

## LETTER TO THE EDITOR

**EVALUATION OF BRONCHIAL HYPERREACTIVITY WITH MANNITOL DRY POWDER CHALLENGE TEST IN A PAEDIATRIC POPULATION WITH INTERMITTENT ALLERGIC ASTHMA OR ALLERGIC RHINITIS**

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We evaluated the bronchial hyperreactivity (BHR) with a new bronchial challenge test, mannitol dry powder, in a paediatric population with intermittent allergic asthma or allergic rhinitis who did not respond to an exercise challenge test. We selected 50 children, aged 9-16 years, with intermittent allergic bronchial asthma (Group 1) or allergic rhinitis without clinical manifestation of asthma for at least 12 months (Group 2). All patients performed the following tests in three different days ( $\geq 48$  hours apart): Day 1: exhaled nitric oxide (FeNO) determination followed by baseline spirometry and reversibility to inhaled  $\beta_2$ -agonists; Day 2: exercise challenge test followed by FeNO determination; Day 3: mannitol challenge test followed by FeNO determination. Forty children completed the study. Eighteen subjects of Group 1 (90%) and 5 subjects of Group 2 (25%) resulted positive to the mannitol test. Positive mannitol challenge subjects showed no statistically significant differences compared to negative subjects as regard baseline spirometry, reversibility to salbutamol and response to the exercise challenge test, but they had significantly higher FeNO values. In conclusion, the mannitol challenge test can be a diagnostic tool more useful than the exercise challenge test to identify BHR in a paediatric population with intermittent allergic asthma or allergic rhinitis because it is better reproducible, quick and easy to perform and well tolerated.

Bronchial asthma is a chronic inflammatory disease of the airways characterized by airflow obstruction, usually reversible either spontaneously or after therapy, bronchial hyperreactivity (BHR) and accelerated deterioration of lung function that can sometimes lead to irreversible airway obstruction. Currently, the up-to-date GINA guidelines classify asthma according to 4 levels of increasing severity: 1) Intermittent 2) Mild Persistent, 3) Moderate Persistent, 4) Severe Persistent, which correspond to different treatments (1).

Intermittent asthma is generally characterized by occasional symptoms that may occur over long periods of time or by symptomatic periods, even relevant and sustained, alternated with long periods of remission. Asthma can be triggered by many factors (physical exercise, allergens, etc.), it occurs especially during early childhood, can remain stable for a long time and usually requires no control therapy, but only as-needed or for short periods (2). Bronchial reactivity should also be monitored in subjects with allergic rhinitis, due to

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the close correlation between the two conditions (3) and because allergic rhinitis may be a risk factor for asthma (4).

Currently BHR can be evaluated through bronchoconstrictor stimuli classified as direct (pharmacological agents such as histamine or methacoline) or indirect (physiological such as exercise, allergens, adenosine monophosphate, hyperosmolar saline solution and inhaled mannitol dry powder) (5).

Inhaler mannitol dry powder, a new indirect osmotic challenge, seems to have very interesting and promising perspectives, especially in paediatric age, due to its diagnostic specificity, high standardization and because it is easy and quick to perform (6).

The aim of this study is to evaluate the presence of BHR with the mannitol dry powder challenge test in children with intermittent allergic asthma or allergic rhinitis without asthma, who did not respond to an exercise challenge test (primary endpoint). We also verified the possibility to correlate the exhaled nitric oxide test (FeNO) to a higher risk of BHR (secondary endpoint).

## MATERIALS AND METHODS

### *Study design*

The study is a cross-sectional population study. Fifty children, aged 9-16 years (24 males/26 females), with intermittent allergic bronchial asthma (Group 1 – 27 subjects), according to GINA guidelines, or with allergic rhinitis without clinical manifestation of asthma by  $\geq 12$  months (Group 2 – 23 subjects), were recruited from the Servizio Autonomo Asma e Fisiopatologia Respiratoria Infantile “Maurizio Miraglia del Giudice”, Department of Paediatrics, Second University of Naples. Demographic characteristics of the subjects are shown in Table I.

All subjects were able to run on a treadmill and to carry out a reproducible spirometry and they had undergone a skin prick test in the six months before the study. The children had not been receiving systemic or inhaled steroids or long-acting  $\beta_2$ -agonists for 4 weeks, montelukast and antihistamines for 2 weeks and short acting  $\beta_2$ -agonists for 48 hours before the tests.

