Clinical, functional and inflammatory evaluation in asthmatic patients after a simple short-term educational program: a randomized trial

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Abstract

This study aimed to evaluate the clinical evolution, functional parameters and inflammatory activity of asthma in patients who submitted to an educational intervention. 58 adult patients over 18 years of age with partly controlled and uncontrolled asthma were randomized into an intervention group (IG) (N = 32) and a control group (CG) (N = 26) and evaluated for 12 weeks. The Asthma Control Test (ACT), Asthma Control Questionnaire (ACQ), Asthma Quality Life Questionnaire (AQLQ) and Beck Depression Inventory (BDI) questionnaires were applied. Spirometry, exhaled nitric oxide (NO), exhaled breath condensate (EBC) and induced sputum (IS), measurement of the peak flow and symptoms were performed. The IG patients received an educational activity for 30 minutes applied by a nurse. Statistical analysis: analysis of variance with repeated intragroup measures. IG presented a decreased number of eosinophils in IS and IL-17A in EBC, an increase in the percentage of FEV$_1$ after bronchodilatation and an improvement in quality of life compared to the CG. There was an improvement in depression levels and a decrease in IL-4 and IL-5 in the IS and in the EBC in the IG compared to the CG. Our results suggest that an educational intervention can bring benefits concerning the control of inflammation, lung function alterations, quality of life and levels of depression in asthmatic patients. Registration: ClinicalTrials.gov; NCT03655392.

Introduction

It is estimated that approximately 300 million individuals worldwide are affected by asthma, and the global prevalence can vary from 1 to 18% $^1$. Chronic inflammation in asthma is a consequence of the participation of several mediators that lead to cell infiltration and remodeling in the bronchial helper (Th) 2-type cytokines are associated with an important role in asthma-related inflammation $^2$.

Thus, the main goal of asthma treatment is to reduce inflammation in the airways and consequently control the disease and its symptoms. However, studies have found that more than 80% of asthmatic patients do not use their inhalation device correctly. The correct use of these drugs is associated with better efficacy and fewer collateral effects related to therapy $^3$.

Inhaled corticosteroids (ICSs) are the basis of treatment for most cases of asthma. Its constant use is associated with an improvement of symptoms and a reduction of morbidity and mortality related to disease $^1$.

The asthma education program is considered one of the fundamental pillars for adherence and proper treatment of the disease. It is estimated that more than half of patients treated for asthma do not have adequate adherence to the prescribed medication or do not perform the treatment properly $^1$.

Thus, the objectives of this study were to assess asthma control, quality of life, levels of depression, lung function and inflammatory parameters of patients with asthma before and after an educational intervention.
Methods

Study design. This study was a prospective, randomized, controlled trial approved by the Hospital Research Ethics Committee of the University of Sao Paulo (protocol number: 11496/14); the recruitment and protocol was carried out between 2015/2017. It was registered at ClinicalTrials.gov, registration number NCT 03655392, first registered in 03/28/2018 (retrospectively recorded) (https://clinicaltrials.gov/ct2/show/NCT03655392).

Individuals with a clinical asthma diagnosis were recruited from an outpatient allergy and immunology clinic at a university hospital. Written informed consent was obtained from all participants included in the study. All participants received explanations, signed and received a copy of the written consent form. Participation was voluntary and participants were free to withdraw from the study at any stage.

Eligibility criteria. Adults between 18-69 years of age with clinical diagnosis of asthma; asthma with partly controlled or uncontrolled symptoms; continuous medical follow-up and patients using ICS with or without LABA (long-acting beta-agonist) for at least one year; increase in FEV₁ of >12% and >200 ml from baseline 10-15 minutes after 400 mcg Salbutamol; dose of ICS stable in the last eight weeks before recruitment; and nonsmokers or ex-smokers less than or equal to 10 pack-years.

Exclusion criteria: clinical asthma diagnosis and upper respiratory tract infection less than 30 days before; systemic steroids within four weeks of enrollment; pregnancy; other lung or uncontrolled chronic disease and chronic obstructive pulmonary disease (COPD) according to the Global Initiative for COPD (GOLD).

Outcome measures. Individuals were recruited after regular medical visits in outpatient clinics, by the researchers and physicians, after routine outpatient medical care. The study protocol was explained to the possible participant, and signed informed consent was requested (day 0 – baseline = T0) within 28 days. Subjects were followed for 56 days at three visits every 4 weeks (day 1=T1, day 28=T2 and day 56=T3) (Fig. 1).

At the first visit (T1), the patients were randomized in the order of inclusion in a 1:1 ratio for the IG or CG groups. The symptom diary and a manual and portable peak flow meter (Mini-Wright, Clement Clark International) were delivered to all patients. Then, they answered the following questionnaires administered by a blind investigator: Asthma Control Test (ACT), Asthma Control Questionnaire, Asthma Quality Life Questionnaire (AQLQ) and Beck Depression Inventory II (BDI II). After, individuals underwent spirometry for the collection of exhaled breath condensate (EBC) and induced sputum (IS). All these data were collected from both groups at the three study visits (T1, T2 and T3).

