



## Original Article

## Bronchial Hyperresponsiveness to Methacholine in Children Under 4 Years with Recurrent Bronchitis

Inés de Mir Messa, \* Antonio Moreno Galdó, Nicolás Cobos Barroso, Sílvia Gartner, Carlos Martín de Vicente, Sandra Rovira Amigo, Alba Torrent Vernetta, Santos Liñán Cortés

Unidad de Neumología Pediátrica y Fibrosis Quística, Hospital Universitari Vall d'Hebron, Barcelona, Spain

## ARTICLE INFO

## Article history:

Received March 13, 2010

Accepted July 29, 2010

## Keywords:

Bronchial hyperresponsiveness

Methacholine

Auscultatory method for infants

Pre-school

## ABSTRACT

**Objective:** To evaluate bronchial hyperresponsiveness in children under 4 years old with recurrent wheezing bronchitis, and to determine if its presence or absence can predict subsequent progression to a transient or persistent wheezing bronchitis phenotype.

**Population and methods:** A bronchial challenge test was performed with methacholine using a modified tidal volume method without sedation, in a group of patients from 8 to 47 months of age with recurrent wheezing bronchitis and a control group of healthy children. A decrease in oxygen saturation of  $\geq 5\%$  or an increase in respiration rate of  $>50\%$  [PCwheeze (PCw)] was considered a positive response. The patients were subsequently clinically followed up to assess their progress.

**Results:** A total of 63 patients and 16 controls were studied (mean age 23.9 compared to 25.2 months). The PCw in 43 (68%) children from the bronchitis group was lower than the control group ( $\leq 4$  mg/mL), ( $P < .001$ ). No significant adverse effects were observed on performing the test. After a mean follow-up of 28.5 months, completed in 49 of the patients, no differences were seen between the presence of bronchial hyperresponsiveness at the beginning of the study and the subsequent progression to transient, infrequent or frequent wheezing ( $P = .63$ ).

**Conclusions:** A high percentage of children under 4 years old with wheezing bronchitis had a bronchial hyperresponse. Subsequent progression to transient or persistent wheezing bronchitis phenotype is not associated with bronchial hyperresponsiveness.

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### Hiperrespuesta bronquial a la metacolina en niños menores de 4 años con bronquitis de repetición

## RESUMEN

## Palabras clave:

Hiperrespuesta bronquial

Metacolina

Método de la auscultación lactantes

Preescolares

**Objetivo:** Valorar la hiperrespuesta bronquial en niños menores de 4 años con bronquitis sibilantes de repetición, y determinar si su presencia o ausencia permite predecir la evolución posterior hacia un fenotipo de bronquitis sibilantes transitorias o persistentes.

**Población y métodos:** Se realizó una prueba de broncoprovocación con metacolina utilizando un método modificado de respiración a volumen corriente, sin sedación, a un grupo de pacientes de 8 a 47 meses de edad, con bronquitis sibilantes recurrentes, y a un grupo control de niños sanos. Se valoró como respuesta positiva la presencia de sibilantes, la disminución de la saturación de oxígeno  $\geq 5\%$  o el aumento de la fre-

\*Corresponding author.

E-mail address: idemir@vhebron.net (I. de Mir Messa).

cuencia respiratoria > 50% (PCwheeze [PCw]). Se realizó un seguimiento clínico posterior de los pacientes para valorar su evolución.

**Resultados:** Se estudiaron 63 pacientes y 16 controles (edad media 23,9 vs. 25,2 meses). La PCw fue inferior a la del grupo control ( $\leq 4$  mg/mL) en 43 niños (68%) del grupo bronquitis ( $p < 0,001$ ). No se observaron efectos adversos significativos con la realización de la prueba. Tras un seguimiento medio de 28,5 meses, completado en 49 de los pacientes, no se observaron diferencias entre la presencia de hiperrespuesta bronquial al inicio del estudio y la evolución posterior a sibilantes transitorios, infrecuentes y frecuentes ( $p = 0,63$ ).

