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Bronchial Allergen Challenge Using the Medicaid Dosimeter

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Key Words

Bronchial allergen challenge · Early asthmatic response · Late asthmatic response · Reproducibility · Exhaled nitric oxide

Abstract

Background: Bronchial allergen provocations are well established in asthma research. We evaluated the reproducibility of single-concentration, single-step allergen challenges in volunteers with grass pollen allergy. ***Methods:*** Forty-seven subjects underwent bronchial challenges using the aerosol provocation system nebulizer (Medicaid Sidestream) with incremental doses of grass pollen to define the individual allergen dose that causes a 20% drop in FEV_1 ($PD_{20}FEV_1$). In 39 subjects this procedure was followed by single-step challenges. Early and late asthmatic responses were monitored, and increases in exhaled nitric oxide were measured before and 24 h after single-step challenges. ***Results:*** After the first single-step challenge, the maximum drop in FEV_1 was 21.3% ± 8.0. A comparison of the drop in FEV_1 to the initial incremental challenge (29.7% ± 7.5) revealed an intraclass correlation of –0.30 ($p < 0.05$). In the second single-step challenge, the mean drop in FEV_1 was 20.9% ± 7.2. Compared with the first single-step challenge, the intraclass correlation was 0.37 ($p < 0.05$) and the 95% limits of agreement according to Bland and Altman were –17.5 to 18.1%. The increases in exhaled nitric oxide revealed substantial agreement in repeated single-step challenges (26.8 ppb ± 27.8 and 21.8 ppb ± 21.9, ICC 0.62, $p < 0.001$). ***Conclusions:*** The use of aerosol provocation system to calculate the $PD_{20}FEV_1$ allergen is a timesaving procedure and is less prone to errors because only one dilution of the allergen is used. The repeatability in well-defined subjects is excellent to study the mechanisms of allergen-induced airway inflammation and the development of new treatments for allergic diseases.

Introduction

Specific bronchial allergen provocation is an established tool in asthma research that can increase our understanding of the pathological mechanisms responsible for allergic asthma and can offer key information concerning the therapeutic potential of new agents [1–8].

However, there are numerous methodological differences in bronchial allergen challenges [1], and it is a matter of debate regarding which standard procedure is safe and reliable for multicenter studies. Many procedures employ a standard dosimeter (SDM) protocol with incre-

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mental allergen concentrations to calculate the concentration that causes a 20% drop in FEV_1 in an individual ($PC_{20}FEV_1$) [1, 9–11].

New commercially available dosimeter methods are emerging and may offer a promising alternative to established procedures. In a recently published paper, we have demonstrated the practical use of the aerosol provocation system (APS; Medicaid dosimeter; Carefusion GmbH, Hoechberg, Germany) in bronchial methacholine challenges [12]. The APS combines the advantages of the tidal breathing and five-breath protocols [7, 12]. A single concentration is delivered in doubling doses, and the cumulative dose that causes a 20% drop in FEV_1 is defined as $PD_{20}FEV_1$ [13]. The advantages include the prevention of dilution errors and the short amount of time required to prepare the dilutions.

For subsequent challenges, there is no universal agreement concerning the best method. Several studies have demonstrated the reproducibility of bronchial challenges. Two studies [1, 18] have compared an incremental challenge followed by an individual concentration (PC_{15}) or dose (PD_{20}) that causes an early (EAR) or a late asthmatic response (LAR). Three studies have compared the correlation of the incremental constant dose [16, 17] and incremental dose challenges [10].

A good correlation was observed between the two procedures. Although safety concerns support an incremental approach in which the allergen response can be monitored between increasing doses [2], the single-step method may provide some distinct advantages, such as the ability to allow for the exact and equivalent timing of allergen administration between subjects. Moreover, in studies of the inflammatory response, the single-step challenge may ensure that a constant allergen dose is delivered at any given time. This is of particular importance in trials in which inflammation markers, such as exhaled nitric oxide (eNO), are critical read-out parameters and in which repeated allergen challenges are necessary to study the kinetics of antiallergic drugs.

