

Effect of Nebulized Beclomethasone on Airway Inflammation and Clinical Status of Children with Allergic Asthma and Rhinitis: A Randomized, Double-Blind, Placebo-Controlled Study

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Key Words

Allergic asthma · Allergic rhinitis · Oxidative stress ·
Airway inflammation · Nebulized beclomethasone

Abstract

We aimed to evaluate the therapeutic effect of nebulized beclomethasone dipropionate (nBDP) on both allergic asthma and rhinitis. In a randomized, double-blind, placebo-controlled study, 40 children (mean age 10.7 ± 2.1 years) with allergic asthma and rhinitis received either nBDP (daily dose of 800 μ g, administered twice daily) or placebo for 4 weeks (with a face mask), after a 2-week run-in period of clinical assessment. Nasal and oral fractional exhaled nitric oxide (FeNO) measurements together with pulmonary function tests, nasal and oral exhaled breath condensate (EBC) collection for pH and interleukin-5 (IL-5) measurements as well as nasal and bronchial symptom scores were obtained at baseline and after 4-week treatment. A significant improvement in oral FeNO, oral and nasal EBC IL-5 and nasal EBC pH was

observed in the nBDP group when comparing the values with baseline, together with an improvement in symptom score of the visual analogue scale, nasal obstruction, sneezing, rhinorrhea, breathing difficulty, cough, wheezing and sleep disturbance (nBDP end treatment vs. baseline, Wilcoxon signed-rank test). nBDP was more effective than placebo (ANCOVA test) in improving [difference Δ = response after treatment at the last visit (active or placebo) – value at baseline] nasal pH, oral IL-5, oral FeNO, forced expiratory volume in 1 s, forced expiratory volume in 1 s/forced vital capacity, peak expiratory flow, visual analogue scale, breathing difficulty, cough, wheezing and sleep disturbance scores. No differences were observed between the nBDP and the placebo group for symptom score of rhinitis. nBDP is a useful treatment for airway inflammation and clinical status in children with concomitant allergic asthma and rhinitis.

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Introduction

Inhaled corticosteroids (ICS) are the most effective anti-inflammatory agents for the management of persistent asthma and are therefore recommended as first-line anti-asthmatic therapies in children of all ages, adolescents and adults. Various studies have documented the anti-asthmatic efficacy of ICS in the following aspects: reduction in symptoms, airway inflammation, hyper-responsiveness, frequency and severity of exacerbations and mortality, as well as improvement in pulmonary function and quality of life (QoL) [1]. The use of nebulized corticosteroids has been shown to be effective in children and adults [2]. The guidelines recommend the use of a compressor-driven nebulizer as an alternative device to pressurized metered-dose or dry powder inhalers for children with asthma [3]. Nebulizers are still frequently used in clinical practice to overcome problems with the technique of the inhalers [4], and as such, nebulization appears to be a useful method in the treatment of children, elderly and severe or steroid-dependent asthmatics [5] mainly for bronchodilators.

Allergic rhinitis (AR) and allergic asthma (AA) are chronic inflammatory disorders with a prevailing Th2 immune response to inhaled allergens leading to hyper-reactivity as well as to the recruitment of eosinophils, mast cells and lymphocytes in the upper and lower airways [6]. The presence of uncontrolled inflammation in the upper and lower airways may compromise the control of AR and AA with an increase in symptoms and a consequent progression of the allergic disease [7, 8]. The pharmacologic rationale of targeting the mucosa, where allergic or inflammatory processes take place, is clear and has been confirmed by the widespread use of nebulization in clinical practice.

Although once-daily nebulized beclomethasone dipropionate (nBDP) is effective in maintaining pulmonary function and improving symptoms in asthmatic children [9], there is no evidence about the effectiveness of nBDP on both upper and lower airway inflammation at the same time and on the correspondent nasal bronchial pulmonary function and symptom score.

In this study, we aim to investigate the advantages of nebulized therapy in positively modifying both the nasal and bronchial markers of inflammation and in the correspondent management of nasal and bronchial symptom scores in children. To address this issue, we conducted a randomized, double-blind, placebo-controlled study to determine whether nBDP positively affects inflammatory parameters, such as fractional exhaled nitric oxide

(FeNO), pH and interleukin (IL)-5 in exhaled breath condensate (EBC), and improves clinical and functional parameters in both children with AA and AR with exclusive exacerbation during the pollen season.

Materials and Methods

Patients

The study was conducted in 40 children recruited among those attending the Pulmunology/Allergy Clinic at the Institute of Biomedicine and Molecular Immunology (Italian National Research) in Palermo, Italy. The study was performed enrolling children aged 8–14 years with allergic mild intermittent asthma (IA) and AR. Asthma diagnosis and assessment of severity were performed according to the Global Initiative for Asthma guidelines [10]. Baseline forced expiratory volume in 1 s (FEV₁) and forced vital capacity (FVC) were measured according to guidelines [10]. Baseline FEV₁ was defined as the best of three reproducible curves. All asthmatic patients were characterized by a reversible airway obstruction assessed by an increase of 12% in FEV₁ after inhalation of 200 µg of salbutamol. Children with IA have been treated with short-acting β₂-agonists on demand during the last 6 months. Patients with intermittent or persistent rhinitis were indifferently recruited according to the ARIA classification [11]. Children were excluded from the study if they had a respiratory tract infection during the last 3 weeks.

Study Design

A randomized, double-blind, placebo-controlled pilot study was performed after a 2-week run-in period of eligible children during which patients were treated with short-acting β₂-agonists on demand, but they refrained from taking this medication for at least 24 h before the test. During the screening visit, an interview-administered questionnaire was completed by parents to collect information on demographic characteristics, age and gender, atopic personal and familiar status, environmental tobacco exposure, pet exposure, mold exposure, place of residence, and breast feeding.

