

Article Type: Original

Exercise-induced bronchospasm diagnosis in children. Utility of combined lung function tests.

Silvia Sánchez-García^{1,2}, Pablo Rodríguez del Río^{1,2}, Carmelo Escudero^{1,2}, Cristina García-Fernández³, M.Dolores Ibáñez^{1,2}.

¹Allergy Section. Niño Jesús University Children's Hospital, Madrid (Spain).

²Instituto de Investigación Sanitaria –IIS. La Princesa University Hospital, Madrid (Spain)

³Preventive Medicine Department, Infanta Sofia Hospital, Madrid (Spain).

Pediatr Allergy Immunol

Running title: Diagnosis of exercise-induced bronchospasm.

Corresponding author:

M. Dolores Ibáñez

Sección de Alergología

Hospital Infantil Universitario Niño Jesús

Avda Menéndez Pelayo 65

28009 Madrid (Spain)

mibanezs@salud.madrid.org

S. Sánchez-García belongs to EAACI Junior Members Group.

Exercise-induced asthma diagnosis in children. Utility of combined lung function tests.

Sánchez-García S^{1,2}, Rodríguez del Río P^{1,2}, Escudero C^{1,2}, García-Fernández C³, Ibáñez M.D.^{1,2}.

¹Allergy Section. Niño Jesús University Children's Hospital, Madrid (Spain).

²Instituto de Investigación Sanitaria –IIS. La Princesa University Hospital, Madrid (Spain)

³Preventive Medicine Department, Infanta Sofia Hospital, Madrid (Spain).

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/pai.12319

This article is protected by copyright. All rights reserved.

ABSTRACT:

Background: The diagnosis of exercise-induced asthma or bronchospasm (EIB) is a complex dare in daily clinical practice. The consensus is that if bronchial hyperresponsiveness (BHR) is demonstrated in a patient with symptoms consistent with EIB, then that patient can be diagnosed with exercise-induced bronchospasm. The aim of this study is to determine which BHR test is the most efficient to diagnose EIB.

Methods: Children under 16, without previous asthma diagnosis, or with stable asthma, complaining of asthma-like symptoms triggered by exercise were included. Bronchodilator, methacoline, mannitol and exercise tests were performed on all patients, following established protocols. The performance of single and combined tests was determined.

Results: 46 patients (median age: 12 years, ranged 8-16 y.o.) were recruited, 30 (70%) previously diagnosed of asthma. BHR was detected in 93.47% of the children. The exercise challenge test detected BHR in 11/46 (23.90%) patients, bronchodilator test in 10/46 (21.70%), mannitol in 36/45 (80%) and methacoline in 41/45 (91.11%). The total number of patients with BHR was detected using a combination of the methacoline and mannitol tests. A combination of the methacoline test performed first, followed by the mannitol test, was able to diagnose BHR in 100% of children with lower number of tests (N=45) than if the order was reversed (N=50).

Conclusions: Methacholine and mannitol tests detect BHR in most children with suspected EIB. Bronchodilator and exercise tests show a low positivity rate. A combination of the methacoline test, followed by the mannitol test, gives the highest return to identify BHR in children for the diagnosis of EIB.

Key words: asthma control questionnaire, bronchodilator test, children, exercise-induced asthma, exercise-induced bronchospasm, exercise-induced diagnosis, exercise test, mannitol test, methacoline test, nitric oxide test.

M. Dolores Ibáñez
Sección de Alergología
Hospital Infantil Universitario Niño Jesús
Avda Menéndez Pelayo 65
28009 Madrid (Spain)

Utility of combined lung function tests for pediatric exercise-induced bronchospasm diagnosis.

INTRODUCTION

Sports and physical activities are a feature of children's daily life, and their restriction results in social limitations (1). Exercise is one of the most important causes of asthma exacerbation among pediatric patients, thus limitation of activities is one of the items used by expert guidelines to classify asthma control (2). Moreover, respiratory symptoms after physical exercise may be the first sign of new-onset asthma in asymptomatic children; so their evaluation in regular clinical practice is crucial.

However, achieving an accurate diagnosis of asthma-like symptoms due to physical exercise remains a challenge in daily clinical practice. Firstly, there is no expert consensus on defining exercise-induced asthma (EIA) or exercise-induced bronchospasm (EIB) (3) (4). The Therapeutic Use Exemptions (TUEs) from the World Anti-Doping Agency (WADA) Asthma Expert Group defines EIA as a transient airway narrowing induced by exercise in an asthmatic individual, whereas EIB is a reduction in baseline lung tests only after exercise, even in the absence of a previous asthma diagnosis (5). Following ATS criteria, exercise-induced bronchoconstriction (EIB) is defined as acute airway narrowing that occurs as a result of exercise, both in patients with asthma and individuals without known asthma (6). For better comprehension, we will include both definitions into EIB, whether or not the child has been previously diagnosed with asthma.

