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Lung function and bronchial hyperreactivity from 11 to 18 years in children with bronchiolitis in infancy

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ABSTRACT

Background

Various trajectories for lung function and bronchial hyperreactivity (BHR) from early childhood to adulthood are described, including puberty as a period with excessive lung growth. Bronchiolitis in infancy may be associated with increased risk of developing chronic obstructive pulmonary disease, but the development of respiratory patterns during puberty is poorly characterized for these children. We aimed to study the development and trajectories of lung function and BHR from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy.

Methods

Infants hospitalized for bronchiolitis at the University Hospitals in Stavanger and Bergen, Norway during 1997-1998, and an age-matched control group, were included in a longitudinal follow-up study and examined at 11 and 18 years of age with spirometry and methacholine provocation test (MPT). The MPT data were managed as dose-response slope (DRS) in the statistical analyses. Changes in lung function and DRS from 11 to 18 years of age were analyzed by generalized estimating equations, including interaction terms.

Results

Z-scores for forced vital capacity (FVC), forced expiratory volume in first second (FEV₁), FEV₁/FVC-ratio and DRS were not different from 11 to 18 years of age in both the post-bronchiolitis and the control group. The trajectories from 11 to 18 years did not differ between the two groups. BHR at age 11 was independently associated with asthma at age 18.

Conclusion

Children hospitalized for bronchiolitis had stable predicted lung function and BHR from 11 to 18 years of age. The lung function trajectories were not different from controls.

Keywords

Bronchiolitis, Asthma, Bronchial hyperreactivity, Child, Adolescent, Spirometry, Bronchial Provocation Tests, Methacholine Chloride.

INTRODUCTION

Longitudinal cohort-studies have shown that the lung function trajectories throughout a life-course vary between individuals, and that abnormal lung function trajectories may origin in early life.¹⁻³ This has been shown both in unselected populations^{2,4} and after infant respiratory disease such as bronchopulmonary dysplasia associated with extreme prematurity.⁵ It has therefore been hypothesized that chronic obstructive pulmonary disease (COPD) may begin in childhood, conceivably precipitated by interactions between genetic predispositions, disadvantageous intrauterine environments or early respiratory insults.⁶

Worldwide, bronchiolitis represents a substantial health burden for infants, and it is the most common cause for hospitalization during infancy in developed countries.⁷ These children have increased risk of developing asthma, low lung function and increased bronchial hyperreactivity (BHR) both during childhood⁸⁻¹¹ and adulthood,¹²⁻¹⁴ and possibly increased risk of developing COPD.⁶ However, we do not know the nature of this association; i.e., if it is the bronchiolitis *per se* that alters the pattern of lung development, or if both disorders are caused by inherent predispositions or vulnerabilities of genetic or antenatal origin. Puberty is the period of life with the most excessive lung growth,^{1,15} but we do not know if bronchiolitis in infancy modulates the development of airway size and hyperreactivity during the pubertal growth spurt.

We have previously reported lung function data in a cohort of 11-year-old children hospitalized for bronchiolitis in 1997-1998.¹⁰ The present study is based on examinations of the same subjects at age 18 years. We aimed to study if lung function and BHR changed from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy, and whether lung function trajectories during this period was different from an age-matched control group.

METHODS

Originally, 131 children hospitalized for bronchiolitis during their first year of life during the winter seasons 1997 and 1998 at the University Hospitals in Stavanger and Bergen, Norway, were included in a longitudinal prospective follow-up study.¹⁶ Bronchiolitis was defined as an acute viral respiratory tract infection during the first year of life with fever, tachypnea, dyspnea, prolonged expiration and wheeze on auscultation.¹⁷ In order to avoid including children with other

conditions such as viral induced wheezing and asthma, only children below 12 months of age were included.⁷ At hospitalization, nasopharyngeal mucus was examined for Respiratory syncytial virus (RSV) by direct immunofluorescence (bioMérieux, Marcy-l'Étoile, France). Children testing positive for RSV were defined as RSV positive, the others were defined as RSV negative.

One-hundred and twenty-one of the children (92 %) participated in a first follow up at 11 years of age, together with an age-matched control group of 141 children. The control group were children born in 1997 with no previous history of hospitalization for bronchiolitis recruited from three different schools in Stavanger, Norway. The 11-year-examination included questionnaires and tests for atopic sensitization, lung function and BHR, as previously reported.¹⁰

All participants from the original study were invited to a second follow up at approximately 18 years of age, including questionnaires and clinical tests of lung function, BHR and atopic sensitization. This study presents results from the children participating at both follow-ups. A total of 108 children from the post-bronchiolitis group and 89 children from the age-matched control group at the 11-year-examination were invited to participate in this substudy (Figure 1).