Patients who met the inclusion criteria were randomly allocated to visit 1 (day 0) to receive either nBDP 400 µg (Clenil per Aerosol; Chiesi Farmaceutici S.p.A., Parma, Italy) or placebo twice a day, i.e. one nebulization each morning and evening for 4 weeks with facial mask, and after that, visit 2 (day 28–30) was performed. Placebo and nBDP were provided blinded by the manufacturer (Chiesi Farmaceutici S.p.A.) to assure the double-blind study design. To standardize treatment administration and to maintain the double-blind reduced variability in the drug delivery design, all patients used the same type of nebulizer (Bimbo-neb; Markos-Mefar, Italy) with a tight-fitting face mask by which nBDP was inhaled by breathing through the mouth and nose. The study was conducted during the pollen season (April and May), when patients were symptomatic for grass pollen. Only patients who met all of the following criteria were eligible for inclusion into the study: (1) a history of AR during the grass pollen season and concomitant IA; (2) monosensitized to grass pollen, identified by positive skin prick test and specific immunoglobulin E (IgE >0.70 kU/l), and (3) no concomitant treatment.

During both visit 1 and visit 2, the investigators verified compliance with the assigned treatment by counting the returned vials and reviewing diary cards. Compliant patients were considered those who had taken at least 75% of the scheduled doses of study medication and had filled out the diary cards consistently since their last visit. The short-acting β_2 -agonists were used as rescue medication on demand during the 4 weeks. Further, during visit 1 and visit 2, children were assessed for pulmonary function test, visual analogue scale (VAS) health-related QoL (HRQoL), assessed by the Rhinasthma score, nasal and oral FeNO measurements, and nasal and oral EBC collections.

The local ethics committee approved the study, and participating subjects gave their written informed consent. The approved study was registered as a randomized, double-blind, placebo-controlled study on the central registration system ClinicalTrials.gov (identifier: NCT01113489).

Clinical Assessment of Patients

Baseline FEV₁ and FVC, as well as peak expiratory flow (PEF) or forced expiratory flow 25–75% (FEF_{25–75}), were measured following ATS/ERS recommendations [12]. Atopic status was established in all patients by clinical history and confirmed by skin prick test. Skin prick testing was performed as previously described [13]. Serum samples were collected, and total serum IgE and specific IgE levels were determined by CAP System (Pharmacia-Upjohn, Uppsala, Sweden).

FeNO Measurement

Nasal and oral FeNO were determined by chemiluminescence (Niox, Aerocrine, Solna, Sweden) following ATS/ERS recommendations [14], as previously described [15]. To evaluate nasal FeNO, the nasal samples were obtained from 1 nostril. Patients were instructed to blow up the cheeks to elevate the mouth pressure and to isolate the nasal cavity from the rest of the respiratory system [15]. The reproducibility of these procedures was previously assessed [15].

EBC Collection

Nasal EBC was collected according to Profita et al. [15, 16]. After applying a nasal mask to the EcoScreen condenser, children were asked to breathe through their noses, with their mouths closed. Before the collection, subjects were asked to rinse out their mouths. An aliquot of at least 1 ml of nasal condensate was collected and stored at -80°C in disposable polypropylene sterile tubes until analyzed. A median volume of 2.3 ml (interquartile range 1.4–2.7) of nasal EBC was collected.

Oral EBC was collected using a commercially available condenser (EcoScreen; Jaeger, Würzburg, Germany). Children were asked to breathe at a normal frequency and tidal volume, wearing a nose clip, for 15 min. A median volume of 3 ml (interquartile range 2.6–3.5) of oral EBC was collected.

The reproducibility of nasal and oral EBC biomarkers with these methods was previously assessed [14, 16]. None of the EBC samples contained detectable levels of amylase activity.

EBC Measurement of IL-5

Determination of the absolute values of IL-5 in EBC was assessed using commercially available specific ELISAs (Amersham Biosciences UK Limited, Little Chalfont, UK), as previously described [15]. Limits of detection were <2 pg/ml [15].

EBC Measurement of pH

A stable pH was achieved in all cases after deaeration/decarbonation of breath condensate specimens by bubbling with argon (350 ml/min) for 10 min. pH was then measured within 15 min of condensate collection by means of a pH meter (Corning 240, Science Products Division, New York, N.Y., USA) with a 0–14.00 pH range [15].

Questionnaires and Symptom Scores

Parents/guardians were trained in the correct use of the nebulizer and instructed to keep daily records of their children's symptoms, about asthma and rhinitis and their impact on QoL at baseline and after treatment. A VAS ranging from 0 cm (extreme of negative events) to 10 cm (extreme of positive events) was used to assess the severity of the general status of asthma and rhinitis symptoms [17, 18], in accordance with our minor modifications regarding the extreme of the scale. The subjects were asked to mark a point on the line of the VAS that represents how they feel about the allergic disease at the time. The specific symptom scores of rhinitis and asthma were monitored using information obtained from the Rhinasthma questionnaires [19, 20]. This instrument utilizes a five-point Likert scale (not at all, a little, fairly, much, and very much, range 0–5) to measure the extent to which patients were bothered by every item during the previous 2 weeks.

Outcome Measures

The primary outcome measures of the study were the evaluation of the effects of nBDP and placebo on the levels of nasal and oral FeNO, on the levels of nasal and oral EBC pH values and IL-5 levels, and on pulmonary function test during the 4-week study period. The secondary outcome measures of the study were the evaluation of (1) the VAS for the general status of disease, and (2) HRQoL assessed using Rhinasthma for the disease-specific symptom score of asthma and rhinitis. The tertiary outcome of the study was to detect the correlation between FeNO and EBC biomarkers with VAS and Rhinasthma symptoms for asthma and rhinitis. Variations were then computed.

Power of the Study

Statistical power analysis was computed considering the 'sample average' of a marker included in the study such as oral FeNO. The 'test value' was referred to differences in oral FeNO (from 42.6 ± 10 to 14.9 ± 3.38 ppb) [21] before and after treatment with ICS. The sample size of 20 subjects for each study group ensured a power of the study of $>95\%$, with an α error of 5% to detect such a difference. The sample size was evaluated using the website http://Hedwig.mgh.harvard.edu/sample_size/quanmeaurr/para-quant.html.

Statistical Analysis

The data are expressed as means (95% confidence intervals), unless otherwise stated, for both active and placebo treatment groups. Demographic and baseline clinical characteristics between the two groups were compared using unpaired Student's *t* test for normal distributed data or the Mann-Whitney *U* test for non-parametric data. Paired analyses within each treatment group were carried out using the Wilcoxon signed-rank test. To compare the effects of active treatment versus placebo, the difference (Δ) was calculated as: Δ = response after treatment in the last

Fig. 1. Study flow chart. nBDP (400 µg) or placebo was administered twice a day with a face mask for 4 weeks.