Moreover, suspected symptoms are usually subjective and are reported by a young child or parents/guardians, which implies an over- or underestimation of the symptoms perceived, and consequently an over- or undermedication. Although we need objective methods for this evaluation, there is a lack of a gold standard test to establish correct diagnosis. Regarding diagnostic tools, the WADA - World Anti-Doping Program - states that the minimum criteria for diagnosing asthma are not known. Indeed, lung tests by themselves are inadequate for diagnosing asthma or EIB in athletes. Recurrent symptoms of bronchial obstruction triggered by hyperventilation, exercise or other stimuli, are a precondition for diagnosing asthma or EIA in athletes (4).

The consensus is that if bronchial hyperresponsiveness (BHR) is demonstrated in a patient with symptoms consistent with EIB, then that patient can be diagnosed with exercise-induced asthma (1). In the pediatric population, a 10% or higher increase in FEV₁ and/or FVC, following the use of inhaled β_2 agonist, is considered to be the standard diagnostic test for the reversibility of bronchospasm (7) (8). A number of bronchial provocation tests are currently available to evaluate BHR. They include the following, in no specific order: Eucapnic Voluntary Hyperpnea, Methacholine Aerosol Challenge, Mannitol Inhalation, Hypertonic Saline Aerosol Challenge, Exercise Challenge, and Histamine Challenge. A positive response to any one of the above provocation tests is required to confirm BHR. If a negative result is obtained, carrying out the remaining test is recommended until one of them shows BHR, or all of them come out negative (4).

Methacoline acts directly on airway smooth muscle, and shows bronchial muscles functionality. Nevertheless, mannitol and exercise challenge are indirect agents, producing bronchospasm as they activate inflammatory cells releasing mediators and cytokines, reflecting the inflammatory compound of the bronchial response to stimuli. Therefore, each of the agents shows a partial vision of the BHR pathophysiology. If combined, a higher percentage of children suffering asthma can be detected in children with suggestive clinical symptoms.

Following these guidelines for elite athletes, we tried to determine what is the most suitable methodology for diagnosing EIB in the pediatric population in daily clinical practice, as reported symptoms are subjective and an objective test is needed. We also describe the clinical characteristics of the patients, of the physical exercise that provoked the asthma symptoms, the degree of bronchial eosinophilic inflammation with indirect methods, and its relationship to BHR tests.

METHODS

Study design

An observational, prospective, single centre and cross-sectional study was carried out at the Allergy Department at Niño Jesús University Children's Hospital (Madrid, Spain), from February to September 2012. Protocol was approved by ethical committee and informed consent was obtained from the children >12 and their parents/guardians.

Study population

Selection was of patients under 16 years old, of both genders, who consecutively attended our outpatient clinic complaining of one or more symptoms after physical exercise. Patients were either not previously diagnosed with asthma, or they had stable asthma control according to GINA guidelines (1). All were free of maintenance therapy for asthma (inhaled corticosteroids, montelukast) during the last 3 months, but were allowed to have short-acting β_2 agonists as rescue medication for exercise-related asthma symptoms. None of them had suffered any asthma exacerbations in the last 3 months. With these criteria authors assume that influence of other triggers rather than exercise would be minimized and positive detection of BHR was caused mainly by physical exercise.

Children that could not perform a spirometry test, suffered a cardiac or other pulmonary disease other than asthma, suffered any disease or event contraindicated for performing the test, or that refused to participate, were excluded.

Baseline visit

All patients were interviewed by an allergist with regard to asthma control, and a general physical examination was performed. They were asked to give a detailed description of symptoms perceived (cough, shortness of breath, wheezing, chest tightness), as well as the type of exercise and the time of the year when symptoms appeared. The exercises triggering symptoms were classified into mild (daily life activities), moderate (games and light sport) or intense (exertion during competition or during school physical exams).

Asthma Control Questionnaire for Children

Every patient and their parents/guardians were asked to fill in a Spanish version of a standardized and validated questionnaire to assess asthma control in pediatric population (CAN). This questionnaire includes 9 items, evaluated from 0 to 4 per item, <8 represents the best possible control and ≥ 8 mirrors a poorly controlled asthma. The higher the score, the worse the asthma control (9). This questionnaire has been validated by GEMA (Spanish Guideline on the Management of Asthma) (10) (11) and is widely applied in Spanish-speaking countries (12).