**Conclusiones:** Un porcentaje elevado de niños menores de 4 años afectos de bronquitis sibilantes presentaron hiperrespuesta bronquial. La evolución posterior a un fenotipo de bronquitis sibilantes transitorias o persistentes no se relacionó con la hiperrespuesta bronquial.

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## Introduction

Recurrent wheezing bronchitis in infants and pre-school children is a highly prevalent problem that may occur at an early age in 29% of children.<sup>1,2</sup>

Bronchial hyperresponsiveness is a key component of the asthmatic phenotype,<sup>3</sup> but its relationship with recurrent episodes of bronchitis in children is not fully clear.

Previous studies<sup>4,5</sup> found no differences in bronchial hyperresponsiveness between a control group of healthy children and infants with a history of wheezing bronchitis. They concluded that the presence of recurrent bronchitis in infants is independent of bronchial hyperresponsiveness. In contrast, other authors<sup>6,7</sup> did find increased bronchial hyperresponsiveness in infants and pre-school children with recurrent bronchitis.

Moreover, the literature provides little data about the effect of possible differences in the presence of bronchial hyperresponsiveness in infants or children of pre-school age developing transient wheezing bronchitis and those with a persistent wheezing bronchitis phenotype. Saga et al. found a relationship between the persistence of asthma symptoms at 10 years old and the presence of bronchial hyperresponsiveness in infants with wheezing bronchitis.<sup>7</sup> Turner et al. also found a relationship between the presence of bronchial hyperresponsiveness during breastfeeding and the presence of wheezing bronchitis 10 years later.<sup>8</sup> Moreover, Delacourt et al<sup>9</sup> found a similar prevalence of bronchial hyperresponsiveness in children with transient and persistent wheezing, but could not find an effective cut-off point to differentiate between children with transient and persistent wheezing.

Generally, for the assessment of bronchial hyperreactivity, bronchial challenge tests are used to determine the forced expiratory volume in the first second (FEV1) as a response variable; but this test is limited to use in adults and older children. Alternative techniques have been used to assess lung function and bronchial hyperreactivity in infants and pre-school children,<sup>8,10,11</sup> such as rapid thoracoabdominal compression, which determines the maximum flow at functional residual capacity, or forced expiratory volumes including the previous insufflation technique, plethysmography, impulse oscillometry and measurement of respiratory resistance by occlusion method. However, the use of these tests is limited by their technical complexity and the need for sedation.

Other techniques have been developed for use in infants and pre-school children that do not require sedation: measurement of resistance by interruption,<sup>11</sup> measurement of transcutaneous O<sub>2</sub> pressure<sup>12</sup> and tracheal auscultation.<sup>13</sup> This last method increases the concentration of methacholine until wheezing is heard in the trachea or thorax.<sup>14</sup> The measurement of bronchial hyperresponsiveness to methacholine by the modified tracheal auscultation method<sup>13</sup> could allow better characterisation of young children under four years old with recurrent wheezing.

The aim of this study is to assess bronchial hyperresponsiveness in children under 4 years old with recurrent wheezing bronchitis, compared with a control group of healthy children of the same age, and to determine whether the presence or absence of bronchial hyperresponsiveness can predict which children will develop a transient or persistent wheezing bronchitis phenotype.

## Patients and Methods

### Patients

A cross-sectional study was conducted on the presence of bronchial hyperresponsiveness in children with recurrent bronchitis. The results were compared with a healthy control group and a long-term follow-up was carried out. Children in both groups came from the same area (Barcelona, Spain). The details of the response to methacholine in healthy children have been published previously.<sup>15</sup>

A group of patients were chosen by consecutive convenience sampling from our pediatric pulmonology outpatient unit. They were aged between 6 months and 4 years old and a doctor from the bronchitis group had diagnosed 3 or more episodes of acute wheezing bronchitis during the previous year. Acute wheezing bronchitis was defined as an acute cough accompanied by wheezing on auscultation, with or without signs of shortness of breath, requiring treatment with a bronchodilator. We accepted treatment with inhaled corticosteroids in a maximum of one-third of the children. We excluded patients with chronic lung disease (cystic fibrosis, bronchopulmonary dysplasia, etc.) or cardiovascular disease, and premature children.