To the best of our knowledge, none of the previous studies has calculated the repeatability of a single-concentration and single-step bronchial allergen challenge using the APS, nor has any study attempted to quantify the increase in eNO between two single-step challenges. In general, an increase in eNO is detected within 24 h after allergen challenge [19] and is clearly associated with an LAR [19, 20].

Using the APS, we challenged 47 young adults with incremental doses of grass pollen extract. The dose that caused a 20% drop in FEV_1 in the EAR ($PD_{20}FEV_1$ allergen) was calculated. This individual dose was employed for two re-challenges as a single-step inhalation to study the safety and repeatability of the allergic response in the EAR with a special focus on the increase in eNO.

Subjects and Methods

Subjects

Forty-seven subjects with a known allergy to grass pollen were recruited for the study. Subjects who used a regular therapy with inhaled or oral corticosteroids, long-acting β-agonists or leukotriene receptor antagonists were excluded from the study. The study was approved by the ethics committee of Goethe University. Written informed consent was obtained from each subject.

Study Design

An open study consisting of three visits was performed. The participants attended an initial visit to assess baseline characteristics, skin prick tests, and pulmonary function tests. During the same visit, a first challenge with incremental dosages of allergen was performed. The endpoint was the dose of grass pollen allergen that caused a 20% drop in FEV_1 from baseline in the EAR ($PD_{20}FEV_1$ allergen). During the second visit, a first single-step inhalation with grass pollen allergen was administered, and the eNO level was measured before and 24 h after challenge. At the third visit, the single-step challenge and eNO measurements were repeated. An interval of at least 7 days separated all of the challenges. Intervals of 1 week or more have not demonstrated systematic changes in the bronchial response [21]. Challenges were performed when the baseline FEV_1 was at least 75% of the predicted value and the patients were free of exacerbations for the last 4 weeks and during non-birch and grass pollen season in Frankfurt, Germany.

Test Materials

Before each challenge, lyophilized grass pollen allergen (Allergopharma KG, Reinbek, Germany) was resolved in 5 ml of 0.9% saline as a solution of 5,000 standardized biological units (SBU)/ml.

Intradermal Skin Prick Test

For the skin prick test, different dilutions containing 5, 50, 500 and 5,000 SBU ml^{-1} were prepared, and the dilutions were applied in the volar skin of the forearm. The wheal response was compared to positive (histamine 1+ 999; Allergopharma) and negative (0.9% saline) controls. A wheal diameter equivalent to histamine (3 mm) was defined as positive.

Incremental Challenge Using the Aerosol Provocation System

The APS dosimeter technique (Carefusion, Hoechberg, Germany) allows the computer-controlled production of aerosol using a jet-type nebulizer (Sidestream Medicaid; Carefusion). The integrated pressure calibration procedure associated with the compressor ensures a highly constant and reproducible nebulizer output. The APS was calibrated to produce a continuous output of 240 mg min^{-1}. Several studies have demonstrated a particle size in terms of the mass median aerodynamic diameter of around 3.2 μm and an average of the fine particle fraction <5 μm of 49.7%

[22]. Using a single allergen dilution of 5,000 SBU ml^{-1}, incremental doses were predefined, and the protocol was programmed by Carefusion technicians in advance. Subjects should inhale slowly without holding their breath and should demonstrate maximal flows of less than 0.5 liters s^{-1}. During tidal breathing, the system exactly and automatically determines the administered dose of allergen. It measures the effective nebulization time and inspired dose for any breath. In continuous nebulization mode, the inhalation time and number of breaths depend on the concentration of the allergen and the ventilation of the subject. If the dose is achieved, the nebulizer stops immediately. Simultaneously, the flow recording is shown on a screen and thus the patient can visualize the flow threshold.

The incremental challenge protocol consistently followed the same algorithm as described previously [7, 23]. The dose of inhaled allergen was doubled beginning with the lowest dose of 10 SBU, according to the following pattern from steps 1–5: 10, 20, 40, 80 and 160 SBU. Thus, the entire protocol delivered cumulative doses of 10, 30, 70, 150 and 310 SBU. Ten minutes after each step-up, FEV_1 was measured. Inhalation was stopped if FEV_1 dropped >20% compared to the baseline values. The individual allergen dose that caused a 20% drop in FEV_1 in the EAR ($PD_{20}FEV_1$ allergen) was calculated using a logarithmic interpolation [24] between the doses before and after the 20% drop in FEV_1 using an integrated program (table 1).