Allergic profile

Skin prick tests to the most common aeroallergens in our area were performed: pollen, dust mites, epithelium and alternaria (LETI, Barcelona, Spain), following European guidelines (13).

Indirect inflammation measurement

Fractional exhaled nitric oxide (FeNO; NIOX MINO, Aerocrine, Sweden) was performed during the baseline visit.

Lung function tests

Baseline spirometry, followed by a bronchodilator test, were carried out. This was deemed positive with an increase in $FEV_1 \geq 12\%$ after inhaling 400 μg of Salbutamol (3). Patients underwent three bronchial challenge tests, each of them carried out on different days, within an interval of at least 2 days and a maximum of 4 days, in no fixed sequence. The exercise test was deemed positive if a decrease in $FEV_1 \geq 10\%$ was observed at 5, 10, 15 or 30 minutes after running for 8 minutes on a treadmill, at 80-90% maximum heart rate, following ATS guidelines (14). It was performed under an average temperature between 20 and 27°C, humidity was 29-35%. The methacholine test, with a decrease of $\geq 20\%$ in FEV_1 by using the tidal volume technique with $PC_{20} < 16 \text{ mg/ml}$ (14) and the Mannitol challenge test was deemed positive with a decrease in FEV_1 of $\geq 15\%$ after inhaling the contents of a capsule, with doses increased step-wise up to 635 μg , or a decrease in FEV_1 of $\geq 10\%$ between successive doses, following the manufacturer's recommendations (15). Children were asked not to practice any sport or physical exercise for 8 hours prior to the challenge, in order to ensure absence of short-acting β_2 -adrenergic agents, following the guidelines' recommendations (2) (3). Since our goal was to measure BHR due to exercise, we minimized the aeroallergen influence by performing the four tests at the time of year with the lowest environmental allergen load for each allergen the patient was sensitized to.

The study design diagram is shown in Figure 1.

Statistical analysis

A 95% binomial confidence interval was calculated for determination of utility of single challenge test.

This article is protected by copyright. All rights reserved.

Accepted Article

Performance of combined challenge examinations, named the First Round Test (FRT) and the Second Round Test (SRT), was determined by calculating the percentage of patients with at least one positive test from the combination of 2, compared to the total of patients with at least one positive test, according to the formula: FRT Positive and/or SRT Positive / Patients with BHR detected by any test. In order to find the most profitable sequence of the two challenges, we determined the number of tests we needed to carry out to detect this percentage of patients.

The relationship between FeNO and CAN results, and the number of positive lung tests, was determined using the Kruskal Wallis test and Spearman correlation. Statistical significance was set at $p < 0.05$.

RESULTS

Demographic, clinical and baseline scan data

A total of 79 patients were recruited, but 33 of them did not fulfil the inclusion criteria (see figure 1) due to rejection to participate or impossibility to obtain evaluable results on challenge tests owing to patient's ability. We selected 46 patients, 24 male, with a median age of 12 (SD 2.20) years. Mean Body Mass Index (BMI) was 18.70 (SD 2.99).

Skin prick tests revealed common sensitization in the central area of Spain: 38 (82.60%) patients were sensitive to at least one type of pollen, 18 (39.13%) to animal epithelium, 13 (28.26%) to alternaria, 4 (8.69%) to dust mites and 6 (13.04%) were non-atopic. EIB symptoms were reported only during spring in 7% of the patients, all of them pollen sensitive, whereas 43 (93%) suffered exercise-related symptoms all year round.

Despite 70% of the children being previously diagnosed with asthma, only 26 (57%) inhaled short-acting β_2 agonists before exercise, as preventive medication.

Regarding the characteristics of the physical exercise that provoked asthma-like symptoms, patients complained most after running (43.47%) or during physical activity at school (28.26%). Swimming was the least reported (2.17%).

Regarding severity, 29 (63%) children reported symptoms from moderate exercise, 12 (26%) from intense exercise, and 5 (11%) from mild activities carried out in normal daily life.

Seventeen percent reported symptoms during or after training at a professional or semi-professional level, regardless exercise intensity.

For symptoms perceived during or after sport, most, 15 (32.60%) reported shortness of breath, 13 (28.26%) cough and shortness of breath, 6 (13%) shortness of breath and wheeze, 5 (10.86%) shortness of breath, cough and wheeze, 4 (8.69%) cough, 2 (4.34%) shortness of breath and chest tightness, 1 (2.17%) cough and wheeze. None of them reported isolated wheeze as symptom.

