ERJ Express. Published on October 10, 2007 as doi: 10.1183/09031936.00158106

Airway Responsiveness-Associated Features in Infants with Recurrent Respiratory Symptoms

Anne Kotaniemi-Syrjänen, MD, L. Pekka Malmberg, MD, Anna S. Pelkonen, MD, Kristiina Malmström, MD, Mika J. Mäkelä, MD.

Department of Allergology, Helsinki University Central Hospital, Helsinki, Finland.

Correspondence and requests for reprints:

Dr Pekka Malmberg

Skin and Allergy Hospital

P.O. Box 160

FIN-00029 HUS

FINLAND

Email: pekka.malmberg@hus.fi

Fax: +358 9 471 86560

Tel: +358 9 471 86217

Short title: Clinical features associated with AR

Presented in part as a poster at the 15th Annual Congress of the European Respiratory Society, Copenhagen, Denmark, September 17-21, 2005. Abstract related to the poster published in *Eur Respir J* 2005; 26: Suppl. 49, 675s.

ABSTRACT

Increased airway responsiveness (AR) is one of the main pathophysiological manifestations of asthma. We aimed to define the clinical features associated with increased AR in infants with recurrent lower respiratory tract symptoms. AR was evaluated by performing a novel dosimetric methacholine challenge test.

Increased AR to methacholine, defined as a methacholine dose of ≤0.90 mg producing a 40% fall (PD_{40}) in the maximal flow at functional residual capacity ($V'_{max,FRC}$), was associated with atopy (OR 4.1; 95% CI 1.3-13.3), a history of physician-confirmed wheezing with respiratory syncytial virus (32.9; 2.5-428.8) or by a non-specified aetiology (4.9; 1.1-22.5), functional residual capacity (FRC) z-score of ≥ 2 (36.8; 2.9-472.6), and $V'_{max,FRC}$ z-score (0.5; 0.2-0.9) at baseline, when compared with infants with only mild or no responsiveness to methacholine ($PD_{40}V'_{max,FRC}$ >0.90 mg).

In conclusion, in recurrently symptomatic infants, increased AR is associated with reduced baseline lung function, an atopic trait of the child, a history of physician-confirmed wheeze, and a viral aetiology of wheeze. Future intervention studies are needed to confirm the role of AR in respiratory morbidity during infancy.

Key words: airways hyperreactivity, bronchial provocation tests, infant, methacholine chloride, pulmonary function tests

INTRODUCTION

Increased airway responsiveness (AR), together with persistent airway inflammation, is one of the main pathophysiological manifestations of asthma [1]. Increased AR to pharmacological stimuli such as methacholine or histamine can be demonstrated in almost all steroid-naïve asthmatics [2,3], and therefore, tests to measure AR are commonly used as part of the clinical assessment of adults and school children with a suspicion of asthma. In contrast, the pathophysiology of increased AR is poorly understood in infants, and there are contradictory data on how increased AR is related to actual symptoms and the clinical characteristics during infancy. Most infants respond to bronchoconstrictor agonists unless dosages have been corrected for their body size [4-6], which complicates the assessment of AR at this age. Previous reports have suggested that these responses are independent of lower respiratory tract symptoms, including wheezing [5,7].

There is, however, some evidence that increased AR during infancy is related to the development of asthma in later childhood. In an Australian follow-up study, increased AR to histamine at 1 month of age in healthy infants was found to associate with asthma, decreased lung function, and lower respiratory tract symptoms at six years of age [8]. Even at 11 years of age, AR was reported to be increased in those children with both increased AR and reduced lung function at 1 month of age [9]. In addition, in children with a history of bronchiolitis or wheezy bronchitis, increased AR to methacholine was found to associate with persistent wheezing and the development of asthma in a 10 year-follow-up [10].

Simple clinical characteristics have also been shown to predict the subsequent development of asthma in young children with reasonable accuracy [11]. Based on

the Tucson Children's Respiratory Study, Castro-Rodríguez *et al.* proposed a clinical index including several predictive features like frequent wheezing during the first 3 years of life, a parental history of asthma, atopy and eosinophilia [11].

We hypothesized that the clinical features associated with the increased risk of the development of asthma are the same as those associated with increased AR during infancy. Therefore, the aim of this study is to clarify the relationship between increased AR and several clinical characteristics and lung function parameters in a cohort of infants with recurrent lower respiratory tract symptoms, in order to identify those infants with increased AR by their clinical characteristics. In the present study, AR was measured with a novel dosimetric methacholine challenge method designed for infants.

METHODS

Study subjects

Altogether 199 consecutive children who had been referred to a tertiary center (Department of Allergology, Helsinki University Central Hospital) for investigation of recurrent lower respiratory tract symptoms (including wheeze, cough, sputum production, or dyspnoea) underwent lung function and provocation tests as part of their clinical assessment between 5th May 2004 and 8th March 2006. These children constituted a part of the recruitment cohort of a prospective intervention study into infant asthma. The intervention study, including the performance of the baseline lung function and bronchial provocation tests during the recruitment phase, was approved by the local ethical committee.