All selected patients performed the following tests on three different days  $\geq 48$  hours apart:

Day 1: exhaled nitric oxide (FeNO) determination followed by baseline spirometry and reversibility to inhaled  $\beta_2$ -agonists (salbutamol 200 mcg MDI + spacer, Aerochamber plus - Gsk);

Day 2: exercise challenge test followed by FeNO

determination;

Day 3: mannitol powder challenge test followed by FeNO determination.

The study was approved by the ethics committee of Second University in Naples and written informed consent was signed by the children and their parents and/or legal guardians.

### *Lung function test*

All subjects were trained to perform a correct spirometry. Lung volumes and flow-volume loops were evaluated by a Microlab Viasys spirometer, calibrated each morning, according to the ATS protocol. Pulmonary function was determined as the best result of at least 3/8 acceptable spirometry with a FVC variability range  $\leq 10\%$  of each other) (7).

### *Exercise challenge*

The exercise challenge test was performed by running on a treadmill with the nose clipped (Pro Form 570) according to the ATS standardized protocol (8) in an air-conditioned room with a controlled temperature (20-25°C) and relative humidity ( $\leq 50\%$ ).

Baseline spirometry was performed and the best of three determinations of FEV1 was recorded. The heart frequency rate was constantly monitored by a device (Polar T31) connected to the chest of the subject. The test started with running at low speed on the treadmill with a 10% slope. The running speed was gradually increased to reach approximately 90% of the predicted maximum heart rate  $[(220 - \text{age}) \times 0.9]$ . This speed was maintained for a maximum of 6 min after which the test was interrupted. FEV1 determination was performed at 1, 5, 10, 15 and 30 minutes after the end of the exercise. The test was considered positive if FEV1 decreased  $\geq 12\%$  from baseline (I.B.).

### *Mannitol challenge*

Osmohale™ kit, packaged in capsules (cps) containing respectively 0, 5, 10, 20, 40 mg of dry powder of mannitol, was used for the challenge test. The powder was delivered by Osmohaler™ disposable inhaler device. The test was performed as recommended by the distributor (Italchimici S.p.A. under exclusive licence of Pharmaxis Ltd, Sydney, Australia). The dose protocol consisted of 0, 5, 10, 20, 40, 80, 160, 160, 160 mg mannitol; the last four doses were given in multiples of 40 mg cps (9).

The test started with inhalation of the cps 0 (empty cps acting as a placebo). Subjects were asked to perform, through the device, a maximal exhalation near to functional residual capacity followed by a maximal inspiration near to total lung capacity; then hold the breath for about 5 seconds. The children had a nose clip during inhalation to reduce as

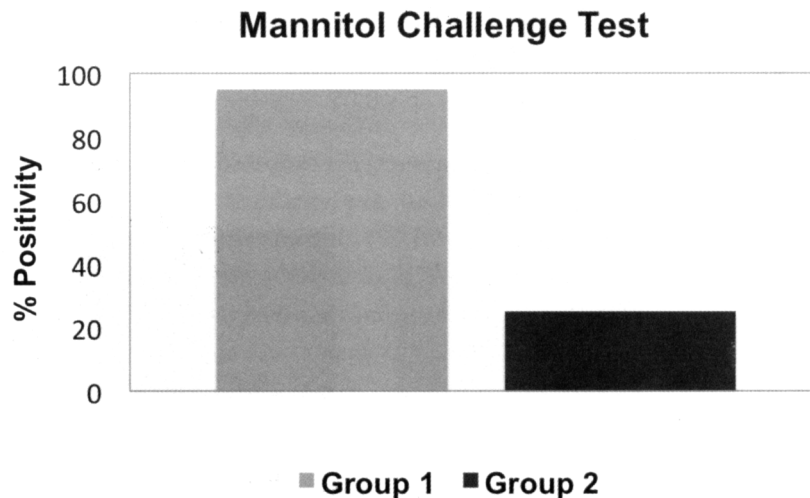


**Fig. 1.** Eighteen subjects of Group 1 (90%) and five subjects of Group 2 (25%) resulted positive to mannitol challenge test.

much as possible the deposition of mannitol powder in the nasopharynx. Three FEV1 measurements were carried out one minute after the end of inhalation and the best value was recorded. The FEV1 recorded after inhalation of capsule 0 was used as a reference value (baseline) to determine the decrease (%) in FEV1 in response to the mannitol challenge.

The test was stopped when FEV1 decreased  $\geq 15\%$  (PD15) vs baseline or  $\geq 10\%$  after two consecutive doses or when a maximum cumulative dose of 635 mg had been given. For any decrease in FEV1, cumulative dose/response ratio was also calculated.

