Pediatr Allergy Immunol 2002: 13: 361–367
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PEDIATRIC ALLERGY AND IMMUNOLOGY
ISSN 0905-6157

Hypertonic saline challenge tests in the diagnosis of bronchial hyperresponsiveness and asthma in children

Mai X-M, Nilsson L, Kjellman N-IM, Björkstén B. Hypertonic saline challenge tests in the diagnosis of bronchial hyperresponsiveness and asthma in children.
Pediatr Allergy Immunol 2002: 13: 361–367. ©2002 Blackwell Munksgaard

The hypertonic saline challenge test is the recommended method to assess bronchial hyperresponsiveness in the International Study of Asthma and Allergies in Childhood (ISAAC). The sensitivity of this procedure to assess asthma symptoms, however, has been reported to vary among study centers. The purpose of our study was to evaluate the value of this provocation test in an epidemiological survey in children, and to relate the degree of bronchial hyperresponsiveness to the severity of asthma symptoms. All 11–13-year-old children from 16 randomly selected schools in Linköping, Sweden received a questionnaire regarding respiratory symptoms and allergic disease. Skin prick tests with eight inhalant allergens were performed. In addition, all children with wheeze over the past 12 months (current wheeze) and a random sample of children without current wheeze were invited to perform hypertonic saline provocation tests. A complete data set was available for 170 children, including 50 with and 120 without current wheeze. Bronchial hyperresponsiveness (BHR) was defined as at least 15% decline in FEV_1. The degree of BHR was represented by the response/dose ratio, i.e. the fall in FEV_1 divided by total dose of inhaled saline. The severity of asthma symptoms was classified by the number of wheezing episodes over the past 12 months. 'Asthma ever' was defined by a combination of symptoms in the questionnaires. Children with 'asthma ever' and current wheeze were considered as having current asthma. Current atopic asthma was defined as current asthma with at least one positive skin prick test. The sensitivity of the procedure to detect 'asthma ever', current asthma and current atopic asthma was 62, 61 and 83%, and the specificity 83, 81 and 60%, respectively. The positive challenge rate was 52, 34, 13 and 7% among current wheezers, previous wheezers, non-wheezers with a history of allergy and healthy children. The degree of bronchial hyperresponsiveness increased with the number of wheezing episodes. Thus, the median and range of the response/dose ratio were 4.8%/ml (2.1–14.8), 2.6%/ml (0.7–8.6) and 1.3%/ml (0.8–2.7), respectively, for children with ≥ 4 episodes, 1–3 episodes and no wheezing episodes over the past 12 months ($p < 0.001$). In conclusion, hypertonic saline provocation test is useful as a tool to detect asthma in epidemiological studies in children. The degree of bronchial hyperresponsiveness, as represented by the response/dose ratio, reflects the severity of asthma symptoms.

Xiao-Mei Mai[1], Lennart Nilsson[1], N-I Max Kjellman[1] and Bengt Björkstén[1,2]

[1]Department of Health and Environment, Division of Paediatrics, Faculty of Health Sciences, Linköping University, Sweden; [2]Center for Allergy Research and Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

Key words: asthma; bronchial hyperreactivity; bronchial provocation tests; child; epidemiological studies; hypertonic saline; sensitivity; specificity

Dr Xiao-Mei Mai, Department of Health and Environment, Division of Paediatrics, Faculty of Health Sciences, S-581 85 Linköping, Sweden
Tel.: + 46 13 22 47 00
Fax: + 46 13 22 47 73
E-mail: xiama@ihm.liu.se

Accepted 4 March 2002

The prevalence rates of asthma in school children have increased over the last 30 years in Western industrialized countries (1, 2). The reported figures may be partly biased, however, due to differences in classification and diagnostic methods (2). To date, there are no generally accepted

uniform criteria. Bronchial hyperresponsiveness (BHR) is a major feature of asthma, although not pathognomonic, and it is closely associated with the presence and severity of disease (3). It can be measured with a variety of pharmacological and physical agents, but there is no strictly standardized procedure allowing world-wide comparisons.

The International Study of Asthma and Allergies in Childhood (ISAAC) was founded to overcome some of the problems in epidemiological research into asthma and allergic disease by establishing a standardized methodology (4). The questionnaires have been validated. Hypertonic saline challenge, employing a standardized protocol was chosen to assess bronchial hyperresponsiveness. However, the relationship between BHR induced by hypertonic saline and asthma symptoms differed considerably in two studies (5, 6). We have therefore assessed the sensitivity and specificity of hypertonic saline provocation test as a tool to identify asthma in an epidemiological survey and also evaluated the association between the degree of bronchial hyperresponsiveness and the severity of asthma symptoms.