Methacholine challenge could not be performed in 47 children due to severe baseline obstruction (flow limitation during tidal breathing and/or resting dyspnoea). In 39 children, technical difficulties (due to e.g. glottic closure, sputum production from upper airways, a leak around the mask during lung function measurements, or age <6 months) interfered with the performance of the challenge test, and in 6 children, awakening during the measurements led to discontinuation of the challenge.

The methacholine challenge was successfully performed in 107 infants. Only full-term (gestational age ≥36 weeks), steroid-free (for at least one month prior to testing) or steroid-naïve infants without any symptoms of acute respiratory infection (for at least 2 weeks prior to testing) were included in the analyses (n=90). The data from these 90 infants comprise the material of the present study.

All the tests were performed as part of the clinical assessment of the children, and were conducted after informed consent to perform these tests had been received from the parents. After lung function testing and the methacholine challenge, some of the children were recruited into an intervention study of infant asthma, and a further written consent to participate the study was obtained from the parents of these children. In addition, parents of the children who were not eligible for the intervention study were asked their consent for the use of lung function and challenge test results for research purposes.

At the hospital visit, the clinical data were collected prospectively from the medical records of the hospital with a standardized form designed for the intervention study (including the data on the occurrence of symptoms, medication, hospital admissions, and the history of respiratory syncytial virus (RSV) bronchiolitis).

Lung function tests

Lung function tests were performed according to the protocol used in our hospital, as follows: Infants were sedated with orally administered chloral hydrate (75-100 mg/kg; maximum dose 1000 mg) prior to testing. During lung function testing, the infant was lying supine with the head supported in the midline and the neck slightly extended to minimize airway or glottic obstruction. All measurements were recorded and calculations performed with commercial paediatric pulmonary function equipment (Babybody Masterscreen, Jaeger GmbH, Germany). Functional residual capacity (FRC) was measured by total body plethysmography as described in detail previously [12]. The maximal partial expiratory flow volume (PEFV) was obtained using the rapid thoracic compression technique (i.e. tidal squeeze) by rapid inflation of a thoracoabdominal jacket at the beginning of expiration [13]. The jacket was wrapped around the infant's chest and abdomen with the arms extended outside the jacket. Flow was measured at the infant's nose and mouth with a pneumotachometer attached to a face mask. A rim of silicone putty was applied around the mouth and nose and to the face mask to provide an airtight seal. The compression pressure was progressively increased until there was no further increase in forced expiratory flow at functional residual capacity ($V'_{max,FRC}$), and the mean $V'_{max,FRC}$ of 3 technically acceptable PEFV curves obtained at that compression pressure was recorded. PEFV curves were considered technically acceptable when the peak flow occurred prior to expiring 50% of the tidal volume, there were no transients in the PEFV curve in the region of FRC, and forced expiration lasted long enough to allow recording of flow at FRC.

The baseline lung function results were expressed as z-scores, which are equivalent to the number of standard deviations by which the measured value deviates from the length- and sex-corrected reference value [14,15].

All infants were studied when they were free from current signs of respiratory infection. Beta 2 –agonists were withheld for 12 hours prior to lung function and challenge tests.

Methacholine challenge

For the dosimetric methacholine challenge, a calibrated nebuliser (Salter Labs 8900, Arvin, CA) was connected to an automatic, inhalation-synchronised dosimeter (Spira Electro II, Spira Respiratory Care Center Ltd, Finland) [16]. The dosimeter was set to be triggered by an inhaled volume of 20 ml, after which a methacholine chloride dose of 50 µg was nebulised within 0.2 seconds in an air volume of 25 ml, in each breath. The dosimeter incorporates an indicator for inspiratory flows and volumes, which were carefully monitored during the administration of methacholine to ensure sufficient tidal flows (>100 ml/s) and volumes (>50 ml). By calculating the number of breaths with nebulised methacholine, a rapid dosage scheme with four non-cumulative dose steps was applied (0.1, 0.3, 0.9 and 1.8 mg), with $V'_{max,FRC}$ being recorded after each dose.

At each phase, the applied compression pressure was the same as that achieved the highest flows at baseline. There were two endpoints in the challenge test; a fall of 40% or more in $V'_{max,FRC}$, or reaching the maximal dose of methacholine.

The provocative dose of methacholine causing a 40% fall in $V'_{max,FRC}$ (PD_{40} $V'_{max,FRC}$) was determined from the dose-response curves. In cases where the maximal dose was reached and $PD_{40}V'_{max,FRC}$ could not be determined from the

dose-response curves, for statistical purposes, $PD_{40}V'_{max,FRC}$ was defined as twice the highest dose of methacholine, 3.60 mg.