A group of healthy children (control group) were also studied at the same time. These were aged between 6 months and 4 years old and were recruited from a paediatric practice in the Barcelona area and from the hospital. These were children born at term in the Barcelona area, with no personal or family history of atopy or passive smoking.

A prospective clinical follow-up was performed on the patients for an average of 28.5 months to determine which of them still had wheezing bronchitis. After the follow-up the patients were classified into transient wheezing bronchitis (no episodes of bronchitis in the previous year), infrequent wheezing bronchitis ( $\leq 3$  episodes per year) and frequent wheezing bronchitis ( $> 3$  episodes/year).

The study was approved by the local Ethics and Clinical Research Committee and the children were included after information was provided to the parents and they gave their written consent.

### Methods

The medical history included family history of atopy, passive smoking and other risk factors for recurrent bronchitis. A group of

children with recurrent bronchitis underwent the following clinical study protocol as part of the study of their underlying pathology: blood count with the total number of eosinophils, total IgE and specific IgE against the following inhaled allergens: *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, cat dander, *alternaria*, *parietaria* and olive tree; skin prick tests with standard inhalant allergens in our environment: mites, dog dander, cat dander, olive tree, *parietaria*, grasses, trees.

A bronchial challenge test was conducted with methacholine, using the tidal breathing method for 2 minutes. In this technique, the aerosol is generated with a continuous flow nebuliser. Three millilitres of methacholine chloride solutions (Provocholine®, Methapharm Inc., Brantford, Ontario, Canada) diluted in saline were nebulised and the child inhaled it (at tidal volume) for 2 min.<sup>16</sup>

The following was required before conducting the methacholine test: lack of upper respiratory tract infection or bronchitis in the three previous weeks, basal oxygen saturation  $\geq 95\%$ , normal respiratory auscultation and absence of tachypnea. Bronchodilators were suspended as recommended by the American Thoracic Society (ATS).<sup>17</sup>

The test was conducted with the child in the arms of his father or mother and in a play environment with books and toys appropriate for the child's age.

ATS recommendations, with some modifications, were followed for conducting the bronchial challenge test with methacholine in collaborating children and adults,<sup>17</sup> as reflected in Table 1. The ATS protocol uses increasing concentrations of methacholine from 0.031 to 8 mg/mL, doubling the concentration of methacholine on each occasion. Thus, a total of 10 sprays was administered, including the solvent used. Given the difficulty of using such a high number of sprays in young children, an abbreviated protocol was designed, with 6 sprays in the control group and 7 in the bronchitis group.

The different sodium chloride and methacholine solutions were administered with a nebuliser and face mask, using a compressed air cylinder with a working pressure of 15 psi (1 bar). A MicroMist® nebuliser (Hudson RCI, Temmecula, Ca, USA) was used, which provides a mass median aerodynamic diameter of 1-3.6 microns. The nebuliser was calibrated to calculate the flow of compressed air required to provide a discharge rate of 0.13 mL/min  $\pm 10\%$ . It was estimated that a flow of 4 L/min produced a discharge rate within the required range (0.13 mL/min  $\pm 10\%$ ).

The assessment of the response to the methacholine stimulus was performed using the modified tracheal auscultation method.<sup>13</sup> It was considered a positive response to a given concentration of methacholine (PCwheeze) when: clear wheezing was heard in the trachea or chest, hemoglobin saturation (SaO<sub>2</sub>) fell by 5% or more above the basal SaO<sub>2</sub> or the respiratory rate increased 50% or more above the baseline.

**Table 1**

Dosing protocols used to perform a bronchial challenge test with methacholine using the tidal breathing method for 2 min, compared with the American Thoracic Society protocol

Methacholine concentrations		
American Thoracic Society protocol	Reduced protocol Recurrent bronchitis group	Reduced protocol Control group
Solvent	Solvent	Solvent
0.031 mg/mL		
0.0625 mg/mL	0.0625 mg/mL	
0.125 mg/mL		
0.250 mg/mL	0.250 mg/mL	
0.500 mg/mL		0.500 mg/mL
1 mg/mL	1 mg/mL	1 mg/mL
2 mg/mL	2 mg/mL	2 mg/mL
4 mg/mL	4 mg/mL	4 mg/mL
8 mg/mL	8 mg/mL	8 mg/mL

After each nebulisation, the trachea was listened to in periods of 20 seconds, as well as the antero-superior and inferior-posterior part of the chest. Also, the respiratory rate, heart rate, and SaO<sub>2</sub> were measured during each of the 3 following minutes. If there was no positive response, the process was continued with the next spray concentration, until the maximum concentration of 8 mg/mL was reached. Nebulised salbutamol was administered in cases with a positive response.