Methods

Subjects

All 11–13-year-old children from a random sample of 16 schools out of all schools in Linköping, Sweden were invited to participate (n = 1115). The parents were encouraged to complete questionnaires regarding respiratory symptoms, eczema and allergic family history, and 911 questionnaires were returned (response rate 82%). Skin prick tests were performed in 857 of these 911 children (94%). All of the 73 children who reported wheeze during the last 12 months (current wheeze, cases) and a random sample of children without current wheeze (n = 207, controls) were invited to participate in bronchial provocation tests. Fifty-seven cases and 131 controls performed the tests. Eighteen of these 188 children were excluded due to incomplete questionnaires (n = 9) or incomplete challenge tests (n = 9). A complete data set was thus available for 170 children, including 50 cases and 120 controls. The characteristics of the two groups are shown in Table 1. There were no significant differences between participants and dropouts either in cases or controls in gender, heredity, positive skin prick tests, prevalence of hay fever and eczema, and 'asthma ever' diagnosis. However, the prevalence of previous wheeze was higher among participants than dropouts in the control group (24% vs. 10%, p = 0.01).

Definitions

Five of the questions in the ISAAC questionnaire (4) were used to identify wheezing, asthmatic and

Table 1. Characteristics of current wheezing and non-current wheezing children

	Current wheeze n = 50		No current wheeze n = 120		
	n	%	n	%	p-value
Boys	22	44	61	51	NS
Provocation (+)	26	52	20	17	<0.001
Asthma ever	31	63	6	5	<0.001
Hay fever ever	21	42	16	13	<0.001
Eczema ever	30	60	43	36	<0.01
SPT(+)	22	50	21	19	0.001
Allergic family history	43	86	79	66	<0.01
Tobacco smoke exposure, ever	17	34	41	35	NS
Poor ventilation, ever	27	54	55	47	NS
Pets, ever	33	66	80	67	NS
FEV_1/Pred. mean ± SD	1.0 ± 0.1		1.0 ± 0.1		NS
FEF_{75}/Pred. mean ± SD	0.9 ± 0.3		1.0 ± 0.3		<0.01
MMEF/Pred. mean ± SD	0.9 ± 0.3		1.0 ± 0.2		0.01
FEV_1%					
Median	88.8		91.0		<0.01
Range	78.2–96.0		84.0–98.0		
RDR (%/ml)					
Median	3.4		1.3		<0.01
Range	0.8–12.5		0.8–2.7		

RDR: response/dose ratio. Statistical comparisons between children with and without current wheeze were made by Chi-squared tests in nominal data. The values of lung function, except FEV_1% were analyzed by *t*-tests (unpaired). The comparison of FEV_1% or RDR was made by Mann–Whitney test.

allergic children, i.e. R1: 'Has your child ever had wheezing or whistling in the chest at any time in the past?'; R2: 'Has your child had wheezing or whistling in the chest in the last 12 months?'; R6: 'Has your child ever had asthma?'; H6: 'Has your child ever had hay fever?'; E7: 'Has your child ever had eczema?'. 'Current wheeze' was defined as a positive answer to R2. 'Previous wheeze only' as a positive answer to R1 and negative to R2. 'Asthma ever' had affirmative answers to R1 and R6. 'Current asthma' was defined as children with 'asthma ever' and current wheeze. 'Current atopic asthma' included children with current asthma and at least one positive skin prick test. The severity of asthma symptoms was graded by the number of wheezing episodes over the past 12 months.

Skin prick test

Skin prick tests were performed in duplicate, using extracts of *D. pteronyssinus*, *D. farinae*, *Alternaria*, birch, grass mixture, dander of cat, dog and horse (ALK, Hørsholm, Denmark). The concentration of the extracts was 10 HEP, except for *Alternaria* with a potency of 1:20 w/v. Histamine 10 mg/ml and 50% glycerin were used as positive and negative controls. The wheals on the forearms were measured after 15 min and the size was recorded as the mean of the longest diameter and the diameter perpendicular at its mid-point. A wheal with a mean diameter of 3 mm or more was regarded as positive (4). Atopy was defined as at least one positive skin prick test.

