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Validated safety predictions of airway responses to house dust mite in asthma

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Summary

Background House dust mite (HDM) is the most common aeroallergen causing sensitization in many Western countries and is often used in allergen inhalation challenges. The concentration of inhaled allergen causing an early asthmatic reaction [provocative concentration of inhaled allergen causing a 20% fall of forced expiratory volume in 1 s (FEV₁)(PC₂₀ allergen)] needs to be predicted for safety reasons to estimate accurately the severity of allergen-induced airway responsiveness. This can be accomplished by using the degree of non-specific airway responsiveness and skin sensitivity to allergen.

Objective We derived prediction equations for HDM challenges using PC₂₀ histamine or PC₂₀ methacholine and skin sensitivity data obtained from patients with mild to moderate persistent asthma and validated these equations in an independent asthma population.

Methods PC₂₀ histamine or PC₂₀ methacholine, skin sensitivity, and PC₂₀ allergen were collected retrospectively from 159 asthmatic patients participating in allergen challenge trials. Both the histamine and methacholine groups ($n = 75$ and $n = 84$, respectively), were divided randomly into a reference group to derive new equations to predict PC₂₀ allergen, and a validation group to test the new equations.

Results Multiple linear regression analysis revealed that PC₂₀ allergen could be predicted either from PC₂₀ methacholine only ($^{10}\log \text{PC}_{20} \text{ allergen} = -0.902 + 0.741 \cdot ^{10}\log \text{PC}_{20} \text{ methacholine}$) or from PC₂₀ histamine and skin sensitivity (SS) ($^{10}\log \text{PC}_{20} \text{ allergen} = -0.494 + 0.231 \cdot ^{10}\log \text{SS} + 0.546 \cdot ^{10}\log \text{PC}_{20} \text{ histamine}$). In the validation study, these new equations accurately predicted PC₂₀ allergen following inhalation of HDM allergen allowing a safe starting concentration of allergen of three doubling concentrations below predicted PC₂₀ allergen in all cases.

Conclusion The early asthmatic response to inhaled HDM extract is predominantly determined by non-specific airway responsiveness to methacholine or histamine, whereas the influence of the cutaneous sensitivity to HDM appears to be rather limited. Our new equations accurately predict PC₂₀ allergen and hence are suitable for implementation in HDM inhalation studies.

Keywords airway responsiveness, asthma, bronchial allergen challenge, Dermatophagoides pteronyssinus, skin sensitivity

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Introduction

The significance of house dust mites (HDM) for allergic respiratory diseases worldwide was first published in 1967 [1]. Since then, it has been reported frequently that the HDM plays a role in both asthma development and symptoms [2–4]. In many Western countries, the HDM is ubiqui-

tous and the most common aeroallergen causing allergic sensitization [5], in particular, in areas with a moderate/coastal type of climate [6]. For this reason, allergen inhalation challenges in research studies during the past decade have often been performed using HDM extract [7].

Allergen inhalation challenges are of great value in research for investigating the pathophysiology of asthma

[8, 9], and studying prophylactic, pharmacological agents [10]. For safety purposes, an accurate estimation of the severity of allergen-induced airway responsiveness is essential. This can be achieved by prior measurements of the degree of atopic sensitization and the sensitivity of the target organ as reflected by the level of airway hyper-responsiveness to e.g. histamine or methacholine. To this end, allergen challenge and histamine or methacholine challenge need to be performed using the same method. In addition, atopic sensitization measured as skin sensitivity has to be determined using the same allergen and dilutions that are being used for the allergen inhalation challenge. In this way, Cockcroft et al. [11, 12] showed that the severity of the early asthmatic response (EAR), expressed as the provocative concentration of inhaled allergen causing a 20% fall of forced expiratory volume in 1 s (FEV₁) (PC₂₀ allergen), can be predicted from the PC₂₀ histamine as a measure of non-allergic airway responsiveness, and skin sensitivity to allergen as an estimate of circulating allergen-specific IgE. Their prediction equation is based on data from a population of 51 asthma patients who were challenged with various antigens including pollen (60.8%), cat (21.6 %), or *Dermatophagoides farinae* (9.8%) [12]. Recently, it was shown that the EAR may also be predicted from the PC₂₀ methacholine in a population of 158 asthma patients who were provoked with animal allergens (34.2%), pollen (31.0%), mite including *D. pteronyssinus* (30.4%) and *D. farinae* (3.8%), or mould (0.63%) [13].