Lung function, BHR and atopic sensitization

At both follow-ups, lung function was measured by spirometry according to established guidelines,¹⁸ using Vmax Encore 229D spirometer (SensorMedics Inc., Anaheim, USA), with data standardized for age, height, and sex.¹⁹ Results were presented as z-scores and % predicted.

BHR was measured by methacholine provocation test (MPT) performed with an inhalation-synchronized dosimetric nebulizer, providing baseline $FEV_1 > 65\%$ predicted.^{20,21} The test continued until a fall in FEV_1 of $> 20\%$ compared with baseline FEV_1 , or until a maximal cumulative dose of 11.5 μmol methacholine had been administered. A dose-response slope (DRS) was calculated as the ratio of maximal percentage decline in FEV_1 from baseline to cumulative administered dose of methacholine ($\%/\mu\text{mol}$).²²

Atopic sensitization was assessed by using skin prick tests according to guidelines²³ and by measuring specific immunoglobulin E (IgE). For details, see appendix.

Data collection and definitions

Asthma symptoms and medications during the last year were reported by the parents (11-year-examination) and study subjects (18-year-examination) from questionnaires based on the International Study of Asthma and Allergies in Childhood (ISAAC).²⁴ In addition, at 18-years more detailed data regarding personal and family history of asthma and atopy were collected through questionnaires and supplemented with information from medical records at hospitalization. For details, see appendix.

Asthma ever was defined as positive answer to *have you ever been diagnosed with asthma?* Current asthma was defined as asthma ever combined with a positive answer to at least one of the two questions: 1) *Have you during the last 12 months had heavy breathing or wheezing/chest-tightness* 2) *Have you during the last 12 months used any asthma medications*(inhaled corticosteroids, long- or short acting beta-2-agonists, montelukast, ipratropium bromide, any combinations).

Ethics

The study was approved by the Regional Committee on Medical Research Ethics. Signed statements of informed consent were obtained from all participants and from parents if the participants were younger than 18 years of age.

Statistical analysis

Continuous variables are presented as group means with 95% confidence intervals, and were compared by Student's t test, or as medians and interquartile range (IQR) and compared by Mann-Whitney U-test, as appropriate. Categorical variables are presented as counts and percentages, and differences were tested by Pearson chi-square test. Lung function and DRS to methacholine at both follow-ups were compared by generalized estimating equations (GEE). Interaction terms (group*time) were applied to test divergent development of lung function and DRS between the post-bronchiolitis and control group from 11-18 years of age, and the analyses were adjusted for atopic sensitization and asthma at 11 years of age as well as family history of asthma or atopy. The distribution of the DRS to methacholine was highly skewed and therefore transformed using the natural logarithm, after negative values were set to zero and 0.1 was added to all DRS values. A Cox regression analysis allowing for correlation between repeated tests of the same individuals, was used to analyze the proportion of non-responders at each cumulative dose of methacholine.

The association between BHR at 11-years and current asthma at 18-years was analyzed by multivariable logistic regression analysis. LnDRS was included as explanatory variable, and the analyses were adjusted for the following covariates measured at 11 years: group variable, gender, z-score FEV₁ and current asthma.

Analyses were carried out using SPSS version 24.0 (IBM Corp. Armonk, N.Y., USA) and Stata version 15.1 (StataCorp LLC, TX, USA). Generally, p-values ≤ 0.05 were considered statistically significant.

RESULTS

Sixty children (56%) in the post-bronchiolitis group and 40 (45%) of the controls consented to participate, and 54 (50%) in the post-bronchiolitis group and 33 (37%) controls consented to clinical tests at the 18 year examination (Figure 1). One control and one in the post-bronchiolitis group failed to complete spirometry according to standard quality criteria, leaving 53 (49%) and 32 (36%) individuals with results from spirometry, respectively. One control had low peak expiratory flow and only acceptable FVC, and not valid MPT. In the post-bronchiolitis group, MPT was not valid in one subject and not performed in three subjects due to contraindications, hence 49 (45%) participants in the post-bronchiolitis group and 31 (35%) controls had acceptable MPT (Figure 1).

Baseline characteristics of both groups are presented in Table 1. There were no differences regarding age, gender, weight and length between the two groups. In the post-bronchiolitis group, 83% had been hospitalized with RSV positive bronchiolitis. Atopic sensitization was more common in the control group than in the post-bronchiolitis group at both follow-ups.

Lung function and bronchial hyperreactivity

Lung function and DRS to methacholine in both groups and at both ages are presented in Table 2. Children in the post-bronchiolitis group had lower FEV₁, FEV₁/FVC, FEF₂₅₋₇₅ and higher DRS than controls at both follow-ups.