During lung function measurements and the challenge test, oxygen saturation and heart rate were continuously monitored with a pulse oximeter (Biox 3700e, Ohmeda, Louisville, KY). Following the challenge test, the children received inhaled salbutamol (0.6 mg) (Ventoline Evohaler 0.1 mg/dos) via Nebunette (manufactured by AstraZeneca Liquid production Sweden AB), and the measurement of $V'_{max,FRC}$ was repeated 15 min after the salbutamol inhalation.

Skin prick tests

Sensitisation to common food and/or inhalant allergens, e.g. egg white, cow's milk, wheat, soy bean, cod, shrimp, peanut, birch pollen, timothy grass pollen, dog epithelial dander, cat epithelial dander, house dust mite *Dermatophagoides pteronyssinus* was tested by skin prick tests (SPT). SPT positivity was defined as a wheal with a diameter of ≥3 mm [17] against at least one of the tested allergens. Physiologic saline was applied as a negative control, and no reaction was allowed against to its injection.

Definitions

A history of wheezing was defined as at least one episode of physician-confirmed wheeze. A family history of asthma was regarded as physician-diagnosed asthma in a 1st degree relative. A family history of allergy was regarded as physician-diagnosed allergy in a 1st degree relative. Food allergy was defined as a diagnosis confirmed by a positive food challenge. Atopic eczema was defined as a current

diagnosis made by a paediatrician. Atopy was defined as the presence of atopic eczema and/or SPT positivity.

AR to methacholine was divided into three categories; increased ($PD_{40}V'_{max,FRC}$ ≤0.90 mg), mild ($PD_{40}V'_{max,FRC}$ >0.90 but <3.60 mg), or no ($PD_{40}V'_{max,FRC}$ 3.60 mg) responsiveness. The categorization based on the aforementioned dose steps applied in the dosimetric methacholine challenge. For statistical purposes, the groups of mild and no responders were combined.

The RSV bronchiolitis was defined as a wheezing lower respiratory tract illness with RSV infection confirmed by antigen detection (Light Diagnostics™ Respiratory DFA Viral Screening and Identification Kit, manufactured by Chemicon International Inc.) in a nasopharyngeal aspirate during an emergency consultation.

Statistics

The data were analysed using SPSS 12.0.2 for Windows (SPSS Inc. Chicago, IL, USA). To evaluate the statistical differences between the groups, χ^2- test or Fisher's exact test (if the expected frequency for any cell was <5) were used for dichotomous variables, and Mann-Whitney U test was used to analyse continuous or ordinal variables. Correlations between variables were analysed using Pearson's correlation test (continuous variables) or Spearman's rank correlation test (ordinal variables). Logistic regression analysis was performed to calculate the adjusted odds ratios (OR) and related 95% confidence intervals (CI) in a multivariate setting. Two-tailed tests were used in all analyses. P-values less than 0.05 were considered statistically significant.

RESULTS

The baseline characteristics of the study children are presented in Table 1. Most infants were Caucasian (n=88 (98%)). None of the children had major congenital cardiac or other malformations. The median duration of respiratory symptoms was 7 months (range 2 to 18 months). A family history of asthma or allergy was present in 78 (87%) children; in the majority, this was a maternal history of asthma or allergy which was present in 55 (61%) infants. Thirty-six (40%) children had atopic eczema or were SPT positive. Eleven (12%) children had a history of proven RSV bronchiolitis, which had been diagnosed at a median 4 months earlier (range 1 to 20 months). In addition, there were 12 children with negative results in RSV antigen testing during a wheezing episode, and 48 children with a history of wheeze confirmed by a physician with no attempts of viral detection made.

Table 1. Baseline characteristics of the study children.

Baseline characteristics	All children (n=90)
Age (months) [median (range)]	14.9 (6.8; 25.8)
Boys:Girls (% boys)	68:22 (76%)
Gestational age (weeks) [median (range)] [a]	40.00 (36.57; 42.43)
Birthweight (g) [median (range)]	3585 (2040; 4600)
Main complaints	
Cough	50 (56%)
Wheeze	13 (14%)
Dyspnoea	17 (19%)
Sputum production	10 (11%)
Duration of symptoms (months) [median (range)]	7 (2; 18)
History of wheezing [b]	71 (79%)
1 episode of wheezing	18 (20%)
2 episodes of wheezing	24 (27%)
3 or more episodes of wheezing	29 (32%)
Hospital admission for wheezing	24 (27%)
History of RSV bronchiolitis [c]	11 (12%)
Family history of asthma [d]	48 (53%)
Family history of allergy [e]	75 (83%)
Atopic eczema [f]	28 (31%)
Skin prick test positive [g]	20 (22%)
Atopy [h]	36 (40%)
Food allergy [i]	21 (23%)
Exposure to tobacco smoke	31 (34%)
Maternal smoking	22 (24%)
Furry animals at home	22 (24%)

[a] Data available for 89 children.

[b] At least 1 episode of physician-confirmed wheeze.

[c] Wheezing lower respiratory tract illness with respiratory syncytial virus infection proven by antigen detection.

