

Effects of immunotherapy on symptoms, PEFr, spirometry, and airway responsiveness in patients with allergic asthma to house-dust mites (*D. pteronyssinus*) on inhaled steroid therapy

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The present study was designed to investigate the effects of immunotherapy (IT) with an extract of *Dermatophagoides pteronyssinus* (Alergo-Merck Depot®) during a 27-month period in patients with allergic asthma to house-dust mites. We included 11 patients (mean age 18 years) treated with a combination of IT and inhaled beclomethasone dipropionate (BDP) in comparison to another 11 (mean age 22 years) treated with BDP alone. We evaluated symptom scores, salbutamol use, peak expiratory flow rates (PEFR), spirometry, and bronchial hyperresponsiveness (BHR) during 18 months of therapy with BDP and in the 9 months after BDP interruption. The two kinds of treatment were efficient and comparable in relation to symptom score, salbutamol use, morning PEFr, FVC, and FEV₁, but patients treated with IT and BDP had a faster improvement of BHR and PEFr variability. The interruption of BDP after 18 months of therapy was linked to an impairment of all end points, which were more pronounced in patients previously treated only with BDP. These findings suggest that in selected asthmatic patients allergic to house-dust mites, the association of IT and BDP is more effective than therapy with this inhaled steroid alone due to a faster and more striking improvement during the first months of treatment and to a lower rate of relapse after the interruption of therapy with BDP.

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Asthma is an inflammatory airway disorder characterized by tissue cellular infiltration, cell activation, and morphologic changes in airway structure (1, 2). These structural changes can be present even in the mild forms of asthma (3–5) and are mainly implicated in chronic airflow obstruction and bronchial hyperresponsiveness (BHR), which are usually related to the severity of asthma and medication requirements (6, 7).

The exposure to nonspecific stimuli can develop or exacerbate symptoms in all asthmatics, whereas inhalant allergens trigger asthma in sensitized patients through IgE-mediated inflammation.

Therapy with inhaled steroids efficiently controls bronchial inflammation and BHR as the mainstay of asthma management (8–11). In allergic asthma with the identification of the implicated allergen(s), it is possible to direct the treatment to the etiologic agent through allergen-avoidance and immunotherapy (IT). The clinical efficacy of IT in house-dust mite (HDM) asthma has been documented in controlled studies (12–15). However, a question not completely answered is whether the combination of drug treatment and IT is more effective than drug treatment alone (16). This is particularly important in HDM asthma, where the long-sustained bron-

chial inflammation induced by these allergens is more difficult to control, and allergen-avoidance measures and inhaled steroids are frequently required throughout the patient's life. IT could thus be of capital importance by decreasing the severity of symptoms and the need for drugs, thereby diminishing the risk, inconvenience, and cost of pharmacotherapy, as well as improving the quality of life for these patients. In this study, we investigated for 27 months the effects of IT in a group of asthmatics allergic to HDM, treated for 18 months with associated beclomethasone dipropionate (BDP), and in the following 9 months after BDP interruption, evaluating their symptom scores, salbutamol use, PEFR, FVC, FEV₁, and PC₂₀ methacholine (M).

Material and methods

Patients

We included 22 asthmatic patients (11 females and 11 males), with a mean age of 20 ± 8.5 years (range: 12–38), consecutively selected from our outpatient clinic. Inclusion criteria were as follows: documented perennial moderate asthma according to the International Consensus Report (9), skin "prick" tests exclusively positive to HDM, and serum specific IgE (Kallestad Allercoat®) to *Dermaphagoides pteronyssinus* with a RAST greater than or equal to class III (≥ 3.50 AEU/ml). We excluded patients with FEV₁ below 70% of predicted values; associated rhinitis, sinusitis, or other active pulmonary diseases; contraindications for IT use; therapy with oral or inhaled steroids in the last 3 months; and previous IT.

