

Histamine challenges discriminate between symptomatic and asymptomatic children

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Histamine challenges discriminate between symptomatic and asymptomatic children. B. Niggemann, S. Illi, C. Madloch, K. Völkel, S. Lau, R. Bergmann, E. von Mutius, U. Wahn, MAS-Study Group. ©ERS Journals Ltd 2001.

ABSTRACT: The aims of this study were to investigate a threshold value for bronchial responsiveness in children aged 7 yrs, which discriminates between symptomatic and asymptomatic children, and to identify determinants of this responsiveness.

Titration bronchial histamine challenges using the reservoir method were performed in 645 children aged 7 yrs, from the birth cohort Multicentre Allergy Study (MAS).

When defining a reference population of healthy children within the MAS cohort, the 95th percentile of the provocative concentration causing a 20% fall in forced expired volume in one second PC₂₀ among these asymptomatic study subjects amounted to 0.60 mg·mL⁻¹. This resulted in a specificity of 93.0% and a sensitivity of 45.9%, for discriminating between "current wheezers" and "non-current wheezers". Determinants of airway responsiveness at this age were pulmonary function, sensitization to indoor allergens, total immunoglobulin E and current wheeze.

The results indicate that a very low cut-off provocative concentration causing a 20% fall in forced expired volume in one second (<1.0 mg·mL⁻¹) defines airway hyperresponsiveness in children aged 7 yrs using the reservoir method. Provocation protocols for histamine challenges in this age group should therefore start with concentrations markedly below 1.0 mg·mL⁻¹.

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Bronchial hyperresponsiveness (BHR) can be defined as an increase above normal in both the ease and the magnitude of airway narrowing on exposure to a number of nonsensitizing bronchoconstrictive stimuli [1]. BHR is commonly thought to be closely related to bronchial asthma [2]. The most important factors influencing BHR are genetic predisposition and atopy [3–7]. The degree of BHR seems to be associated with asthma severity [2], although recent work shows that there is a dissociation between airway inflammation and airway hyperresponsiveness in allergic asthma [8].

BHR can already be measured in early infancy [9]. There is evidence that BHR decreases with age from childhood to adolescence [10–14] and adulthood [15, 16]. Several methods have been used to assess BHR in children: pharmacological substances (methacholine, histamine, carbachol), physical exercise, isocapnic hyperventilation of cold air and hyper- or hypobaric solutions. There is only partial correspondence between these methods.

Provocation protocols so far suggest histamine threshold values of 8 mg·mL⁻¹ to indicate BHR.

However, this threshold value has never been evaluated in young children. Furthermore, it strongly depends on the methods used. Aims of this study were to investigate bronchial responsiveness to histamine in children aged 7 yrs, and to define a threshold value which discriminates between symptomatic and asymptomatic children. Furthermore, determinants of this responsiveness were identified.

Methods

Study sample

The German Multicentre Atopy Study (MAS), a prospective birth cohort study, recruited 1314 newborns in five German cities during 1990. A detailed description of study subjects and methods is given elsewhere [17]. Briefly, of all 7609 children born in 1990 in five study centres, information on family history of atopy was available for 5,863 children. Of these, 499 were included in the study with risk factors for atopy

(elevated cord blood immunoglobulin E (IgE; $\geq 0.9 \text{ kU}\cdot\text{L}^{-1}$) and/or ≥ 2 atopic family members) and 815 newborns with none of these risk factors. At the age of 7 yrs, all children were invited into the clinic within 3 months of their birthday. The study protocol was approved by the local ethics committee and parents gave their informed consent.

Lung function tests

Lung function tests were performed using the same full body plethysmograph in all five study centres (Master-Lab, E. Jaeger, Würzburg, Germany). Forced expired volume in one second (FEV₁) manoeuvres were repeated until maximal reproducible results were achieved (usually 3–5 manoeuvres). Beta-2-mimetic agents were withdrawn for at least 12 h and sodium cromoglycate for 48 h. No other drugs which might interfere with lung function were used by the children. After measuring height and weight, a baseline lung function test was performed. Results were expressed as per cent predicted (% pred) according to ZAPLETAL *et al.* [18]. FEV₁, forced vital capacity (FVC), and maximal expiratory flow when 75%, 50%, or 25% of FVC remains to be exhaled (MEF_{75/50/25}) were measured; FEV₁/FVC was also calculated.