[d] Physician-diagnosed asthma in a 1st degree relative.

[e] Physician-diagnosed allergy in a 1st degree relative.

[f] Current diagnosis made by a paediatrician.

[g] A wheal with a diameter of ≥3 mm against at least one of the tested allergens.

[h] Presence of atopic eczema and/or skin prick test positivity.

[i] Diagnosis confirmed by a positive food challenge.

Data on baseline lung function are presented in Table 2. Median coefficients of variation for FRC and $V'_{max,FRC}$ were 2.6% and 4.0%, respectively. At baseline, there was a weak but positive correlation (r=0.223; p=0.035) between FRC and $V'_{max,FRC}$, expressed as z-scores.

Table 2. Baseline lung function in the study children.

Lung function parameters	**All children (n=90)**
FRC (ml) [median (range)]	228.0 (127.0; 412.7)
FRC z-score [median (range)]	0.4 (-1.8; 4.8)
≥ 2 [n (%)]	14 (16%)
$V'_{max,FRC}$ (ml/s) [median (range)]	208 (72; 506)
$V'_{max,FRC}$ z-score [median (range)]	-1.1 (-3.0; 1.4)
≤ -2 [n (%)]	16 (18%)

FRC, functional residual capacity
$V'_{max,FRC}$, maximal expiratory flow at functional residual capacity

During the methacholine provocation, the median changes in $V'_{max,FRC}$ and oxygen saturation were -51% and -3%, respectively. At its lowest, oxygen saturation varied from 86% to 97% (median 93%). There were three children with a fall of oxygen saturation below 90%. None of these three children had reduced lung function at baseline, and thus, the fall in saturation could not have been anticipated. In each case, the fall in saturation was transient, improving in a few minutes. No serious adverse events were encountered, and there was a rapid improvement in oxygen saturation and lung function after administration of bronchodilator in all but two infants who exhibited prolonged bronchoconstriction (15 min after the salbutamol inhalation $V'_{max,FRC}$ was still >40% lower than at baseline) and required an additional

salbutamol inhalation. All the infants were discharged from the hospital as clinically healthy and stable after a follow-up of 2 to 5 hours.

AR to methacholine with regard to baseline characteristics is presented in Table 3, and with regard to baseline lung function parameters in Table 4. AR to methacholine was considered increased in 56 (62%) children, and mild in 14 (16%) children. Twenty children (22%) did not respond significantly to the maximum dose of methacholine.

Table 3. Airway responsiveness to methacholine with regard to baseline characteristics.

	Airway responsiveness (AR) to methacholine		
	Mild or no AR $PD_{40}V'_{max,FRC}$ **>0.90 mg**	**Increased AR** $PD_{40}V'_{max,FRC}$ **≤0.90 mg**	
Baseline characteristics	**(n=34)**	**(n=56)**	**p[a]**
Age (months) [median (range)]	14.2 (7.6; 25.8)	13.5 (6.8; 25.4)	0.535
Male gender [n (%)]	26 (76%)	42 (80%)	0.875
Atopic eczema [n (%)] [b]	6 (18%)	22 (39%)	0.032
Skin prick test positive [n (%)] [c]	5 (15%)	15 (27%)	0.181
Atopy [n (%)] [d]	9 (26%)	27 (48%)	0.041
Exposure to tobacco smoke [n (%)]	13 (38%)	18 (32%)	0.555
History of wheezing [n (%)] [e]	21 (62%)	50 (89%)	0.002
with RSV aetiology [n (%)] [f]	1 (3%)	10 (18%)	0.003 [g]
with non-specified aetiology [n (%)]	20 (59%)	40 (71%)	0.003 [g]
No. of wheezing episodes [median (range)] [h]	1 (0; 5)	2 (0; 7)	0.009

[a] Univariate analyses, performed by χ^2-test or Fisher's exact test (dichotomous variables), or by Mann-Whitney U test (continuous variables).

[b] Current diagnosis made by a paediatrician.

[c] A wheal with a diameter of ≥3 mm against at least one of the tested allergens.

[d] Presence of atopic eczema and/or skin prick test positivity.

[e] At least 1 episode of physician-confirmed wheeze.

[f] Respiratory syncytial virus infection proven by antigen detection.

[g] No history of wheezing as a reference.

[h] Physician-confirmed episodes of wheezing.

Table 4. Responsiveness to methacholine with regard to baseline lung function.

	Airway responsiveness (AR) to methacholine		
	Mild or no AR	**Increased AR**	
	$PD_{40}V'_{max,FRC}$ >0.90 mg	**$PD_{40}V'_{max,FRC}$ ≤0.90 mg**	
Baseline lung function	**(n=34)**	**(n=56)**	**p[a]**
FRC z-score [median (range)]	0.4 (-1.5; 4.8)	0.3 (-1.8; 4.8)	0.809
≥ 2 [n (%)]	1 (3%)	13 (23%)	0.010
$V'_{max,FRC}$ z-score [median (range)]	-0.5 (-2.7; 0.8)	-1.4 (-3.0; 1.4)	0.002
≤ -2 [n (%)]	3 (9%)	13 (23%)	0.083

[a] Univariate analyses, performed by χ^2-test (dichotomous variables), or by Mann-Whitney U test (continuous variables).