To date, prediction equations for PC₂₀ allergen specific for HDM inhalation challenge in a Western asthma population have not yet been developed. Therefore, the aims of the present study were to determine such prediction equations for HDM challenges using PC₂₀ histamine or PC₂₀ methacholine and skin sensitivity obtained from patients with mild to moderate persistent asthma and to validate these equations in an independent asthma population.

Methods

Subjects

For this study, data from non-smoking patients with mild to moderate persistent asthma were selected [14]. All subjects experienced episodic chest tightness, dyspnoea and/or wheezing, showed airway hyper-responsiveness (PC₂₀ methacholine or PC₂₀ histamine < 8 mg/mL), and were atopic to HDM as determined by the skin prick test. They were clinically stable, only used short-acting β_2 -agonists, and had no history of a recent (< 2 weeks) upper respiratory tract infection or other relevant diseases.

Study design

A retrospective analysis of nine allergen challenge research studies in the Leiden University Medical Center

from 1992 to 2005 using HDM extract was performed [15–21]. All data used were collected during screening visits for clinical trials. Asthmatic subjects were included in the analysis if the following data were available: PC₂₀ histamine or PC₂₀ methacholine, PC₂₀ allergen and skin sensitivity determined from a dose–response allergen skin prick test. In most cases PC₂₀ methacholine or PC₂₀ histamine and skin sensitivity were determined within 1–2 weeks before the allergen challenge. For subjects who had participated in multiple trials, the first data collected were used in the analysis to avoid any possibility of a selection bias.

Histamine and methacholine inhalation test

Bronchoprovocation tests with histamine (0.03–8 mg/mL; Sigma Chemicals, St Louis, MO, USA), methacholine bromide (0.03–8 mg/mL; Janssen Pharmaceutica, Beerse, Belgium), or methacholine chloride (0.048–12.5 mg/mL; Provocholine[®], Methapharm Inc., Brantford, ON, Canada) were performed by standardized methodology [22]. Serial doubling concentrations were aerosolized with the use of a jet-nebulizer (model 646; DeVilbiss, Somerset, PA, USA) (output 0.13 mL/min) and inhaled by tidal breathing for 2 min at 5-min intervals with the nose clipped, until FEV₁ declined by $\geq 20\%$ from post-saline baseline. FEV₁ was determined at 30 and 90 s after each inhalation. The lowest value of a technically satisfactory recording was used in the analysis. PC₂₀ was calculated as the concentration causing a 20% fall in FEV₁ from post-saline baseline using a log-linear interpolation of two adjacent data points.

Skin tests

Atopy was determined using a skin prick test for the following common air-borne allergen extracts (Soluprick, ALK, Benelux): HDM (*D. pteronyssinus*) (10 000 BU/mL), cat (10 000 BU/mL), dog (10 000 BU/mL), horse (10 000 BU/mL), tree mix (3) (10 000 BU/mL), grass mix (5) (10 000 BU/mL), mugwort (10 000 BU/mL), aspergillus (1 : 20 g/v), and alternaria (1 : 20 g/v). Histamine (10 mg/mL) and skin prick test solution (phosphate-buffered saline with 0.03% human serum albumin and 0.5% phenol as a preservative) were used as positive and negative control, respectively.

To determine skin sensitivity, a multi-dose skin prick test was performed in duplicate with doubling concentrations of purified aqueous allergen extract of *D. pteronyssinus* (SQ 503, Vivodiagnost, ALK, Benelux) from 1 : 1 (2000 BU/mL) to 1 : 1024 (1.95 BU/mL) and skin prick test solution as a negative control. The weal diameter was measured in two perpendicular directions at 15 min, and the mean weal diameter was determined for each dilution. The skin sensitivity expressed as skin test endpoint (SS) was defined as the weakest allergen concentration

producing a weal 2 mm in diameter relative to the negative control on each of two duplicate prick tests [12, 22].