Baseline lung function test and hypertonic saline provocation test

Hypertonic saline provocation tests were performed during the winter and autumn in 1998. In accordance with the ISAAC protocol (4), provocation tests were not done in children who had a cold or had FEV_1 less than 75% of the predicted value. The baseline and post-challenge lung function were tested by MasterScope spirometer (JAEGER). At least two baseline spirograms were performed and the highest of two reproducible (within 5%) measures of FEV_1 was recorded as the baseline FEV_1. If the first two baseline FEV_1 readings were not within 5% of each other, a third spirogram was done. All baseline FEV_1% (FEV_1/FVC) values less than 80% were regarded as indicative of baseline airway obstruction (16 of the 170 children). Hypertonic saline (4.5%) was nebulized via a Devilbiss Ultraneb 2000 connected to 60-cm tubing (Devilbiss no. 8885) and a two-way valve (Laerdal valve No 560 200/850 500, Devilbiss, manufactured by Dahlhausen, Cologne, Germany). The child was encouraged to maintain tidal breathing. The exposure time was progressively increased from 30 s to 1, 2, 4 and 8 min each. After each exposure, two or three reproducible (within 5%) measurements of FEV_1 were made. The exposure time was doubled if the fall in FEV_1 was less than 10%. The same dose was repeated if the fall was between 10 and 15%. The challenge test was stopped and considered to be positive when the decline in FEV_1 was more than 15%. The maximum inhalation period was 15.5 min. The canister with tubing was weighed before and after the challenge test to measure the total dose of inhaled saline (grams). Response/dose ratio (RDR) was used to represent the degree of bronchial hyperresponsiveness, which was defined as the percentage fall in FEV_1 divided by total dose of inhaled saline (%/ml, 1 g/ml used as density for saline) (7).

Statistical analysis

Statistics were performed with Stat View 5.0 for Macintosh (Abacus Concepts Inc., Berkeley, California, USA). Chi-squared tests were employed to compare the nominal data between groups with and without current wheeze. Lung function comparisons were done by unpaired Student's *t*-tests, except for FEV_1%. The difference of RDR among groups was analyzed by Mann–Whitney test and Kruskal–Wallis test. The correlation between RDR and the baseline lung function was tested by Spearman rank correlation. Factors associated with BHR were analyzed in a multivariate logistic regression model. Sensitivity = true positives/(true positives plus false negatives); specificity = true negatives/(true negatives plus false positives); positive predictive value = true positives/all positives; negative predictive value = true negatives/all negatives; efficiency = (true positives + true negatives)/all tested.

Ethical aspects

The study was approved by the Human Research Ethics Committee of the Medical Faculty at Linköping University. All parents of the participating children gave their informed consent.

Table 2. Hypertonic saline provocation test as a screening test for asthma

	Current wheeze n = 50	'Asthma ever' n = 37	Current asthma n = 31	Current atopic asthma n = 18
Sensitivity percentage	52	62	61	83
Specificity percentage	83	83	81	60
PPV percentage	57	51	42	79
NPV percentage	81	89	90	67
Efficiency percentage	74	79	78	75

PPV: positive predictive value. NPV: negative predictive value.

Results

Of the 188 children that performed the bronchial challenge tests, two cases and seven controls did not complete the process, due to unwillingness or cough or FEV_1 less than 75% of the predicted value. The maximal fall in FEV_1 for a positive test was 51% (median: 17.7%, range: 15–51%). No severe asthma attacks occurred during the challenges.

The sensitivity of the procedure for screening 'asthma ever', current asthma and current atopic asthma according to the responses in the questionnaires was 62, 61 and 83%, and specificity 83, 81 and 60%, respectively. The positive predictive value (PPV), negative predictive value (NPV) and efficiency are given in Table 2.

The prevalence of a positive challenge was 52% (26/50) among current wheezers, as compared to 17% (20/120) among the control children ($p < 0.001$). Among the 20 controls who had a positive provocation test, 10 had a history of previous wheeze and eight had never wheezed, but had allergic symptoms and/or a family history of allergy. Thus only two healthy children with no history of wheeze or allergy, nor allergic family history had a positive response, giving a 93% (28/30) specificity of this test in healthy children.

The rate of positive challenge was 34% (10/29) in children with only previous wheeze and 11% (10/91) among children with no history of wheeze. In the latter group, 61 children had a history of allergy and/or allergic family history. Eight (13%) of them responded positively, as compared to 7% (2/30) of those with no history of allergy.