Additionally, IGs were submitted to educational intervention at all three visits (T1, T2 and T3) and received phone calls every two weeks, reinforcing the educational content and the date of return. CGs were only advised as to the date of return and received phone calls two days before to confirm attendance.
Educational intervention was applied by a trained nurse in a 30-minute session. Its main objective was to verify whether the patient was using the ICS properly and, if necessary, to teach the correct inhalation technique. It included the asthma education components recommended in the Global Initiative for Asthma (GINA) main report\(^1\) and a simplified explanation of this disease and its main characteristics, guidance on inhalation technique using illustrations, a script and an explanatory leaflet.

Skills for correct medication inhalation from a metered-dose inhaler and for peak flow measurement were taught and practiced. The inhalation technique was explained and demonstrated to the patients. As a complementary material, a four-minute explanatory video addressing the correct inhalation technique was shown (available at http://www.incor.usp.br/sites/incor2013/videos/asma-dpoc/). Environmental control was also briefly addressed, and IG subjects were guided on measures to avoid exposure to allergens and irritants.

Finally, patients were invited to demonstrate the inhalation technique. When necessary, the technical errors were corrected by the nurse until the inhalation technique was performed correctly. This activity was performed considering the particularities of each patient.

The symptoms of asthma and peak flow measurement were recorded in a diary of symptoms already used by patients in hospital outpatient clinics\(^10\). Patients registered these data twice a day in the morning and evening before using ICS. Symptom-free days were accounted for by each asthma symptom individually (cough, wheezing, shortness of breath, waking up at night for asthma and use of rescue medication). At each new visit (T2 and T3), patients returned the symptom diary and the annotation of the peak flow values.

**Clinical evaluation.** To access the level of asthma control, the ACT questionnaire scores were used\(^5,6\). To evaluate clinical parameters, the ACQ-7 was applied\(^7\) considering the week before patients fill it out.

Asthma quality of life was assessed using the AQLQ; a higher score indicates a better quality of life\(^11\). A minimal clinically important difference (MCID) of 0.5 was used. The MCID indicates the minimal difference in mean scores that is regarded as important\(^11\).

The BDI was used to assess depression levels, it's a scale that has been used to evaluate related symptoms of depression in the last four weeks. The higher the score, the worse the severity of the symptoms\(^9,12,13\).

**Functional evaluation.** Spirometry was also performed at the three study visits and was analyzed according to the acceptability and reproducibility criteria recommended by ATS/ERS (2005)\(^14\); a Koko spirometer (N Spire Health, Inc; Longmont, CO, USA) was utilized.

Peak expiratory flow (PFE) measurement was performed after NO collection and after each inhalation. The patients were also instructed to perform this measurement three times, twice a day, morning and evening, before the use of the ICS, and to note down the three measurements in the symptom's diary.
**Inflammatory parameters.** Sputum was induced by standard methodology\(^{15,16}\). Patients were asked to inhale 400 \(\mu\)g Salbutamol via a metered-dose inhaler 20 minutes before induction and then inhaled hypertonic saline (3\% NaCl) for seven minutes; peak flow and symptoms were evaluated before and after each inhalation. A Devilbiss Ultraneb 99 (Devilbiss Corp., Somerset, PA, USA) ultrasonic nebulizer was used.

The measurement of FeNO (fractional exhaled nitric oxide) was performed by chemiluminescence (Sievers 280) according to the recommendations of the ATS\(^{17}\). The patients were instructed to blow into a Mylar balloon with an expiratory pressure supported in 12 cmH\(_2\)O and a stable flow of 200 ml/s. This procedure and analysis were performed by a blinded investigator.

EBC (exhaled breath condensate) was collected for 15 to 20 minutes in the current volume to measure cytokines\(^{18}\). A Turbo capacitor (DECCS® Medivac SRL, Italy) cooled to -20°C for at least thirty minutes was used. The individuals were instructed to perform oral breathing using the mouthpiece of the equipment and with the aid of a nasal clip.

The levels of interleukin (IL)-4, IL-5 and IL-17A were quantified in IS supernatant and EBCs. ELISA (enzyme-linked immunosorbent assay) (EL Human ISA Max Deluxe, Biolegend, San Diego, CA)\(^{19}\) was utilized. The tests were performed according to the manufacturer's guidelines; the samples were analyzed in an ELISA reader (Polaris model, Celer Biotechnology SA, Brazil).