Allergen inhalation test

Allergen challenges were performed according to a standardized protocol [12, 15, 22]. At the time of the studies, PC₂₀ allergen was predicted from PC₂₀ histamine and skin test endpoint (SS), according to the available formula by Cockcroft: $^{10}\log \text{PC}_{20} \text{ allergen} = 0.68 \cdot ^{10}\log(\text{PC}_{20} \text{ histamine} \cdot \text{SS})$ [12]. When methacholine challenges were performed, PC₂₀ methacholine was substituted in the histamine-based equation. Before allergen challenge, the diluent was inhaled to assure FEV₁ stability (< 10% fall). Purified aqueous allergen extract of *D. pteronyssinus* (SQ 503; Vivodiagnost, ALK Abello, Nieuwegein, the Netherlands) was aerosolised by a jet-nebulizer (model 646; DeVilbiss, Somerset, PA) calibrated to an output of 0.13 mL/min. Starting three to four concentrations below the predicted PC₂₀ allergen for safety requirements, consecutive doubling concentrations of allergen ranging from 1 : 1024 (1.95 BU/mL) to 1 : 1 (2000 BU/mL) were inhaled by tidal breathing for 2 min with the nose clipped at 10 min intervals, until a fall in FEV₁ of $\geq 20\%$ from post-diluent baseline was reached. The response to allergen was measured in duplicate 10 min after each inhalation of allergen, and repeated at 10, 20, 30, 40, 50, 60, 90, and 120 min, and then hourly until 7–8 h after the last inhalation. At each time-point, the highest technically satisfactory FEV₁ value was applied in the analysis and expressed as percentage change in FEV₁ from post-diluent baseline. The EAR was determined as the maximum percent fall in FEV₁ during the interval of 0–3 h post-allergen.

Analysis

PC₂₀ allergen was defined as the final concentration of allergen causing a fall in FEV₁ $\geq 20\%$ 10 min after inhalation. PC₂₀ allergen, PC₂₀ methacholine, PC₂₀ histamine, and allergen skin test endpoint data were log-transformed before the analysis. Linear regression was performed to calculate new equations predicting PC₂₀ allergen. Subsequently, a separate external validation study was performed to test the diagnostic accuracy of the newly derived equations [23]. To this end, both the histamine and the methacholine group were divided randomly into a reference group to derive a new equation by regression analysis ($n = 50$ and $n = 54$, respectively), and a validation group to test the performance of this new equation ($n = 25$ and $n = 30$, respectively). The estimated values of PC₂₀ allergen based on the new equations were compared with those based on the formulas previously published by Cockcroft et al. [12, 13]

$$^{10}\log \text{PC}_{20} \text{ allergen} = 0.68 \cdot ^{10}\log(\text{PC}_{20} \text{ histamine} \cdot \text{SS})$$

$$(r^2 = 0.67)$$

or

$$^{10}\log \text{PC}_{20} \text{ allergen} = -0.42 + 0.50 \cdot ^{10}\log \text{SS}$$

$$+ 0.41 \cdot ^{10}\log \text{PC}_{20} \text{ methacholine}$$

$$(r^2 = 0.37)$$

Results

Subjects

We identified 159 patients with mild to moderate persistent asthma who met the inclusion criteria. In 75 subjects, a PC₂₀ histamine was determined before allergen challenge and in 84 subjects a PC₂₀ methacholine. Patient characteristics are described in Table 1. There were no significant differences with respect to age, lung function, airway hyper-responsiveness, skin sensitivity, or EAR between the reference and the validation group for either histamine or methacholine ($P > 0.05$).

New equations

A multiple linear regression analysis of log PC₂₀ allergen vs. log PC₂₀ histamine and log SS revealed the following regression formula (Table 2):

$$^{10}\log \text{PC}_{20} \text{ allergen} = -0.494 + 0.231 \cdot ^{10}\log \text{SS}$$

$$+ 0.546 \cdot ^{10}\log \text{PC}_{20} \text{ histamine.}$$

The regression coefficients for log PC₂₀ histamine and log SS were both significant.

A multiple linear regression analysis of log PC₂₀ allergen vs. log PC₂₀ methacholine and log SS revealed the following regression formula (Table 2):

$$^{10}\log \text{PC}_{20} \text{ allergen} = -0.673 + 0.177 \cdot ^{10}\log \text{SS}$$

$$+ 0.704 \cdot ^{10}\log \text{PC}_{20} \text{ methacholine.}$$

The regression coefficient was significant for log PC₂₀ methacholine, but only showed a trend towards significance for log SS. Excluding skin sensitivity as a predictor of PC₂₀ allergen yielded the following equation:

$$^{10}\log \text{PC}_{20} \text{ allergen} = -0.902 + 0.741$$

$$\cdot ^{10}\log \text{PC}_{20} \text{ methacholine.}$$

Validation of new equations

Subsequently, the accuracy of predicting the allergen PC₂₀ by Cockcroft's formulas and our new regression formulas was assessed. Figure 1 shows that PC₂₀ allergen could be predicted from PC₂₀ histamine and SS in all subjects within 2 DD using our new formula and within 3 DD using Cockcroft's formula. PC₂₀ allergen could be predicted