FRC, functional residual capacity

$V'_{max,FRC}$, maximal expiratory flow at functional residual capacity

Children with a history of RSV bronchiolitis (n=11) had a median $PD_{40}V'_{max,FRC}$ of 0.43 mg (range 0.11 to 1.13 mg), and those with a history of wheezing due to a non-specified aetiology (n=60) had a median $PD_{40}V'_{max,FRC}$ of 0.62 mg (range <0.10 to 3.60 mg) (p=0.003 and 0=0.006, respectively, with regard to $PD_{40}V'_{max,FRC}$ of children with no history of wheezing (n=22) (median 3.60 mg, range 0.10 to 3.60 mg)) (Figure 1). The difference in $PD_{40}V'_{max,FRC}$ between the children with a history of RSV bronchiolitis and those with a history of wheezing due to a non-specified aetiology was not statistically significant (p=0.150). The number of wheezing episodes was significantly associated with increased AR (Table 3); there was a negative correlation between the number of wheezing episodes and $PD_{40}V'_{max,FRC}$ (r= -0.310; p=0.003).

Atopy, defined as atopic eczema and/or skin prick test positivity, was also associated with increased responsiveness to methacholine (Table 3). In contrast, gender, age, weight, height, birth weight, prematurity, a family history of asthma or allergy, or exposure to tobacco smoke did not have any significant effect on a child's responsiveness to methacholine (data not shown for most of the aforementioned variables).

Maternal smoking was more common in those with a history of RSV bronchiolitis (n=6/11 (55%)) than in those without such a history (n=16/79 (20%)) (p=0.022).

There was a positive correlation between $V'_{max,FRC}$ z-score and $PD_{40}V'_{max,FRC}$ (r=0.392; p<0.001), and the infants with low lung function (decreased $V'_{max,FRC}$) or signs of hyperinflation (increased FRC) were more prone to exhibit increased AR (Table 4).

Table 5 presents the results of the multivariate analyses, showing that atopy, a history of physician-confirmed wheezing, and viral aetiology of wheeze were all independently associated with increased AR in recurrently symptomatic infants. In

addition to these clinical characteristics, diminished baseline lung function was also associated with increased AR.

Table 5. Increased airway responsiveness to methacholine with regard to clinical characteristics and baseline lung function; results of the multivariate analyses. In Model 1, only clinical characteristics are considered; in Model 2, baseline lung function data are also included.

Clinical characteristics and lung function parameters	**Model 1**		**Model 2**	
	OR [a]	**95% CI** [a]	**OR** [a]	**95% CI** [a]
Age (months)	1.0	0.9; 1.1	1.0	0.9; 1.1
Male gender	0.6	0.2; 2.0	0.7	0.2; 2.6
Atopy [b]	3.2	1.1; 9.3	4.1	1.3; 13.3
History of wheezing [c]	6.7 [d]	2.0; 21.9 [d]	6.4 [d]	1.4; 28.6 [d]
with RSV aetiology [e]	26.7	2.5; 279.5	32.9	2.5; 428.8
with non-specified aetiology	5.6	1.7; 18.5	4.9	1.1; 22.5
FRC z-score of ≥2	-	-	36.8	2.9; 472.6
$V'_{max,FRC}$ z-score	-	-	0.5 [f]	0.2; 0.9 [f]

[a] The multivariate analyses were performed by logistic regression; mild or no airway responsiveness to methacholine (PD_{40} $V'_{max,FRC}$ >0.90 mg) as a reference.

[b] Presence of atopic eczema and/or skin prick test positivity.

[c] At least 1 episode of physician-confirmed wheeze.

[d] OR and related 95% CI for the history of wheezing were obtained from a separate multivariate logistic regression analysis excluding viral aetiology of wheeze. Adjustments were performed for age, gender, and atopy in Model 1; for age, gender, atopy, FRC z-score of ≥2, and $V'_{max,FRC}$ z-score in Model 2.

[e] Respiratory syncytial virus infection proven by antigen detection.

[f] Increase in $V'_{max,FRC}$ z-score reduces airway responsiveness to methacholine.

DISCUSSION

We found that there were several clinically important features which were significantly associated with increased AR in infants and toddlers with recurrent lower respiratory tract symptoms. These features include a history of physician-confirmed wheeze, especially if this was due to RSV infection, atopic characteristics, and decreased baseline lung function. Although the present study design differs from earlier studies in the same field [8,10], the results point in the same direction; future intervention studies are needed to confirm the role of AR in respiratory morbidity during infancy. In addition, the infants likely to present with increased AR seemed to have similar clinical features as those associated with symptom persistence and asthma later in childhood.