### Sample Size

The following calculation was performed to determine the sample size required to estimate the bronchial hyperresponsiveness of children in the control group and bronchitis group: the control group PCwheeze was estimated at around 8 mg/mL.<sup>20</sup> A difference in PCwheeze of 4 mg/mL (methacholine dilution) between the control group and the wheezing group was considered a clinically significant result. From literature data we assumed a standard deviation of 3.55.<sup>18</sup> Given a .05 alpha risk, beta risk of .15 and a unilateral hypothesis, 13 patients were needed in each group, but considering a 20% loss, we would require 16 children in each group.

In addition, to discern if there were differences in bronchial hyperresponsiveness in children with persistent and transient wheezing, published data on the frequency of transient and persistent wheezing in this age group were taken into account. It was expected that two-thirds of the wheezing bronchitis group would be transient wheezing and one-third would be persistent wheezing.<sup>19</sup> Therefore, having 20 children in the latter group required an initial sample of 60 children in the group of patients with recurrent bronchitis.

### Statistical Analysis

To perform the PCwheeze numerical calculations, it was assumed that the methacholine concentration for those children with no response to 8 mg/mL should be 16 mg/mL.

The Kolmogorov-Smirnov test was applied to study the normality of the distribution of the variables. To compare 2 groups following a normal distribution, the *t* test was applied. To compare 2 groups of qualitative variables, the nonparametric Mann-Whitney U-test was applied. To compare 3 or more groups, a one-way analysis of variance test was used, followed by the Bonferroni test, or Kruskal-Wallis test if the variables did not follow a normal distribution. The comparison between qualitative variables was performed using the chi-squared test. The relationship between quantitative variables was studied using the Pearson correlation coefficient.

The univariate analysis was completed with the multivariate analysis using the logistic regression model when it was appropriate for the analysis of the influence of different variables on the results. Values of *P* < .05 were considered statistically significant.

Statistical analysis of the data obtained was performed using the MedCalc® software package, version 8.0.0.0 (MedCalc Software, Mariakerke, Belgium).

### Results

The bronchial challenge test with methacholine was performed in 16 children from the control group and 63 children from the group with wheezing bronchitis. Table 2 summarises the demographic characteristics of both groups, which were comparable for most traits, such as sex, age, birth weight, gestational age, breastfeeding, and child care attendance. They differed significantly only in the number of siblings, which was higher in the control group.

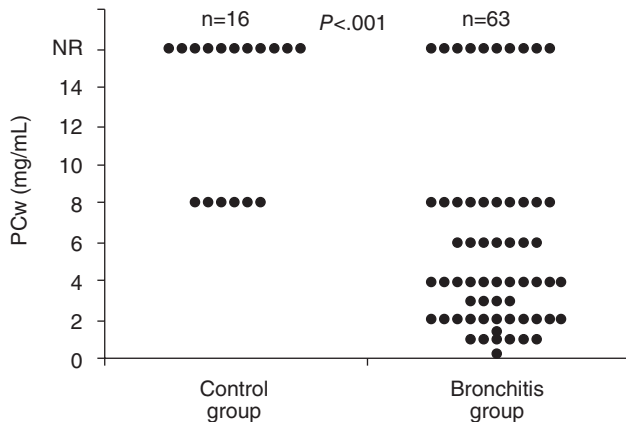
In the wheezing bronchitis group, the average age of the first bronchitis was 6 to 8 months (SD 0.78 months). The number of bronchitis episodes for the year preceding the study was between 3 and 5 in 27 children (42.8%), between 6 and 9 in 30 children (47.6%) and 10 or more episodes in 6 children (9.6%). Eighteen out of the 63