Study design

After a 4-week run-in period during which inhaled β_2 -agonists were the only allowed medication, patients included in this study were randomly allocated to either treatment group by a previously determined sequence of random numbers: 11 patients (mean age 18, range 13–38) treated with BDP and IT (IT group), and another 11 (mean age 22, range 12–38) treated only with BDP (control – C group). Then, they were evaluated after 6, 12, and 18 months of therapy. After the last visit, all patients stopped BDP and were assessed again, 6 and 9 months later. In each one of these visits, we attended to: 1) the recording of daily symptom scores, salbutamol intake, and PEFR; 2) spirometry; 3) bronchial challenge test with methacholine (M). After the run-in period, the patients were instructed in environmental control measures to reduce exposure to HDM and irritants. All were treated with a daily dose of 600–800 μg of powder-inhaled BDP and used inhaled salbutamol on

demand. One month later, patients were observed to adapt this dose in accordance with the salbutamol intake. Two patients from the C and one from the IT group had to raise the dose from 600 to 800 $\mu\text{g}/\text{day}$. Thereafter, all patients kept the dose of BDP constant until its interruption after 18 months of therapy.

Recording symptom scores, salbutamol demand, and PEFR

All patients were provided with diaries in which they recorded a subjective daily evaluation of their respiratory symptoms (wheeze, cough, chest tightness, shortness of breath, or awakening by respiratory symptoms), salbutamol use (number of inhalations per day), and PEFR. Patients had to assign each symptom a score based on a scale according to the intensity from zero (absent) to three (severe). Patients were trained in the use of the Mini-Wright peak-flow meter and instructed to record in the diary the highest value of three measurements performed before taking medication and on each of the following occasions: in the morning after rising, at approximately 1700 h, and just before bedtime. We analyzed the highest, lowest, daily mean, and PEFR daily variability (highest–lowest reading/mean reading of the day $\times 100$). The diary was completed during the run-in period and thereafter for a 4-week period before each protocolized visit.

Lung function and methacholine challenge test

Forced expiratory volume in the first second (FEV₁) and forced vital capacity (FVC) were evaluated by spirometry (Microlab 3300®). The methacholine challenge test was performed according the method of Cockcroft et al. (17), and PC₂₀ M was defined as the provocative concentration of M that causes a fall in FEV₁ of 20% or more. Lung function and methacholine challenge tests were always performed on two different occasions before each expected visit.

Immunotherapy

We used a standardized extract of *D. pteronyssinus* adsorbed in aluminum hydroxide (Alergo-Merck Depot®-Allergopharma, Germany). The potency of this extract is titrated in therapeutic units (TU) in reference to an internal standard. One UT is roughly equivalent to 4 BU (biologic units) as determined by RAST inhibition assay between the in-house reference extract and the international standards for this allergen (18). The injection schedule recommended by the manufacturer was followed. An

initial dose of 5 TU (0.1 ml of a vial with a concentration of 50 TU/ml) was followed by weekly increment of doses (10, 20, 40, 50, 100, 200, 400, 500, 1000, and 2000 TU), until the maintenance dose of 3000 TU (0.6 ml of 5000 TU/ml), equivalent to 12 000 BU, was reached by the 12th week. We repeated this dose every 2 weeks for 2 months and then at monthly intervals. Local and general side-effects were recorded in each visit.

Statistical analysis

Descriptive parameters (median, mean, standard deviations, and range) were estimated for the studied variables. There were no missing data, as every patient completed the predetermined evaluations. Differences between the two groups at baseline and at different time points were analyzed with the Mann-Whitney test, while the time-related differences within each group were tested by the Wilcoxon signed-rank test. Results are expressed as mean ± SD for convenience of presentation. For all comparisons, statistical significance was considered to be present if $P < 0.05$.

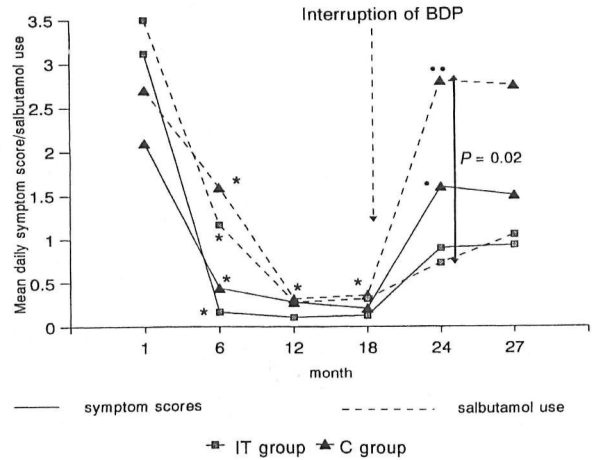
Results

Baseline characteristics of both groups

Table 1 summarizes the initial characteristics of the two groups, which did not show any significant differences according to clinical parameters, sensitivity to *D. pteronyssinus*, spirometry, and BHR.