Single-Step Challenge Using the Aerosol Provocation System

Before each single-step challenge, the APS was programmed to deliver the individual dose of allergen that caused a 20% drop in FEV1 ($PD_{20}FEV_1$) by a technician or physician (table 1). The APS delivered the dose similarly to that described above. A source of errors could be due to the entry of an incorrect, or in the worst cases, a very high dose of allergen, which could lead to overdosing. At 10, 15 and 30 min after each challenge, spirometry was performed to determine the maximum decrease in FEV_1 (EAR) compared to the initial values and the area under the curve (AUC).

Procedures Common to Both Methods

All lung function tests were performed according to American Thoracic Society guidelines [25]. Prior to the allergen challenge, all subjects inhaled 0.9% saline; a maximum drop in FEV_1 of 10% was considered acceptable [1, 11]. The postsaline FEV_1 was set as the baseline value, and a drop in FEV_1 after allergen challenge was expressed as the percentage change from baseline. During the challenge, oxygen saturation and heart rate were monitored. Each challenge ended with the inhalation of at least 0.2 mg salbutamol. After full recovery from the EAR, the subjects documented peak-flow values hourly for 12 h [11], and the LAR was defined as a drop of at least 15% in the peak flow. In this case, the subjects were instructed to inhale 0.2 mg salbutamol.

Measurement of Exhaled Nitric Oxide

Measurements of exhalative NO were conducted using NIOX1 (Aerocrine, Solna, Sweden). NIOX1 measures eNO in exhaled air according to American Thoracic Society guidelines [26]. This chemiluminescence gas analyzer is sensitive to eNO at concentrations ranging from 1.5 to 200 ppb and demonstrates a deviation from the mean value of +2.5 ppb at NO <50 ppb or +5% of the measured value at >50 ppb.

Statistical Analysis

For the statistical analyses, GraphPad Prism 5.01 (GraphPad Software Inc., La Jolla, Calif., USA) and BiAS for Windows™ (version 8; Epsilon-Publisher, Frankfurt, Germany) were used. The maximum percentage decrease in FEV_1 after each challenge, AUC and eNO after single-step challenges are expressed as means and standard deviations (SDs). The AUC for the EAR was calculated as an integral from point zero to 30 min. The maximum decreases in FEV_1 after the incremental and first single-step challenges and after both single-step challenges resembled one another and were described by the intraclass correlation (ICC). Repeatability was evaluated using the method described by Bland and Altman [27]. The means and SD of the differences between both measurements were calculated. Among the differences, 95% should lie between the mean difference ±1.96 SD (95% limits of agreement). The calculations included the maximum decreases in FEV_1, AUC and eNO.

Results

Participant Characteristics

All subjects had baseline FEV_1 values of more than 80% of the predicted. For the FEV_1 and FVC maneuvers, ATS/European Respiratory Society test criteria for acceptability and repeatability [25] were met in 93.2% of all measurements. Forty-seven subjects underwent an incremental challenge with grass pollen; 7 did not meet the criteria of a drop of 20% in FEV_1. One participant was excluded due to an asthma exacerbation between the first and second single-step challenge. In total, 39 subjects (19 female and 20 male) ranging in age from 18 to 40 years (mean 24.8 ± 4.3 years) underwent a first single-step challenge. Six subjects were monosensitized to grass pollen, 7 additionally to birch pollen and 26 to further allergens (house dust mite, cat and moulds). Thirty-five subjects were nonsmokers and 4 were smokers.

Safety

The bronchial allergen challenges caused no severe obstruction in the EAR (decrease in FEV_1 of more than 50%). The oxygen saturation was consistently higher than 92%. The mean of the maximum fall in FEV_1 in the incremental challenge was 29.7 ± 7.5%. Although some participants had cough and chest tightness, they were all able to complete the entire study protocol. No participant required rescue medication prior to the last FEV_1 measurement at 30 min. In general, the rescue medication caused an immediate recovery of asthma symptoms.