Table 1. Demographics

	Histamine		Methacholine	
	Reference group	Validation group	Reference group	Validation group
<i>n</i>	50	25	54	30
Gender (M/F)	26/24	14/11	13/41	8/22
Age (years)	22.5 (0.54)	22.8 (0.70)	22.4 (0.54)	23.5 (0.93)
FEV ₁ (L)	3.78 (0.10)	4.00 (0.14)	3.55 (0.08)	3.67 (0.15)
FEV ₁ % predicted	93.5 (1.42)	95.4 (2.46)	94.6 (1.35)	97.5 (2.09)
PC ₂₀ histamine (mg/mL)	1.00 (0.06–6.18)	0.65 (0.14–5.20)		
PC ₂₀ methacholine (mg/mL)			0.78 (0.09–7.41)	0.67 (0.06–8.75)
SS (dilution 1:x)	20.5 (2–128)	24.9(4–256)	20.7 (2–256)	22.1 (8–512)
EAR at PC ₂₀ allergen (% fall FEV ₁)	28.5 (0.93)	29.0 (1.52)	27.7 (0.77)	26.6 (0.87)
Atopy				
<i>D. pteronyssinus</i>	50 (100)	25 (100)	54 (100)	30 (100)
Mixed grass pollen	29 (58.0)	19 (76.0)	32 (59.2)	26 (86.7)
Mixed tree pollen	18 (36.0)	11 (44.0)	21 (38.9)	20 (66.7)
Cat	32 (64.0)	18 (72.0)	42 (77.8)	25 (83.3)
Dog	33 (66.0)	21 (84.0)	39 (72.2)	25 (83.3)
Horse	10 (20.0)	2 (8.0)	7 (13.0)	2 (6.7)

Data are represented in the format mean (SEM) except that for PC₂₀ histamine, PC₂₀ methacholine, and skin test endpoint (SS) which are in the format geometric mean (range). For atopy the format is number of subjects (% of group).

FEV₁, forced expiratory volume in 1 s; PC₂₀, provocative concentration causing a 20% fall in FEV₁; SS, skin test endpoint; EAR, early asthmatic reaction.

Table 2. Multiple linear regression of PC₂₀ allergen vs. log PC₂₀ histamine or PC₂₀ methacholine, and log skin test endpoint

Model		<i>B</i>	SE	<i>P</i> -value
<i>Histamine</i>				
1	Adjusted <i>R</i> ²	0.394		< 0.001
	Constant	− 0.494	0.147	0.002
	Log PC ₂₀	0.546	0.106	< 0.001
	Log SS	0.231	0.107	0.035
<i>Methacholine</i>				
1	Adjusted <i>R</i> ²	0.573		< 0.001
	Constant	− 0.673	0.127	< 0.001
	Log PC ₂₀	0.704	0.091	< 0.001
	Log SS	0.177	0.092	0.059
2	Adjusted <i>R</i> ²	0.551		< 0.001
	Constant	− 0.902	0.047	< 0.001
	Log PC ₂₀	0.741	0.091	< 0.001

from PC₂₀ methacholine and SS in all subjects within 3 DD using either our new formula or Cockcroft's formula. When leaving out SS from our new equation, PC₂₀ allergen was predicted equally well within 3 DD in all subjects. Figure 2 shows the identity plots for the measured PC₂₀ allergen and the predicted PC₂₀ allergen that was estimated with either our new equations or Cockcroft's formulas.

Discussion

In this study we present new equations to predict the PC₂₀ allergen following inhalation of HDM extract, based on

either PC₂₀ methacholine, or PC₂₀ histamine and skin sensitivity to HDM. In addition, we demonstrated that these new equations could accurately predict the PC₂₀ HDM in atopic patients with mild to moderate persistent asthma, thus allowing a safe starting concentration. Hence, our novel formulae complement the standard by Cockcroft et al. [12] recommended by the ERS [22].