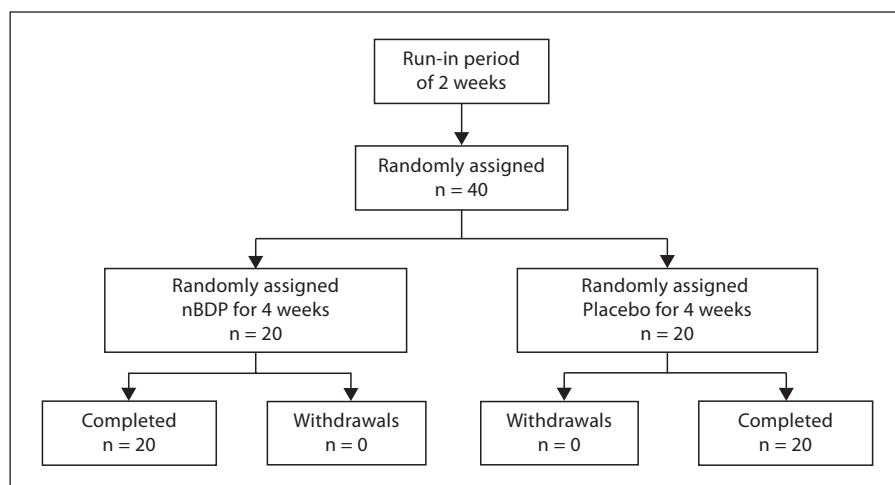


Table 1. Demographic and baseline clinical characteristics of 40 patients with allergic IA and AR enrolled in the study

	nBDP (400 µg b.i.d.)	Placebo	p values
ARIA classes	20 [50]	20 [50]	
Intermittent rhinitis	11 [27.5]	13 [32.5]	
Persistent rhinitis	9 [22.5]	7 [17.5]	
Age, years	11.1 (10.1–12.1)	10.4 (9.6–11.2)	n.s.
Sex	20	20	
Male	14	14	
Female	6	6	
Body mass index	13.7 (12.7–14.7)	14.3 (13.1–15.5)	n.s.
FEV ₁ , % predicted	95.1 (90.1–100.1)	97.9 (93.8–102.0)	n.s.
FVC, % predicted	96.1 (91.4–100.8)	95.5 (91.5–99.8)	n.s.
FEV ₁ /FVC, ratio %	84.6 (81.1–88.1)	88.6 (85.8–91.4)	n.s.
PEF, % predicted	77.8 (67.8–87.71)	80.6 (77.51–84.6)	n.s.
Total IgE, IU/ml			
Geometric mean	280.60	344.67	n.s.
Mean log total IgE ± SD	2.45 ± 0.39	2.54 ± 0.34	
Grass pollen specific IgE			
Classes	II–V	II–V	n.s.
kU/l	28.8 (14.7–42.9)	24.7 (15.0–34.4)	
Wheezing during last 6 months	1.95 (0.28–1.62)	2.5 (1.40–3.60)	n.s.

Data are means, with 95% confidence intervals in parentheses, unless otherwise stated. Figures in brackets are percentages. p values are calculated with unpaired Student's t test or with the Mann-Whitney U test.

ARIA = Allergic Rhinitis and its Impact on Asthma; n.s. = not significant.

visit (active or placebo) – value at baseline. The differences between active and placebo treatment groups were evaluated using an analysis of covariance model for the mean values of calculated Δ , including the baseline value as a covariate. All statistical analyses were performed using StatView® 5 software (SAS Institute Inc.). A p value <0.05 was considered to indicate statistical significance in these analyses.

Results

Subject Characteristics

The children (mean age 10.7 ± 2.1 years) with seasonal allergic IA randomized in the nBDP and placebo groups had similar demographic characteristics and dis-