**Statistical analysis.** Descriptive analysis was performed on all patient data (means, standard deviations and medians). Differences between groups were analyzed using Student's t-test and one-way repeated measures analysis of variance (ANOVA). Intragroup analysis was compared by means of repeated measurement ANOVA. The confidence interval was 95\% (\(p<0.05\)). A statistical package was used for tests (Sigma Stat 3.11, San José, CA, USA) – descriptive analysis, differences between groups and intragroup, sample calculation) and SPSS 20.0 (odds ratio (OR) and chi-square). To calculate the odds ratio, the rates of change were compared between the groups (IG/CG) at different times of assessment: T1-T2, T2-T3, T1-T3 (95\% confidence interval).

Sample size estimates were based on the estimated effect of the intervention on eosinophils in induced sputum according to a previous study\(^{20}\). The sample size calculation took into account a minimum mean difference of 67\% for eosinophils, with an expected difference in means of 8 and a standard deviation of 10, test power of 80\% and alpha of 0.05, thus resulting in a requirement of 13 patients in each group.

**Results**

**Patient disposition.** A total of 140 individuals who met the inclusion criteria were assessed for eligibility. During the protocol, 76 patients were excluded or gave up the protocol due to difficulties in scheduling the exams or related problems. Three patients were removed from the study for having presented slight
respiratory discomfort after inhalation with hypertonic saline solution, and 6 were removed for insufficient sputum samples. Fifty-eight patients completed the entire study (IG; n=32) (CG; n=26) (Fig. 2).

**Clinical parameters.** In the evaluation of quality of life (AQLQ), we found an improvement in IG patients when comparing T1 with T2 or T3 moments (Fig. 3A, Table 2) (*p<0.05). There were no differences between T1, T2 or T3 in CG.

Regarding depression levels, IG patients presented improvement at T2 (one month). In the CG, this improvement occurred only in the T3 (two-month) analysis (*p<0.05) (Fig. 3B, Table 2).

No significant differences were observed in relation to asthma control when the ACT questionnaire was applied, although an increase of 2.5 points was observed in IG patients. In the ACQ analysis, no significant differences were found in symptom-free days or daily measurement of peak flow (Table 2).

**Functional evaluation.** A significant difference was observed in the pre-BD FEV$_1$ between T1 and T3 (p=0.010) and post-BD FEV$_1$ between T1 and T3 (p=0.014) (Table 3).

In the analysis of post-BD VEF$_1$ (%) at T2 and T3, a difference was observed (*p<0.05) (Fig. 3D, Table 3).

**Inflammatory parameters.** Both groups presented similar patient characteristics at baseline (T0). When classified according to cellularity in the induced and endotype sputum, most patients had paucigranulocytic asthma (69%) (Table 1).

A significant decrease in the eosinophil number was observed at T3 (two months) in the IG (*p<0.05) (Fig. 3C, Table 3). About NO measurement, there was no difference between the two groups (Table 3).

Regarding the IG, we observed improved IL-4 and IL-5 values in the evaluations at T2 and T3 in both the IS and EBC (Fig. 4, A, B, C and D). In the CG, the same occurred (Fig. 4, A, B and D), with the exception of the IL-4 EBC measurement, where an improvement was observed only at T3 (Fig. 4C) (*p<0.05) (Table 3).

Regarding the EBC analysis of interleukin IL-17A, there was a decrease in the IG group in the T3 evaluation when compared with T2 (Fig. 4E, Table 3) (*p<0.05). In the IS supernatant, it was not possible to analyze the IL-17 data in either group since the values were lower than the detection curve.

**Effects of the educational intervention – odds ratio.** The effect of the educational intervention was compared between the groups (IG and CG) through the odds ratio and chi-square test among the 3 study visits (T1, T2, T3), including clinical, functional and inflammatory parameters (Table 4).

A significant difference was observed in the percentage of: ACQ between T1 - T3 (1.293 – 11.383, OR 3.911, p= 0.014); FEV$_1$ before BD among T1 - T3 (1.382 – 12.493, OR 4.156, p=0.010); FEV$_1$ after BD among T1 - T2 (1.806 – 18.080, OR 5.714, p= 0.002) and T1 - T3 (1.042 – 8.953, OR 3.055, p= 0.039); eosinophils in IS between T1 – T2 (1.060 – 10.482, OR 3.300, p= 0.036), T2 - T3 (1.742 – 17.714, OR 5.556, p= 0.003) and T1 -T3 (1.627 – 17.921, OR 5.400, p= 0.004) (Table 4).
Discussion

In the present study, we found that asthmatic patients with the disease partly controlled and uncontrolled who underwent an educational intervention showed improved clinical control of asthma, pulmonary function, markers of airway inflammation, and quality of life and decreased levels of depression.

Some previous studies have shown that educational activities and self-management related to asthma features have been associated with better control of the disease in both adults and children. Boulet et al. (2015) showed significant improvements after an educational program, such as a reduction in unscheduled visits, inappropriate use of medications and an increase in FEV$_1$ in mild to moderate asthmatic patients after one year of follow-up. In another study, asthmatic and COPD patients showed better clinical control and lung function after submitting to a 6-8 follow-up educational program. In our study, we also found an increased FEV$_1$ after 1 month of educational intervention follow-up.