Baseline lung function showed a mean percentage predicted FEV₁ 94.57 (SD 14.86) (Table 1).

The FeNO results had a mean of 40.77 ± 26.51 ppb (9-123 ppb). We observed that 31 (67%) patients obtained a result >30 ppb. In 12 patients (26%), FeNO was >70 ppb, all whom had concomitant rhinitis.

Analysis of the CAN questionnaire results showed that 47.80% of children had not well-controlled asthma, scoring more than 8 points. Detailed results of previously asthma-diagnosed patients are shown in Table 2.

Lung function tests

Table 1 shows the results of the four lung tests carried out and analyzed separately. All four tests were done for every selected subject, except methacholine which was rejected by one child, and mannitol, the test for which had to be prematurely ended in another patient due to adverse reaction (irritative cough) before a result could be obtained. BHR was detected in 43/46 (93.47%) of the children. The methacholine test had the highest percentage of positive results in detecting BHR, but, when 95% binomial confidence interval was calculated both the methacholine and mannitol tests obtained similar results (Table 1). Only 4 of the subjects recruited had a negative methacholine result. From these, 1 (25%) had a positive mannitol result, while the other 3 patients had negative results. Conversely, of all 9 patients having a negative mannitol result, all of them had a positive methacholine test.

The exercise challenge test detected a low percentage of BHR (23.90%), with the maximum decrease in FEV₁ occurring between 5 and 15 minutes afterwards. The bronchodilator test only detected BHR in 21.70% of the patients evaluated.

BHR was detected in 93.75% (30/32) of those patients previously diagnosed as “asthmatic patients” and in 92.85% (13/14) of “non-asthmatics”. In 3 (6.50%) children, all tests came out negative. One non-asthmatic, who complained of shortness of breath and chest tightness after exercise, was finally diagnosed with anxiety. Of the two remaining patients who had been previously diagnosed with asthma, and who complained of shortness of breath after sport, one of them was an elite athlete and the other one showed physiological intolerance to exercise during the exercise challenge. In 5 (10.86%) all tests were positive. More than half of the studied population (56.52%) showed 2 positive challenge tests.

A correlation was found between the number of positive tests and FeNO results ($p=0.049$) but not with the CAN questionnaire score (table 2). Spearman correlation between number of test and FeNO resulted: 0.371 ($p=0.012$), showing a weak positive correlation statistically significant between number of tests and FeNO which could not be found with CAN: 0.11 ($p=0.943$). Non-asthmatic patients’ data are also shown in Table 2.

Carrying out the combined challenge tests resulted in 100% of patients having any positive test being detected by the combination of methacholine and mannitol. If the mannitol test is performed before the methacholine test we require a total of 50 challenges, whereas if the methacholine test is performed before, we require 45 examinations to detect 100% of patients with BHR (see table 3).

DISCUSSION

Exercise is one of the most important features of childhood for evaluating asthma, both with regard to control and severity (2).

Our findings supported the relevance of assessing exercise tolerance in asthma control in the pediatric population. According to current guidelines, all included patients were either well controlled asthmatics or non-asthmatic subjects. However, 11% of them suffered symptoms during mild exercise in their regular life despite a normal baseline spirometry, reflecting truly uncontrolled asthma. Indeed, this may be the only sign of uncontrolled asthma even though baseline spirometry is normal (16)(17). We found that running the CAN questionnaire in our population revealed half of them perceiving not well-controlled asthma. This could be explained by the addition in that questionnaire of specific items addressing symptoms during or after exercise, leading to a final score leaning towards not well-controlled asthma.

We found out a low average BMI, ruling out asthma phenotype associated with overweight. A high percentage of patients were atopic, as also a high proportion (70%) were previously asthma-diagnosed. Although 82.60% of the total of patients were pollen-sensitized, it is remarkable that only 7% reported symptoms exclusively during springtime, whereas the remaining 93% reported symptoms all year round.

Regarding the FeNO results, 31 patients obtained a result >30 . Singer et al. (18) recently identified FeNO in pre-school children as a predictor of later asthma.

If BHR is detected in a child with no cardiac disease or other lung disorders, asthma diagnosis is highly probable. Symptoms only after exercise, and a positive BHR, may define the onset of asthma (19). We found that 93.47% of children complaining of symptoms after physical exercise suffered from BHR.