Symptom scores and salbutamol demand

At the first follow-up visit (6 months), both groups had significantly lower daily symptom scores and salbutamol use in relation to the baseline evaluation ($P < 0.01$) (Fig. 1), and these differences were kept constant after 12 and 18 months with BDP therapy. During this period, the trend of changes was similar among the IT and C groups, with no significant



	Baseline	6 months	12 months	18 months	24 months	27 months
Symptom scores	IT group	3.1 ± 2.2	0.2 ± 1.2	0.1 ± 1.8	0.1 ± 3.9	0.9 ± 0.8
	C group	2.1 ± 1.1	0.4 ± 0.7	0.3 ± 0.8	0.2 ± 0.7	1.6 ± 1.1
Salbutamol use	IT group	3.5 ± 1.6	1.2 ± 3.0	0.3 ± 4.3	0.3 ± 2.1	0.7 ± 3.5
	C group	2.7 ± 2.0	1.6 ± 5.5	0.3 ± 4.9	0.4 ± 5.0	2.8 ± 2.7

Fig. 1. Symptom score and salbutamol demand in both groups during 27 months of study. Daily symptom score (—) and salbutamol demand (---) were significantly different after 6, 12, and 18 months of therapy with BDP, in IT (□) and C (Δ) groups (* $P < 0.01$). Six months after BDP interruption, we observed significantly higher values of these two parameters (● $P = 0.03$; ●● $P < 0.01$) in C group and significant difference ($P = 0.02$) between groups in relation to salbutamol use (solid arrow).

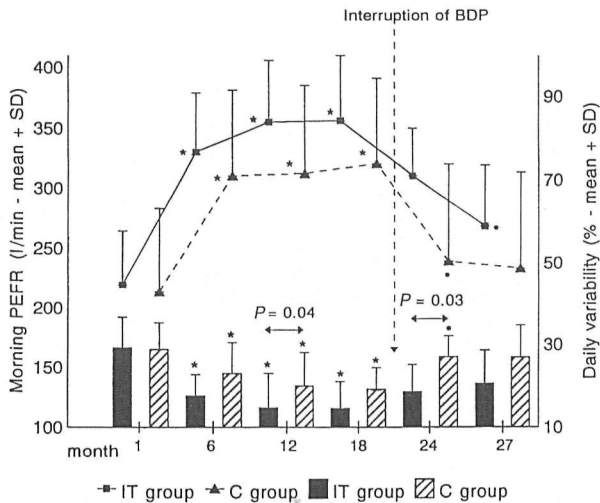
differences between them. After BDP interruption, both groups showed a clinical deterioration which seemed to be more severe for the C group, particularly within the first 6 months, showing a significant increase in the mean values of these two parameters when compared to the 18-month evaluation ($P = 0.03$; $P < 0.01$) (Fig. 1). Moreover, at this visit (24 months), the use of salbutamol was also significantly different between the two groups ($P = 0.02$) (Fig. 1). By the end of this study, the IT group had significantly lower symptom scores and salbutamol use ($P = 0.03$) than the baseline evaluation. This statistical significance was not found for the C group.

PEFR

We observed in both groups a significant improvement in the morning PEFR and daily variability, after 6, 12, and 18 months of therapy with BDP ($P = 0.02$) (Fig. 2). At the 12-month visit, the IT group had significantly lower daily variability ($P = 0.04$), but 6 months later, this significant difference was no longer present (Fig. 2). With the suspension of BDP, we observed a decline of these parameters in both groups. Six months later (24-month visit), the C group experienced a significant impairment in the morning PEFR and daily variability ($P = 0.05$) which was significantly higher in relation to

Table 1. Baseline characteristics of IT and C groups (mean ± SD) with no significant differences observed between them

	IT group	C group
Age (years)	18 ± 7	22 ± 9
Total IgE (kU/l)	310 ± 196	298 ± 150
Skin reactivity to <i>D.pt.</i> (wheal diameter-mm)	13.0 ± 0.7	11.8 ± 0.3
Daily symptom scores	3.1 ± 2.2	2.1 ± 1.1
Daily salbutamol demand	3.4 ± 1.6	2.7 ± 2.0
Morning PEFR	219 ± 45	213 ± 70
Daily PEFR variability	29.2 ± 7.7	28.8 ± 6.5
FVC (%)	71.6 ± 10.5	78.4 ± 7.3
FEV ₁ (%)	71.8 ± 11.0	72.2 ± 5.3
PC ₂₀ M (mg/ml)	0.30 ± 0.21	0.21 ± 0.18