For both groups, there were no significant changes in z-scores for forced vital capacity (FVC), forced expiratory volume in first second (FEV₁) or FEV₁/FVC-ratio between 11 and 18 years of age (Table 3). In the post-bronchiolitis group, but not in the control group, z-scores for forced expiratory flow between 25-75% of FVC (FEF₂₅₋₇₅) was higher at 18 than 11 years of age. Change in absolute lung function variables from 11 to 18 years of age are presented in Supplemental table 1. We found no significant interaction between age and group, meaning that the trajectories of lung function and DRS from 11 to 18 years of age did not differ between the post-bronchiolitis and the control group neither in the unadjusted nor in the adjusted analyses (Table 3, Supplemental table 1, Figures 2 and 3). Furthermore, there were no significant interactions between age, group and gender, i.e. we found no differences between boys and girls in how bronchiolitis in infancy affected the trajectories of lung function and BHR from 11 to 18 years. Figure 3 displays the methacholine responsiveness at 11 and 18 years of age. There was no

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significant interaction effect between group and age by Cox regression ($p=0.988$) or from the GEE analysis (Table 3), and we found no differences in the trajectories for DRS from 11 to 18 years of age between the post-bronchiolitis and control groups.

In the multivariable logistic regression analysis, BHR at age 11 was independently associated with current asthma at age 18 (OR 1.88; 95% CI 1.22-2.89, $p=0.004$).

DISCUSSION

The present study shows that lung function z-scores and BHR was stable from 11 to 18 years of age in children hospitalized for bronchiolitis during their first year of life, and that the trajectories for lung function and BHR from 11 to 18 years of age were not different from the control group. These results applied for both boys and girls. BHR at age 11 was independently associated with asthma at age 18.

In this rather small cohort, lung function was lower and BHR was higher after bronchiolitis in infancy compared to age-matched controls with no such history both at 11 and at 18 years of age. This is in line with previous follow-up studies during childhood,^{10,11,25} and a similar pattern has also been shown in adults with a history of bronchiolitis in early childhood.^{13,14,26} However, this issue was not the focus of the present study, our aim was to study the trajectories of lung function and BHR during the important pubertal growth spurt.

Although there are suggestions that childhood respiratory illnesses, such as viral bronchiolitis, predispose to subsequent asthma and COPD,⁶ only a few longitudinal studies include repeated measurements of lung function and/or BHR during childhood and up to young adulthood in children hospitalized for bronchiolitis in infancy.¹²⁻¹⁴ As far as we know, none of these previous post-bronchiolitis studies have investigated lung function and/or BHR longitudinally during the important transitional period from childhood to young adulthood.

During childhood and adolescence, the lung function trajectories are characterized by a growth phase reaching a peak after puberty at 18-25 years of age, followed by a plateau phase and finally a decline linked to physiological aging.¹ Some children following a trajectory below normal, may have catch-up of lung function during childhood and adolescence.¹ Unselected population cohort studies have shown that lung function trajectories contributing to COPD include both early and persistent low lung function as well as accelerated decline in adulthood.³ The results from the present study suggest that children with former bronchiolitis during puberty follow a lung function trajectory below normal peak values, but that the development between 11 and 18 years of age is parallel to healthy controls with no catch up nor decline. The results support a notion that low lung function and increased BHR, which has been observed at different ages after bronchiolitis, are features that are established in early life, either due to airway damage caused by the respiratory insult during bronchiolitis or that these abnormalities are already present

prior to the respiratory event, as also suggested by others.⁶ The accelerated growth and pubertal period is also characterized by a shift from male dominated childhood asthma to female dominated adult asthma.^{15,27} We did not find that gender affected the impact of former bronchiolitis on the lung function trajectories during puberty.

Similar lung function trajectories as observed in the present study, have been found in young adults/adolescents after other early respiratory insults such as repeated episodes of viral wheeze and extreme prematurity.^{4,5} The Tucson cohort included children with viral wheeze up to the age of 3 years, and found that patterns of wheezing prevalence and levels of lung function were established by the age of six and did not change significantly by the age 16 years.^{4,28} Similarly, a Norwegian longitudinal cohort study from mid-childhood to adulthood showed that individuals born extremely preterm consistently had lower lung function and increased BHR compared to term born controls, and that the trajectories from 10 to 25 years of age were parallel and irrespective of the degree of bronchopulmonary dysplasia.⁵

We found a tendency for increased z-scores for FEF₂₅₋₇₅ from 11 to 18 years in the post-bronchiolitis group, but when tested in interaction terms, this development did not differ from the control group. The finding must be further elaborated in larger longitudinally studies, but may at least support that lung function does not decline in this group during puberty.