In our experience, both methacholine and the mannitol tests were the best test used in isolation, detecting the highest number of patients suffering BHR; diagnosing 97.60% of them. To enhance and optimize the diagnostic procedure, we analysed the combination of different tests and demonstrated that performing the methacholine test followed by the mannitol test increased detection of BHR to 100%.

Barben et al. (20) found that exercise and mannitol were concordant in 83% of 111 children with respiratory symptoms, with a higher sensitivity for mannitol, which is consistent with our higher positivity rate. Andregnette-Roscino et al. (21) have published a series on 23 athletes with respiratory symptoms. They did not find a correlation between mannitol and methacholine, and a superior sensitivity for methacholine was demonstrated. Conversely, Anderson et al. (22) determined, after a multicentre study of over 500 patients, that mannitol and methacholine were equivalent in sensitivity and specificity. However, the population surveyed included both children and adults. Gold standard is defined by a positive exercise test in this group, but this is performed together with dry air inhalation, thus results are not comparable to exercise tests performed by other investigators.

More than 20 years ago, Clough et al. (23) performed both methacholine and exercise tests in 60 children between 7 and 8 years old, who attended the clinic due to respiratory symptoms after physical exercise. They found that exercise resulted positive in 29% of patients, thus

methacholine was more sensitive and exercise more specific, with no correlation between both tests. They concluded that both tests studied different components of airway dysfunction. We obtained a similarly low rate for the exercise test in detecting BHR (23.90%), which implies that this should not be the test of first choice. Effectiveness of exercise challenge is improved if simultaneous dry air is inhaled, as previously described by Anderson et al. (22), which is not usually effectuated in general clinical practice. We did not either and this is one of the reasons that can explain our low rate of positive tests. Another reason may be that exercise challenge does not exactly reproduce the sport as practised by children. Sports and physical exercise in the field provoke a change in environment conditions (i.e., exposition to pollen, cold air if it is open air and other factors such as stress in competition, etc) which may confound the results.

As there is no gold standard test for diagnosing asthma or EIB, we need to be careful when evaluating sensitivity and specificity results. In some studies, clinical diagnosis is used as a reference for comparing the results of the different lung tests. With no diagnostic test to confirm/discard the results of our tests, from a methodological point of view, we could not calculate the sensitivity or specificity of the aforementioned tests. We therefore felt that providing the BHR detection raw data was the most accurate way to report our results.

Asthma-like symptoms after exercise, in children with BHR, leads to EIB, (93.47% in our population), which means that overdiagnosis and consequent overmedication is not a general rule among a pediatric population. BHR may be detected with a methacholine or mannitol test. Both challenges measure different physiopathological characteristics of asthma.

The bronchodilator test showed a low positivity rate. However, it may be a useful tool if we take into account that it is cheap, not time-consuming, and is easy to perform. Moreover, if it comes out as positive, more complex additional tests will be avoided.

According to our data, a methacholine challenge test performed first, followed - if negative – by a mannitol test, are the two best challenges for detecting BHR in children with exercise-related asthma-like symptoms.

Clinical limitations of the study: The active selection of patients with stable or without previous asthma diagnose was performed in order to study a homogeneous population. However, our results should be carefully evaluated when extrapolated to other patients.