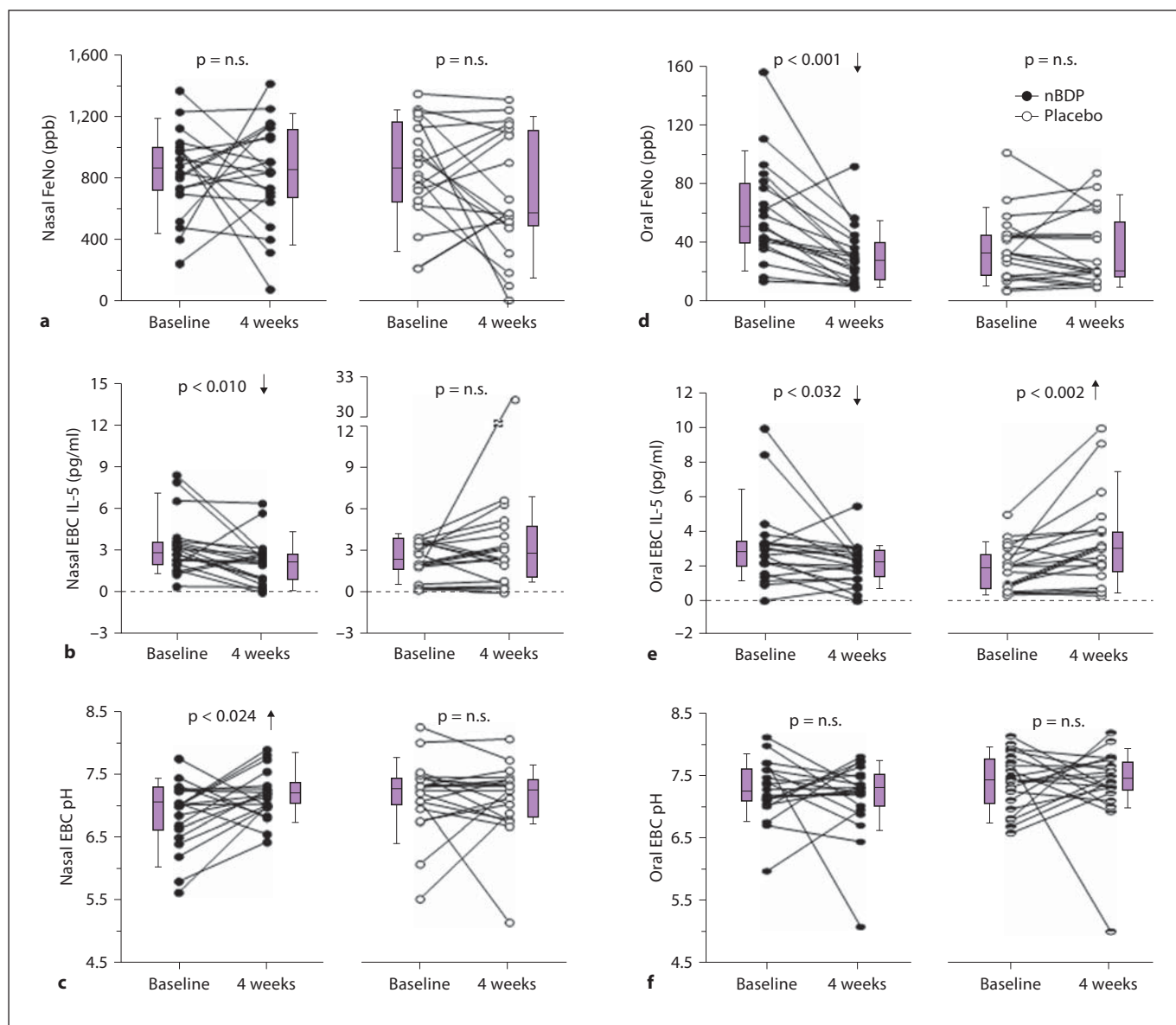


Fig. 2. Effects of nBDP (400 µg; $n = 20$) or placebo ($n = 20$) administered twice a day for 4 weeks with a face mask before and after treatment in patients with concomitant seasonal allergic IA and AR on EBC biomarkers: nasal FeNo (a), nasal EBC IL-5 (b), nasal EBC pH (c), oral FeNo (d), oral EBC IL-5 (e), and oral EBC pH (f). Results are expressed as individual data points and the lateral bars represent the median and interquartile range. Statistical analysis was performed by the Wilcoxon signed-rank test. n.s. = Not significant.

tribution of intermittent or persistent rhinitis (table 1). All children selected for study in the nBDP and placebo groups completed treatment using the nebulizer for 9.2 ± 3.7 min. All children completed the 4-week period of treatment following the same medication regimens (nBDP or placebo; fig. 1).

Primary Outcomes

The levels of nasal FeNo were not reduced after treatment with nBDP and placebo compared with baseline, respectively (fig. 2a), while the concentrations of nasal EBC IL-5 were significantly reduced ($p < 0.01$; fig. 2b) and nasal EBC pH levels were significantly increased ($p < 0.024$; fig. 2c). The levels of oral FeNo were significantly

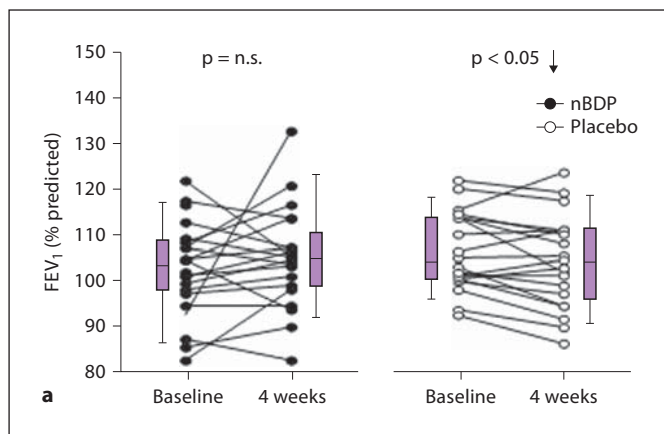


Fig. 3. Effects of nBDP (400 μ g; n = 20) or placebo (n = 20) administered twice a day for 4 weeks with a face mask before and after treatment in patients with concomitant seasonal allergic IA and AR on FEV₁ (a), FEV₁/FVC (b) and PEF (c). Results are expressed as individual data points and the lateral bars represent the median and interquartile range. Statistical analysis was performed by the Wilcoxon signed-rank test. n.s. = Not significant.

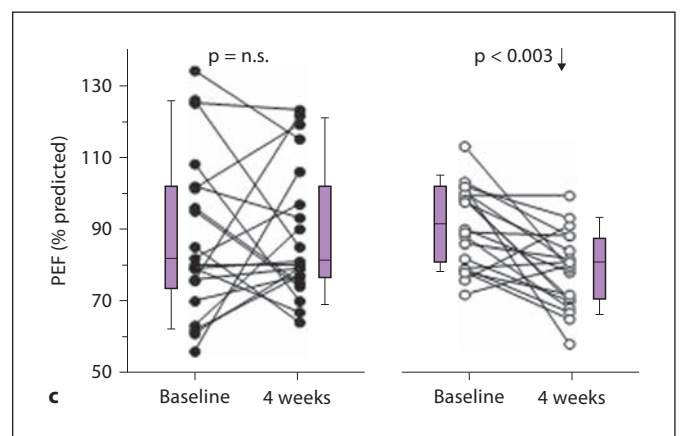
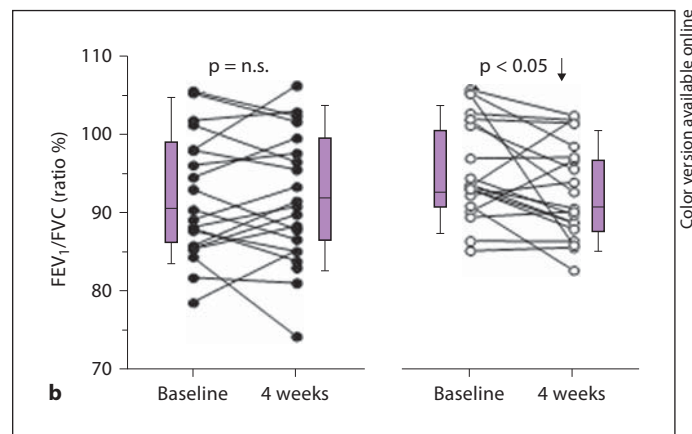
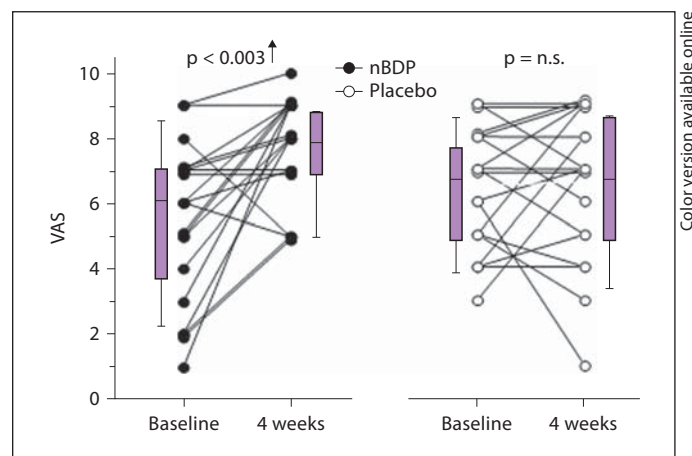