A late asthmatic response developed in 13 of the 39 participants (33.3%) after the incremental challenge. After the first and second single-step challenges, the LAR was recorded in 6 of 39 (15.3%) and in 3 of 26 (11.5%) challenges, respectively.

Table 1. Patient characteristics

Patient	Age years	FEV_1 % pred	Cumulative allergen dose, SBU	Individual $PD_{20}FEV_1$ SBU	1st single-step challenge fall FEV_1% pred	2nd single-step challenge fall FEV_1% pred	1st single-step challenge Δ eNO, ppb	2nd single-step challenge Δ eNO, ppb
1[a]	22	86	310	159	14.5	16.4	31.5	55.1
2[a]	25	97	70	37	16.3	20.3	18.0	19.5
3	29	90	70	48	29.0	20.7	42.0	52.0
4[a]	24	80	70	42	27.5	16.5	1.0	0.0
5[a]	27	98	150	86	22.9	24.8	23.0	28.0
6	22	107	30	27	9.6	12.1	89.0	75.0
7[a]	18	102	310	201	18.8	21.4	34.0	31.0
8	23	85	70	39	38.9	17.8	17.0	26.0
9	26	88	30	19	31.9	43.4	17.0	22.0
10[a]	22	113	310	247	22.7	27.1	62.0	37.0
11[b]	26	110	70	42	11.7	14.8	43.3	33.0
12[a]	23	94	310	237	26.8	30.4	37.0	14.0
13[a, b]	23	118	310	225	12.5	16.3	21.0	–21.0
14	24	113	150	100	33.9	14.8	61.7	57.0
15[a]	22	99	70	40	19.0	18.7	132.0	66.0
16	22	105	70	44	31.2	17.3	17.0	87.0
17[a]	40	82	10	20	20.1	14.9	–5.0	–9.0
18[a]	21	125	30	23	20.4	17.1	40.0	52.0
19	27	107	70	53	33.1	35.9	42.0	21.0
20[a]	26	107	30	27	24.6	28.3	29.0	3.0
21[a]	24	103	150	87	12.7	14.6	12.0	36.5
22[a]	25	102	10	3	14.4	20.5	22.0	31.0
23	20	102	30	21	7.4	16.7	50.0	48.1
24[a]	36	80	30	14	18.0	30.7	3.4	2.6
25[a, b]	28	109	30	20	25.0	28.9	29.0	28.7
26[a]	29	106	30	11	20.1	16.3	22.4	17.1
27[b]	26	108	150	89	29.6	32.4	34.6	36.3
28	26	101	150	98	32.1	22.0	35.5	31.7
29[a]	24	103	30	28	19.3	28.3	43.0	44.2
30	21	95	70	56	8.1	27.1	53.9	91.8
31[a]	29	120	70	38	26.5	16.2	30.0	37.4
32	30	109	70	54	33.7	17.3	51.3	15.0
33[a]	23	95	30	18	12.1	9.9	13.2	11.0
34[a]	27	96	10	10	12.2	14.6	5.9	–3.0
35[a, b]	19	84	10	5	14.0	14.2	28.0	–6.0
36[a]	24	102	70	70	23.1	13.6	38.0	21.0
37[a]	20	120	70	40	17.6	17.7	46.0	48.0
38[a]	20	98	10	7	16.4	19.0	–15.0	9.0
39[a]	26	95	310	157	21.6	28.0	–4.0	13.0
Mean	24.8	101	99.8	65.2	21.3	20.9	31.5	29.8
SD	4.3	11.1	99.4	65.9	8	7.2	27.4	25.5

$PD_{20}FEV_1$, SBU = Dose of standardised allergen units that produces a 20% decrease in the FEV_1.
[a] $Group_{12–28}$. [b] Smoker.