The study design represents a subset of patients referred to a tertiary center for further investigation of recurrent lower respiratory tract symptoms within a period of 22 months. Due to the present setting, it is likely that there was a bias with regard to the characteristics of the study subjects; it is reasonable to presume that there was an enrichment of the atopics and those with more troublesome symptoms in these study subjects than would be the case in paediatric patients presenting at primary health care units.

For ethical reasons, we did not have a control group of healthy children, and thus comparisons of AR had to be done within the cohort. Therefore, we could not assess the relative risks for increased AR in various subgroups of the cohort with respect to asymptomatic infants. Predicted values from healthy infants used in this paper were obtained from studies utilizing identical equipment and procedures as used in our

laboratory, and represent infants of similar age and origin (Caucasian): therefore our findings and interpretation regarding z-scores are likely to be valid.

We used a modified and shortened dosimetric protocol for methacholine challenge [18]. We found the method to be safe, although special caution with infants with reduced lung function at the baseline is needed, since two infants displayed prolonged bronchoconstriction after the challenge. Based on this experience, we do not encourage bronchial challenge testing in infants with baseline $V'_{max,FRC}$ below a z-score of -2. Inhalation synchronized dosimetry permitted accurate and rapid dosing of the test agonist and determination of AR. At the moment, there is no consensus about the standardization of bronchial challenge tests in infants, and the comparison of AR between infants and children with different ages and size is difficult due to methodological reasons [6]. We did not make any size correction for the delivered dose, because the design was cross-sectional, and the sample rather homogeneous as regards the size of the children. Furthermore, within the sample, there was no association between AR and the dimensions of the infant. Since there is no “golden standard” $PD_{40}V'_{max,FRC}$ value to indicate an increased AR, an arbitrary value of 0.90 mg, based on the doses of methacholine administered in our method, was chosen as the cut-off value. Although arbitrary, the value was shown to be useful in differentiating between the children with the characteristics usually associated with symptom persistence from those without such features.

Little is known on the mechanisms of AR in infancy. Structural changes in the airways associated with increased contractile force or decreased load to airway narrowing may result in increased AR [19]. However, in the developing lungs of infants, airway-parenchymal interdependence is complex, and the lungs are susceptible to many pre- and postnatal factors [20]. Recently, we found that a

thickened basement membrane, and eosinophilic airway inflammation, two factors which in older children and adults are typically associated with increased AR, are usually absent in infants with recurrent respiratory symptoms [21]. Reduced baseline lung function was found to be associated with increased AR in infants, as has been observed in infants also by others [22,23], and is consistent with findings in older subjects [9]. In our study, high FRC was also significantly associated with increased AR. Mechanistically, high FRC should oppose airway narrowing; therefore, the observed association with AR is likely to be due to mechanisms leading to hyperinflation rather than high FRC *per se*. Notably, both high FRC and low $V'_{max,FRC}$ were independent predictors of increased AR. This may be due to biased $V'_{max,FRC}$ recordings in cases of elevated FRC, and it indicates that this group of infants with baseline lung function abnormalities may actually consist of two different subcategories, i.e. volume elevators and flow decreasers.

The measurement of $V'_{max,FRC}$ is by definition, dependent on the lung volume (i.e. FRC) where the measured flow takes place. Biased $V'_{max,FRC}$ values in cases of elevated FRC may partly explain the unexpected positive correlation between FRC and $V'_{max,FRC}$ at baseline. Furthermore, methodological problems causing underestimation of lung volumes in infants recovering from bronchiolitis have been previously recognised [24], and may account for the findings in our series.

The findings of previous follow-up studies suggest that increased AR in early childhood associates with the subsequent occurrence of lower respiratory tract symptoms and asthma up to school age, both in previously healthy [8] and in symptomatic [10] infants and toddlers. Therefore, it may be useful to identify symptomatic infants who exhibit increased AR. Their symptoms are likely to persist, and this may necessitate anti-asthmatic treatment. However, the routine

measurement of AR during infancy is not feasible, and cut-off points for provocative concentrations or doses of methacholine determining clinical significance have not been established [25]. Therefore the infants likely to present with increased AR have to be identified from their clinical characteristics. Although increased AR is an important feature of asthma in adults and older children [1], the relationship between AR, actual respiratory symptoms, and clinical presentation is not obvious in infants. Previous reports have suggested that AR is independent of lower respiratory tract symptoms, including wheezing [5,7]. Our results with a larger cohort are at odds with the aforementioned findings since we were able to identify several clinical features associated with increased AR in infants with recurrent lower respiratory tract symptoms. These clinical features, i.e. recurrent wheeze and atopy, are rather similar with those listed in a clinical index to define risk of asthma in young children [11].