Bronchial histamine challenges

Bronchial histamine challenge was performed using the reservoir method described by MATTHYS [19]. Briefly, a high quality nebulizer system (Pari Provocation Test 2, Pari, Starnberg, Germany) was used, combined with a 10-L storage bag, allowing standardized pulmonary aerosol deposition at saturated ambient temperature and pressure conditions. Provocation tests were performed in a titrated manner starting with $0.5 \text{ mg}\cdot\text{mL}^{-1}$ histamine dihydrochloride (Merck, Darmstadt, Germany). After measuring FEV₁ and FVC, increasing provocation doses were applied (2.0 and $8.0 \text{ mg}\cdot\text{mL}^{-1}$) every 10 min. In children with a history of bronchial asthma and/or bronchial hyperreactivity, lower starting doses were chosen ($0.125 \text{ mg}\cdot\text{mL}^{-1}$ in 21.4%, and $0.25 \text{ mg}\cdot\text{mL}^{-1}$ in 14.6% of the children). Provocation was stopped if the highest concentration ($8 \text{ mg}\cdot\text{mL}^{-1}$) was tolerated, or if a 20% fall in FEV₁ was induced; hence the provocative concentration causing a 20% fall in FEV₁ (PC₂₀) was recorded.

Parental questionnaire

Parents were invited into the clinic within 3 months of the 7th birthday of their child. In an interview, the study doctor asked parents whether their child had ever had "a wheezing or whistling noise in the chest while breathing" ("wheezing ever") and whether the child had "wheezed in the past 12 months" ("current wheeze"). Parents were also asked whether their child had had "a dry nocturnal cough in the past 12 months apart from colds" and whether a doctor had ever diagnosed asthma, hay fever or eczema.

"Current asthma" was defined as a doctor diagnosis with asthma and "current wheeze". Several questions about environmental exposure were also asked, for example, about cigarette smoke exposure, pet ownership, the heating system and the fuel used for heating. Passive smoking was defined as 10 or more cigarettes smoked in the home of the child per day. Parental history of atopy (asthma, hay fever or eczema), number of older siblings (defined as the number of previous deliveries), social status according to the highest school education of the parents (low, medium, high) and smoke exposure during pregnancy (yes/no) had been assessed at the time of birth.

Total and specific immunoglobulin E

Serum total IgE levels and specific IgE antibodies to food allergens (cow's milk, egg white, soya bean and wheat), indoor allergens (house dust mites *Dermatophagoides pteronyssinus*, latex, cat and dog dander), and outdoor allergens (mixed grass and birch pollen) were determined by CAP-RAST FEIA (Pharmacia & Upjohn, Freiburg, Germany) [20]. Since blood samples at the age of 7 yrs were not available for all participants ($n=546$, 84.7%), measurements of the two previous study years were also considered in the definition of atopic sensitization in order to maximize the number of children with available measurements of specific IgE ($n=609$, 94.1%). Hence, sensitization to a specific allergen was defined as a concentration of $0.70 \text{ kU}\cdot\text{L}^{-1}$ (or \geq CAP-RAST FEIA class 2) of the respective specific IgE at the age of 5, 6, or 7 yrs.

A child was defined as "sensitized" if it was sensitized to any one of the indoor, outdoor or food allergens, respectively. For bivariate analysis, total IgE was dichotomised using the cut-point of $100 \text{ kU}\cdot\text{L}^{-1}$ to define elevated total IgE levels. For multivariate regression analysis, total IgE measurements were log-transformed and used as a continuous variable.