**Table 2**

Comparison of baseline characteristics between the control group and patients with recurrent wheezing bronchitis. values are expressed as mean (SD) or percentages

	Control	Bronchitis	P
Age, mo	23.9 (12.2)	25.22 (11.44)	.693
Sex	8M/8F (M 50%)	45M/18F (M 71.4%)	.103
Birth weight, g	3240.6 (465.6)	3163.4 (524.4)	.593
Gestational age, w	39.6 (1.8)	39.2 (1.3)	.374
Breastfeeding	14/16 (87.5%)	45/63 (71.4%)	.076
Breastfeeding, mo	5.7 (2.7)	4.2 (4.3)	.188
Childcare attendance	9/16	38/63	.767
Childcare attendance (entry age), mo	9.3 (11.1)	9.6 (10.6)	.913
Number of siblings	2.25 (1.84)	0.75 (0.6)	.005

Abbreviations: F, females; M, males.

**Figure 1.** Comparison of the response to methacholine between the control group and the recurrent wheezing bronchitis group by the modified tracheal auscultation method. Results expressed as PCwheeze (concentration of methacholine that produces audible wheezing in the trachea or lung area, a  $\geq 5\%$  drop in  $\text{SaO}_2$  or an increased respiratory rate of  $\geq 50\%$ ). NR indicates no response; PCw, PCwheeze.

children (28.5%) were treated with inhaled corticosteroids. Fifty-five of the 63 children had their first bronchitis episode in their first year of life. A syncytial respiratory virus test for this first bronchitis was conducted in 24 cases, which was positive in 12 (19%) and negative in the other 12 (19%).

All the children in both groups were able to perform the bronchial challenge test without significant difficulty and without sedation.

Two-thirds of healthy children ( $n=10$ ) did not respond to methacholine, and 6 did so only at the maximum concentration (8 mg/mL). In the wheezing bronchitis group, there were 10 who did not respond (negative challenge test), 10 who reacted at the maximum concentration and 43 (68%) who responded to lower concentrations than the control group ( $\leq 4$  mg/mL). The PCw in the wheezing bronchitis group (5.8 mg/mL; SD 3.9) was significantly lower than the control group (13.3 mg/mL; SD 5.02); ( $P < .001$ ), Figure 1.

A positive test was determined in 48/58 cases (82.7%) by the presence of wheezing on auscultation, either isolated or associated with a decrease in  $\text{SaO}_2$  or tachypnea. In 10 cases, no wheezing was heard, but there was a  $\geq 5\%$  drop in  $\text{SaO}_2$  (Table 3).

With the administration of methacholine, the respiratory rate increased from 27.4 (4.9) breaths/min to 42.1 (10.4) breaths/min ( $P < .0001$ ) and  $\text{SaO}_2$  decreased from 97% (0, 9%) to 93.2% (2.4%) ( $P < .0001$ ). The increase in the respiratory rate was higher in the bronchitis group than in the control group, with no significant differences between the groups in the  $\text{SaO}_2$  mean decrease (Table 3).

In total, a decrease in  $\text{SaO}_2$  higher than 5% was observed in 30 children (51.7% of the positive tests), a decrease between 93% and 95% in 7 children and between 90% and 92% in 20 children. Saturation

fell below 90% (between 88% and 89%) in only 3 cases. The decrease in  $\text{SaO}_2$  was self-limited as it spontaneously increased immediately after stopping the spray, and completely normalised after nebulisation with salbutamol.

Regarding other nebulisation effects that were not considered as positive criteria, 35 children had intercostal, subcostal or suprasternal retraction. Forty-one children had a cough. The cough appeared in 36 children with a positive test and 5 of them did not respond to methacholine. Seventeen children with a positive bronchial challenge test did not have a cough.