Table 3. Factors associated with positive provocation test to hypertonic saline in a multivariate logistic regression model

Variables	OR	95% CI
Current wheeze	5.7	2.0–15.3
Previous wheeze	4.0	1.3–12.0
Airway obstruction	2.7	0.6–11.5
Hay fever ever	2.3	0.7–6.9
Positive skin prick test	1.7	0.6–4.8
Eczema ever	1.7	0.6–4.1
Allergic family history	1.3	0.4–3.8

Current wheeze and only previous wheeze were significantly associated with the presence of bronchial hyperresponsiveness, with an increased risk of 5.7 and 4.0, respectively (Table 3).

The median RDR in the 46 children with BHR was 1.9%/ml. Sixty-nine per cent (18/26) of children with current wheeze had an RDR higher than 1.9%/ml, as compared with 30% (6/20) among controls ($p < 0.001$). As shown in Fig. 1, the median RDR was 4.8%/ml (range: 2.1–14.8), 2.6%/ml (range: 0.7–8.6) and 1.3%/ml (range: 0.8–2.7), respectively ($p < 0.001$) in children with ≥ 4, 1–3 episodes and no wheezing episodes over the past 12 months. Thus, recurrent wheeze was associated with a high RDR. Children who had baseline airway obstruction tended to be more hyperresponsive than children without baseline airway obstruction (median RDR 4.4 vs. 1.8%/ml, $p = 0.09$). Furthermore, children with less than one night attack of wheezing per week during the last year tended to be more

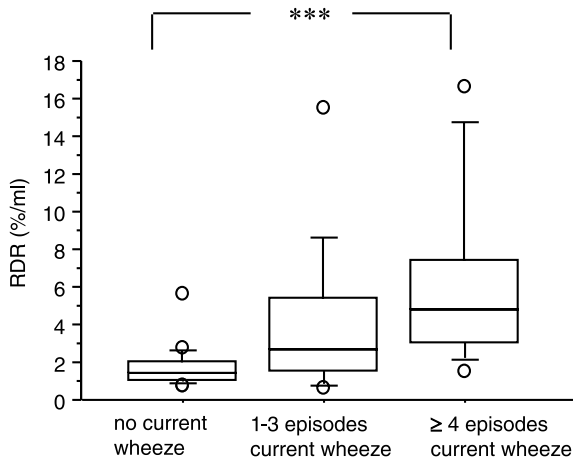

Fig. 1. Response/dose ratio (RDR) in relation to frequency of wheeze in 46 children with positive hypertonic saline tests. ***$p < 0.001$, Kruskal–Wallis test. RDR was defined as the percentage fall in FEV_1 divided by the total dose of hypertonic saline inhaled (%/ml).

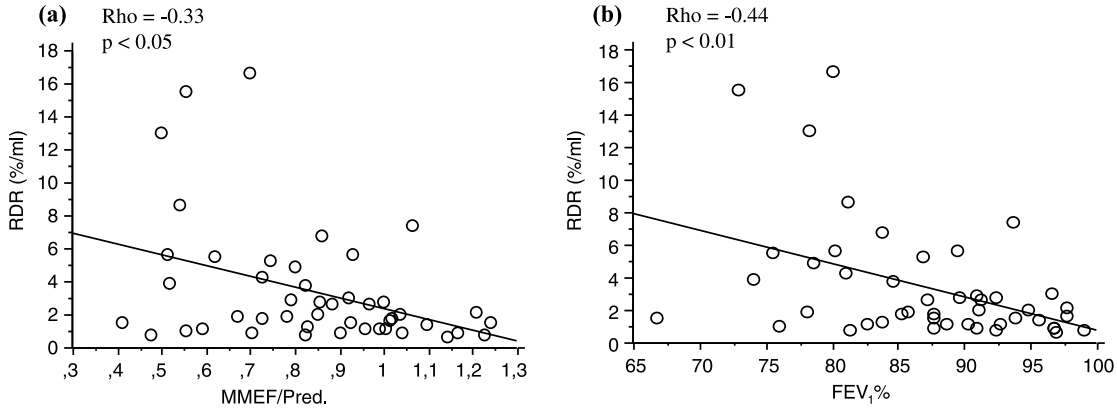

Fig. 2. Correlation between response/dose ratio (RDR) and (a) baseline MMEF/Pred.; (b) FEV_1%, as analyzed by Spearman rank correlation. MMEF/Pred.: maximal midexpiratory flow compared with the predicted value. FEV_1%: the percentage of forced expiratory volume at 1 s compared with forced vital capacity.

hyperresponsive than children without any night attacks (3.7 vs. 1.8/ml, $p = 0.08$). There was a weak, negative correlation between RDR and baseline MMEF/Pred., as well as between RDR and FEV_1% in children with bronchial hyperresponsiveness (Fig. 2).