In our study, we observed better results in the percentage of FEV$_1$ before and after BD, between T1 and T3 and after BD also among T1 and T2, that is, the improvement of this parameter after the educational activity.

Lv et al. (2012) compared an SMS with a traditional educational program and an outpatient ordinary service (control group). They found that both groups that received an educational program presented increased scores on perceived control asthma questionnaires, although scores in the SMS group were higher than those in the traditional educational program. Continuing medical program education in asthma care was shown to improve parent-reported provider communication skills, the number of days affected by asthma symptoms, and asthma health care use.

Other authors have evaluated, quality of life as an auxiliary measure of asthma treatment and its control. In our study, asthmatic patients from IG presented better quality of life after 1 and 2 months (T2 and T3) of protocol intervention. Janson et al. (2009) showed that individualized asthma self-management education resulted in a decrease in nighttime awakenings, improved perceived control of asthma, better adherence to treatment, and improved clinical parameters of the disease and quality of life.

However, we did not observe differences when comparing ACT and ACQ scores in either group. In the same way, Maricoto et al. also did not find differences between groups after an educational intervention regarding proper use of ICS in ACT scores. França-Pinto et al. (2015) did not show a difference in ACQ after an aerobic training program of asthmatic patients, although they presented a better quality of life and better scores of depression. However, when the effect of the educational intervention was compared between the groups (IG and CG) through the odds ratio and chi-square test among the 3 study visits (T1, T2, T3) we observed a significant difference in the percentage of ACQ between T1 - T3.
One strength of our study is that GC showed better IL-4, IL-5 and depression levels after the protocol. We hypothesized that these positive results could be motivated by the use of peak flow to fill a diary of symptoms and by some calls received from a health professional; thus, it was enough to help them feel self-confident to better manage the disease.

Depression is an important asthma comorbidity and has been associated with worsening disease control in adults. Therefore, Plourde et al. (2016) validated the BDI, one of the most widely used questionnaires to screen depression in health research areas, to be used in asthmatic adult patients. We presented better scores of depressions in the IG after 1 month (T2) of educational intervention and after 2 months (T3) in the CG.

Regarding inflammatory parameters, the exact role of FeNO in asthma is still not clear. Some studies have associated FeNO with eosinophilia in asthmatic patients. However, according to GINA, this measure has not been established as useful for ruling in or ruling out a diagnosis of asthma; among other factors, it is not elevated in some asthma phenotypes, such as neutrophilic asthma. The majority of our patients had paucigranulocytic asthma, which may explain the decreased values of FeNO after educational intervention (Table 2).

Eosinophils have an important role in the immunological response in asthma. Studies have demonstrated a relationship between eosinophils and airway hyperresponsivity and remodeling as well as its relationship with asthma severity. A retrospective study of asthmatic adult patients followed up for 2 years showed a relationship between better disease control and a decrease in the number of eosinophils. They suggested that the control of asthma could be a consequence of intensifying treatment with ICS.

In our study, patients who submitted to an educational program also presented a decrease in the number of eosinophils in the sputum, which may be due to better adherence to ICS.

Another mediator related to eosinophils and the inflammatory process of asthma is T helper (Th) cytokines, such as IL-4 and IL-5. The ERS/ATS Task Force made recommendations on the use of novel therapies for severe asthma, specifically biological for type 2 high asthma, among which they suggest using anti-interleukin (IL)-5 and anti-IL5 receptor α for severe uncontrolled adult eosinophilic asthma phenotypes and anti-IL-4/13 for adult patients with severe eosinophilic asthma and for those with severe corticosteroid-dependent asthma regardless of blood eosinophil levels. Thus, decreasing the levels of these cytokines with only a short-term educational intervention can bring benefits to patients.

We found decreased expression of IL-4 and IL-5 in EBCs and IS in both groups. CG presented a decrease in these interleukins but they did not present improvement in other parameters that could represent disease control, such as lung function, the number of eosinophils and quality of life. Although these cytokines are involved in asthma pathogenesis, other mediators are also important to achieve the control of asthma symptoms.
Although allergic asthmatic features are linked to Th2 cytokines, recent studies have associated Th2 cytokines with Th17 cytokines. Bullens et al. (2006) found an increase in the expression of IL-17 mRNA in the sputum of asthmatic patients compared to healthy controls. Barczyk et al. (2002) also presented a correlation of increased levels of IL-17 in asthmatic patients with bronchial hyperreactivity. Camargo et al. (2018) showed that therapy with anti-IL-17 could be used to control the inflammatory process in an exacerbated asthma model. In addition, Fattahi et al. (2016) found that lower levels of IL-17+ cells in asthmatic patients were associated with atopy and ICS use. We observed decreased expression of IL-17A at EBC in GI, suggesting a possible pathway to explain reduced inflammation in these patients.

