells, not on effector cells, so they probably reflect more properly the inflammatory status of the Airways. The decrease of airway responsiveness to

### Table 1 Characteristics of the subjects

<table>
<thead>
<tr>
<th></th>
<th>BUD group</th>
<th>FORMO group</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>43.0 (10.6)</td>
<td>40.1 (14.0)</td>
<td>0.50</td>
</tr>
<tr>
<td>Sex (male/female), n</td>
<td>4/6</td>
<td>6/4</td>
<td>0.65</td>
</tr>
<tr>
<td>FEV$_1$, (%)</td>
<td>81.2 (9.3)</td>
<td>85.8 (9.2)</td>
<td>0.16</td>
</tr>
<tr>
<td>Morning PEF, (%)</td>
<td>83.6 (5.6)</td>
<td>93.4 (22.0)</td>
<td>0.37</td>
</tr>
<tr>
<td>Night PEF, (%)</td>
<td>85.8 (8.7)</td>
<td>91.8 (21.7)</td>
<td>0.86</td>
</tr>
<tr>
<td>$\Delta$PEF, (%)</td>
<td>2.2 (6.1)</td>
<td>1.8 (3.8)</td>
<td>0.08</td>
</tr>
<tr>
<td>PD20 HS (g)</td>
<td>19.3 (10.7)</td>
<td>13.1 (10.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>Symptom-free days (%)</td>
<td>71.4 (40.4)</td>
<td>77.1 (31.5)</td>
<td>0.50</td>
</tr>
<tr>
<td>Sputum eosinophils (%)</td>
<td>8.2 (15.7)</td>
<td>11.2 (18.8)</td>
<td>0.36</td>
</tr>
<tr>
<td>BD response (%)</td>
<td>10.8 (6.6)</td>
<td>7.3 (7.3)</td>
<td>0.24</td>
</tr>
</tbody>
</table>

All data, except sex, are presented as means (standard deviation).

FEV$_1$, (%): forced expiratory volume in one second (percentage of predicted) on visit 2; PEF, (%): mean peak expiratory flow (percentage of predicted) in the 7 days before visit 2; $\Delta$PEF: mean daily variation of PEF in the 7 days before visit 2; PD20 HS: hypertonic saline mass that provoked a 20% fall in FEV$_1$ in visit 3; Symptom-free days (%): percentage of days free of asthma symptoms during the 14 days that preceded visit 2; Sputum eosinophils (%): percentage of eosinophils in sputum in visit 2; BD response: FEV1 percent increase after albuterol use in visit 2.
HS during the use of corticosteroids has been reported previously, but in our study there was not such an effect in any group. Perhaps that happened because the ICS and/or LABA doses were not sufficient to provide more bronchoprotective effect than the dose of the run-in period. We do not believe that the length of the treatment period was too short since other authors have shown that 8 weeks of therapy (or even less) are enough to achieve an effect on hyperresponsiveness.

The action of LABAs on inflammation of the airways is still a matter of debate. McIvor et al. have demonstrated that when the dose of ICSs was gradually reduced, asthmatic patients taking high doses of ICSs plus salmeterol (a LABA) suffered from little clinical deterioration despite a marked rise in sputum eosinophils, indicating that LABAs may “mask” ongoing inflammation. Such a masking effect may be the reason why our FORMO group had fewer exacerbations; i.e., perhaps the symptomatic relief prevented them from noticing a worsening of airway inflammation.

Nevertheless, other authors have shown some anti-inflammatory properties of LABAs, both in vitro and in vivo. In one of these studies, patients who were taking only an ICS as controller medication were randomized to one of three groups, in which they took, in addition to the dose of ICSs they were already taking, respectively: salmeterol, fluticasone, or placebo for 12 weeks. Endobronchial biopsies before and after the treatment regimens evidenced that only the ones who took salmeterol had eosinophilic cationic protein (ECP)-positive eosinophil counts decrease on the bronchial wall. In another study, asthmatic subjects treated with a fixed dose of ICS underwent treatment with an equivalent dose of fluticasone plus salmeterol for 12 weeks; this intervention resulted in a lowering of mast cells number on the bronchial submucous layer, as assessed by comparison of endobronchial biopsy specimens before and after the treatment period.