Discussion

Most (61%) children with current asthma were hypersensitive to hypertonic saline and the specificity was 81%. Even more (83%) children with current atopic asthma were detected to have BHR, but the specificity was only 60%. The sensitivity of this procedure to identify 'ever diagnosed asthma' was 62%, which was similar to the sensitivity of pharmacological provocation tests in previous epidemiological studies (8, 9). However, hypertonic saline offers some benefits over pharmacological tests in population-based surveys (10). It is more widely available, cheaper and less irritating for the staff than pharmacological agents (4). Hypertonic saline induces bronchial hyperresponsiveness by transiently increasing the osmolarity of the periciliary fluid. The resulting osmotic gradient across the mucosal surface is believed to cause bronchoconstriction via an activation of responsive cells, such as mast cells, to release endogenous mediators (11, 12). This procedure is more similar to the mechanism of asthma disease than the procedure induced by pharmacological agents and it can give answers that pharmacological tests cannot. Pharmacological challenges using jet nebulizers to deliver aerosol pose unresolved problems of size correction owing to different ages of subjects (13). Consequently, challenge results cannot be accurately compared over a wider size and age range of subjects. In addition, provocation with hypertonic saline is preferable to the inhalation of pharmacological substances from an ethical point of view. Furthermore, the 4.5% saline challenge test yields good reproducibility in children with asthma (14) and there is no late asthmatic response in subjects with mild to moderate bronchial hyperreactivity (15).

Hypertonic saline provocation test has a higher sensitivity than challenges by other physical agents in population-based studies. The sensitivity of cold air hyperventilation and distilled water to screen asthma is 31% and 36%, respectively (16, 17). Although the sensitivity of the exercise challenge test is similar to the hypertonic saline challenge (5), the environment has to be more carefully controlled and the test requires more co-operation from the children.

The sensitivity of hypertonic saline challenge for screening current wheeze was 52%, which agreed with a previous study in Australia using the same ISAAC protocol (5). A possible reason for the moderate sensitivity was that some of the current wheezing children with a negative response regularly used inhaled steroids and cromoglycate. If the negative responses in children with current wheeze were assumed to be induced by medication ($n = 10$), the adjusted sensitivity would increase from 52% to 72%. In an Austrian study employing the ISAAC protocol, only 33% of children with current wheeze had a positive response (6). This could perhaps be explained by the lack of a precise word for wheeze in German. When current asthma was defined as a combination of a diagnosis and current symptoms, the sensitivity of the hypertonic saline challenge was consistent in two previous studies, i.e. 51% (5), 53% (6) and 61% in our study.

A positive hypertonic saline challenge was present in 11% of children with no history of wheeze. Pathologic changes in the bronchial mucosa, similar to the inflammation in chronic asthmatics in remission, have been found in nine of 17 children with a positive challenge test but no history of wheeze/asthma who were subject to bronchial biopsies (18). Furthermore, asymptomatic BHR is also associated with airway remodelling (19) and nearly half of these children will develop asthma within 2–6 years (20, 21).

The degree of bronchial hyperresponsiveness increased with the number of wheezing episodes over the past 12 months. In a previous report, children who had more than three episodes of wheezing in the last 12 months and a diagnosis of asthma had significantly greater airway responsiveness, as indicated by the bronchial responsiveness index (22). A relationship between the degree of airway response, as expressed by RDR value, and the frequency of symptoms was also found in school children challenged with histamine (23). RDR values contribute additional information to $PD20FEV_1$ regarding the degree of airway hyperresponsiveness. Moreover, RDRs are easier to calculate in population-based studies and they can be obtained even in subjects who have a negative response (24). Therefore, we suggest that RDR values should be used in preference to $PD20FEV_1$ values to express the degree of bronchial responsiveness in epidemiological surveys.

In conclusion, bronchial hyperresponsiveness to hypertonic saline is a valuable tool for asthma screening in population-based studies in children, because it yields acceptable sensitivity and has benefits over other provocative agents. Furthermore, the degree of bronchial hyperresponsiveness reflects the severity of asthma symptoms and is well represented by RDR.

Acknowledgments

The authors are grateful to research nurses Ing-Marie Sandberg and Lena Lindell for their excellent work of collecting questionnaires, interviewing children and performing skin prick tests. This work was supported by a grant from the Swedish Foundation for Health Care Sciences and Allergy Research (Vårdal).

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