We believe that one of the limiting factors of this study may have been the use of dithiothreitol (DTT) for processing sputum supernatant. It is indicated for better preparation of slides; however, its use has already been described as potentially harmful for differential cell analysis. This procedure may have influenced the analysis of cytokines, a fact that was observed only after the freezing of samples. Another factor to be considered is the sample size, which could be expanded in future studies. It should also be considered that the CG may have influenced some of the results despite not having received the educational intervention in the first two visits. The fact that CG participated in the research may have influenced the results in a beneficial way and may have inferred some of the results. Despite these limitations, our study has several strengths. We showed that an educational intervention performed for a short period of time, applied by a trained professional, can produce benefits to patients in relation to inflammation, lung function, quality of life and depression levels.

In summary, we showed that an educational program directed to asthmatic patients in addition to physician care has an important role in disease control. This was evidenced by a reduction in airway inflammatory markers, such as IL-4, IL-5, IL-17A, FeNO and lung function, which consequently resulted in better quality of life and decreased depression levels. Finally, we suggest that health educational programs should be present as a part of treatment to help the control of diseases.

**Declarations**

**Acknowledgments**

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**Author contributions**
S.N.F. and B.M.S.R. take full responsibility for the integrity of the work. S.N.F., B.M.S.R., R.C.A and M.V.A. designed the study and wrote the protocol. F.M.A., R.C.A., J.C.C., P.G.B.J. included the patients in the protocol. S.N.F., R.C.A., J.C.C., P.G.B.J. and B.M.S.R. performed the follow-up of patients. S.N.F., F.M.A., M.A.M. and B.M.S.R. conducted the statistical analysis. S.N.F., C.R.O., I.F.L.C.T., M.A.M. and B.M.S.R. analyzed and interpreted the data. S.N.F and B.M.S.R. wrote the manuscript, and all authors critically reviewed and approved the final version of the manuscript. All authors contributed to the study conception and design.

**Availability of data and material**

All relevant data are within the paper and; the main data can also be accessed at: https://clinicaltrials.gov/ct2/show/results/NCT03655392. Clinical Trial Registration: ClinicalTrials.gov; number: NCT03655392; URL: www.clinicaltrials.gov. It was first registered in 03/28/2018 (retrospectively recorded). Any additional data can be requested from the corresponding author or the last author.

**Conflicts of interest/Competing interests.** We have read the journal’s policy and the authors of this manuscript have declared no competing interests.

**Consent to participate**

Informed consent was obtained from all individual participants included in the study. All participants received explanations, signed and received a copy of the written consent form.

**Consent for publication**

The authors affirm that human research participants provided informed consent for publication of the study data without personal identification. In the written consent form, all participants authorized the publication of the data for this study, without any individual identification related to the participants. The confidentiality and privacy of any personal data were respected in this study.

**Ethics approval**

This study was approved by the HCFMUSP Research Ethics Committee, protocol number 639.895 in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki. The study was registered and approved according to the country’s standards on the Brazil Platform (Plataforma Brasil), number CAEE 25319213.5.0000.0068 (http://plataformabrasil.saude.gov.br/login.jsf).

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**Tables**
|                               | IG            | CG            | Total         | p    |
|-------------------------------|---------------|---------------|---------------|------|
|                               | Number (%) or mean ± SD |               |               |      |
| **Gender**                    |               |               |               |      |
| Female                        | 23 (72)       | 17 (65)       | 40 (69)       | NS   |
| Male                          | 9 (28)        | 9 (35)        | 18 (31)       | NS   |
| Total                         | 32 (100)      | 26 (100)      | 58 (100)      | NS   |
| **Age (mean ± SD)**           | 55 ± 11       | 51 ± 14       | NS            |      |
| Education (years)             |               |               |               |      |
| Illiterate – 0                | 3 (9)         | 2 (8)         | 5 (9)         | NS   |
| ≤ 8                           | 13 (41)       | 13 (50)       | 26 (45)       | NS   |
| ≤12                           | 12 (38)       | 5 (19)        | 17 (29)       | NS   |
| >12                           | 4 (12)        | 6 (23)        | 10 (17)       | NS   |
| Total                         | 32 (100)      | 26 (100)      | 58 (100)      | NS   |
| **Lung function**             |               |               |               |      |
| FEV\textsubscript{1} after β2 (%) | 85.21±10,72   | 89.53 ±11,00  | NS            |      |
| **Treatment**                 |               |               |               |      |
| Formoterol + budesonide (capsules) | 23 (72)       | 21 (80)       | 44 (76)       | NS   |
| Salmeterol + Fluticasone (diskhaler spray) | 2 (6)        | 2 (8)        | 4 (7)         | NS   |
| Formoterol + budesonide liquid (spray) | 7 (22)       | 2 (8)        | 9 (15)        | NS   |
| Beclometasone (spray)         | 0             | 1(4)          | 1 (2)         | NS   |
| **Onset of asthma**           |               |               |               |      |
| Childhood                     | 14 (44%)      | 11 (42)       | 25 (43)       | NS   |
| Adult                         | 18 (56)       | 15 (58)       | 33 (57)       | NS   |
| **Level of asthma symptom control** |           |               |               |      |
| Well controlled               | 0             | 0             | 0             | NS   |
| Partly controlled             | 8 (25)        | 14 (54)       | 22 (38)       | NS   |
| Uncontrolled                  | 24 (75)       | 12 (46)       | 36 (62)       | NS   |
| Total                         | 32 (100)      | 26 (100)      | 58 (100)      | NS   |
| **Endotype (induced sputum)** |               |               |               |      |
| Paucigranulocytic             | 21 (66)       | 19 (73)       | 40 (69)       | NS   |
| Neutrophilic                  | 3 (9)         | 7 (27)        | 10 (17)       | NS   |
| Eosinophilic                  | 6 (19)        | 0             | 6 (10)        | NS   |
| Mixed                         | 2 (6)         | 0             | 2 (4)         | NS   |
| Former smoking |   |   | NS |
|---------------|---|---|----|
| > 5 years     | 0 | 4 (15) | NS |
| > 10 years    | 10 (31) | 6 (23) | NS |
| Total         | 10 (31) | 10 (38) | 20 (34) | NS |