Statistical analysis

The concentration of histamine that would produce PC₂₀ was estimated by linear interpolation between the last two measurements. If a decline of 20% was not reached by $8 \text{ mg}\cdot\text{mL}^{-1}$, the value $>8 \text{ mg}\cdot\text{mL}^{-1}$ was assigned as PC₂₀. Since 5% of the children had a fall in FEV₁ of $\geq 20\%$ after the first concentration administered, baseline FEV₁ and $0 \text{ mg}\cdot\text{mL}^{-1}$ histamine were used as the first data point for interpolation in these cases. Hence, the histamine concentrations were not log-transformed [21].

The sensitivity and specificity to detect "current wheeze" and "current asthma" for several cut-off values of PC₂₀ (varying between $0.05 \text{ mg}\cdot\text{mL}^{-1}$ and $8 \text{ mg}\cdot\text{mL}^{-1}$ in steps of 0.05) were calculated and plotted as receiver operator characteristic (ROC) curves in order to assess the optimum cut-off value for PC₂₀.

In order to assess the relevance of this cut-off value with optimum specificity and sensitivity, it was compared with the 95th percentile of the distribution of PC₂₀ in a healthy reference population. This

Table 1. – Study population and response rates

	Numbers	Per cent
Children in the MAS birth cohort 1990	1314	(100.0%)
Participation in the study:	939/1314	(71.5%)
Parental questionnaire	939/939	(100.0%)
Parental questionnaire and lung function	805/939	(85.7%)
Parental questionnaire, lung function and histamine challenge	645/939	(68.7%)

MAS: Multicentre Allergy Study.

reference population was defined within the study population according to the following criteria [22]: 1) no history of doctor diagnosed asthma; 2) no nocturnal cough without colds; 3) no history of wheeze; 4) no respiratory infection at the time of examination according to the study doctor; 5) no premature birth (<38 weeks of gestation) or low birth weight (<2500 g); 6) no history of doctor diagnosed hay fever; 7) no sensitization to any allergen (specific IgE <0.35 kU·L⁻¹) if exposed to the respective allergen at the time of testing, *i.e.* no sensitization to cat or dog allergen if at home, no sensitization to grass pollen if the child was tested between March and July, and no sensitization to house dust mite; 8) not a member of the 'high-risk group' at birth, *i.e.* no elevated cord blood IgE levels (>0.9 kU·L⁻¹) and <2 atopic first-degree relatives.

The dose-response slope (DRS) was calculated as the gradient of the line connecting the last data point of the dose-response curve, with the origin of the curve, *i.e.* the percentage fall in FEV₁ at the last concentration, divided by the last concentration. This expresses the percentage change in FEV₁ per mg·mL⁻¹ histamine.

In order to identify potential determinants of bronchial responsiveness, the distribution of the DRS was analysed with the Wilcoxon rank sum test in the case of binary variables, and the Kruskal-Wallis test for variables with >2 categories.

All variables with a p-value of ≤0.08 in the bivariate analysis were considered as covariates in a multivariate regression analysis, with the log-transformed DRS as dependent variable. Additionally, the continuous variables height, weight, baseline-FEV₁ and baseline-FEV₁ in per cent of FVC, were included as independent variables in the analyses. A stepwise forward variable selection procedure was used to obtain an optimum regression model, using a p-value of 0.08 as criterion for variables to stay or be removed from the model. All multivariate analyses were adjusted for study centre, season of testing and high risk for atopy at birth.

Results

Response rates

Of the 1314 children in the MAS birth cohort, 939 (71.5%) participated in the follow up study at age 7 yrs, *i.e.* parents responded to the questionnaire (table 1). Of these 939 children, 645 (68.7%) completed the bronchial histamine challenge. Of the 645 participating children,

351 (54.5%) had a positive parental history of asthma and/or atopy.

In order to assess potential participation bias, these 645 participating children were compared with all other children in the MAS birth cohort with respect to data collected at birth. Participants of the histamine challenge were more likely to have mothers smoking during pregnancy (26.8% *versus* 18.7%, *p*<0.001), but did not differ significantly from nonparticipants with respect to gender, parental history of atopy at birth, cord-blood IgE levels, cord-blood cotinine, or parental education at birth.