Bronchiolitis is associated with subsequent asthma both in children and adults, and BHR is a fundamental characteristic of asthma.^{10,13} We found that BHR was stable from 11 to 18 years of age in the post-bronchiolitis group, and the trajectory did not differ from controls. However, we found that BHR at 11-years was associated with current asthma at 18-years, also when adjusting for asthma at age 11. The association between BHR and subsequent asthma is in line with the results from a Norwegian unselected birth cohort study.²⁹ The finding supports the speculation that BHR might be an independent and possibly inborn feature that can be causally related to bronchiolitis as well as to subsequent development of asthma.^{30,31} The clinical implication could be that children with severe BHR may benefit from regular clinical follow-ups to monitor if asthma develops later in life.

Strength and limitations

The main strength of this study is the longitudinal design, and the main weakness is the modest participation rate and power, increasing the risk of selection bias and false negative results. The

lack of lung function data from infancy and early childhood precluded assessment of studying tracking from early life.

Conclusion

This longitudinal study shows that lung function z-scores and BHR were stable from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy, following trajectories that were significantly lower, but parallel to those of the control group in both boys and girls. BHR at age 11 was associated with asthma at age 18. Further long-term follow-up studies are needed to study if, and possibly to what extent, children with former bronchiolitis have increased risk of developing COPD.

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- Accepted Article
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- Accepted Article
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Table 1. Clinical characteristics at 11 and 18 years in children hospitalized for bronchiolitis in infancy and an age-matched control group.

Data are presented as medians (interquartile ranges) unless otherwise stated.

	Post-bronchiolitis group N=60	Control group N=40	P-value*
Boys (%)	29 (48.3)	21 (52.5)	0.683
Age at hospitalization, months	4.0 (1.8, 6.2)		
RSV positive bronchiolitis, n (%)	50 (83.3)		
Personal smoking, n (%)	4 (6.7)	1 (2.5)	0.349
Ever household smoking, n (%)	21 (35.0)	8 (20.0)	0.105
Family history of atopy, n (%)	45 (75.0)	22 (55.0)	0.037
First follow up at 11 years			
Age, years	11.3 (11.0, 11.7)	11.8 (11.3, 12.1)	0.001
Height, cm	148.5 (144.0, 153.8)	149.0 (146.0, 155.8)	0.459
Weight, kg	38.3 (35.0, 45.4)	40.0 (35.3, 44.9)	0.625
Current asthma, n (%)	9 (15.0)	5 (12.5)	0.724
Allergic sensitization, n (%)	12 (20.0)	17 (42.5)	0.015
Second follow up at 18 years			

Age, years	18.0 (17.0, 18.0)	18.0 (17.0, 18.0)	0.100
Height, cm	172.4 (164.4, 180.9)	173.8 (166.5, 181.2)	0.635
Weight, kg	65.9 (59.9, 75.9)	63.2 (57.8, 72.4)	0.233
Current asthma, n (%)	18 (30.0)	9 (22.5)	0.408
Asthma ever, n (%)	23 (38.3)	10 (25.0)	0.249
Allergic sensitization, n (%) [†]	13 (24.1)	20 (60.6)	0.001
Allergic rhinoconjunctivitis, n (%)	28 (46.7)	24 (60.0)	0.191
Atopic dermatitis ever, n %	15 (25.0)	14 (35.0)	0.280

Abbreviations: RSV: Respiratory syncytial virus.

*P-values from Mann Whitney U-test for continuous variables and Pearson Chi Square test for dichotomous variables.

[†]54 subjects in the post-bronchiolitis group and 33 controls underwent allergy tests at the second follow up at 18 years

Table 2. Lung function and bronchial hyperreactivity at 11 and 18 years in children hospitalized for bronchiolitis in infancy and an age-matched control group.

			Post-bronchiolitis group		Control group		P-value*
			N	Observed mean (95% CI)	N	Observed mean (95% CI)	
FEV ₁							
	z- score	11y	60	-0.44 (-0.67, -0.21)	40	0.20 (-0.20, 0.61)	0.004
	% of predicted			94.9 (92.3, 97.5)		102.2 (97.5, 107.0)	0.004
	z- score	18y	53	-0.57 (-0.83, -0.30)	31	0.15 (-0.28, 0.57)	0.003
	% of predicted			93.3 (90.1, 96.5)		101.6 (96.7, 106.6)	0.003
FVC							
	z- score	11y	60	0.15 (-0.13, 0.43)	40	0.41 (-0.02, 0.83)	0.302
	% of predicted			102.0 (98.7, 105.3)		105.1 (99.9, 110.2)	0.293
	z- score	18y	53	-0.13 (-0.41, 0.15)	32	0.23 (-0.12, 0.59)	0.107
	% of predicted			98.5 (95.2, 101.8)		102.8 (98.6, 107.0)	0.112
FEV ₁ /FVC,							
	z- score	11y	60	-0.86 (-1.17, -0.54)	40	-0.36 (-0.69, -0.03)	0.037
	% of predicted			93.1 (90.7, 95.5)		97.1 (94.8, 99.4)	0.023
	z- score	18y	53	-0.66 (-0.98, -0.34)	31	-0.20 (-0.53, 0.13)	0.058