**Table 1.** Baseline data. Data are expressed as the numbers and percentages – N (%) or means ± SD (standard deviation). FEV$_1$= Forced Expiratory Volume in One Second. β2= beta2-adrenérgico. Statistical analysis: T-test. **Values are expressed as the percentages of patients with sputum samples sufficient for analysis. Eosinophilic: eosinophils > 2.5%, neutrophils ≤ 54%; neutrophilic: eosinophils ≤ 2.5%, neutrophils > 54%; mixed: eosinophils > 2.5%, neutrophils > 54%; paucigranulocytic: eosinophils ≤ 2.5%, neutrophils ≤ 54%.**
| Group | T1         | T2         | T3         | p       |
|-------|------------|------------|------------|---------|
| Median (25-75%) | Group | T1         | T2         | T3         | p       |
| Questionnaire | AQLQ | IG       | 4.7(3.7-6.0) | 5.2(4.3-6.3)* | 5.2(4.0-6.0)* | <0.05* |
|         |       | CG       | 5.7(4.6-6.2) | 6.0(4.8-6.4) | 6.0(5.2-6.2) | NS     |
|         | BDI   | IG       | 14.0(4.6-20.0) | 11.0(2.5-24.0)* | 12.5(2.0-20.0)* | <0.05* |
|         |       | CG       | 9.0(5.0-19.0) | 7.5(4.0-14.0) | 9.0(3.0-15.0)* | <0.05* |
|         | ACT   | IG       | 19.0(16.0-21.5) | 19.5(17.0-22.0) | 21.5(16.0-24.0) | NS     |
|         |       | CG       | 22(20.0-24.0) | 23.0(20.0-23.0) | 22.0(18.0-24.0) | NS     |
|         | ACQ   | IG       | 1.4(1.0-2.1) | 1.2(0.5-2.0) | 1.1(0.5-1.8) | NS     |
|         |       | CG       | 1.1(0.7-1.2) | 0.9(0.7-1.5) | 1.0(0.5-1.7) | NS     |
| Free-symptom days | Cough | IG       | 28.5(10.5-50.5) | 30.5(19.5-58.0) | NS     |
|         |       | CG       | 29.5(5.0-49.0) | 42.5(6.0-51.0) | NS     |
|         | Wheezing | IG   | 31.5(15.0-53.0) | 38.0(21.5-58.5) | NS     |
|         |       | CG       | 40.0(14.0-51.0) | 44.5(22.0-54.0) | NS     |
|         | Dyspnea | IG    | 34.0(15.5-53.0) | 38.5(21.5-59.0) | NS     |
|         |       | CG       | 41.0(16.0-51.0) | 42.0(22.0-53.0) | NS     |
|         | Wake up (asthma) | IG | 36.5(17.5-56.0) | 40.5(23.5-59.0) | NS     |
|         |       | CG       | 39.5(15.0-52.0) | 42.5(22.0-55.0) | NS     |
|         | Emergency (SABA) | IG | 37.0(16.5-54.5) | 41.0(24.0-62.0) | NS     |
|         |       | CG       | 41.0(20.0-51.0) | 45.5(22.0-56.0) | NS     |
|         | Peak flow | IG   | 309.3(244.8-377.4) | 316.1(271.1-367.2) | NS     |
|         |       | CG       | 315.6(287.1-385.2) | 328.2(293.5-390.0) | NS     |