		Baseline	6 Months	12 Months	18 Months	24 Months	27 Months
Morning PEFR	IT group	219 ± 45	330 ± 49	355 ± 51	356 ± 54	310 ± 40	268 ± 51
	C group	213 ± 70	310 ± 71	312 ± 73	320 ± 71	239 ± 81	233 ± 80
Daily variability	IT group	29.2 ± 7.7	17.6 ± 5.2	14.7 ± 8.3	14.5 ± 6.6	18.6 ± 6.6	20.6 ± 8.0
	C group	28.8 ± 6.5	23.0 ± 7.4	20.0 ± 8.0	19.2 ± 5.2	27.0 ± 5.1	26.9 ± 7.8

Fig. 2. Daily morning PEFR (l/min - Mean + SD) of IT (—) and C (---) groups during study. PEFR daily variability (% - mean + SD) is represented by dark bars for IT and traced bars for C group. Significant differences in relation to initial visit are represented as * ($P=0.02$) during therapy with BDP, and as • ($P=0.05$) thereafter. Significant difference in relation to PEFR daily variability was observed between groups after 12 months of therapy and 6 months after BDP interruption (solid arrows).

the IT group ($P=0.03$). Three months later, the IT group had a significantly lower morning PEFR in comparison to the 18-month evaluation ($P=0.05$) (Fig. 2), but, at the end of the study, no important differences were observed between the two groups. No significant differences were present in the C group when we compared the PEFR daily variability between the baseline and the 27-month evaluation.

Spirometry

During the first 18 months of this study, we observed in both groups a similar and significant improvement of FEV₁ and FVC, which was apparent already within the first 6 months ($P=0.02$; $P<0.01$) (Fig. 3). A similar trend of FEV₁ and FVC decline was observed in the IT and C groups with significantly lower mean values ($P<0.01$), 6 and 9 months after the interruption of BDP (Fig. 3). No significant differences were present between the two groups throughout the 27 months of the study.

Bronchial responsiveness

Despite the significant increase in PC₂₀ M in both groups after 6, 12, and 18 months of therapy

($P<0.01$), the pattern of this increase was different between them (Fig. 4). At the 6-month visit, patients treated with IT plus BDP (IT group) had a significantly higher PC₂₀ M than those treated only with BDP (C group) ($P=0.02$). Six months later, this difference was further increased ($P<0.01$), but, by 18 months, it was no longer present. The interruption of BDP led to a deterioration of BHR in both groups with a significantly lower PC₂₀ M in relation to the 18-month visit ($P<0.01$). This fall was more severe for the C group in the first 6 months, with a significant difference observed at this evaluation in relation to the IT group ($P<0.01$). Similar PC₂₀ M mean values were observed in the C group between the baseline and the 27-month visit, while the IT group had a significantly higher PC₂₀ M ($P=0.02$) at the end of the study.

Discussion

Allergen avoidance is always the first recommendation in the management of HDM asthma. When avoidance is not practical and appropriate medication, including the use of inhaled steroids, fails to control symptoms, IT should be considered. Despite placebo-controlled studies confirming the clinical improvement of HDM asthma in patients treated with potent high-quality extracts (12–15), there are still some reservations about its use, especially in view of the potentially severe systemic reactions and doubts about its clinical efficacy (19). On the other hand, the control of bronchial inflammation induced by these allergens often requires therapy for long periods with high doses of inhaled steroids. This has a significant economic impact and carries the risk of adverse effects, particularly in children, in whom the use of doses greater than 400 µg/day may be associated with impaired growth and bone formation (20).