FEF 25-75				% of predicted	94.3 (91.8, 96.8)	98.2 (95.8, 100.6)	0.039
	z- score	11y	60	-1.00 (-1.28, -0.72)	40	-0.38 (-0.69, -0.06)	0.004
	% of predicted			79.8 (74.2, 85.5)		92.1 (85.0, 99.2)	0.007
	z- score	18 y	53	-0.75 (-1.03, -0.48)	31	-0.09 (-0.46, 0.28)	0.004
	% of predicted			84.8 (79.2, 90.4)		99.1 (91.0, 107.3)	0.003
DRS [†]							
	Geometric mean	11y	58 [†]	5.86 (3.76, 9.12)	39 [†]	2.28 (1.33, 3.89)	0.008
	Geometric mean	18y	49	7.17 (4.31, 11.93)	31	2.58 (1.40, 4.75)	0.012

Abbreviations: CI: Confidence interval. 11y: First follow up at median 11 years of age. 18y: Second follow up at median 18 years of age. FEV₁:

Forced expiratory volume in first second. FVC: Forced vital capacity. FEF₂₅₋₇₅: Forced expiratory flow between 25-75% of the forced vital capacity.

DRS: Methacholine dose response slope. DRS (%/μmol) is the ratio of maximum percentage decline in FEV₁ from baseline to cumulative administered dose (μmol) of methacholine.

*P-values from Student's T- test.

[†]Due to contraindications in two subjects in the post-bronchiolitis group and one control, methacholine provocation test were only performed in 58 and 39 subjects respectively at 11y.

Table 3. Change in lung function variables from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy and an age-matched control group, presented as mean change with 95% CI.

	Post-bronchiolitis		Controls		Interaction
	Mean change (95% CI)	P-value*	Mean change (95% CI)	P-value*	P-value*
FEV ₁ , z-score	-0.09 (-0.31, 0.13)	0.442	-0.14 (-0.53, 0.25)	0.484	0.816
FEV ₁ , z-score [†]	-0.08 (-0.30, 0.14)	0.454	-0.15 (-0.55, 0.24)	0.451	0.765
FVC, z-score	-0.24 (-0.51, 0.03)	0.076	-0.24 (-0.61, 0.13)	0.203	0.997
FVC, z-score [†]	-0.23 (-0.49, 0.04)	0.089	-0.26 (-0.63, 0.12)	0.182	0.911
FEV ₁ /FVC, z-score	0.18 (-0.14, 0.50)	0.263	0.10 (-0.22, 0.42)	0.529	0.731
FEV ₁ /FVC, z-score [†]	0.17 (-0.15, 0.49)	0.301	0.12 (-0.20, 0.43)	0.471	0.819
FEF ₂₅₋₇₅ , z-score	0.25 (0.00, 0.50)	0.046	0.21 (-0.13, 0.54)	0.236	0.834

FEF ₂₅₋₇₅ , z-score [†]	0.24 (-0.00, 0.49)	0.054	0.21 (-0.13, 0.55)	0.230	0.873
LnDRS	0.17 (-0.29, 0.64)	0.465	0.11 (-0.42, 0.64)	0.697	0.851
LnDRS [†]	0.17 (-0.29, 0.63)	0.471	0.10 (0.43, 0.62)	0.721	0.835

Abbreviations: CI: Confidence interval. FEV₁: Forced expiratory volume in first second. FVC: Forced vital capacity. FEF₂₅₋₇₅: Forced expiratory flow between 25-75% of the forced vital capacity. DRS: Methacholine dose response slope. DRS (%/μmol) is the ratio of maximum percentage decline in FEV₁ from baseline to cumulative administered dose (μmol) of methacholine. Due to highly skewed distribution, DRS was transformed using the natural logarithm. The group-wise mean changes were estimated in generalized estimating equations (GEE) models including interaction terms group*time to test for unequal trajectories in controls and post-bronchiolitis. A positive mean change indicates that z-scores were higher at 18 than 11 years of age.

*P-values from Wald test.