Fig. 4. Effects of nBDP (400 μ g; n = 20) or placebo (n = 20) administered twice a day for 4 weeks with a face mask before and after treatment in patients with concomitant seasonal allergic IA and AR on VAS. Results are expressed as individual data points and the lateral bars represent the median and interquartile range. Statistical analysis was performed by the Wilcoxon signed-rank test. n.s. = Not significant.



reduced after treatment with nBDP ($p < 0.001$) compared to baseline while they were not affected by placebo treatment (fig. 2d). The levels of oral EBC IL-5 were significantly reduced after treatment with nBDP ($p < 0.032$) and were increased after treatment with placebo during the period of seasonal AR ($p < 0.002$; fig. 2e), while the levels

of oral EBC pH were not affected in either the nBDP or the placebo group (fig. 2f).

No increase in FEV₁ (% of predicted), FEV₁/FVC (ratio %) and PEF (% of predicted) were detected after nBDP treatment, but all these parameters were significantly decreased in the placebo group (fig. 3). Furthermore, no

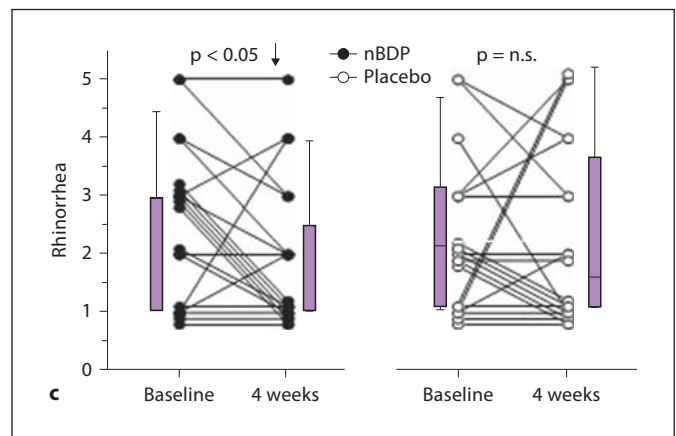
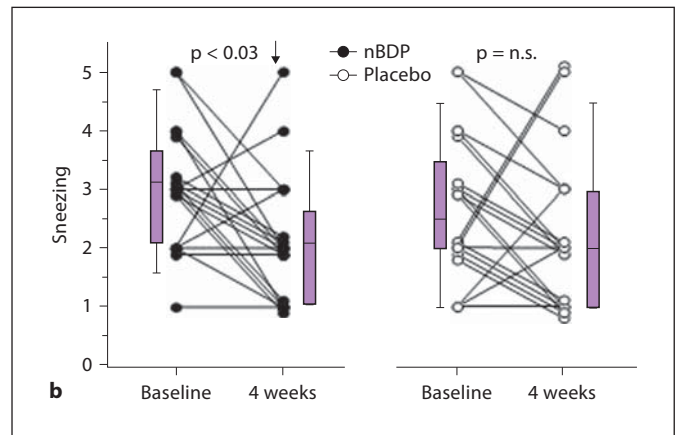
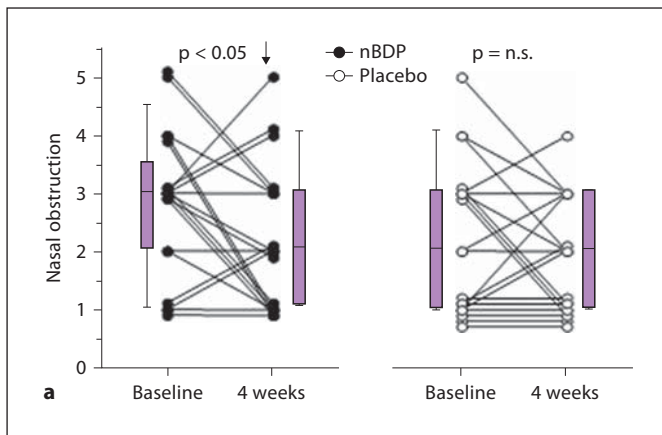


Fig. 5. Effects of nBDP (400 μ ; n = 20) or placebo (n = 20) administered twice a day for 4 weeks with a face mask before and after treatment in patients with concomitant seasonal allergic IA and AR on symptom score, obtained by the Rhinasthma questionnaire, on nasal obstruction (a), sneezing (b) and rhinorrhea (c). Results are expressed as individual data points and the lateral bars represent the median and interquartile range. Statistical analysis was performed by the Wilcoxon signed-rank test. n.s. = Not significant.

changes were observed for FEF_{25-75} in either the nBDP or the placebo group.

Secondary Outcomes

The VAS score was significantly increased after the 4-week treatment with nBDP ($p < 0.003$), showing an improvement in the general status of children with concomitant seasonal allergic IA and AR. The VAS score was not affected after placebo treatment (fig. 4). Measurements of the symptom score for rhinitis during seasonal allergen exposure using the Rhinasthma questionnaire showed a significant reduction in nasal obstruction ($p < 0.05$; fig. 5a), sneezing ($p < 0.03$; fig. 5b) and rhinorrhea ($p < 0.05$; fig. 5c) after treatment with nBDP, while treatment with placebo had no effect. Evaluation of the symptom score for asthma during the period of allergen exposure, using Rhinasthma questionnaires, showed a significant reduction in breathing difficulty ($p < 0.03$; fig. 6a), cough ($p < 0.02$; fig. 6b), wheezing scores ($p < 0.003$; fig. 6c) and sleep disturbance ($p < 0.003$; fig. 6d)

after treatment with nBDP, while treatment with placebo had no effect.