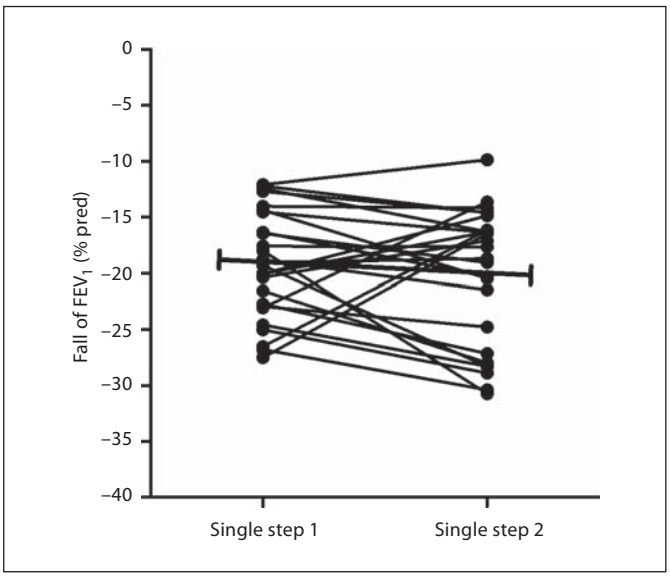

Fig. 1. Individual maximal decreases in FEV_1 (mean and SD) in the early asthmatic response after the first (19.2 ± 4.8%) and second single-step challenges (20.2 ± 6.1%) with grass pollen in 26 subjects ($group_{12–28}$).

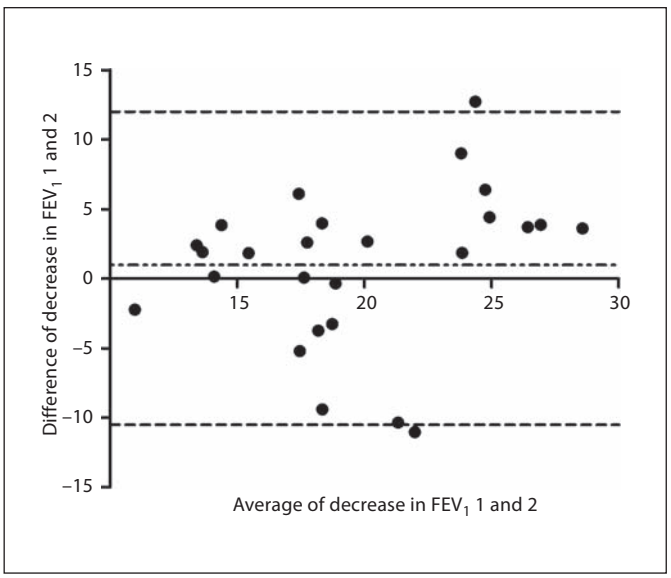

Fig. 2. Difference between the average differences in the maximal fall in FEV_1 (% pred) in the first and second single-step challenges in $group_{12–28}$ (Bland and Altman plot).

As shown in table 2, the patients differed only with respect to the LAR during challenge. All other parameters, the maximal drop in FEV_1, the increase in eNO and the $PD_{20}FEV_1$, were similar.

Skin Testing

A comparison of the endpoint concentration in the prick test and $PD_{20}FEV_1$ revealed no correlation according to Spearman's coefficient (rho = 0.08, p = 0.61, data not shown).

Bronchial Challenges

The time interval between challenges comprised a median of 12 days. Single-step challenges could be performed without interruptions caused by cough or chest tightness. The mean duration of the incremental challenge was 33.7 ± 13.8 min, and a median of 3 step-ups (range 1–5) was required. The initial eNO was 22.6 ± 13.8 ppb. The cumulative mean dose of allergen in the incremental challenge was 99.8 ± 99.4 SBU, and the mean dose of allergen in the first single-step challenge was 65.2 ± 65.9 SBU (table 1). In the first single-step challenge, the mean drop in FEV_1 in the EAR was 21.3 ± 8.0%. A comparison of the fall in FEV_1 to the initial incremental challenge (29.7% ± 7.5) revealed an ICC of –0.30 ($p < 0.05$). In the second single-step challenge, the mean decrease in FEV_1 was 20.9 ± 7.2%. Compared with the first single-step challenge, the ICC was 0.37 ($p < 0.05$), and the 95% limits of agreement according to Bland and Altman were –17.5 to 18.1%. Next, we defined a group that demonstrated a decrease in FEV_1 ± 8 of approximately 20% in the first single-step challenge ($group_{12–28}$) to increase the reproducibility of the single bronchial allergen challenge. Twenty-six of 39 patients (66.6%) were within the range ($group_{12–28}$); 9 patients demonstrated a drop of more than 28% and 4 had a drop of less than 12% in FEV_1.