Another important point is that IT is often considered inferior to drug treatment, but this view is based on a misunderstanding. The efficacy of IT as a single treatment should not be compared with that of drug treatment since IT and pharmacotherapy should be integrated in the treatment strategy for allergic asthma. Nevertheless, as recognized by the EAACI Immunotherapy Subcommittee, it is not fully proven that the combination of drug treatment and IT is more effective than drug treatment alone (16). The present study, designed to answer some of these questions, investigated the effects of IT in asthmatic patients allergic to HDM who were treated with BDP for 18 months and then in the following 9 months after its interruption. A random, double-blind clinical trial would be the best investigational approach to this problem. However, in the present study, due to the long

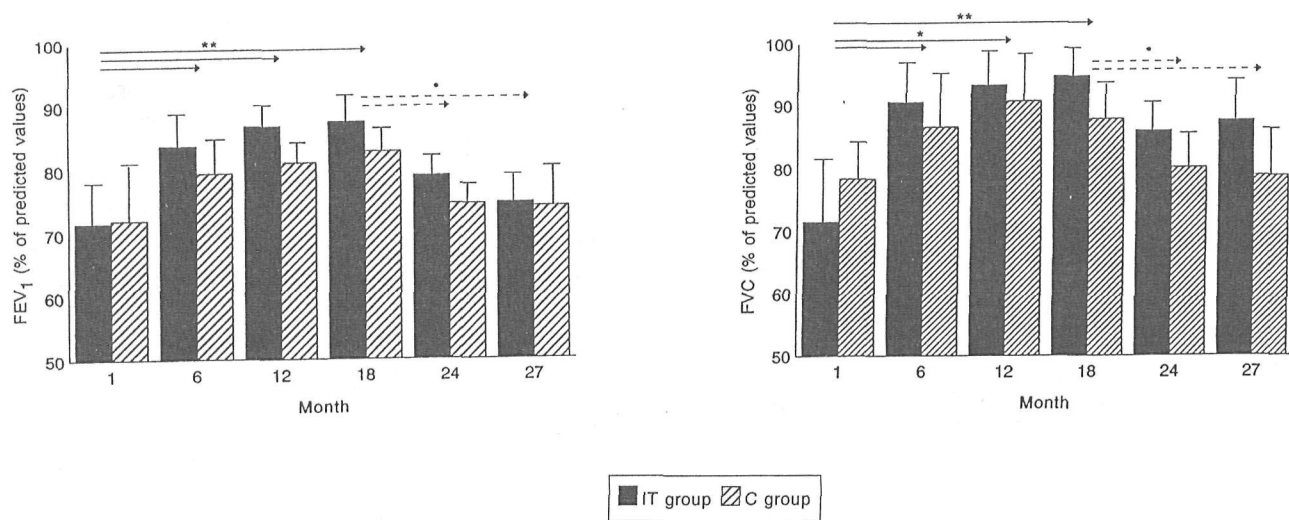


Fig. 3. Significant changes in BHR (mean PC₂₀ M – mg/ml) in IT (dark bars) and C (traced bars) groups are indicated as solid arrows (* $P<0.01$; ** $P<0.05$). Differences between groups in relation to patterns of change are also represented as dotted arrows (● $P=0.02$; ●● $P<0.01$; NS=no significant differences).

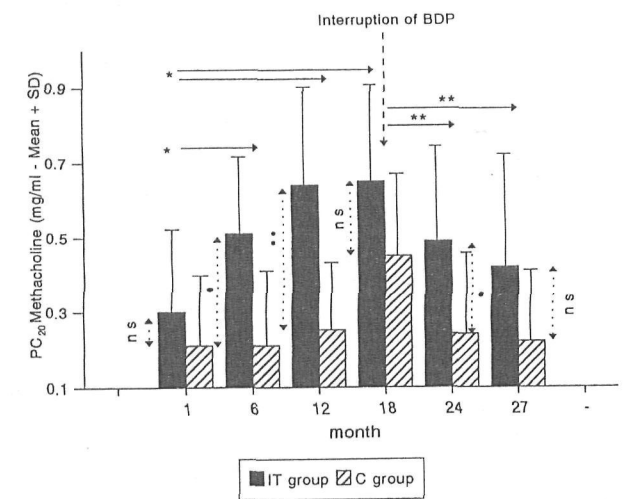


Fig. 4. FVC and FEV₁ (% – mean+SD) in patients treated with IT+BDP (IT group – dark bars) and only with BDP (C group – traced bars). Significant changes are represented for both groups by solid arrows (* $P=0.02$; ** $P<0.01$) during therapy with DBP, and by dotted arrow (● $P<0.01$) after BDP interruption.

follow-up period and the nature of the disease, we could not enrol patients in such a trial. Therefore, a major concern in our open study was to include objective measurements that are probably not biased by knowledge of the patient treatment status. The end points were symptom scores, salbutamol

use, PEFR, FVC, FEV₁, and PC₂₀ M, combining patient self-evaluations with our objective evaluations, a method which in clinical practice is the most convenient way to monitor the clinical efficacy of IT (16).