Furthermore, the 645 participating children were compared with the other 294 children with data from parental questionnaires, pulmonary function testing and/or blood sampling, but who had not participated in the bronchial challenge, with respect to data collected at the age of 7 yrs. Participants had a higher FEV₁ (% pred) than those unable or unwilling to participate (107.5±12.9 *versus* 102.6±12.9, *p*<0.001), but the ratio FEV₁/FVC (in %) did not differ significantly (92.4±5.9 *versus* 91.8±7.4, *p*=0.95). Additionally, no difference at the age of 7 yrs was found with respect to sensitization to any inhalant allergen, total IgE, wheeze (ever and current), passive smoking, and pets at home.

In order to assess the generalizability of the reference population, it was compared to the study base from which the MAS study population was recruited, *i.e.* the 5863 children born in 1990 in the five study centres with complete data on family history of atopy at birth. No

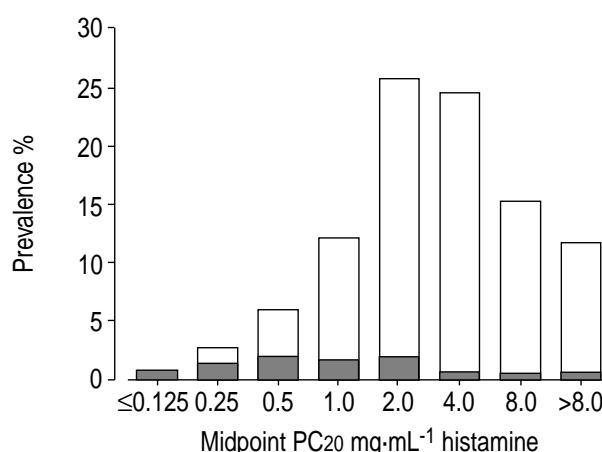


Fig. 1. – Distribution of provocative concentration causing a 20% fall in forced expired volume in one second (PC₂₀) in children with (■) and without (□) current wheeze during 7th year of life.

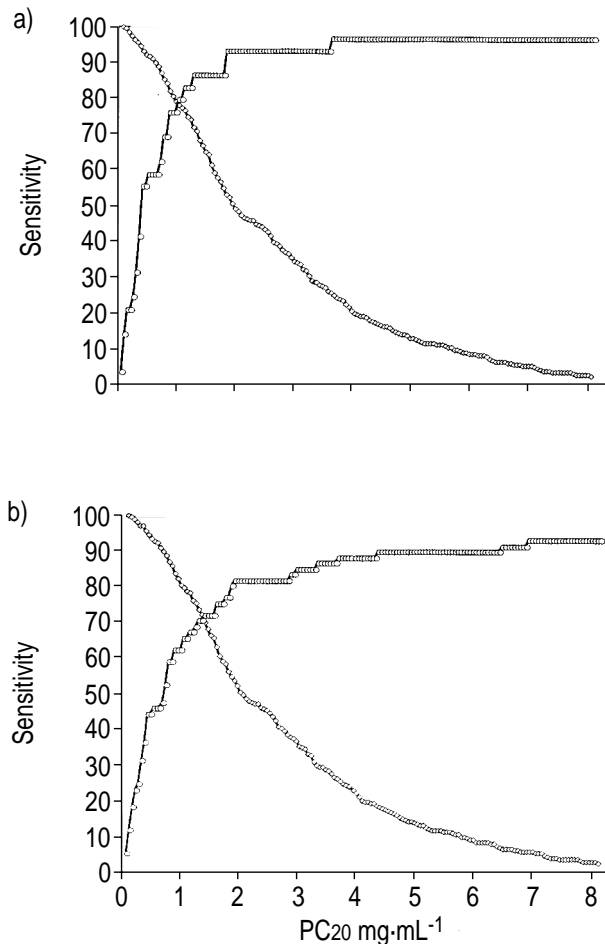


Fig. 2. – Histamine reactivity: sensitivity and specificity for various cut-off points for provocative concentration causing a 20% fall in forced expired volume in one second, in steps of $0.05 \text{ mg}\cdot\text{mL}^{-1}$, to discriminate between a) current asthmatics and non-current asthmatics; and b) current wheezers and non-current wheezers. ○: Sensitivity; ◇: Specificity.

significant difference was seen with respect to parental history of atopy (reference population 34.4% versus study base 36.9%, $p=0.53$).