Repeatability of Single-Step Challenges in $Group_{12–28}$

In the first single-step challenge, the mean decrease in FEV_1 in the EAR was 19.2 ± 4.8%; in the second challenge, it was 20.2 ± 6.1% (fig. 1). A comparison of the results revealed an ICC and repeatability according to Bland and Altman of 0.46 ($p < 0.005$) and 95% limits of agreement of –10.5 to 12.0% (fig. 2).

After the first and second challenge, the AUC was 6.9 ± 1.9 and 6.9 ± 2.2, respectively. The ICC was 0.53 ($p < 0.001$) with 95% limits of agreement of –5.1 to 5.1. Before the first and second single-step challenge, the eNO levels were 37.6 ± 21.6 ppb and 42.1 ppb ± 22.8, respectively (p = 0.24, 95% CI –3.5 to 13.6). Within 24 h, the changes in eNO levels demonstrated an increase of 26.8 ± 27.8 ppb after the first and an increase of 21.8 ± 21.9 ppb after

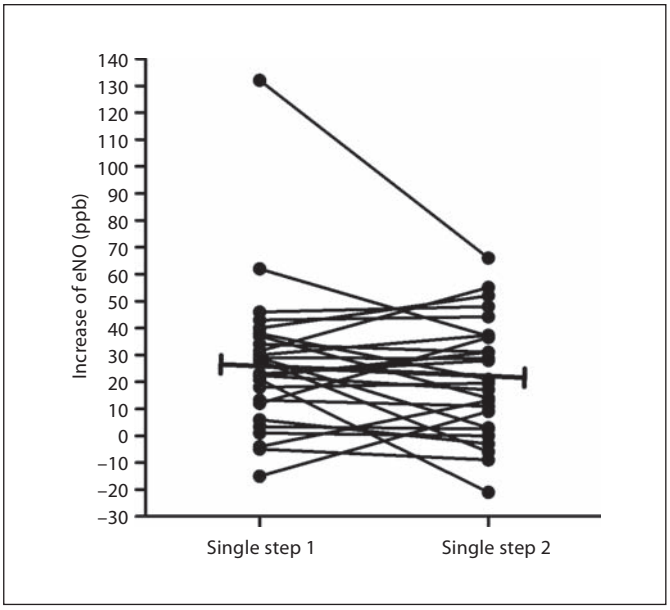

Fig. 3. Individual increases in eNO levels (mean and SD) in the early asthmatic response 24 h after the first (26.8 ± 27.8 ppb) and second single-step challenges (21.8 ± 21.9 ppb) with grass pollen in group$_{12–28}$.

Table 2. Mean values of the patient groups with and without LAR

	Patients with LAR	Patients without LAR	p value
Number of patients	13	26	
Baseline measurement values			
FEV_1, %	98.3	103.1	0.21
eNO, ppb	25.1	21.4	0.44
LAR (PEF decrease)			
Incremental test, %	20.5	5.6	<0.0001
Single-step test 1, %	13.0	8.6	0.07
Single-step test 2, %	13.8	7.8	0.02
FEV_1 decrease			
Incremental test, %	28.0	30.6	0.32
Single-step test 1, %	21.1	21.3	0.94
Single-step test 2, %	23.1	19.9	0.21
eNO increase			
Single-step test 1, %	31.4	32.5	0.90
Single-step test 2, %	37.6	25.9	0.18
$PD_{20}FEV_1$			
Incremental test, SBU/ml	59.9	67.8	0.73

the second single-step challenge (fig. 3). The ICC was 0.62 ($p < 0.001$) with 95% limits of agreement of –39.38 to 49.47 ppb.