The long-term outcome of a wheezy infant is also affected by a history of RSV bronchiolitis [26], a family history of asthma [27] or a family history of atopy [25], and smoke exposure in infancy [8,27]. There is little (and contradictory) evidence on the association of these characteristics and increased AR in infancy. We found one controlled study stressing the association of bronchiolitis and increased AR [28]; one cross-sectional study in healthy infants, showing that the family history of asthma and parental smoking were significantly associated with increased AR [29]; and one controlled follow-up study with no association between AR and a history of RSV lower respiratory tract illness or exposure to tobacco smoke [23]. In agreement with the findings of the first mentioned study [28], we observed that a history of physician-confirmed wheeze was strongly associated with increased AR in our study setting. Furthermore, it was shown that the AR increased with a growing number of wheezing episodes. In contrast to the earlier findings, the history of RSV bronchiolitis indicated

increased AR. In addition, the infants with a smoking mother were more likely to have a history of RSV bronchiolitis. However, as viral studies had been performed as a clinical basis, viral aetiology had been sought only for one third of the children with a history of physician-confirmed wheeze, and it is thus probable that the role of RSV as a triggerer of wheeze was underestimated in the present study.

At present, there is no evidence that any medical interventions, for instance, could influence the clinical course of AR in at-risk infants [30].

In summary, in addition to poor baseline lung function, several clinical characteristics are associated with increased AR in recurrently symptomatic infants. These clinical characteristics, which also resemble the clinical features known to be predictive of the development of childhood asthma, include atopy, and a history of physician-confirmed wheeze -- especially if due to RSV bronchiolitis. Future intervention studies are needed to confirm the role of AR in respiratory morbidity during infancy.

COMPETING INTERESTS

None of the authors has any competing interests to declare.

ACKNOWLEDGEMENTS

We wish to thank Tuija Rito, RN, and Helena Punkari, RN, for their skill and care with the infants and their parents. The personnel of Spira Respiratory Care Center Ltd, is acknowledged for their help in the development of the inhalation-synchronized dosimetric equipment used in the methacholine challenge.

FUNDING

The study was financially supported by Helsinki University Central Hospital Research Fund, Sigrid Juselius Foundation, Foundation for Paediatric Research in Finland, Nummela Sanatorium Foundation, and Finska Läkaresällskapet. An unrestricted research grant was received from Merck Sharp & Dohme Ltd.

REFERENCES

1. Global Initiative for Asthma (GINA) workshop report. Global strategy for asthma management and prevention 2006. www.ginasthma.org/Guidelineitem.asp??l1=2&l2=1&intId=60. Date last upadated: November 13 2006. Date last accessed: November 13 2006.

2. Joos GF. Do measures of bronchial responsiveness add information in diagnosis and monitoring of patients with asthma? *Eur Respir J* 2001: 18: 439-441.

3. Turner SW, Palmer LJ, Rye PJ, Gibson NA, Young S, Goldblatt J, Landau LI, LeSouëf PN. Determinants of airway responsiveness to histamine in children. *Eur Respir J* 2005: 25: 462-467.

4. LeSouëf PN, Geelhoed GC, Turner DJ, Morgan SE, Landau LI. Response of normal infants to inhaled histamine. *Am Rev Respir Dis* 1989: 139: 62-66.

5. Clarke JR, Reese A, Silverman M. Bronchial responsiveness and lung function in infants with lower respiratory tract illness over the first six months of life. *Arch Dis Child* 1992: 67: 1454-1458.

6. Le Souëf PN. Can measurements of airway responsiveness be standardized in children? *Eur Respir J* 1993: 6: 1085-1087.

7. Stick SM, Arnott J, Turner DJ, Young S, Landau LI, LeSouëf PN. Bronchial responsiveness and lung function in wheezy infants. *Am Rev Respir Dis* 1991: 144: 1012-1015.

8. Palmer LJ, Rye PJ, Gibson NA, Burton PR, Landau LI, LeSouëf PN. Airway responsiveness in early infancy predicts asthma, lung function, and respiratory symptoms by school age. *Am J Respir Crit Care Med* 2001: 163: 37-42.

9. Turner SW, Palmer LJ, Rye PJ, Gibson NA, Judge PK, Young S, Landau LI, LeSouëf PN. Infants with flow limitation at 4 weeks. Outcome at 6 and 11 years. *Am J Respir Crit Care Med* 2002: 165: 1294-1298.

10. Saga R, Mochizuki H, Tokuyama K, Morikawa A. Relationship between bronchial hyperresponsiveness and development of asthma in wheezy infants. *Chest* 2001: 119: 685-690.

11. Castro-Rodríguez JA, Holberg CJ, Wright AL, Martinez FD. A clinical index to define risk of asthma in young children with recurrent wheezing. *Am J Respir Crit Care Med* 2000: 162: 1403-1406.

12. Malmberg LP, Pelkonen A, Hakulinen A, Hero M, Pohjavuori M, Skyttä J, Turpeinen M. Intraindividual variability of infant whole-body plethysmographic measurements: effects of age and disease. *Pediatr Pulmonol* 1999: 28: 356-362.