Although previous studies have addressed the role of non-specific airway hyper-responsiveness and the degree of sensitization to HDM in the prediction of the early asthmatic response [24–26], the present study not only determined equations based on either PC₂₀ methacholine or PC₂₀ histamine to predict the airway response to HDM

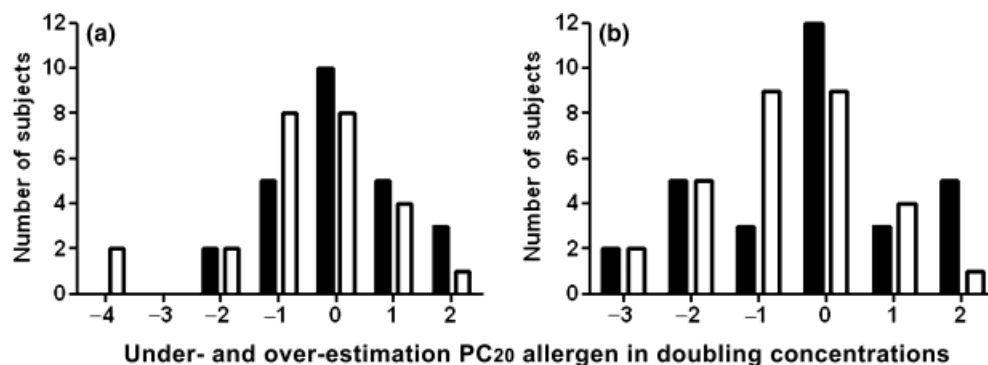


Fig. 1. Under- and overestimation of PC_{20} allergen in doubling concentrations by our new equations (■) and Cockcroft's formulas (□), based on either PC_{20} histamine (a) or PC_{20} methacholine (b). Negative values indicate a prediction that is more dilute than the measured PC_{20} allergen.