Changes in Data in the nBDP and Placebo Groups

We selected two groups of patients with no statistically significant differences at baseline in terms of pulmonary function test, VAS and symptoms. Statistically significant differences were observed in the following nasal and oral biomarkers: nasal pH, oral EBC IL-5 and oral FeNO. When analysis of covariance (ANCOVA) was applied to correct for the imbalances at baseline and to evaluate the statistical significance of change in biomarkers, statistically significant differences were observed in oral FeNO ($p < 0.001$), concentrations of IL-5 in nasal and oral EBC ($p < 0.05$ and 0.001 , respectively), and in the levels of nasal pH ($p < 0.029$) in the nBDP compared to the placebo group. The changes in pulmonary function parameters were statistically significant for FEV_1 ($p < 0.05$), FEV_1/FVC ($p < 0.05$) and PEF ($p < 0.01$). While the changes in VAS and Rhinasthma scores of asthma were

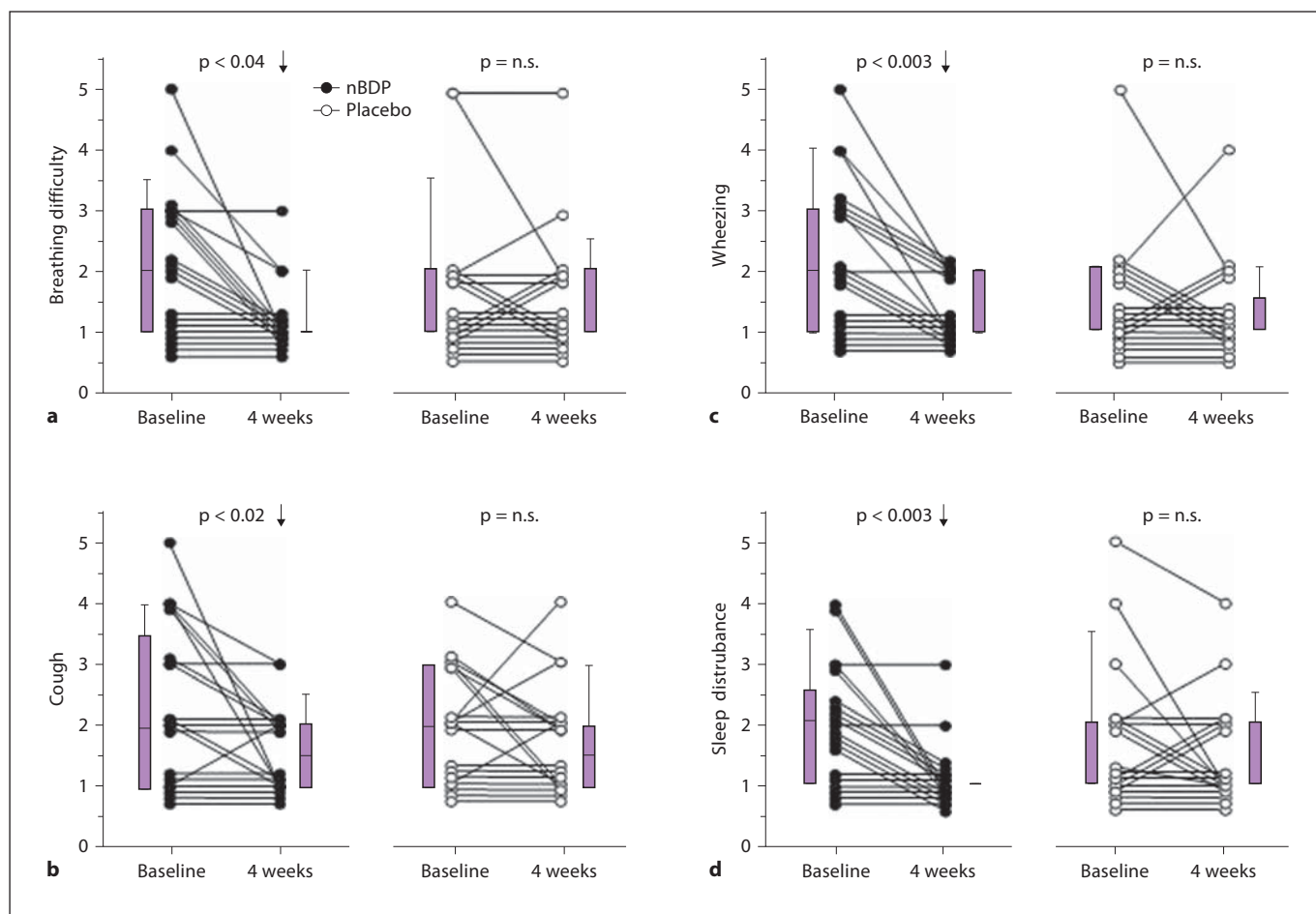


Fig. 6. Effects of nBDP (400 µg; $n = 20$) or placebo ($n = 20$) administered twice a day for 4 weeks with a face mask before and after treatment in patients with concomitant seasonal allergic IA and AR on symptom score, obtained by the Rhinasthma questionnaire, on breathing difficulty (a), cough (b), wheezing (c) and sleep disturbance (d). Results are expressed as individual data points and the lateral bars represent the median and 25–75 percentiles. Statistical analysis was performed by the Wilcoxon signed-rank test. n.s. = Not significant.

significantly different, no changes were detected for Rhinasthma scores of rhinitis in the nBDP and placebo groups (table 2).

Discussion

Our results provide evidence that the administration of nBDP (400 µg twice a day for 4 weeks) with a facial mask might be an effective treatment to control airway inflammation and to obtain control of clinical symptoms in children with concomitant allergic IA and AR, age matched between 8 and 14 years, during the season of allergen exposure.