[†] Adjusted for family history of asthma or atopy, atopic sensitization 11 years of age and asthma 11 years of age

FIGURE LEGENDS

Figure 1. Flowchart of the study population, consisting of children hospitalized for bronchiolitis in infancy and an age matched control group. The invited children participated in the 11-year-examination. *Abbreviations:* 18y: Second follow up at median 18 years of age. MPT: Methacholine provocation test. †For one participant, only forced vital capacity (FVC) was considered valid, making n=31 in calculations of other lung function variables.

Figure 2. Z-scores for lung function variables presented as estimated marginal means with 95% confidence intervals at 11 and 18 years of age in subjects hospitalized for bronchiolitis in infancy and controls. Results from generalized estimating equations (GEE) analysis. The x-axis depicts age, and the y-axis depicts mean z-scores. The black lines represent spirometric scores for the post-bronchiolitis group and the grey lines represent spirometric scores for the control group. *Abbreviations:* 11y: First follow up at median 11 years of age. 18y: Second follow up at median 18 years of age. FEV₁: Forced expiratory volume in first second, FVC: Forced vital capacity. FEF₂₅₋₇₅: Forced expiratory flow between 25-75% of the forced vital capacity.

Figure 3. Bronchial hyperreactivity to methacholine at 11 and 18 years of age in subjects hospitalized for bronchiolitis in infancy and controls. The x-axis depicts total cumulative dose of methacholine given to each subject, censored at the maximum given dose of 11.5 μ mol. The y-axis depicts the proportion of non-responders at each given dose. *Abbreviations:* 11y: First follow up at median 11 years of age. 18y: Second follow up at median 18 years of age.

APPENDIX 1

Atopic sensitization

Atopic sensitization at 11 years of age was defined by a positive skin prick test (SPT) for at least one allergen (wheal diameter ≥ 3 mm larger than the negative control), and at 18 years of age as a positive SPT or specific immunoglobulin E (IgE) ≥ 0.35 kU/l for at least one allergen. Both tests included the following allergens: *Dermatophagoides pteronyssinus*, dog and cat dander, *Cladosporium herbarium*, birch, timothy, egg white, milk, peanut, hazelnut and codfish. The SPT was performed with Soluprick® allergens (ALK Albello, Hørsholm, Denmark). Histamine (10 mg/ml) was used as a positive control and a 0.9% saline solution as a negative control. For analysis of specific IgE, blood was drawn and serum stored at -70 degrees Celsius and analyzed by Phadiatop®, fx5E® and by specific IgE when positive at the department of medical biochemistry, Stavanger University Hospital.