In the wheezing bronchitis group patients, the relationship between different variables and the presence of bronchial hyperresponsiveness was studied. It was noted that sex, family and atopy history, smokers at home and breastfeeding had no influence on the presence or absence of bronchial hyperresponsiveness. Nor was there any relationship with a history of respiratory syncytial virus infection or the number of bronchitis episodes in the previous year (Table 4). There was no relationship between the age of children when carrying out the test and the PCw ( $r=0.11$ ;  $P=.37$ ) (Figure 2). However, there was a slight correlation between the age at which children first had bronchitis and the PCw ( $r=0.26$ ,  $P=.04$ ). The difference in PCw between the untreated group (5.51 mg/mL [SD 4.85]) and the group receiving inhaled corticosteroids [7.0 mg/mL (SD 5.55)] was not significant ( $P=.308$ ).

It was possible to carry out a follow-up for 28.5 months (range, 21.6-41.8 months) until an average age of 53.8 months (range, 35-90 months) in 49 out of the 63 children in the bronchitis group. There were no significant differences between the presence of bronchial hyperresponsiveness in the initial evaluation and evolution to transient wheezing (PCw 4.5 mg/mL), infrequent wheezing (PCw 6.1 mg/mL) and frequent wheezing (PCw 5.5 mg/mL) ( $P=.63$ ). Bronchial hyperresponsiveness was seen in 10/16 (62.5%) of children with transient wheezing, 15/23 (65.2%) of children with infrequent wheezing and 7/10 of children with frequent wheezing (70%) (Figure 3).

## Discussion

The study showed methacholine-induced bronchial hyperresponsiveness in 68% of infants and pre-school children with recurrent wheezing bronchitis. However, there were no differences in the prevalence of bronchial hyperresponsiveness between the children who stopped having bronchitis (transient wheezing) and those who continued having bronchitis (frequent or infrequent persistent wheezing).

Our results showed that bronchial hyperresponsiveness seems to be an important part of the pathophysiology of wheezing bronchitis in children with transient and persistent bronchitis, but it is not a differential marker of its evolution. Other factors may be involved, such as the maturation of the immune system, growth of the bronchial tree or allergic sensitisation.

**Table 3**

Response criteria to methacholine bronchial challenge test and comparison of changes in respiratory rate and hemoglobin saturation between the control group and the group of patients with recurrent bronchitis

	Control group (n=16)	Bronchitis group (n=63)	Total (n=79)
Isolated wheezing	3 (18.7%)	22 (34.9%)	25 (31.6%)
+↓ SaO <sub>2</sub>	1 (6.2%)	17 (27%)	18 (22.8%)
+ Tachypnea		3 (4.8%)	3 (3.8%)
+↓ SaO <sub>2</sub> +tachypnea		2 (3.2%)	2 (2.5%)
Total wheezing	4 (25%)	44 (69.8%)	48 (60.7%)
↓ SaO <sub>2</sub>	1 (6.2%)	4 (6.3%)	5 (6.3%)
↓ SaO <sub>2</sub> +tachypnea		5 (7.9%)	5 (6.3%)
Respiratory rate	+9.3 (6.5)	+16.0 (9.0)	.008
SaO <sub>2</sub> (%)	-3.2 (2.2)	-4.3 (2.4)	.118

**Table 4**

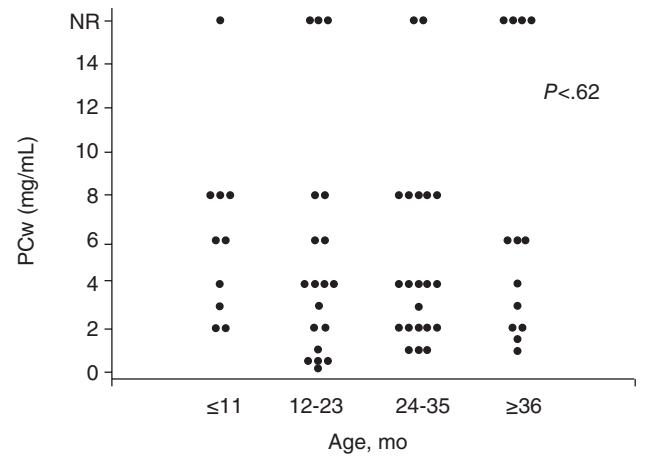
Influence of the different study variables on bronchial hyperresponsiveness in the group of children with recurrent bronchitis