Discussion

Specific bronchial allergen provocation is an established tool in asthma research for proof-of-concept studies of new anti-inflammatory agents. However, numerous methodological differences are observed in bronchial allergen challenges, and there is an ongoing debate concerning whether to use a protocol with incremental concentrations of allergen or the newly available dosimeter techniques to deliver a defined amount of a given allergen dilution. This is the first study to investigate the repeatability of single-step, single-dose bronchial allergen challenges in the EAR using a new APS. A constant-dose allergen challenge is a sensitive tool for detecting changes in the EAR and the LAR after the use of an antiasthmatic medication, and a relatively small number of subjects is required to demonstrate significant differences [16]. Published investigations have compared incremental and bolus challenges [1, 18] as well as different kinds of incremental challenges [10, 16, 17].

Although both procedures correlated well with one another [1, 10, 16–18], the single-step method has been superior in proof-of-concept studies because the APS dosimeter technique allows a constant-dose allergen administration, which is of particular importance in monitoring inflammation markers.

During the first visit, we performed an incremental challenge to select the appropriate allergen doses for the subsequent single-step challenges. As expected, a correlation between incremental and single-step challenges was shown, but after the incremental challenge, the mean fall in FEV_1 in the EAR was slightly higher (29.7 ± 7.5%) than that in the single-step challenge (21.3 ± 8.0%). Interestingly, the LAR occurred more often after incremental compared to single-step challenges. These findings are most likely explained by a higher cumulative allergen dose in the incremental challenge. The administration of a single-step dose represents the PD_{20} interpolated from the log-dose response curve derived from the initial (incremental) challenge and may provide a diminished bronchoconstrictor response compared to the screening challenge, because the latter challenge usually produces a decrease greater than 20% during the EAR [1]. Another explanation is that progressive bronchoconstriction with increasing doses causes a progressively greater deposition

of the inhaled particles in the airways and increases the airway response.

The evaluation of the LAR using peak-flow meters might be disadvantageous because small changes in airway obstruction might be missed. However, two previous studies [28, 29] showed that peak-flow measurements are a valuable tool to detect an LAR. An absolute decrease in the PEF of ≥80 liters min^{-1} correlated well with an FEV_1 decrease of ≥15% between challenges. Indeed, in our study, all decreases of ≥15% of PEF corresponded with an absolute decrease of ≥80 liters min^{-1}. Therefore, the cutoff of a 15% decrease in PEF represents a significant reaction in LAR, but values of less than 15% might be less sensitive than FEV_1 monitoring. In the overall response, the mean fall in the PEF was 10.1 ± 7.2% in the first single-step challenge and 9.8 ± 7.4% in the second challenge (ICC 0.80, $p < 0.0001$).

There are two other issues that may contribute to the deficit of LAR. Grass pollen might not be the optimal allergen to produce LAR. In the study of Hatzivlassiou et al. [30], asthmatics with dual sensitization to the house dust mite allergen and the grass pollen allergen underwent an inhalation allergen challenge with these separate allergens. Interestingly, despite a comparable decrease in the percent of FEV_1 of the group mean EAR, the LAR was statistically greater after the house dust mite challenge. Because EAR and LAR are directly related to the cumulative dose of inhaled allergens [31], these data indicate that LAR is dependent on the specific allergen used and on the dose of the allergen given.

This is the first study to investigate the repeatability of two subsequent single-step, single-dose allergen challenges, and an ICC of 0.46 was determined with respect to the maximum fall in FEV_1 in the EAR. A correlation between repeated challenges has been confirmed in the literature. In a study using the Mefar® dosimeter, the mean decrease in FEV_1 in the EAR was 33.1 ± 1.8% in the incremental and 29.9 ± 2.2% in the bolus challenges [1]. The agreement of the decrease in FEV_1 was poorer during the EAR (ICC 0.55) compared to the LAR (ICC 0.75) [1]. Another study in 17 subjects showed an ICC of 0.40 in the EAR and an ICC of 0.32 in the LAR, with respect to the maximum drop in FEV_1 [16].