**Table 2.** Questionnaires and free-symptom days. The data are expressed as the medians and percentiles 25-75. SABA=short action beta-agonist; AQLQ=Asthma Quality Life Questionnaire; ACQ = Asthma Control Questionnaire; BDI =Beck Depression Inventory; ACT= Asthma Control Test. *Symptom Diary was delivered to patients on day 0 and day 28 and returned on day 56. *p<0.05 compared to baseline. One-way ANOVA.
| Outcome                      | Group | T1     | T2     | T3     | p       |
|------------------------------|-------|--------|--------|--------|---------|
| A. Differential count of cells (%) |       |        |        |        |         |
| Eosinophils                  | IG    | 0.60 (0.20-3.00) | 0.20 (0.00-0.65) | 0.20 (0.00-0.85)* | <0.05*  |
|                              | CG    | 0.00 (0.00-0.20) | 0.10 (0.00-0.60) | 0.20 (0.00-0.20) | NS      |
| Neutrophils                  | IG    | 35.40 (10.72-56.10) | 31.80 (13.55-57.65) | 40.60 (6.60-62.85) | NS      |
|                              | CG    | 17.30 (4.40-65.20) | 26.60 (2.282-54.40) | 14.70 (4.20-47.00) | NS      |
| Lymphocytes                  | IG    | 1.20 (0.800-2.90) | 1.40 (0.80-2.05) | 1.60 (0.75-2.90) | NS      |
|                              | CG    | 1.00 (0.668-1.40) | 1.00 (0.40-1.60) | 0.80 (0.60-1.40) | NS      |
| Macrophages                  | IG    | 54.80 (36.10-87.02) | 60.20 (40.00-82.45) | 56.40 (35.15-90.55) | NS      |
|                              | CG    | 80.40 (32.60-94.60) | 72.30 (44.80-96.88) | 82.90 (49.80-95.20) | NS      |
| B. Exhaled nitric oxide (NOex) (ppb) |       |        |        |        |         |
|                              | IG    | 24.6 (17.3-39.1) | 26.7 (23.0-36.1) | 25.5 (15.4-40.3) | NS      |
|                              | CG    | 26.2 (17.8-39.2) | 21.0 (17.4-23.0) | 22.5 (17.6-30.8) | NS      |
| C. Cytokines - induced sputum supernatant |       |        |        |        |         |
| IL-4                         | IG    | 1.335 (1.197-1.543) | 0.995 (0.656-1.139)* | 0.858 (0.501-1.092)* | <0.05*  |
|                              | CG    | 1.140 (0.569-1.464) | 0.786 (0.671-0.962)* | 0.419 (0.300-0.590)* | <0.05*  |
| IL-5                         | IG    | 2.965 (0.967-3.101) | 2.428 (1.918-3.126)* | 1.039 (0.967-1.334)* | <0.05*  |
|                              | CG    | 1.840 (1.195-3.200) | 3.398 (1.627-4.815)* | 0.600 (0.380-0.810)* | <0.05*  |
| D. EBC cytokines             |       |        |        |        |         |
| IL-4                         | IG    | 0.928 (0.800-1.254) | 0.730 (0.456-0.982)* | 0.858 (0.450-1.076)* | <0.05*  |
|                              | CG    | 1.124 (0.906-1.376) | 0.691 (0.454-1.147) | 0.432 (0.388-0.695)* | <0.05*  |
| IL-5                         | IG    | 2.529 (1.709-2.903) | 1.415 (1.306-1.735)* | 0.733 (0.535-1.025)* | <0.05*  |
|                              | CG    | 3.324 (1.937-4.380) | 1.223 (0.841-1.381) | 0.535 (0.433-0.765) * | <0.05*  |
| IL17A                        | IG    | 0.855 (0.633-1.054) | 0.832 (0.681-11.29) | 0.705 (0.268-871)** | <0.05*  |
|                              | CG    | 0.448 (0.296-1.181) | 0.761 (0.665-1.009) | 0.583 (0.444-0.661) | NS      |