Bronchial challenge

During bronchial challenge, all children in the study sample had a fall in FEV₁ 0.7–76.4% of baseline FEV₁, except for one child that remained stable and one child with an increase in FEV₁ of 8.3% of baseline FEV₁. A fall in FEV₁ of $\geq 20\%$ was observed in 569 children (88.2%). In the 76 children (11.7%) whose FEV₁ fell $< 20\%$, the maximum value of $> 8.0 \text{ mg}\cdot\text{mL}^{-1}$ was assigned as PC₂₀.

Provocative concentration causing a 20% fall in forced expired volume in one second

The distribution of PC₂₀ on a doubling scale is shown in figure 1, stratified for wheeze in the 7th year of life

("current wheeze"). The children with "current wheeze" tended to have lower PC₂₀ values, *i.e.* higher bronchial responsiveness, whereas children with "non-current wheeze" tended to have higher values of PC₂₀. However, no unambiguous distinction between "current wheezers" and "non-current wheezers" was observed. Considering all children, about a quarter (22%) had a PC₂₀ of $\leq 1 \text{ mg}\cdot\text{mL}^{-1}$. There was a statistically significant difference between the group of "current wheezers", with 62% of the children having a PC₂₀ below $1 \text{ mg}\cdot\text{mL}^{-1}$, and the group of "non-wheezers" with 18% ($p < 0.001$).

The resulting sensitivity and specificity of a PC₂₀ of varying cut-off points (0.05 to $8 \text{ mg}\cdot\text{mL}^{-1}$ in steps of 0.05) are shown in figure 2. A PC₂₀ of as low as $0.60 \text{ mg}\cdot\text{mL}^{-1}$ had a satisfying specificity of 93.0% and sensitivity of 45.9% for discriminating between "current wheezers" and "non-current wheezers". This corresponds to the 95th percentile of the healthy reference population defined within the MAS study population. If equal sensitivity and specificity is taken as the basis, as proposed by other authors [23, 24], a corresponding cut-off point value of $1.3 \text{ mg}\cdot\text{mL}^{-1}$ resulted (figure 2). For "current asthma", all cut-off points achieved even higher specificity at the expense of only slightly lower sensitivity. If those children only were included in the analyses that had not been at high risk for atopy at birth, the sensitivity of a PC₂₀ of $0.60 \text{ mg}\cdot\text{mL}^{-1}$ for "current wheeze" was slightly lower (39.3%) with equal specificity (93.6%). When all children with any family history of atopy and/or asthma were excluded from the reference population, the 95th percentile of PC₂₀ remained unchanged. Choosing the point of equal sensitivity and specificity resulted in a corresponding cut-off point value of $1.0 \text{ mg}\cdot\text{mL}^{-1}$ (figure 2).

Dose-response slope

The distribution of the dose-response slope DRS on a doubling scale is shown in figure 3, stratified for "current wheeze". The children with "current wheeze" tended to be in the right tail of the distribution, whereas children with, "non-current wheeze" tended to be in the left tail, with an overlap between the groups.