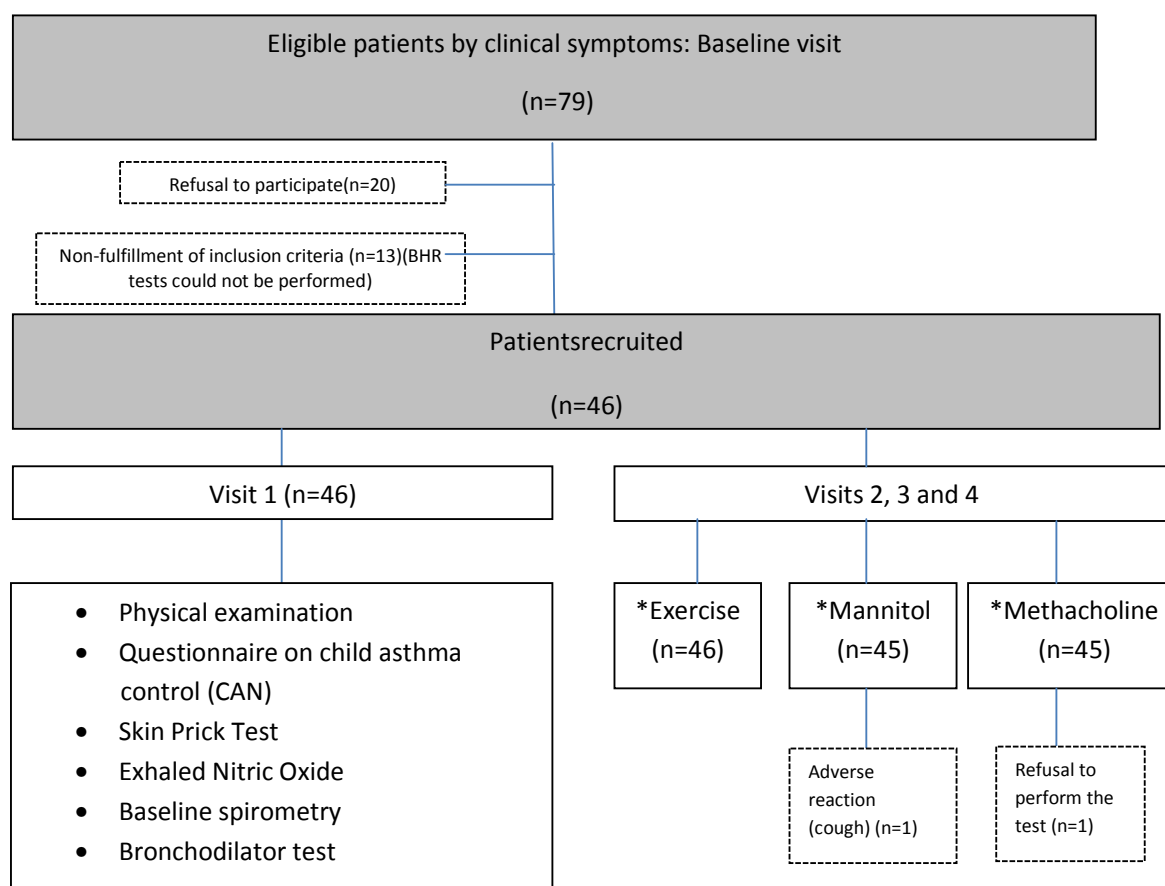
REFERENCES

1. Johansson H, Norlander K, Hedenström H, et al. Exercise-induced dyspnea is a problem among the general adolescent population. *Respir Med*. 2014 Jun;108(6):852-8.
2. GINA Global Initiative for Asthma. Global strategy for asthma management and prevention NHBLI/WHO Workshop Report. Updated 2012. Available from <http://www.ginasthma.com>
3. Weiler JM, Bonini S, Coifman R, et al; Ad Hoc Committee of Sports Medicine Committee of American Academy of Allergy, Asthma & Immunology. American Academy of Allergy, Asthma & Immunology Work Group report: exercise-induced asthma. *J Allergy Clin Immunol* 2007; 119(6):1349-58.
4. Carlsen KH, Anderson SD, Bjermer L, et al; European Respiratory Society; European Academy of Allergy and Clinical Immunology. Exercise-induced asthma, respiratory and allergic disorders in elite athletes: epidemiology, mechanisms and diagnosis: part I of the report from the Joint Task Force of the European Respiratory Society (ERS) and the European Academy of Allergy and Clinical Immunology (EAACI) in cooperation with GA2LEN. *Allergy* 2008; 63(4):387-403.
5. Fitch K, Sue-Chu M, Anderson S, et al. "Asthma and the elite athlete: Summary of the International Olympic Committee's Consensus Conference Lausanne, Switzerland, January 22-24, 2008" *J Allergy Clin Immunol* 2008; 122(2):254-60.
6. Parsons JP, Hallstrand TS, Mastrorade JG, et al. An official American Thoracic Society Clinical Practice Guideline: Exercise-induced Bronchoconstriction. *Am J Respir Crit Care Med* 2013; 187 (9): 1016-1027
7. Sharma S, Litonjua AA, Tantisira KG, et al. Childhood asthma management program research group. Clinical predictors and outcomes of consistent bronchodilator response in the childhood asthma management program. *J Allergy Clin Immunol*. 2008; 122:921-8.
8. Fernández Benítez M, Gómez Ruiz F, López Viña A, et al. GEMA 2009. Spanish guideline on the management of asthma. *J Investig Allergol Clin Immunol* 2010; 20 (Suppl 1):17-25.
9. Barrett A, Clark M, Demuro C, Esser D. Proxy-reported questionnaires for young children with asthma: a structured review. *Eur Respir J*. 2013;42(2):513-26.