#### *Exhaled nitric oxide (FeNO)*

The determination of exhaled nitric oxide (FeNO) was performed with 'single-breath on line' method by electrochemical analyser (Medisoft - EXPA), according to the European Respiratory Society (ERS) (10) and the American Thoracic Society (ATS) guidelines (11).

The patients were instructed to inhale deeply for 2-3 seconds near to the total lung capacity and then to exhale immediately for at least 10 seconds to achieve reproducible and flat-expiratory plateau. The test was repeated twice more, with an interval of 30 seconds and was considered the average value achieved from three measurements. Cut-off value of 20 ppb was considered.

#### *Statistical analysis*

Statistical analysis was performed using SPSS version 18.0 program. Average values, average standard deviations

and the geometric average values were determined. Student's paired *t* test was used to calculate differences between groups. Variations with  $p \leq 0.05$  were considered significant.

## RESULTS

Fifty subjects were selected; 6 subjects were excluded from the trial because they were positive to exercise challenge test (I.B.  $\geq 12\%$ ) and 4 subjects for the onset of persistent cough during the challenge with mannitol dry powder. Forty children (20 with intermittent allergic bronchial asthma and 20 with allergic rhinitis) completed the study.

#### *Lung functions test*

No statistical differences between groups were observed on baseline spirometry, reversibility to salbutamol ( $\Delta\%$  FEV1) and exercise challenge test results (I.B.) (Table II).

#### *Mannitol Test*

Eighteen subjects of Group 1 (90%) and 5 subjects of Group 2 (25%) resulted positive to mannitol test (Fig. 1).

Positive mannitol challenge subjects showed no statistically significant differences compared

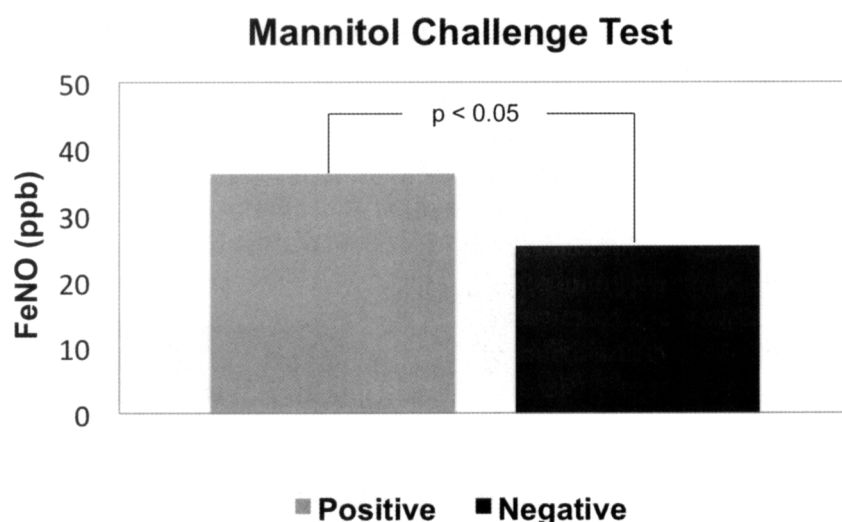


**Fig. 2.** Positive subjects to mannitol challenge test showed significantly higher FeNO values than negative subjects ( $p < 0.05$ ).

**Table I.** Demographic characteristics.

|                            | Group 1 | Group 2 | Significance    |
|----------------------------|---------|---------|-----------------|
| <b>Subjects (n°.)</b>      | 27      | 23      |                 |
| <b>Males</b>               | 15      | 13      |                 |
| <b>Females</b>             | 12      | 10      |                 |
| <b>Average age (Years)</b> | 11.4    | 12.1    | $p = \text{NS}$ |
| <b>Atopy (n°.)</b>         | 27      | 23      | $p = \text{NS}$ |

**Table II.** Lung function tests.

|                                   | Group 1           | Group 2           | Significance    |
|-----------------------------------|-------------------|-------------------|-----------------|
| <b>FEV1 (%)</b>                   | 94.66 (SD 8.23)   | 100.28 (SD 9.87)  | $p = \text{NS}$ |
| <b>FEF 25-75 (%)</b>              | 101.91 (SD 12.43) | 101.63 (SD 13.88) | $p = \text{NS}$ |
| <b><math>\Delta\%</math> FEV1</b> | 3.66 (SD 1.23)    | 4.63 (SD 1.92)    | $p = \text{NS}$ |
| <b>EXERCISE TEST (I.B.%)</b>      | 5.18 (SD 2.53)    | 5.90 (SD 2.24)    | $p = \text{NS}$ |