13. Sly PD, Tepper R, Henschen M, Gappa M, Stocks J, on behalf of the ERS/ATS Task Force on Standards for Infant Respiratory Function Testing. Tidal forced expirations. *Eur Respir J* 2000: 16: 741-748.

14. Hoo A-F, Dezateux C, Hanrahan JP, Cole TJ, Tepper RS, Stocks J. Sex-specific prediction equations for V'max$_{FRC}$ in infancy. A multicenter collaborative study. *Am J Respir Crit Care Med* 2002: 165: 1084-1092.

15. Hülskamp G, Hoo A-F, Ljungberg H, Lum S, Pillow JJ, Stocks J. Progressive decline in plethysmographic lung volumes in infants. *Am J Respir Crit Care Med* 2003: 168: 1003-1009.

16. Sovijärvi AR, Malmberg LP, Reinikainen K, Rytilä P, Poppius H. A rapid dosimetric method with controlled tidal breathing for histamine challenge. Repeatability and distribution of bronchial reactivity in a clinical material. *Chest* 1993: 104: 164-170.

17. The European Academy of Allergology and Clinical Immunology (EAACI). Position paper: Allergen standardization and skin tests. *Allergy* 1993: 48 Suppl 14: 48-82.

18. Benoist MR, Brouard JJ, Rufin P, Delacourt C, Waernessyckle S, Scheinmann P. Ability of new lung function tests to assess methacholine-induced airway obstruction in infants. *Pediatr Pulmonol* 1994: 18: 308-316.

19. James AL, Pare PD, Hogg JC. The mechanics of airway narrowing in asthma. *Am Rev Respir Dis* 1989: 139: 242-246.

20. Stick S. Pediatric origins of adult lung disease. 1. The contribution of airway development to paediatric and adult lung disease. *Thorax* 2000: 55: 587-594.

21. Saglani S, Malmström K, Pelkonen AS, Malmberg LP, Lindahl H, Kajosaari M, Turpeinen M, Rogers AV, Payne DN, Bush A, Haahtela T, Mäkelä MJ, Jeffery PK. Airway remodelling and inflammation in symptomatic infants with reversible airflow obstruction. *Am J Respir Crit Care Med* 2005: 171: 722-727.

22. Prendiville A, Green S, Silverman M. Bronchial responsiveness to histamine in wheezy infants. *Thorax* 1987: 42: 92-99.

23. Adler A, Long Ngo, Tager IB. Association of tobacco smoke exposure and respiratory syncytial virus infection with airways reactivity in early childhood. *Pediatr Pulmonol* 2001: 32: 418-427.

24. Godfrey S, Beardsmore CS, Maayan C, Bar-Yishay E. Can thoracic gas volume be measured in infants with airways obstruction? *Am Rev Respir Dis* 1986: 133: 245-251.

25. Delacourt C, Benoist M-R, Waernessyckle S, Rufin P, Brouard J-J, de Blic J, Scheinmann P. Relationship between bronchial responsiveness and clinical

evolution in infants who wheeze. A four-year prospective study. *Am J Respir Crit Care Med* 2001: 164: 1382-1386.

26. Sigurs N, Gustafsson PM, Bjarnason R, Lundberg F, Schmidt S, Sigurbergsson, Kjellman B. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am J Respir Crit Care Med* 2005: 171: 137-141.

27. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ, and the Group Health Medical Associates. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995: 332: 133-138.

28. Tepper RS, Rosenberg D, Eigen H. Airway responsiveness in infants following bronchiolitis. *Pediatr Pulmonol* 1992: 13: 6-10.

29. Young S, LeSouëf PN, Geelhoed GC, Stick SM, Turner KJ, Landau LI. The influence of a family history of asthma and parental smoking on airway responsiveness in early infancy. *N Engl J Med* 1991: 324: 1168-1173.

30. Stick SM, Burton PR, Clough JB, Cox M, LeSouëf PN, Sly PD. The effects of inhaled beclomethasone dipropionate on lung function and histamine responsiveness in recurrently wheezy infants. *Arch Dis Child* 1995: 73: 327-332.

LEGENDS FOR FIGURES

Figure 1. Airway responsiveness to methacholine and its relationship with a history of wheezing and RSV identification. Methacholine dose producing a 40% fall in the maximal flow at functional residual capacity (PD_{40} $V'_{max,FRC}$) was significantly lower in children with a history of RSV bronchiolitis (median 0.43 mg (range 0.11 to 1.13 mg)) (p=0.003), and in those with a history of physician-confirmed wheeze due to non-specified aetiology (median 0.62 mg (range <0.10 to 3.60 mg)) (p=0.006), compared to those without any episodes of physician-confirmed wheezing (median 3.60 mg (range 0.10 to 3.60 mg)). There were no differences in PD_{40} $V'_{max,FRC}$ between children with RSV and those with a non-specified aetiology for wheeze (p=0.150).