- Accepted Article
10. GEMA 2009 (Spanish guideline on the management of asthma). Executive Committee GEMA 2009. *J Investig Allergol Clin Immunol*. 2010;20 Suppl 1:1-59
 11. Pérez-Yarza EG, Badía X, Badiola C, Cobos N, Garde J, Ibero M, et al. On behalf of the CAN Investigator Group. Development and validation of a questionnaire to assess asthma control in pediatrics. *Pediatr Pulmonol*. 2009; 44: 54-63.
 12. Vidal G, Escobar AM, Medina ME. Comparison of asthma control questionnaire in children (CAN) and GINA control recommendations. *Rev Chil Enf Respir* 2013; 29: 75-80
 13. Dreborg S, Frew A. EAACI Subcommittee on Skin Tests. Allergen standardization and skin tests. *Allergy* 1993;48:48-82.
 14. Crapo RO, Casaburi R, Coates AL, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. *Am J Respir Crit Care Med*. 2000; 161(1):309-29.
 15. Anderson SD, Brannan J, Spring J, et al. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med*. 1997;156(3 Pt 1):758-65.
 16. Hedlin G. Management of severe asthma in childhood-state of the art and novel perspectives. *Pediatr Allergy Immunol*. 2014 Mar; 25(2):111-21.
 17. Barranco P, Perez-Frances C, Quirce S, et al. Consensus Document on the Diagnosis of Severe Uncontrolled asthma. *J Investig Allergol Clin Immunol* 2012; 22(7): 460-475.
 18. Singer F, Luchsinger I, Inci D, et al. Exhaled nitric oxide in symptomatic children at preschool age predicts later asthma. *Allergy*. 2013;68(4):531-8.
 19. Schulze J, Reinmüller W, Herrmann E, Rosewich M, Rose MA, Zielen S. Bronchial allergen challenges in children - safety and predictors. *Pediatr Allergy Immunol*. 2013;24(1):19-27.
 20. Barben J, Kuehni CE, Strippoli MP, Schiller B, Hammer J, Trachsel D; Swiss Paediatric Respiratory Research Group. Mannitol dry powder challenge in comparison with exercise testing in children. *Pediatr Pulmonol* 2011;46(9):842-8.
 21. Andregnette-Roscigno V, Fernández-Nieto M, Arochena L, García Del Potro M, Aguado E, Sastre J. Methacholine is more sensitive than mannitol for evaluation of bronchial hyper-responsiveness in youth athletes with exercise-induced bronchoconstriction. *Pediatr Allergy Immunol*. 2012;23(5):501-3.
 22. Anderson SD, Charlton B, Weiler JM, Nichols S, Spector SL, Pearlman DS; A305 Study Group. Comparison of mannitol and methacholine to predict exercise-induced bronchoconstriction and a clinical diagnosis of asthma. *Respir Res*. 2009; 23(10):4.
 23. Clough JB, Hutchinson SA, Williams JD, Holgate ST. Airway response to exercise and methacholine in children with respiratory symptoms. *Arch Dis Child*. 1991;66(5):579-83.

TABLES AND FIGURES

Figure 1. Study design diagram.



**Challenge tests carried out on different days within an interval of minimum 2 days and maximum 4 days, in a non-fixed sequence*

Table 1. Results of single lung function tests

	Positivity Ratio (positive results/ number of children tested) (Percentage)	CI	Result values	
			Mean	SD
Baseline spirometry (% pred FEV ₁)			94.57	14.86
Bronchodilator (% ↑FEV ₁)	10/46 (21.7%)	10.95 - 36.36	14.81	7.16
Exercise (% ↓FEV ₁)	11/46 (23.9%)	12.59 - 38.77	18.47	14.18
Mannitol (PD15 in µg)	36/45 (80%)	65.40 - 90.42	107.11	177.82
Methacholine (PC20 in mg/ml)	41/45 (91.11%)	78.78 - 97.52	0.50	3.21