**Table III.** Mannitol challenge test.

|                                     | Positive        | Negative          | Significance    |
|-------------------------------------|-----------------|-------------------|-----------------|
| <b>FEV1 (%)</b>                     | 93.85 (SD 9.52) | 102.55 (SD 10.28) | $p = \text{NS}$ |
| <b>FEF 25-75 (%)</b>                | 101 (SD 11.26)  | 103 (SD 13.76)    | $p = \text{NS}$ |
| <b><math>\Delta</math> FEV1 (%)</b> | 3.85 (SD 1.03)  | 5.1 (SD 1.57)     | $p = \text{NS}$ |
| <b>EXERCISE TEST (I.B. %)</b>       | 4.41 (SD 2.08)  | 7.27 (SD 2.67)    | $p = \text{NS}$ |

to negative subjects as regard baseline spirometry, reversibility to salbutamol and response to exercise challenge test (Table III), but they had significantly higher FeNO values (Fig. 2).

## DISCUSSION

Our study showed that 90% of the subjects with intermittent allergic asthma (Group 1) was

positive to the mannitol challenge test, confirming the presence of abnormal lung function in subjects with mild or occasional asthmatic symptoms. In this regard, Horner and coll. have highlighted that, during their growth, asthmatic children show a progressive improvement of respiratory symptoms, despite persistent deterioration of the lung function, BHR and airways inflammation (12). Therefore in these subjects, due to the poor perception of symptoms, the course of the disease and presence of asthma deterioration control markers such as reduction of physical activity, persistent nocturnal dry cough etc., are often underestimated (2).

This finding is still more surprising if we consider that all of the selected subjects did not respond to an exercise challenge test. A possible explanation could be related to the intrinsic characteristics of the two tests. Mannitol and exercise are 'indirect challenges' able to cause an increase in airways osmolarity, with consequent release of mediators such as leukotrienes, prostaglandins, and histamine by inflammatory cells and airway smooth muscle constriction. Thus, the indirect challenge reflects the level of inflammation in the airways (13). It should be stressed that the intracellular events, occurring during the execution of these tests, are the same whether the increase of airways osmolarity is due to bronchial epithelium dehydration, as in the exercise challenge test, or directly due to a hyperosmolar stimulus like mannitol inhalation. However, as recently demonstrated by Kersten et al. (14), the main difference between the two tests is represented by the power of the cascade of broncho-obstructive events, which seems to be much greater in the mannitol challenge test compared to the exercise challenge test.

Besides this, the mannitol challenge test seems to represent a viable alternative to the exercise challenge test since it is better reproducible by using a diagnostic protocol which is well standardized, and quick and easy to perform; it does not require specifically trained personnel and does not use expensive equipment such as a treadmill.

As noted by other authors (15-16), in our study the mannitol challenge test was well tolerated and there were no significant adverse events, except the onset of a mild cough. Only 4 subjects stopped the challenge test for a very prolonged cough.

We also evaluated the presence of BHR in

children with allergic rhinitis without clinical manifestations of asthma for at least 12 months and we demonstrated that 25% of these patients was positive to mannitol challenge. As shown by several epidemiological and pathophysiological studies, this finding confirms that asthma and rhinitis often coexist in the same patient as evidence of a strong relationship between these conditions (3, 17). In this regard subjects with allergic rhinitis without asthma symptoms had a low FEF 25-75 and an increased bronchial reactivity, both predictive factors of an increased risk of asthma (18-19).

We verified the possibility to correlate the exhaled nitric oxide test (FeNO) to a higher risk of BHR. As shown in Fig. 2, the mean baseline FeNO values in the mannitol-positive subjects are significantly higher than in those mannitol-negative. This finding seems to be in accordance with that reported by other authors who demonstrated the presence of a close correlation between FENO values and bronchial hyperresponsiveness to mannitol or methacholine in asthmatic subjects (20-21). These observations therefore enhance the hypothesis that a correlation probably exists between the degree of responsiveness to the mannitol challenge test and the level of eosinophilic airway inflammation, as recently demonstrated by Wood et al. (22).

In conclusion, the mannitol challenge test can be a more useful diagnostic tool than the exercise challenge test to identify BHR in a paediatric population with intermittent allergic asthma or allergic rhinitis because it is better reproducible, quick and easy to perform and well tolerated. Further studies are needed to evaluate in a larger population of asthmatic children the diagnostic validity of the mannitol challenge test and its clinical utility in monitoring the asthma treatment.

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