**Table 3.** Inflammatory and functional profiles. The data are expressed as the medians and percentiles 25-75 (SD). NOex= Nitric Oxide exhaled. ppb= parts per billion. EBC = exhaled breath condensate. p<0.05. One-way ANOVA. * compared to T1. **compared to T2.
| Outcome                  | Time Interval | Odds Ratio (IG/CG) | 95% confidence interval | Between Group (p value) |
|--------------------------|---------------|-------------------|-------------------------|------------------------|
| Eosinophils (%)          | T1 - T2       | 3.300             | 1.060 - 10.482          | 0.036                  |
|                          | T2 - T3       | 5.556             | 1.742 - 17.714          | 0.003                  |
|                          | T1 - T3       | 5.400             | 1.627 - 17.921          | 0.004                  |
| NO (ppb)                 | T1 - T2       |                   |                         |                        |
|                          | T2 - T3       | 2.000             | 0.716 - 5.590           | 0.184                  |
|                          | T1 - T3       | 0.699             | 0.250 - 1.949           | 0.492                  |
|                          | T1 - T2       | 9.143             | 0.859 - 97.265          | 0.062                  |
| IL-4 (sputum)            | T2 - T3       | 0.198             | 0.033 - 1.200           | 0.115                  |
|                          | T1 - T3       |                   |                         |                        |
|                          | T1 - T2       | 0.286             | 0.022 - 3.669           | 0.543                  |
|                          | T2 - T3       | 0.198             | 0.033 - 1.200           | 0.115                  |
|                          | T1 - T3       | 0.913             | 1.326                   | 0.393                  |
| IL-5 (sputum)            | T1 - T2       | 2.857             | 0.405 - 20.141          | 0.371                  |
|                          | T2 - T3       | 0.400             | 0.068 - 2.337           | 0.400                  |
|                          | T1 - T3       | 0.800             | 0.149 - 4.297           | 1.000                  |
|                          | T1 - T2       | 1.167             | 0.862 - 1.579           | 0.318                  |
| IL-4 (EBC)               | T2 - T3       | 0.083             | 0.068 - 2.337           | 0.063                  |
|                          | T1 - T3       | 0.733             | 0.149 - 4.297           | 0.263                  |
|                          | T1 - T2       | 0.286             | 0.022 - 3.669           | 0.543                  |
|                          | T2 - T3       | 0.083             | 0.068 - 2.337           | 0.063                  |
|                          | T1 - T3       | 0.733             | 0.149 - 4.297           | 0.263                  |
| IL-5 (EBC)               | T1 - T2       | 0.800             | 0.587 - 0.995           | 0.150                  |
|                          | T2 - T3       | 0.917             | 0.256 - 3.286           | 0.894                  |
|                          | T1 - T3       | 0.405             | 0.149 - 4.297           | 0.432                  |
|                          | T1 - T2       | 1.167             | 0.862 - 1.579           | 0.318                  |
| IL-17A (EBC)             | T2 - T3       | 0.083             | 0.068 - 2.337           | 0.063                  |
|                          | T1 - T3       | 0.733             | 0.149 - 4.297           | 0.263                  |
|                          | T1 - T2       | 0.286             | 0.022 - 3.669           | 0.543                  |
| FEV1 before BD (%)       | T2 - T3       | 0.917             | 0.256 - 3.286           | 0.894                  |
|                          | T1 - T3       | 0.405             | 0.149 - 4.297           | 0.432                  |
| FEV1 after BD (%)        | T2 - T3       | 1.778             | 0.471 - 6.711           | 0.394                  |
|                          | T1 - T3       | 4.156             | 1.382 - 12.493          | 0.001                  |
|                          | T1 - T2       | 2.556             | 0.861 - 7.590           | 0.088                  |
|                          | T2 - T3       | 0.600             | 0.210 - 1.715           | 0.339                  |
|                          | T1 - T3       | 4.156             | 1.382 - 12.493          | 0.001                  |
|                          | T1 - T2       | 5.714             | 1.806 - 18.080          | 0.002                  |
|                          | T2 - T3       | 0.960             | 0.331 - 2.788           | 0.940                  |
|                          | T1 - T3       | 3.055             | 1.042 - 8.953           | 0.039                  |
| AQLQ                     | T1 - T2       | 1.625             | 0.554 - 4.762           | 0.375                  |
|                          | T2 - T3       | 0.917             | 0.331 - 2.538           | 0.867                  |
|                          | T1 - T3       | 1.297             | 0.462 - 3.646           | 0.621                  |
| BDI (depression)         | T1 - T2       | 1.203             | 0.411 - 3.525           | 0.736                  |
|                          | T2 - T3       | 0.446             | 0.159 - 1.252           | 0.123                  |
|                          | T1 - T3       | 0.889             | 0.301 - 2.626           | 0.831                  |
|                          | T1 - T2       | 1.131             | 0.393 - 3.254           | 0.819                  |
| ACT                      | T2 - T3       | 0.961             | 0.337 - 2.736           | 0.940                  |
|                          | T1 - T3       | 3.911             | 1.293 - 11.383          | 0.014                  |
| ACQ                      | T2 - T3       | 0.961             | 0.337 - 2.736           | 0.940                  |
|                          | T1 - T3       | 3.911             | 1.293 - 11.383          | 0.014                  |

Table 4. Odds ratios of the main outcomes among study’s measurements dates. T1 = day 1, T2 = day 28, T3 = day 56.
Figure 1

Study design*. * Phone calls were made to the GI every two weeks during the study to remind them about the correct use of medication and to remember the date of the study visit.
Figure 2

Enrollment.
Figure 4

Total cytokine count in the three evaluations (T1, T2 and T3). a. and b. IL-4 and IL-5 in EI. c. and d.: IL-4 and IL-5 in ACE. e. IL-17A in ACE. * Compared to T1 (baseline) in their respective groups. ** Compared to T1 in the same group. The boxes represent the 25th to 75th percentiles (SD), the lines inside the boxes represent the median values, and the bars represent the 10th and 90th percentiles. Statistical analysis: One-way ANOVA (intragroup analysis).