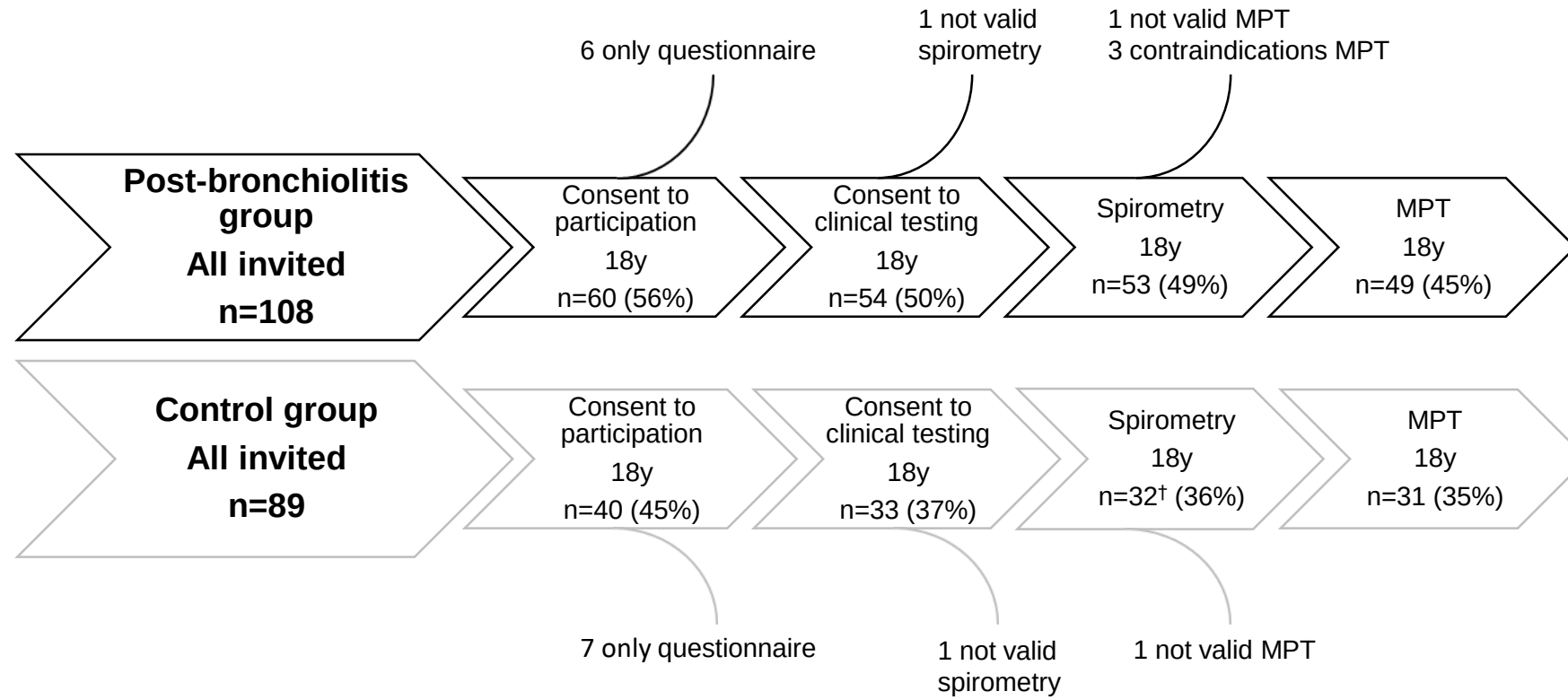
Definitions and questionnaires

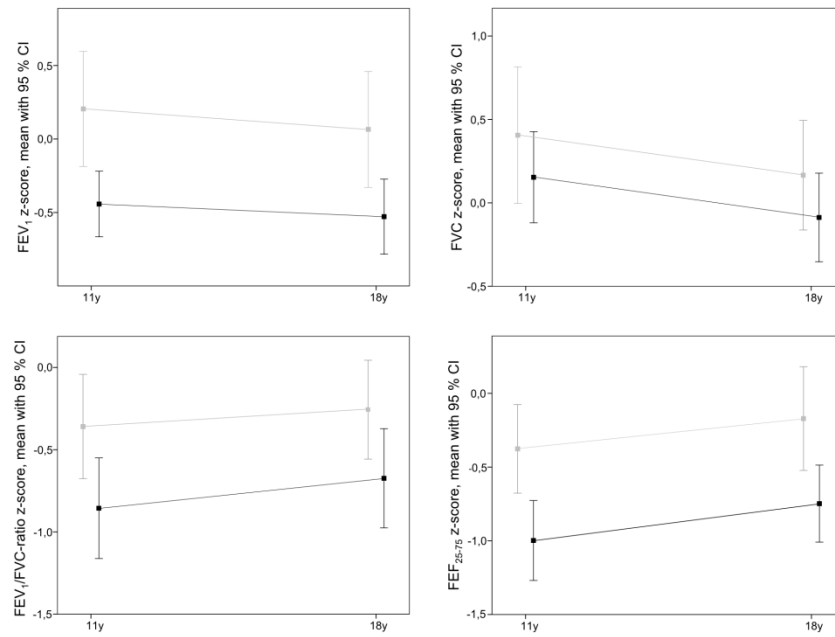
At the 18-year-examination, more detailed data regarding personal and family history of asthma and atopy were collected through questionnaires and supplemented with information from medical records at hospitalization. Atopic dermatitis was defined as a positive answer to *have you ever had atopic dermatitis*. Family history of asthma or allergic diseases was defined as a positive answer to *do you know if your mother, father or siblings have or have had atopic dermatitis, asthma or positive allergy tests*. Ever household smoking was defined as a positive answer to *do/did anyone smoke in your home*. Smoking in participants was defined as a positive answer to *do you smoke*. Allergic rhinoconjunctivitis was defined as a positive answer to *have you ever had runny or itching nose and/or eyes apart from colds*.