During therapy with BDP, both groups experienced improvement of all the end points. However, the time needed to reach a plateau differed for both groups and for the end points. Symptom score/salbutamol intake, morning PEFR, FVC, and FEV₁ stabilized after 12 months of therapy, and no significant differences were observed between the IT and C group in relation to the pattern of this improvement (Figs. 1–3).

It is recognized that BHR and PEFR variability reflect asthma severity and medication requirements (6, 7); according to some investigators, they are interrelated (7, 21). This was apparent in our study, which observed similar changes for PC₂₀ M and PEFR variability and a significant correlation between both parameters ($r=0.627$; $P<0.01$). We found a continuous increase of their values over the 18 months of therapy, suggesting that further improvement is still possible thereafter. It was also evident that the IT group had a faster improvement of these two parameters with significantly lower PEFR daily variability at 12 months ($P=0.04$) and higher PC₂₀ M at 6 ($P=0.02$) and 12 months ($P<0.01$) than the C group. These results suggest that the association of IT and BDP accelerates the improvement of BHR and PEFR variability in the

first 12 months. This could be particularly important in unstable and more severe forms of HDM-allergic asthma, representing a faster decrease in the number of exacerbations, need of emergency department visits or hospitalizations, and use of oral or parenteral corticotherapy, and thus reducing the morbidity of this illness.

However, the study of BHR in patients undergoing IT has yielded conflicting results especially in perennial asthma. Bousquet et al. found a decreased BHR in patients treated with a standardized mite extract (22), while Murray et al. (23) observed an increase in seven children after 1 year of mite IT. Mosbech et al. (24) noticed a decrease in BHR after 1 year of treatment, but it returned to normal values during the next year of therapy. In our study, none of the 22 patients had an increase of BHR during the 18 months of treatment with BDP, and it is likely that this anti-inflammatory agent could have a protective effect. All the 22 patients initially had PC₂₀ M levels below 2 mg/ml, which is considered an indication of severe asthma (25). Nevertheless, PC₂₀ M was the only parameter linked to this classification, and it has been recognized that patients with relatively mild asthma may have high levels of BHR (6). We consider that the association of BDP and regular visits gave us enough security to continue IT treatment in these patients.

The effects of the interruption of inhaled steroids after the therapeutic periods has been studied. When regularly used for 4–6 weeks in doses of 600–1200 µg/day, they reduce respiratory symptoms and airway responsiveness, but their interruption is associated with a deterioration of clinical parameters, lung function tests, and BHR (26). This deterioration is dependent on the daily dose and the duration of therapy (10), and it is related to the resumption of the inflammatory process (26, 27), confirming, on the other hand, that IT is insufficient as a single treatment in mite-induced asthma. In our study, this was evident in the 6 months immediately after the suspension of inhaled steroids and was present in both groups, in spite of the long-term therapy with BDP. However, this deterioration seems to be more pronounced in patients previously treated only with BDP (C group), as this group had a significant impairment for all end points in relation to the evaluation done at 18 months. During this period, patients from the IT group had no significant differences in symptom scores and salbutamol use, and showed significantly lower daily variability of PEFR ($P=0.03$) and higher PC₂₀ M ($P=0.02$) than the C group. After 27 months, we decided to stop the study because of the marked deterioration of all end points as well as the withdrawal of two patients from the C group

due to the severity of symptoms and high salbutamol demand. At the end of this study, there were no significant differences between the groups, but patients from the C group presented the worst values for all end points, and these values were similar to those observed in the baseline assessment (Figs. 1–4).

With the suspension of BDP, some “protective” effect of IT was apparent and may be related to its anti-inflammatory properties (28–30), as IT has been shown to reduce the late-phase reaction after bronchial challenge with allergens (31), decrease blood eosinophil counts and serum eosinophil cationic protein (30), and block the tissue infiltration and activation of eosinophils (29, 30).

In conclusion, the present study suggests that in selected asthmatic patients allergic to HDM, the association of IT and BDP is more effective than therapy with this inhaled steroid alone. These advantages are related to a faster and more striking improvement during the first months of treatment and to a lower relapse rate after the interruption of therapy with BDP, thereby reducing the disease severity and morbidity, improving the quality of life of these patients, and diminishing the risk, inconvenience, and costs of pharmacotherapy.

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