CI: 95% binomial confidence interval

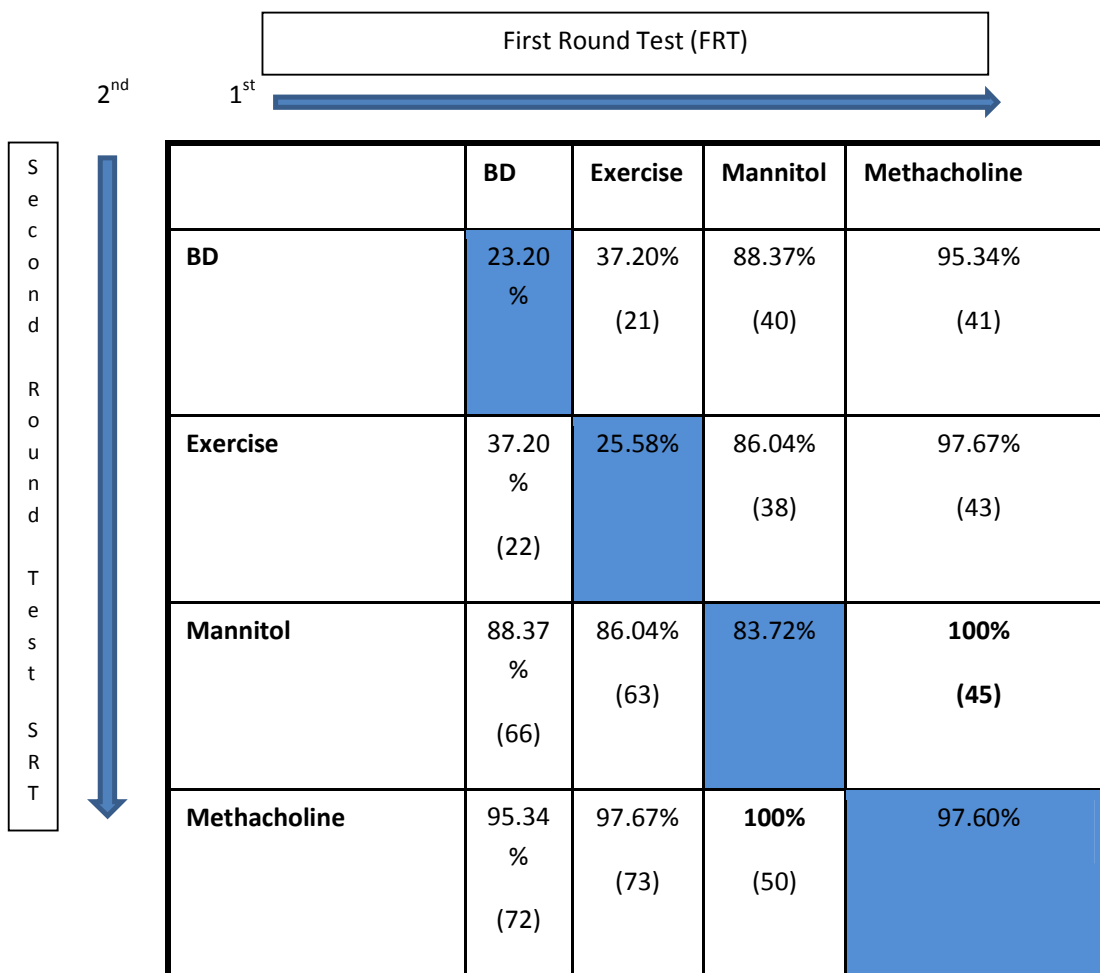
Table 2. Number of positive tests per patient, and correlation with CAN and FeNO results.

	N (%) Total / Asthmatics*	FeNO (ppb)(mean) Total / Asthmatics*	CAN (score value) (mean) Total / Asthmatics*
0 tests	3 (6.52%) / 2 (6.25%)	25 / 35	4 / 4
1 test	5 (10.58%) / 4 (12.50%)	34 / 38.72	8 / 8.75
2 tests	26 (56.52%) / 16 (50%)	30 / 32.43	9 / 6.75
3 tests	7 (15.21%) / 5 (15.62%)	74 / 51.26	5 / 6.93
4 tests	5 (10.86%) / 5 (15.62%)	81 / 66.47	6 / 7.30
S.S.		0.371 p=0.012	0.11 p=0.943

S.S.: Statistically Significant

*Asthmatics: Results according to the group of previously asthma diagnosed patients

Table 3. Results of the combined lung function tests. Results are shown in percentage of children with BHR detected by the combination of the 2 tests, calculated as follows: First Round Test (FRT) Positive and/or Second Round Test (SRT) Positive / Patients with BHR detected by any test. Absolute numbers (in parentheses) indicate the total number of tests needed to demonstrate BHR in our population of 43 children with BHR. The columns show the tests performed first, and the rows show the tests performed second. The blue cells show the percentage of children with BHR detected by a single test.



	First Round Test (FRT)			
	BD	Exercise	Mannitol	Methacholine
BD	23.20 % (21)	37.20% (21)	88.37% (40)	95.34% (41)
Exercise	37.20 % (22)	25.58% (38)	86.04% (43)	97.67% (45)
Mannitol	88.37 % (66)	86.04% (63)	83.72% (45)	100% (72)
Methacholine	95.34 % (72)	97.67% (73)	100% (50)	97.60% (43)