	PCwheeze (SD) (mg/mL)	P
Sex		.28
Masculine	5.4 (4.8)	
Feminine	7.0 (5.7)	
History of asthma		.25
No	6.5 (5.6)	
Yes	5.0 (4.0)	
History of rhinitis		.65
No	6.0 (5.3)	
Yes	5.3 (3.9)	
History of atopic dermatitis		.75
No	5.8 (5.1)	
Yes	6.4 (5.0)	
Maternal smoking in pregnancy		.85
No	5.8 (4.8)	
Yes	6.1 (5.9)	
Postnatal smoking exposure		.23
No	4.8 (4.2)	
Yes	6.4 (5.4)	
Breastfeeding		.15
No	7.3 (6.2)	
Yes	5.3 (4.4)	
Starting age of childcare		.57
No	5.4 (5.3)	
Yes	6.1 (4.9)	
1 or more siblings		.30
No	6.7 (5.7)	
Yes	5.4 (4.6)	
RSV bronchiolitis		.30
Negative	3.9 (4.8)	
Positive	6.0 (4.9)	
Corticosteroid treatment		.31
No	5.5 (4.8)	
Yes	7 (5.5)	
Episodes of bronchitis in the last year, n		.76
3-5	5.7 (4.5)	
5-9	5.6 (5.2)	
≥10	7.2 (6.6)	

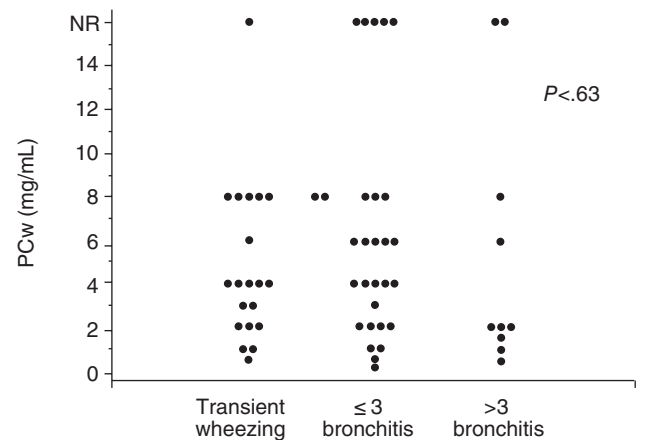
Abbreviations: SD, standard deviation; RSV, respiratory syncytial virus.

Although in contrast with our results some previous studies<sup>4,5</sup> found no differences between the presence of bronchial hyperresponsiveness and healthy controls, our findings agree with those of Saga et al,<sup>7</sup> as well as those of Guirau et al,<sup>5</sup> who also found increased bronchial hyperresponsiveness in children between 4 and 24 months with recurrent bronchitis, compared with the control group.

From epidemiological studies conducted in infants with recurrent wheezing bronchitis, the Tucson group described 3 different phenotypes in the first 6 years of life (transient wheezing, persistent non-atopic and persistent atopic asthma<sup>19</sup>). They considered that infants with transient wheezing did not have bronchial



**Figure 2.** Response to methacholine in the group of patients with recurrent bronchitis by the modified tracheal auscultation method. Patients are classified by age group at the time of conducting the test. Results expressed as PCwheeze (concentration of methacholine that produces audible wheezing in the trachea or lung area, a  $\geq 5\%$  drop in SaO<sub>2</sub> or an increased respiratory rate of  $\geq 50\%$ ). NR indicates no response; PCw, PCwheeze.



**Figure 3.** Comparison of the presence of bronchial hyperresponsiveness at the initial examination and subsequent follow-up classification into transient wheezing bronchitis (with no bronchitis episodes in the last year), infrequent persistent wheezing bronchitis ( $\leq 3$  episodes of bronchitis in the last year) and frequent persistent wheezing bronchitis ( $> 3$  episodes of bronchitis in the last year). Results are expressed as PCwheeze (concentration of methacholine that produces audible wheezing in the trachea or lung area, or  $\geq 5\%$  drop in SaO<sub>2</sub> or an increased respiratory rate of  $\geq 50\%$ ). NR indicates no response; PCw: PCwheeze.