APPENDIX 2

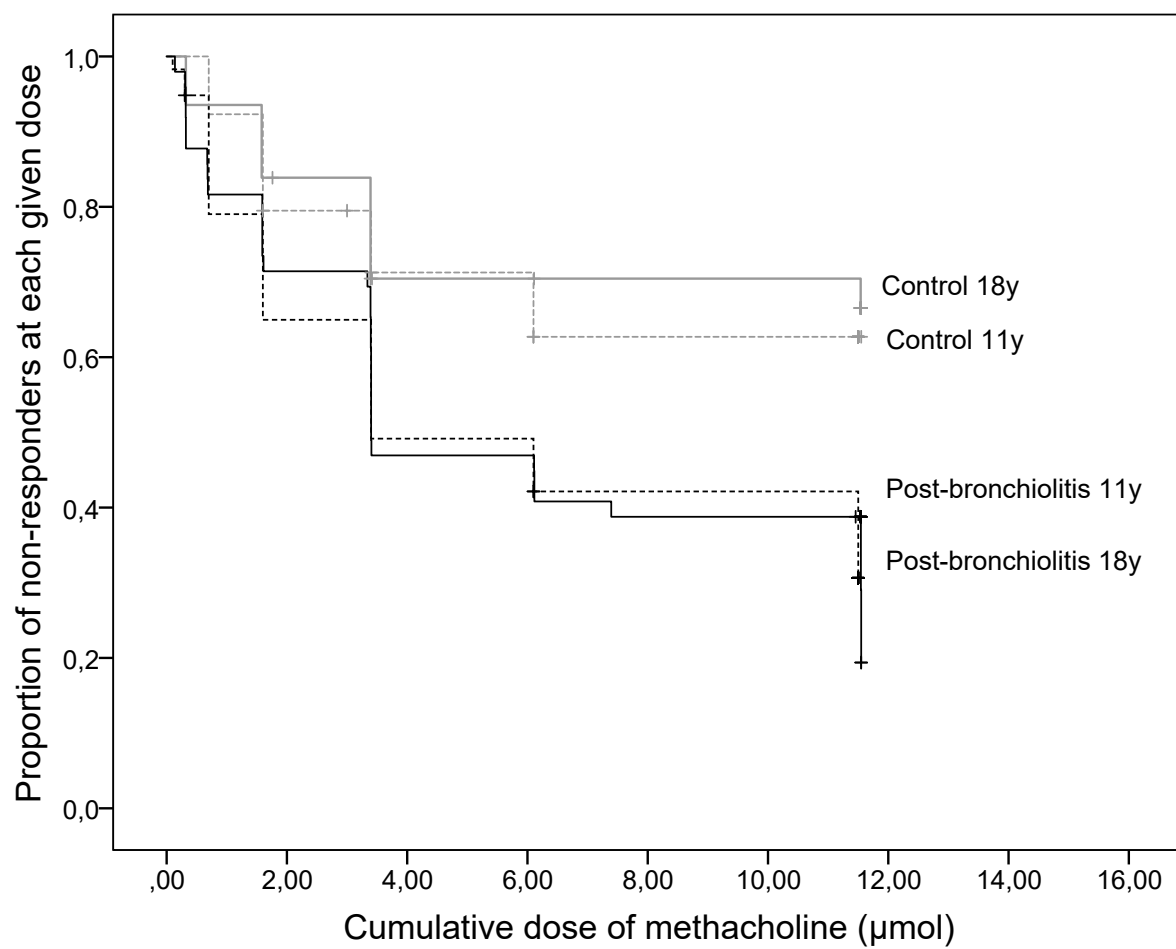
Change in absolute lung function

The change in lung function between 11 and 18 years adjusted for gender, age and height is best expressed by z-scores as given in the article (Table 3). As the changes in absolute lung function may add some clinical information, these are presented in Supplemental table 1. The results show that the absolute lung function values apart from the ratio for FEV1/FVC were higher at 18 than 11 years of age. In line with the results from the analyses using z-scores, there were no significant interaction effects between age and group, underlining that the changes in lung function from 11 to 18 years did not differ between the control and post-bronchiolitis group.





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Supplemental table 1. Change in absolute lung function variables from 11 to 18 years of age in children hospitalized for bronchiolitis in infancy and an age-matched control group, results from unadjusted analyses, presented as mean change with 95% CI.

	Post-bronchiolitis		Controls		Interaction
	Mean change (95% CI)	P-value*	Mean change (95% CI)	P-value*	P-value*
FEV ₁ , liter	1.50 (1.32, 1.68)	<0.001	1.63 (1.37, 1.90)	<0.001	0.421
FVC, liter	1.79 (1.56, 2.02)	<0.001	1.92 (1.63, 2.20)	<0.001	0.508
FEV ₁ /FVC, ratio	0.016 (-0.00, 0.04)	0.087	-1.85 (-5.43, 1.74)	0.313	0.309
FEF ₂₅₋₇₅ , liter/second	1.57 (1.32, 1.82)	<0.001	1.78 (1.40, 2.15)	<0.001	0.363

Abbreviations: CI: Confidence interval. FEV₁: Forced expiratory volume in first second. FVC: Forced vital capacity. FEF₂₅₋₇₅: Forced expiratory flow between 25-75% of the forced vital capacity. The group-wise mean changes were estimated in generalized estimating equations (GEE) models including interaction terms group*time to test for unequal trajectories in controls and post-bronchiolitis.

*P-values from Wald test.