ERJ Express. Published on July 3, 2014 as doi: 10.1183/09031936.00009314

Decreased lung function precedes severe respiratory syncytial virus infection and post-respiratory syncytial virus wheeze in term infants

Kim Zomer-Kooijker[1], Cuno S.P.M. Uiterwaal[2], Anne C. van der Gugten[1], Berry Wilbrink[3], Louis J. Bont[4] and Cornelis K. van der Ent[1]

Affiliations: [1]Dept of Paediatric Pulmonology and Allergology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, The Netherlands. [2]Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, Utrecht, The Netherlands. [3]Laboratory of Infectious Diseases and Perinatal Screening, National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands. [4]Dept of Paediatric Immunology and Infectious Diseases, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, The Netherlands.

Correspondence: Kim Zomer-Kooijker, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Dept of Paediatric Pulmonology and Allergology, room number KB.01.0551, PO Box 85090, 3508 AB Utrecht, The Netherlands. E-mail: k.zomer-kooijker@umcutrecht.nl

ABSTRACT It is unknown why respiratory syncytial virus (RSV) causes mild disease in some children and severe disease, requiring hospitalisation, in others. We aimed to assess whether diminished premorbid lung function in healthy term infants predisposes to hospitalisation during RSV bronchiolitis, and to post-RSV wheeze.

In a prospective birth cohort study of unselected term healthy children, neonatal lung function was measured before the age of 2 months (n=2133). From birth through the first year of life, respiratory symptoms were recorded in a diary, and general practitioner consultations and hospitalisations were documented. In a subgroup (n=417) repeated nose and throat swabs were collected for PCR to detect RSV infections.

Median neonatal respiratory system compliance (C_{rs}) was significantly lower (41.2 *versus* 47.4 mL·kPa^{-1}, p=0.03) and resistance (R_{rs}) was higher (8.2 *versus* 6.3 kPa·s·L^{-1}, p=0.10) in hospitalised RSV patients (n=18) compared with nonhospitalised RSV-positive infants (n=84). Every 10 mL·kPa^{-1} increase in C_{rs} was associated with 55% less post-RSV wheeze (OR 0.56, 95% CI 0.35–0.90), and each kPa·s·L^{-1} increase in R_{rs} was associated with 42% more post-RSV wheeze, which was only marginally explained by pre-RSV wheeze or severity of the RSV disease.

This unselected birth cohort study shows for the first time that decreased lung function at birth predisposes to severe RSV disease, and to post-RSV wheeze.

@ERSpublications
Impaired neonatal lung function precedes a severe course of RSV infection in unselected children
http://ow.ly/xlkcL

This article has supplementary material available from erj.ersjournals.com

Received: Jan 13 2014 | Accepted after revision: May 22 2014

Support statement: C.K. van der Ent received grants for this study from the Netherlands Organisation for Health Research and Development (ZonMw), from the University Medical Centre Utrecht and an unrestricted research grant from GlaxoSmithKline. C.S.P.M. Uiterwaal received grants for this study from the Netherlands Organization for Health Research and Development (ZonMw) and from the University Medical Centre Utrecht.

Conflict of interest: Disclosures can be found alongside the online version of this article at erj.ersjournals.com

Introduction

Respiratory syncytial virus (RSV) is the most common cause of lower respiratory tract infection (LRTI) in children in the first year of life [1], and the most frequent cause for hospitalisation in infancy [2]. By 2 years of age almost all children have been infected with RSV [3]; however, mechanisms underlying the variability in the severity of RSV infections are unclear.

Several risk factors, including premature birth [1, 4], have been identified; however, the largest number of RSV infections occur in otherwise healthy infants without any known risk factors. Lower levels of lung function shortly after birth are associated with the occurrence of respiratory illness during the first year of life [5–8]. STEIN *et al.* [9] found that school age children who had suffered from RSV LRTI before the age of 3 years had lower levels of lung function than children without such history. Based on this finding, they hypothesised that RSV lower respiratory tract illnesses might be associated with pre-existing lower lung function [9]. The latter association has been found in prematurely born infants [10] and in a high risk population [11]; however, these associations have never been studied in term infants.

To our knowledge, no prospective study has assessed the association between neonatal lung function and subsequent RSV infection severity and post-RSV wheeze in term infants. We questioned whether diminished neonatal lung function was associated with subsequently increased severity of RSV infection. It was recently shown that palivizumab treatment for RSV prevention in otherwise healthy preterm infants reduced recurrent wheeze in the first year of life [12]. This implies that RSV infection plays a causal role in post-infection recurrent wheeze. It does not preclude the possibility that other factors predispose children for both the severity of RSV infection and post-RSV wheeze. Therefore, our second question was whether neonatal lung function was associated with the presence of post-RSV wheeze. We conducted a community-based birth cohort study, investigating the role of neonatal lung function on the severity of RSV infection and on post-RSV wheeze in the first year of life.

Methods

Study population

All infants participated in the Wheezing Illnesses Study Leidsche Rijn (WHISTLER), an ongoing, population-based, prospective birth cohort study on determinants of wheezing illnesses in children [13], which started in December 2001. Exclusion criteria are gestational age $<$36 weeks, major congenital abnormalities and neonatal respiratory disease. Information about pre- and post-natal risk factors and about the health status of the parents was obtained by questionnaires. The medical ethics committee of the University Medical Centre Utrecht (Utrecht, the Netherlands) approved the study (project approval number 01/176) and all parents gave written informed consent.