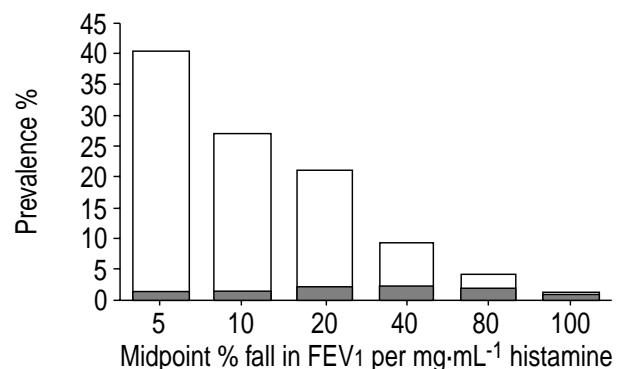


Fig. 3. – Distribution of the dose-response slope in children with (■) and without (□) "current wheeze". FEV₁: forced expired volume in one second.

Table 2. – Median, first and third quartile of the dose-response slope, stratified for risk factors

Total	Tot n 645	Strata	n	Dose-response slope (% fall in FEV ₁ per mg·mL ⁻¹ histamine)		p-value*
				Median 10.4	(1st; 3rd quartile) (5.2; 19.5)	
Cord blood IgE > 0.9 kU·L ⁻¹	622	Yes	112	10.1	(5.3; 19.4)	0.91
		No	510	10.2	(5.0; 19.5)	
Parental history of atopy at birth	644	Yes	351	10.5	(4.9; 20.7)	0.48
		No	293	10.0	(5.4; 18.2)	
Current wheeze (last 12 month)	645	Yes	61	29.4	(12.8; 66.7)	<0.00001
		No	584	9.5	(4.9; 17.4)	
Wheeze ever	645	Yes	111	18.3	(8.6; 48.7)	<0.00001
		No	534	9.3	(4.9; 16.9)	
Nocturnal cough without a cold (last 12 month)	643	Yes	77	12.3	(5.4; 22.2)	0.060
		No	566	10.1	(5.1; 18.7)	
Doctor diagnosis asthma ever	645	Yes	40	30.7	(14.9; 57.5)	<0.00001
		No	605	9.6	(5.0; 18.1)	
Doctor diagnosis hay fever ever	645	Yes	49	18.3	(8.6; 38.6)	0.00002
		No	596	10.0	(5.0; 18.6)	
Doctor diagnosis eczema ever	645	Yes	109	12.5	(5.8; 23.4)	0.013
		No	536	10.1	(5.0; 18.7)	
Sensitization to indoor allergens	607	Yes	129	15.3	(7.7; 47.8)	<0.00001
		No	478	9.2	(4.9; 16.5)	
Sensitization to outdoor allergens	607	Yes	147	13.6	(6.4; 30.6)	<0.00001
		No	460	9.5	(4.9; 17.2)	
Total IgE ≥ 100 kU·L ⁻¹	548	Yes	175	13.3	(6.0; 26.1)	0.00003
		No	373	9.3	(5.0; 16.9)	
Heating with lignite/coal/wood fired stoves	596	Yes	64	14.2	(6.8; 25.2)	0.0099
		No	532	10.1	(5.0; 19.0)	

*: p-value of the Wilcoxon rank sum test. FEV₁: forced expired volume in one second; IgE: immunoglobulin E.

Median, 1st and 3rd quartiles of the dose-response slope are shown in table 2, for the total sample and stratified for "current wheeze" and various other factors. A significantly higher DRS was found for atopic and asthmatic subjects, *i.e.* subjects with wheeze ever or in the past 12 months, nocturnal cough apart from colds in the past 12 months, doctor's diagnosis of asthma, hay fever or eczema, elevated total IgE levels and for children with atopic sensitization. Sensitization to indoor allergens (*e.g.* house dust mite) showed a greater association with the DRS than sensitization to outdoor allergens, whereas sensitization solely to food allergens showed no significant association with the DRS (data not shown).

Analysing indoor allergens separately, sensitization to house dust mites (*D. pteronyssinus*) showed the strongest effect on the DRS (data not shown). Children with lignite, coal or wood-fired stove heating in their home had a higher DRS, whereas other environmental factors such as parental education, maternal smoking in

pregnancy, passive smoking and pets at home had no significant effect on the median DRS. Furthermore, gender, elevated cord-blood IgE levels, number of older siblings, family history of atopy and high risk for atopy at birth had no significant effect on the DRS (data not shown).