hyperresponsiveness, and that the bronchitis was related to a small airway. However, in our study, 62% of children with transient wheezing had bronchial hyperresponsiveness, but this was not predictive of the children who would develop persistent wheezing during the follow-up. Delacourte et al<sup>9</sup> also found a similar prevalence of bronchial hyperresponsiveness, in the initial examination of children with transient and persistent wheezing, in a study of a group of 129 children under 2 years old with  $\geq 3$  episodes of bronchitis. They could not find an effective cut-off point to differentiate between children with transient and persistent wheezing. We do not know if a longer follow-up of our patients would have changed the results, since the follow-up lasted only until an average age of 53.8 months, and some studies have reported a relationship between bronchial hyperresponsiveness during infancy and persistence of asthma at the age of 10.<sup>7,8</sup>

Some authors suggest that bronchial hyperresponsiveness is present in normal and healthy infants, probably due to their smaller airway and the inhalation of a proportionately greater dose of methacholine in relation to body weight.<sup>21,22</sup> Thus, bronchial hyperresponsiveness would be expected to decrease with the growth of the child,<sup>23</sup> therefore, it is important to use a control group of the same age and a standardised technique to study bronchial hyperresponsiveness in small children,<sup>11</sup> as we did. In our study we only observed hyperresponsiveness to methacholine in healthy individuals at the maximum dose. We also excluded healthy children with a history of atopy and exposure to smoking, as these factors may influence bronchial hyperresponsiveness.<sup>19</sup> This could have introduced some bias by creating a control group which was "better than normal", and not fully representative of the general population.

The methacholine bronchial challenge protocol, using a modified tracheal auscultation method,<sup>13,24</sup> seems a safe one for young children and has the advantage of not requiring sedation. We did not observe any serious case of bronchial constriction or any significant decrease in SaO<sub>2</sub> in our series. Decreases in SaO<sub>2</sub> were slight and reversible at the end of the test, either spontaneously or with  $\beta_2$ -agonist inhalation. These safety data are consistent with most studies in the literature.

Little is known about the mechanism of bronchial hyperresponsiveness in infants and pre-school children. In our study, recurrent bronchitis was related with the presence of bronchial hyperresponsiveness in two-thirds of children with this disease. However, other mechanisms must be relevant in other children, since the bronchial challenge test was negative. Although we studied a large number of variables in relation to bronchial hyperresponsiveness, we only found a relationship with the age of onset of the first bronchitis: those who suffered the first bronchitis at a younger age responded to a lower dose of methacholine. This could be related to a muscular or neurogenic injury caused by the bronchitis virus, as greater bronchial constriction was found with lesser stimuli.

Some authors have questioned the sensitivity of the test for detecting the presence of bronchial hyperresponsiveness.<sup>25,26</sup> However, other authors,<sup>27</sup> as our group, found that tracheal auscultation after bronchial challenge detected wheezing in a high percentage of children with recurrent wheezing, and this method has been validated with an objective, computerised system using an acoustic sonogram.<sup>24</sup> These systems could be used to make bronchial challenge tests in small children easier.<sup>28</sup>

Bentur et al<sup>29</sup> compared the PCw with PC<sub>20</sub> determined by spirometry in pre-school children. The moment when the children reached the PCw coincided with an average decline of 44.7% in the FEV1. Therefore, bronchial hyperresponsiveness was detected earlier by spirometry than by tracheal auscultation. In that study, however, the PCw was able to be determined in all children (n=56), but the PC<sub>20</sub> was determined in only 11 children because of the lack of collaboration of this age group.

In summary, by assessing bronchial hyperresponsiveness to methacholine by the tracheal auscultation method, we found that bronchial hyperresponsiveness is present in a high percentage of children under 4 years old suffering from recurrent wheezing bronchitis, although subsequent development is not related to the presence or absence of this condition.

## Funding

The study was funded by the following grants: *Beca SEPAR 2001*, *Beca Fundació Oscar Ravà de la FUCAP 2001* and *Beca Fundació Gol i Gorina*.

## Acknowledgements

Thanks to Caroline Beardsmore (Leicester University, UK) for reviewing the manuscript.

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