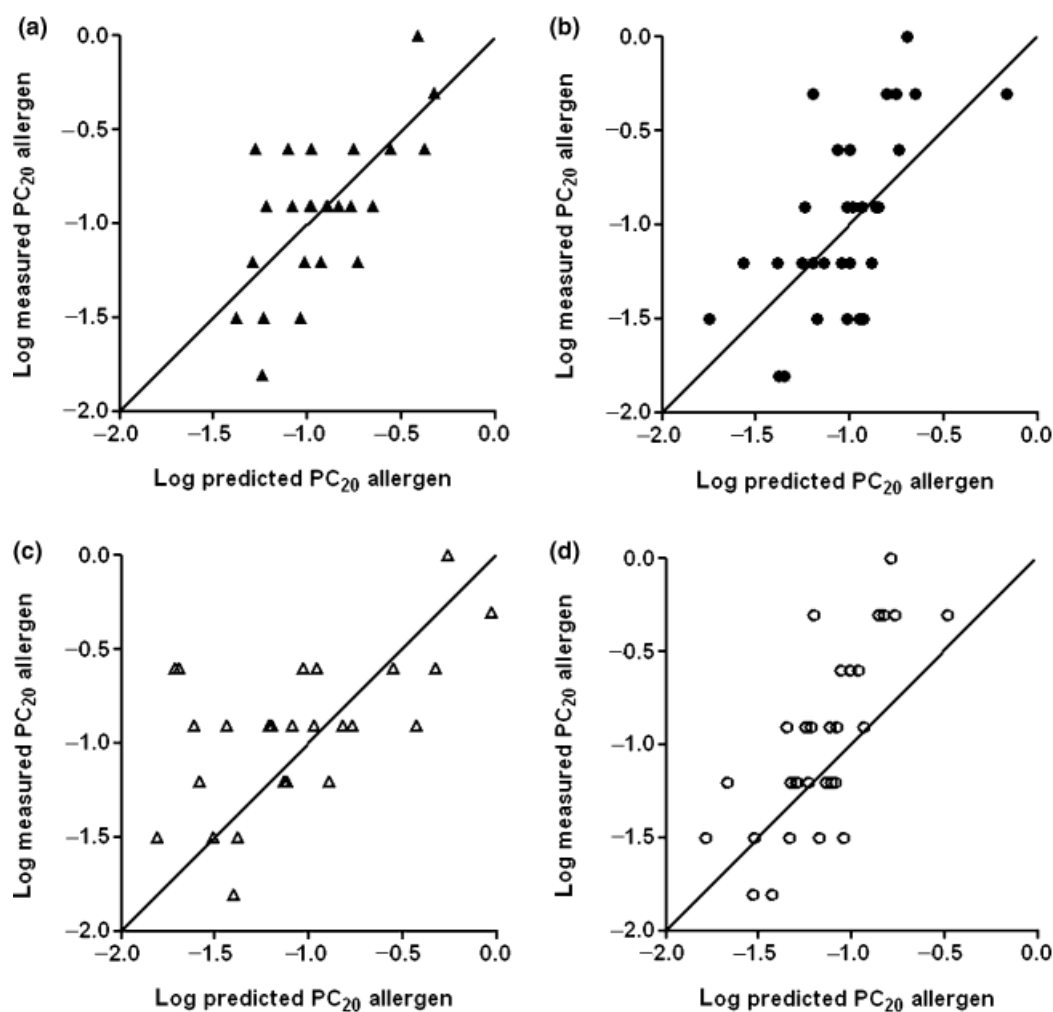


Fig. 2. Identity plots of the measured PC_{20} allergen (log) and predicted PC_{20} allergen (log) based on: (a, b) our new equations with PC_{20} histamine (▲) or PC_{20} methacholine (●), or (c, d) Cockcroft's formulas with PC_{20} histamine (Δ) or PC_{20} methacholine (○).

challenge but also validated these equations in an independent population of patients with asthma.

Our findings have demonstrated for the first time that airway hyper-responsiveness to methacholine is sufficient by itself to predict accurately the EAR after HDM inhala-

tion in a Western European asthma population, explaining 55% of the variance of the PC_{20} allergen. It could be debated whether skin sensitivity should also be included in this equation as earlier studies found that the degree of sensitization expressed as skin sensitivity or RAST could

significantly predict the airway response to inhaled allergens [13, 24, 27] and in some cases contributed even more to the prediction of the EAR than PC₂₀ methacholine. Although skin sensitivity showed a trend towards significance as a predicting factor of the EAR in our study, the explained variance was increased only by 2% when adding this parameter as a predicting factor, and the accuracy of predicting the clinical PC₂₀ allergen did not improve. Differences in methodology and study populations may explain the discordance with the above-mentioned study results. First of all, Bowton and co-workers only found a significant correlation between cutaneous and airway reactivity to a given allergen after exclusion of subjects with marked cutaneous sensitivity, suggesting that there is a population of patients in whom prediction of inhaled responses from cutaneous reactivity is inaccurate. Furthermore, our patients were solely challenged with HDM, whereas the majority of Bowton's population was challenged with cat allergen [27]. Cockcroft et al. [13] used a wide variety of allergens including *D. pteronyssinus*, ragweed, mixed grass, cat, and horse. The use of various allergen sources with possible differences in allergenic potency could have increased the range of allergen thresholds in both skin test and bronchial challenge, thereby strengthening the relationship between the degree of sensitization and airway responsiveness to allergen in these studies. The fact that our population of asthma patients was more responsive to methacholine (PC₂₀: 0.78 mg/mL) than Cockcroft's subjects (PC₂₀: 1.6 mg/mL) may further explain the observed discrepancies. Finally, the outcome parameters of methacholine and allergen challenges in the studies performed by Bowton et al. [27] and Crimi et al. [24] were different, which may not allow a full comparison with the present study.

The results of our study confirm those of other studies that have established the central role of histamine reactivity in the prediction of the airway response following inhalation of HDM allergen [12, 25, 26]. We demonstrated that PC₂₀ histamine and the degree of sensitization expressed as skin sensitivity both contributed significantly to a model predicting PC₂₀ allergen, explaining 39% of its variance. This is in line with previous results by Haugaard et al. [25], who showed that PC₂₀ histamine and allergen-specific IgE could explain 35% of the variance in PD₂₀ allergen in a population of non-steroid-treated patients with asthma who were challenged with HDM. In contrast, van der Veen et al. [26], who investigated predictive factors of the EAR after inhalation of isolated major allergen of *D. pteronyssinus* (Der p 1 or Der p 2), found that both PC₂₀ histamine and the type of allergen contributed significantly to the prediction of PD₂₀ allergen, whereas addition of skin sensitivity and specific IgE to this model did not. However, it must be mentioned that the number of subjects ($n = 27$) in the latter study was not sufficient to perform a multiple regression analysis with