However, despite good correlations, the authors describe relatively wide between-subject variabilities [1]. In another study, 73% of the patients who demonstrated a 20% drop in FEV_1 in the incremental challenge produced a similar drop in FEV_1 in the EAR during a single-dose inhalation. Among the entire group, the repeatability according to Bland and Altman was poor (limits of agreement of –29.5 to 22.5%) [18]. In repeated incremental challenges with the same allergen concentrations, the results obtained for 14 patients revealed great variability in the intrasubject reproducibility, and the PD_{20} in the EAR demonstrated significant differences [10].

The correlation coefficients (for example ICC) do not aid in the interpretation of measurements in a given subject. To prove this, a consideration of the variability between repeated measurements in the same subject is needed. This objective is provided by the repeatability coefficient, which is directly comparable to the 95% limits of agreement [27].

Among the total group in the single-step challenges, the repeatability according to Bland and Altman was moderate, and the 95% CI for the drop in FEV_1 in repeated challenges was between –2.5 and –38.1%. In the medication test, this range will not sufficiently discriminate between treatment and control subjects, because controls might demonstrate a decrease in FEV_1 of less than 10%.

For safety reasons, it is appropriate to adjust the allergen dose in proof-of-concept studies prior to the start of treatment to avoid the overdosing of patients, and we arbitrarily defined a group represented by a drop in FEV_1 of 20% ± 8 in the first single-step challenge ($group_{12–28}$). The results showed that two thirds of the subjects will meet this range, demonstrating adequate limits of agreement with a 95% CI for the decrease in FEV_1 in the EAR of –32.8 to –9.2%. In repeated measurements and medication testing, this range will provide significant discriminatory power.

To overcome the variations in FEV_1, some authors recommend the measurement of the AUC as a read-out parameter [1, 16, 17]. This was verified in the present study and demonstrated an ICC of 0.53 for the AUC. However, there was a broad variation according to Bland and Altman [27], and the limits of agreement revealed broad variations. We believe that this result is due to the use of serial measurements of FEV_1 to determine the AUC, and the variance of each measurement will affect the AUC. The results are consistent with a previous study suggesting that the $PD_{20}FEV_1$ is the best representative index of the EAR [10].

Because the APS technique permits the administration of a constant dose, this is the first study to compare the increase in eNO levels after repeated bronchial challenges with the same amount of allergen. The ICC results demonstrated substantial agreement, whereas the limits of agreement revealed intraindividual variations. In contrast to the results of previous studies [19, 20], the increase

in eNO levels was independent of the LAR. This difference is not completely explained by the biological variation of the investigated patients. The subjects in our study population presented allergic rhinitis and mild seasonal asthma. Several epidemiological studies have confirmed that eNO levels are increased in atopic subjects irrespective of whether they present significant lower respiratory tract symptoms [32]. In addition, natural pollen exposure causes a significant elevation of FeNO in patients with intermittent allergic rhinitis [33].

A limitation of the study is that the majority of the studied patients suffered from allergic rhinitis with mild episodic asthma only. However, recent data suggest that the inflammatory response in the lower airways of subjects with allergic rhinitis is similar to that observed in asthmatic subjects during a natural allergen exposure [34] and after a low-dose allergen challenge [35]. Both groups demonstrated a significant increase in lymphocytes and eosinophils in bronchial biopsies or in sputum, whereas persistent asthmatic patients differed from the subjects with rhinitis alone with respect to their capacity to release a greater number of mediators following the antigen challenge [36]. To address this important question in more detail, we compared 13 patients (33.3%) with an LAR to those demonstrating an EAR following bronchoprovocation. As shown in table 2, patients with LAR versus EAR showed similar reactions with respect to the maximal drop in FEV_1, increase in eNO and the $PD_{20}FEV_1$ allergen. Thus, our data suggest that patients with grass pollen allergy serve as an appropriate model for allergic asthma.

In summary, our results show that use of the APS dosimeter to calculate the $PD_{20}FEV_1$ allergen is a timesaving procedure and is less prone to errors because only one allergen dilution is used. The repeatability determined in well-defined subjects is fairly good and permits the study of the mechanisms responsible for allergen-induced airway inflammation, especially the induction of eNO. Our method provides a reliable tool for testing new anti-allergic and anti-inflammatory agents in asthma and in allergy research.

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