Multivariate analysis

Considering all variables of the previous analyses with a p-value <0.08 in a stepwise forward variable selection procedure adjusted for study centre, season of histamine challenge, and high risk for atopy at birth, five independent variables remained in the final model with the log-transformed dose-response slope as dependent variable, resulting in a multiple correlation coefficient (R^2) of 0.427: baseline-FEV₁, baseline FEV₁/FVC ratio, sensitization to indoor allergens, log-transformed total IgE, and "current wheeze" (table 3).

Table 3. – Results of multiple regression modeling with log-transformed dose-response slopes as criterion

	Parameter estimate	Standard error	p-value
Baseline FEV ₁ L	-1.126	0.2509	0.0000
FEV ₁ /FVC %	-0.032	0.0077	0.0000
Sensitization to indoor allergens	0.359	0.1160	0.0021
Log total IgE	0.227	0.0761	0.0030
Current wheeze	0.760	0.1457	0.0000

n = 542 ($R^2 = 0.427$) adjusted for high risk for atopy (elevated cord blood immunoglobulin E (IgE) and/or ≥2 atopic family members), study centre, season of testing, height and weight. FEV₁: forced expired volume in one second.

Discussion

These findings indicate that in children aged 7 yrs, a cut-off value of a PC₂₀ of $<1.0 \text{ mg}\cdot\text{mL}^{-1}$ is appropriate for the definition of BHR when performing bronchial histamine challenges in children of this young age. Several methods have been used to assess BHR in large epidemiological studies in children: pharmacological substances (*i.e.* histamine, methacholine, carbachol) [12, 25–27], physical exercise (treadmill, ergometer or free running) [12, 25–27], isocapnic hyperventilation of cold air [22, 28, 29] as well as hyper- or hypo-osmolar aerosols [30–32]. The histamine challenge, as a direct bronchial provocation, was chosen for this study, because it represents a very sensitive method which was thought to be suitable for defining threshold values for BHR in our cohort study. The disadvantages of histamine challenges are the propensity for increased side effects such as coughing, vomiting, and flush. However, comparing different methods of measuring BHR is difficult because there is only partial correspondence between these methods concerning relation to clinical symptoms [33]. Hence, the generalizability of the present results obtained with the reservoir method to other techniques needs further evaluation.

The titrated procedure of the histamine challenge used in this study was similar to that described by COCKROFT *et al.* [34], except that a reservoir method was used [19] which offers the advantage of administering a defined dose by inhaling a defined concentration in a defined volume. Furthermore, because children breathed through a valved mouthpiece, the applied dosage was widely independent of the child's cooperation: irrespective of the number of breaths or the time needed, 10 L aerosol with a given concentration were inhaled. Due to the stability of the aerosol in the bag, time for inhaling does not play a significant role; nevertheless, care was taken that children emptied the bag within one min.

The degree of hyperresponsiveness may be influenced by several factors, with respiratory infections being one of the most important in childhood. Therefore, great care was taken to exclude children with a history of upper or lower airway infections during the 4 weeks before challenge. Additionally, sensitization to seasonal allergens may play a role: the statistical analysis showed that the season of testing influenced the PC₂₀/DRS, but adjusting for season of testing as a potential confounder did not change the magnitude of the effects of interest.

The observed low cut-off value for the definition of BHR corresponded to the 95th percentile of a healthy reference population with no increased risk for atopy, hence it may be used in further clinical and epidemiological studies. This reference population did not differ from the study base with respect to a family history of atopy, supporting the generalizability of the findings. Even when defining a super normal reference population by excluding all children with any family history of atopy, the cut-off value, defined as the 95th percentile of the distribution of PC₂₀ in this reference population, remained unchanged.