more than two explanatory variables. We found that PC₂₀ histamine is the major determinant of the predicted PC₂₀ allergen to HDM inhalation in our new equation (regression coefficient 0.55) and not skin sensitivity (regression coefficient 0.23). As skin sensitivity did not contribute significantly to the prediction of the EAR based on PC₂₀ methacholine, we wondered whether exclusion of this parameter from the PC₂₀ histamine-based equation would result in major alterations. As a consequence, the explained variance in PC₂₀ allergen decreased from 39% to 35% (regression coefficient PC₂₀ histamine = 0.57, $P < 0.001$). Nevertheless, PC₂₀ allergen could still be accurately predicted within two doubling concentrations in all patients using the equation based on PC₂₀ histamine only. This indeed underlines the limited role of skin sensitivity in the prediction of the EAR after HDM challenge when the range of airway responsiveness and the range of skin sensitivity are equivalent, as was the case in our population of patients with asthma. Cockcroft et al. [12] found that skin sensitivity and PC₂₀ histamine contributed equally to the prediction of PC₂₀ allergen in their formula (regression coefficient 0.68). This discrepancy may be explained by the fact that they challenged with various allergens, which may have increased the range of skin sensitivity, thereby enhancing the role of cutaneous sensitivity in the prediction of the airway response to allergens. Although airway responsiveness to histamine and allergic hypersensitivity explained a higher degree of the variance of the predicted PC₂₀ allergen in Cockcroft's formula (67%) when compared with our new equation (39%), both performed equally regarding overestimation of the clinical PC₂₀ allergen.

We believe that the current analysis was appropriate given the available data. Even though we cannot exclude non-linearity of the relationship between skin sensitivity, airway hyper-responsiveness, and PC₂₀ allergen, we chose to use a linear model based on inspection of the univariate plots and the models published previously [11–13]. In the present study sufficient numbers of subjects were included to perform multiple regression analyses for two explanatory variables. However, these two variables could not fully explain the variance in PC₂₀ allergen, which leaves us to question what other factors are involved. The optimal way to test the performance of the new equations is to design prospective external validation studies in independent populations [23]. As we did not have the opportunity to perform such studies yet, we decided to test the accuracy of the new equations retrospectively by validation in separate groups, which is second best and an acceptable explorative approximation [23].

According to Tiffeneau [28] the degree of bronchial sensitivity to certain inhalation allergen appears to be dependent on two factors: the degree of bronchial allergy (a given dose of inhalation allergen results in the release of a given quantity of various mediators in the bronchial

mucosa) and the degree of the bronchial sensitivity to the various mediators, the so-called non-specific bronchial sensitivity that is independent of allergy. Already in 1969 Zuidema [29] suggested that if the bronchial sensitivity to histamine, and the result of the skin test with allergen to be tested, are known, it should be possible to predict the result of the inhalation test with reasonable certainty. Subsequent studies have indeed confirmed this hypothesis. Interestingly, it has been reported that the correlation between cutaneous reactivity and airway reactivity/responsiveness is weak or absent [25, 26, 30]. This may be explained by so-called 'shock organ specificity', a term that was introduced by Feinberg *et al.* [31], based on their observation that the bronchi of allergic asthmatics respond differently to ragweed exposure than those of patients with isolated allergic rhinitis. Further support for organ specificity comes from studies comparing allergen sensitivity between different organs of allergic asthmatics, showing that bronchial allergen challenge could not be replaced by challenges of skin, nose, and eyes [32]. The 'shock-organ' specificity is pathophysiologically plausible. It has been proposed that skin and bronchial reactivity may not parallel each other because of differences in mast cell populations among organs, and differential levels of mast cell-associated IgE [33–35].

The results of this study support the hypothesis that the occurrence of bronchial immediate allergic reactions on exposure to a given amount of allergen is predominantly modulated by non-specific airway responsiveness, whereas the influence of the degree of sensitization by specific IgE is limited. Our new equations accurately predict PC₂₀ allergen following inhalation of HDM allergen in a Western asthma population using either PC₂₀ methacholine or PC₂₀ histamine and skin sensitivity, allowing a safe starting concentration of allergen of three doubling concentrations below prediction. These equations are suitable for implementation in allergen challenge studies in Western countries with a high prevalence of HDM sensitization.

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