The presence of an uncontrolled inflammation in the upper and lower airways may compromise the control of rhinitis and asthma with an increase in symptoms and a consequent progression of the allergic disease [6, 22, 23]. More recently, data from clinical studies in patients with rhinitis and coexistent asthma have highlighted that an improved control of nasal symptoms frequently results in improved asthma symptom scores [24]. The measurement of inflammatory parameters in the nasal and oral compartments may provide information about the general inflammatory status and about the effect of anti-inflammatory therapy in the absence of clinical evidence [15, 16]. The definition of both rhinitis and asthma severity by current guidelines is mainly based on symptoms

Table 2. EBC biomarkers, pulmonary function tests and symptom scores in children with allergic IA and AR before treatment and difference after 4 weeks in each group

	nBDP (400 µg b.i.d.)		Placebo		ANCOVA p values ¹
	before	change	before	change	
Nasal FeNO, ppb	835.1 (713.2; 956.9)	−9.3 (−212.1; 193.6)	858.8 (711.9; 1005.6)	−149.8 (−343; 44.309)	n.s.
Oral FeNO, ppb	59.5 (44.5; 74.5)	−29.5 (−41.6; −17.5)	34.0 (24.0; 43.9)	−1.2 (−9.7; 7.4)	<0.001
Nasal EBC IL-5, pg/ml	3.32 (2.40; 4.24)	−1.10 (−2.01; −0.19)	2.29 (1.70; 2.88)	1.86 (−1.05; 4.77)	<0.05
Oral EBC IL-5, pg/ml	3.12 (2.11; 4.14)	−1.04 (−1.98; −0.97)	1.90 (1.34; 2.46)	1.45 (0.60; 2.29)	<0.001
Nasal pH	6.85 (6.62; 7.08)	0.31 (0.05; 0.56)	7.15 (6.89; 7.41)	−0.04 (−0.34; 0.26)	<0.029
Oral pH	7.26 (7.05; 7.47)	−0.11 (−0.47; 0.25)	7.29 (7.09; 7.48)	−0.03 (−0.40; 0.34)	n.s.
FEV ₁ , % predicted	95.1 (90.1; 100.1)	3.86 (−2.02; 9.73)	97.9 (93.8; 102.0)	−2.52 (−4.80; −0.23)	<0.05
FVC, % predicted	96.1 (91.4; 100.8)	1.98 (−2.04; 6.00)	95.5 (91.5; 99.8)	−1.42 (−4.58; 1.74)	n.s.
FEV ₁ /FVC, ratio %	84.6 (81.1; 88.1)	0.95 (−0.89; 3.33)	88.6 (85.8; 91.4)	−2.87 (−5.48; −0.27)	<0.05
PEF, % predicted	77.8 (67.8; 87.71)	0.86 (−9.94; 11.65)	80.6 (77.51; 84.6)	−12.28 (−18.51; −6.05)	<0.01
VAS	5.40 (4.35; 6.45)	2.30 (1.14; 3.46)	6.45 (5.23; 7.27)	0.20 (−0.97; 1.01)	<0.002
Nasal obstruction	2.85 (2.31; 3.39)	−0.70 (−1.34; −0.06)	2.20 (1.64; 2.76)	−0.30 (−0.83; 0.23)	n.s.
Rhinorrhea	2.50 (1.97; 3.13)	−0.65 (−1.24; −0.06)	2.20 (1.64; 2.76)	0.05 (−0.71; 0.81)	n.s.
Sneezing	2.90 (2.41; 3.39)	−0.85 (−1.51; −0.19)	2.70 (2.17; 3.23)	−0.50 (−1.19; 0.19)	n.s.
Breathing difficulty	2.15 (1.63; 2.67)	−0.95 (−1.43; −0.47)	1.70 (1.17; 2.23)	−0.10 (−0.50; 0.30)	<0.001
Wheezing	2.25 (1.72; 2.78)	−0.90 (−1.32; −0.48)	1.50 (1.08; 1.92)	−0.15 (−0.61; 0.31)	<0.001
Cough	2.35 (1.78; 2.92)	−0.75 (−1.23; −0.22)	1.95 (1.54; 2.36)	−0.25 (−0.67; 0.17)	<0.036
Sleep disturbance	2.06 (1.64; 2.47)	−0.96 (−1.37; −0.54)	1.75 (1.26; 2.24)	−0.25 (−0.70; 0.20)	<0.001

The data are expressed as means, with 95% confidence intervals in parentheses. Difference Δ = response after treatment at the last visit (active or placebo) – value at baseline; n.s. = not significant. ¹ ANCOVA test: baseline values as a covariate.

[10, 11] and no inflammatory parameters are presently taken into account, although often symptoms are not specific and not closely related to the presence and severity of the upper and lower airway inflammation [25, 26]. In this scenario, we aim to underline that FeNO and EBC are noninvasive techniques supporting the assessment of nasal and oral markers of airway inflammation and their relationship with the clinical status in atopic children with and without asthma [15, 16, 22, 27].

To clarify the relationships between upper and lower airway disorders and to establish the potential benefits of an integrated airway management approach in children with IA and AR, although GINA guidelines do not take into account the use of steroids for the treatment of mild IA, we treated allergic children with nBDP 400 µg twice a day for a limited period of 4 weeks to reduce the user risk of side effects, with the aim to obtain a control of disease during the pollen season. On the other hand, it has been observed that a daily dose of 800 µg of nBDP administered with a nebulizer either once or twice daily for 12 weeks, rather than for 4 weeks, provides similar efficacy in maintaining pulmonary function and symptoms of mild-moderate asthmatic children, showing a good

tolerability profile [9] that encourages the use of nBDP also in children with allergic IA and intermittent rhinitis to reduce the risk of exacerbations during allergen exposure. Nevertheless, we underline that although nebulizer treatment as a rescue device of asthma attacks is great, since the daily treatment of asthma with a nebulizer takes longer than with the other devices, it reduces the adherence of pediatric patients to treatment and the reliance on doctors.