On the other hand, several studies failed to demonstrate pro- or anti-inflammatory properties of the association of drugs, similarly to our data. Some authors conducted a study in which asthmatic subjects took a high dose of budesonide daily during a run-in period and were then randomly assigned to low dose of budesonide plus formoterol or medium dose of budesonide. There was no deterioration of inflammatory parameters in any of the groups, so the authors concluded that the association of drugs does not lead to worsening of inflammatory status.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>BUD group</th>
<th>FORMO group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Run-in</td>
<td>Treatment</td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td>81.2 (9.3)</td>
<td>81.5 (9.1)</td>
</tr>
<tr>
<td>Morning PEF (% predicted)</td>
<td>83.6 (5.6)</td>
<td>90.4 (12.7)</td>
</tr>
<tr>
<td>Night PEF (% predicted)</td>
<td>85.8 (8.7)</td>
<td>91.4 (15.3)</td>
</tr>
<tr>
<td>Morning PEF (L/min)</td>
<td>348.0 (72.9)</td>
<td>375.1 (84.9)</td>
</tr>
<tr>
<td>Night PEF (L/min)</td>
<td>356.2 (74.7)</td>
<td>380.0 (99.2)</td>
</tr>
<tr>
<td>ΔPEF (%)</td>
<td>2.2 (6.1)</td>
<td>0.6 (6.6)</td>
</tr>
<tr>
<td>PD20 HS (g)</td>
<td>19.3 (10.7)</td>
<td>20.5 (11.8)</td>
</tr>
<tr>
<td>Symptom-free days (%)</td>
<td>71.4 (40.4)</td>
<td>1.9 (4.1)</td>
</tr>
<tr>
<td>Sputum eosinophils (%)</td>
<td>8.2 (15.7)</td>
<td>3.9 (3.4)</td>
</tr>
<tr>
<td>BD response (%)</td>
<td>10.8 (6.6)</td>
<td>11.8 (9.5)</td>
</tr>
</tbody>
</table>

All data are presented as mean (standard deviation).

FEV₁ (%): forced expiratory volume in one second (percentage of predicted) on visit 2 (run-in) and on visit 5 (treatment); PEF (%): mean peak expiratory flow (% predicted) in the 7 days before visit 2 (run-in) and before visit 5 (treatment); Δ PEF: mean daily variation of PEF in the 7 days before visit 2 (run-in) and before visit 5 (treatment); PD20 HS: hypertonic saline mass that provoked a 20% fall in FEV₁ on visit 3 (run-in) and on visit 6 (treatment); Symptom-free days (%): percentage of days free of asthma symptoms during the 14 days that preceded visit 2 (run-in) and visit 5 (treatment); Sputum eosinophils (%): percentage of eosinophils in sputum on visit 2 (run-in) and on visit 5 (treatment); BD response: FEV₁ percent increase after albuterol use on visit 2 (run-in) and on visit 5 (treatment).

*p < 0.01 in the run-in period vs. treatment period.

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**Figure 1**

Individual values of eosinophil counts (in percentage of leukocytes) in the Run-in and Treatment periods in (A) BUD group and (B) FORMO group. Horizontal lines indicate means, $p > 0.3$ in both groups for the comparison between Run-in and Treatment periods (Wilcoxon).
In another study, asthmatics on medium dose of fluticasone for 4 weeks were randomly assigned to low dose of fluticasone plus salmeterol or to the same medium dose of fluticasone for 24 weeks. The evaluation of specimens obtained by endobronchial biopsies performed at the end of the run-in period and at the end of the treatment period failed to demonstrate changes of inflammatory parameters in any group.24

In our study, there was no change of sputum eosinophil counts when the ICSs dose was increased or LABA was added to low dose of ICSs. It is possible that the low dose of budesonide had already exerted its maximal anti-inflammatory effect during the run-in period, so there was no benefit in increasing the anti-inflammatory treatment. This is in concordance with previous studies that demonstrated that ICSs have a flat dose-response curve, providing maximal benefits on low or moderate doses.25–27

The differential sputum cell counts revealed that both treatment groups had their neutrophil counts increased after the treatment period. The role of neutrophils in the pathophysiology of asthma is uncertain, although it is believed that they are involved in exacerbations and that some corticosteroid-resistant populations have higher levels of neutrophilic inflammation. Some authors concluded that LABAs have some antineutrophilic action,28,29 but our findings do not corroborate that hypothesis.