When analysing sensitivity and specificity of the bronchial histamine challenge for current wheeze and asthma, the high proportion of children at increased

risk of atopy in the study population might have biased the results. If sensitivity and specificity were calculated only for children not at high risk, the specificity of a cut-off of $0.60 \text{ mg}\cdot\text{mL}^{-1}$ remained almost unchanged (93.6%), but sensitivity was slightly lower than for the total study population (39.3%), pointing towards a potentially lower sensitivity when using bronchial histamine challenges in epidemiological as compared to clinical studies. However, using a cut-off of $0.75 \text{ mg}\cdot\text{mL}^{-1}$ yielded a satisfactory specificity of 90.6% and a sensitivity of 50.0%, even in the not at high risk population, suggesting that a cut-off level for PC₂₀ $<1.0 \text{ mg}\cdot\text{mL}^{-1}$ can define BHR in all populations of this age group. The achieved sensitivity and specificity are comparable to those found in other studies using histamine challenge [35] or cold air challenge [22].

Using the point of equal sensitivity and specificity, as proposed by other authors [23, 24], revealed slightly higher cut-off values for the histamine PC₂₀. However, even if this method is chosen, the cut-off values were around $1.0 \text{ mg}\cdot\text{mL}^{-1}$ histamine. Moreover, the advantage of this study was that a reference population could be defined, which allowed direct comparison of healthy and asthmatic/wheezing children.

BHR has been shown to be present early in life, and is seen in most infants [36–40] and young children [41–44]. While most of the studies were performed in infants or young children with wheezing, there is little information on healthy infants. In a study using oxygen saturation as a monitoring parameter, histamine challenges were performed in newborns and infants at 4 and 26 weeks, showing a marked decrease in S_{a,O_2} in most of these healthy infants [40]. Similar results were obtained with histamine [37], methacholine [38] and cold, dry air challenges [36] in healthy infants.

In one of these studies, also using a histamine challenge [39, 40], BHR was arbitrarily defined as a postchallenge fall in maximal expiratory flow at functional residual capacity of $\geq 40\%$ from baseline, up to a concentration of $8.0 \text{ mg}\cdot\text{mL}^{-1}$. When using a similar definition of BHR in this study, *i.e.* a fall of FEV₁ of at least 20% from baseline, up to a histamine concentration of $8.0 \text{ mg}\cdot\text{mL}^{-1}$, most children (88.2%) would have been classified as hyperresponsive. At high enough concentrations of histamine, most subjects will eventually react with a fall in FEV₁ of 20%. To identify a meaningful threshold value for defining BHR, we assessed the relationship between airway responsiveness and the presence of symptoms to identify the cut-off level with the best sensitivity, specificity and accuracy for detecting asthmatic symptoms as in previous studies [38, 45–47]. When applying such a definition (PC₂₀ $<1 \text{ mg}\cdot\text{mL}^{-1}$ histamine) the prevalence of BHR amounted to only 22%. This is in accordance with an Australian study which showed 17.8% of children aged 8–11 yrs being hyperresponsive, despite using a different methodology for histamine challenge [35].

In the present study cohort, there was a substantial number of children with a low PC₂₀ to histamine but without clinical symptoms. A Dutch study reported that children and adolescents with asymptomatic BHR (also determined by histamine challenges) showed similar characteristics to those without BHR, but

differed strongly from subjects with symptomatic BHR [47]. The authors concluded that asymptomatic BHR does not seem to be a risk factor for asthma in later life. Using an exercise challenge procedure, a Danish group reported that asymptomatic bronchial hyperresponsiveness indicated increased risk of developing wheezing [48].

The present study's data showing that total IgE, as well as specific IgE to indoor and outdoor allergens, represent an important risk factor for the degree of BHR, is in line with previous studies [11, 26]. Furthermore, the fact that FEV₁ is a significant risk factor may underline the role of baseline lung function for BHR.

In conclusion, the present study's results indicate that a very low cut-off of a provocative concentration causing a 20% fall in forced expired volume in one second below 1.0 mg·mL⁻¹ defines airway hyperresponsiveness in children at 7 yrs of age, using the reservoir method. Provocation protocols for histamine challenges in this age group should therefore start with concentrations markedly below 1.0 mg·mL⁻¹.

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