In this study, we focused our attention on nasal and oral FeNO and on the levels of nasal and oral EBC pH and IL-5 in children with allergic IA and AR showing exclusive exacerbations during the pollen season. We showed that nBDP reduced the levels of oral FeNO and oral EBC IL-5, while it did not affect the levels of oral pH. Furthermore, nBDP reduced the levels of nasal EBC IL-5 and increased nasal EBC pH, while it did not affect nasal FeNO. These results might suggest that the 4-week treatment with nBDP does not control the levels of nasal FeNO and oral EBC pH despite its positive effect on oral FeNO, nasal EBC pH and nasal and oral EBC IL-5. This evidence might suggest extending the use of nBDP for more than 4 weeks in children with concomitant asthma and rhini-

tis to maintain not only the functional and clinical parameters, but also to completely control the markers of inflammation in the nose and bronchi. This explanation was further supported by studies showing that subjects with AR and AA, receiving the same dose of orally inhaled fluticasone propionate, plus intranasal aqueous fluticasone for a period of 6 weeks, show significantly improved levels of oral and nasal FeNO as well as improved scores for AR and AA [28]. On the other hand, while the nasal biomarkers FeNO, IL-5 and pH as well as oral FeNO did not change, we observed an oral IL-5 increase in the placebo group during the period of allergen exposure. This suggests that the immunological response associated with allergic disease of the upper and lower airways, involving an increase in biomarkers, is actively initiated by oral IL-5 [29]. However, to further validate these observations, an appropriate clinical trial extending the follow-up period with nBDP might be necessary. Since the children enclosed in the study showed quite normal values at baseline and exacerbation during the period of allergen exposure affecting the pulmonary function at random during the 4 weeks of the study, as expected, we did not observe changes in spirometric parameters before and after treatment, including FEV₁, FEV₁/FVC and PEF and FEF_{25–75}. However, the children enclosed in the placebo group, having the same demographic and baseline clinical characteristic as those in the nBDP group (table 1), showed a reduction in pulmonary function test, FEV₁, FEV₁/FVC and PEF during the season of pollen exposure. These findings suggest that nBDP treatment contributes to controlling the inflammation, avoiding deterioration in the lung function of the airways, and to reducing the risk of exacerbation exclusively during the period of allergen exposure. This evidence was further underlined by statistical analysis, with the ANCOVA test showing statistical differences for FEV₁, FEV₁/FVC and PEF between active and placebo treatment (table 2). In this scenario, treatment of children with concomitant IA and AR with nBDP during the allergy season supports the goal of asthma therapy (suggested by the international guidelines) to achieve and maintain a good level of disease control [11, 23]. Furthermore, although the use of steroids in allergic diseases of children remains controversial (although it was observed that nBDP and inhaled BDP are equally effective in pediatric patients with moderate to severe asthma exacerbations) [30–33], our findings open new perspectives to support the concept that therapy with nBDP might be able to control all grades of allergic diseases, including mild IA during the period of seasonal exacerbation. Although we decided to compare

the effect of nBDP only with the placebo group, since it was observed that nebulizers are frequently used in clinical practice to overcome problems with the technique of the inhalers [4], to better define the perspective of our results, further studies, including an extra group using the inhaler, are necessary.

Rhinitis and asthma are frequently concurrent diseases. This link has been established in clinical observations and epidemiological studies and also on the basis of immunological observations and therapy outcomes [34]. Recent pathophysiological findings have identified both disorders as manifestations of chronic inflammatory respiratory syndrome of the common airways or as united airways disease [8, 6]. Thus, AR or AA cannot be limited to a specific site but should be considered a disorder of the whole respiratory tract, with a range of clinical manifestations and relevant diagnostic and therapeutic implications. Patients with asthma and rhinitis have similarities in nasal and bronchial inflammation [6], with a relationship between nasal and bronchial compartments in allergic children [16]. The inflammatory nose-lung interaction has functional complementarity [34, 35], and thus, to characterize the interactions between the upper and lower airways, it is important to determine an appropriate treatment of rhinitis which would in turn promote a correct management of asthma [5, 36]. In this scenario, concomitant control of the upper airway inflammation is a crucial point also for the control of asthma in patients with concomitant AR [23].

Rhinitis and asthma influence each other in terms of QoL [37]. Control of rhinitis in asthmatic patients can lead to an optimization of HRQoL related to the upper and lower airways, suggesting that asthmatic patients must be evaluated for rhinitis and vice versa [23]. Up to now, the relationship between HRQoL, as measured by the questionnaires currently used, and either allergen exposure or upper and lower airway inflammation has partially been previously examined. The concomitant united airway diseases have manifestations that include more than one system, and these single-system questionnaires are unable to provide a valid global measure of HRQoL for allergic diseases in children. In this context, to assess the effect of treatment with nBDP on the clinical status, we used the VAS score to define a global status index and the Rhinasthma score for asthma and rhinitis, currently used and validated in the evaluation of allergic diseases in adults [17, 19, 20, 37]. We found that nBDP improves the global VAS and the specific Rhinasthma scores. These findings suggest that nebulized steroids might ameliorate the general status of symptoms of asthma and rhinitis in

children with concomitant seasonal IA and AR despite their pulmonary function. Interestingly, using the ANCOVA model for the mean values of calculated Δ , including baseline values as a covariate, we showed differences for Rhinasthma symptoms of breathing difficulty, cough, sleep disturbance and VAS, while no differences were found for symptoms of nasal obstruction, rhinorrhea and sneezing between changes in the nBDP and placebo groups. This apparent contradiction with statistical significance obtained using the Wilcoxon signed-rank test further underlines the evidence that concomitant seasonal allergic IA and AR are associated with a different impact on the disease-specific QoL. Interactions between the upper and lower airways (AR and IA considered as one disease) have to be taken into account in the management of asthma [36, 38], mainly in the seasonal sensitive patients. Nevertheless, if the patient's QoL for rhinitis does not reach a satisfactory point during the 4 weeks of treatment with nBDP, doctors should add a nasal steroid spray to increase the impact of upper airway inflammation on nasal symptoms [23]. The nose is made of larger and thicker tissue than the bronchus, and this might be

the reason why more concentrated local anti-inflammatory agents are needed to further control the inflammation and symptoms in nasal allergy. However, further investigations are required to determine whether the appropriate treatment (time and/or dose) of concomitant seasonal AR and IA with nBDP would be effective in the control of asthma comorbidity.

In conclusion, we have reported for the first time that a medium-term treatment with a daily dose of 800 μ g of nBDP administered twice daily with a tight-fitting face mask might be effective in the control of airway inflammation, maintaining the pulmonary function during the period of pollen exposure and improving the nasal and bronchial symptoms of children with concomitant AR and IA.

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