Sputum cell culture is not routinely used to assess inflammation in asthma and, to our knowledge, this is the first study in which it was employed to compare inflammation under distinct inhalation therapies. Some authors and ourselves have used the method to assess production of inflammatory mediators in vitro30–36; nevertheless, untreated patients were compared to treated ones, or stable asthmatics to those who underwent bronchial challenge or suffered from an exacerbation. So, it is still unknown the role of cell culture to compare two treated groups.

We observed that cells from individuals in the FORMO group demonstrated enhanced vitality. This may reflect some pro-inflammatory action of formoterol. This hypothesis is in accordance with the findings of Kankaanranta et al., who studied blood cell cultures and concluded that the eosinophils derived from asthmatic patients not taking corticosteroids survived longer than the ones derived from normal subjects. They also reported that incubation of asthmatics’ blood cell cultures with salmeterol inhibited eosinophil apoptosis (programmed cell death), therefore prolonging the survival of these important inflammatory cells and potentially exerting some pro-inflammatory effect.27

The levels of inflammatory mediators in sputum and cell culture medium did not rise or decrease in any treatment group, suggesting that no further anti-inflammatory effect was obtained with any treatment strategy compared to the run-in treatment regimen, perhaps because of the flat dose-response curve of ICSs and a lack of anti-inflammatory effect of LABAs.

In conclusion, our findings suggest that adding a LABA to low-dose ICS or doubling the ICS dose does not modify significantly the inflammatory status of asthmatics with moderate to severe disease. This may be due to the flat dose-response curve of ICS or to the fact that these patients need even higher doses. The sputum cell culture evaluations led to some concern about pro-inflammatory effects of LABAs, but further studies are necessary to assess action of commonly used drugs on inflammatory airway cells. We also showed that the add-on therapy protects from exacerbations and improves functional parameters.

### Conflict of interest

All authors report no financial or other potential conflicts of interest.

### Acknowledgements

We gratefully acknowledge Luciana Cristina Straccia for technical assistance and Dr. Margaret de Castro for loaning of laboratory resources.

### References


### Table 3 Differential cell counts in the run-in and treatment periods

<table>
<thead>
<tr>
<th>Protein Group</th>
<th>Run-in</th>
<th>Treatment</th>
<th>Run-in</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cells (%)</td>
<td>12.8 (8.5)</td>
<td>12.6 (8.7)</td>
<td>16.0 (13.9)</td>
<td>17.3 (16.8)</td>
</tr>
<tr>
<td>Eosinophils (%)</td>
<td>8.2 (15.7)</td>
<td>3.9 (3.4)</td>
<td>11.2 (18.8)</td>
<td>4.3 (2.9)</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>29.0 (14.1)</td>
<td>39.2 (11.0)*</td>
<td>23.0 (12.6)</td>
<td>39.3 (18.1)*</td>
</tr>
<tr>
<td>Macrophages (%)</td>
<td>55.4 (20.0)</td>
<td>49.1 (13.0)</td>
<td>56.9 (19.2)</td>
<td>44.9 (18.2)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>7.3 (4.7)</td>
<td>7.1 (5.8)</td>
<td>7.7 (4.3)</td>
<td>9.29 (4.6)</td>
</tr>
</tbody>
</table>

Data are presented as means (standard deviation). Values are percentage of total cells (for squamous cells) or percentage of leukocytes (for leucocytes).

*p < 0.05 in the run-in period vs. treatment period.