Lung function measurement and respiratory symptoms

From our cohort, we selected all children who had a successful lung function measurement shortly after birth. Lung function measurement was performed in healthy neonates before the age of 2 months during natural sleep. Lung function was assessed from measurement of passive respiratory mechanics (resistance (R_{rs}) and compliance (C_{rs}) of the total respiratory system) using the single occlusion technique. Lung function measurements were performed in the health centre in the Leidsche Rijn district (Utrecht, the Netherlands) where all the children lived, consistently using the same device on all participants. Further details about lung function measurements have been previously reported [14]. In all children from the birth cohort, respiratory symptoms in the first year of life were documented on a daily basis, using diaries completed by the parents.

RSV positivity

In two periods of the cohort study (2003–2005 and 2006–2007) we collected respiratory virus samples on a regular basis in children aged $<$1 year.

In the first period respiratory virus samples were collected on the second day of each episode with wheeze or cough (n=311), with the aim of assessing whether neonatal lung function predisposes to wheezing during viral respiratory tract infections [15]. In the second period samples were collected at the start of every month regardless of respiratory symptoms (n=166), with the aim of describing the occurrence and clinical impact of respiratory pathogens in infants with and without a LRTI episode [16]. Viral samples were collected by the parents, using a cotton-tipped swab, from both the nose and posterior oropharynx. Both swabs were collected into a single vial containing GLY medium with 0.1 $mg \cdot mL^{-1}$ pimaricin as the viral transport medium and sent to our laboratory *via* standard mail. Samples were stored at -20°C until analysis [17]. RSV positivity was defined as having a RSV PCR-positive swab in the first year of life. Of the 477

DOI: 10.1183/09031936.00009314

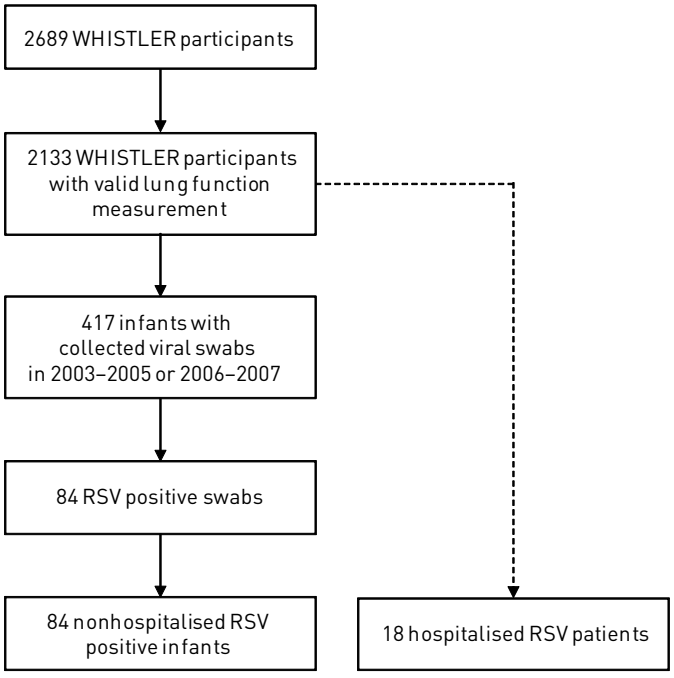

FIGURE 1 Flow chart of the infants included in this study. In total, 102 infants were included in the analysis with a positive respiratory syncytial virus (RSV) test and valid neonatal lung function. WHISTLER: Wheezing Illnesses Study Leidsche Rijn.

children sampled in both study periods, 417 had a successful lung function test. Of these 417 children, 84 children were positive for RSV but did not require hospitalisation (fig. 1).

Outcome variable: RSV hospitalisation

We selected all infants from the complete cohort with a successful lung function measurement (n=2133) that had been hospitalised for bronchiolitis at any point during the first year of life. 26 infants were hospitalised for viral bronchiolitis. In eight of those 26 patients, the immunofluorescence test or PCR was negative for RSV, while the remaining 18 patients had a positive test result. Other reasons for hospital admission in the remaining patients were pneumonia (n=1), pertussis (n=1), bronchial hyperreactivity (n=3) and laryngomalacia/bronchitis (n=3). 18 patients, with a proven RSV bronchiolitis were selected for further analyses (fig. 1).

Outcome variable: post-RSV wheeze

Days with respiratory symptoms were obtained from the diaries, which were filled in during the first year of life. The total number of days with wheeze was calculated before and after the occurrence of the RSV infection up to 1 year of age, with exclusion of the month in which the RSV infection occurred. Post-RSV wheeze was expressed as the median number of days with wheeze per month following the documented RSV infection.

Analysis

Missing values for birth length (9.1%), birth weight (0.2%) and weight and length at measurement (5.5% and 7.0%) were imputed by their mean values, as these values did not differ from the multiple imputations. For our first research question, we selected all RSV-positive infants, both hospitalised and nonhospitalised. Absolute (unadjusted) values of C_{rs} and R_{rs} were compared using the Mann–Whitney U-test. We used logistic regression analysis to explore the relationship between neonatal lung function and the severity of RSV (defined as hospitalisation: ''yes'' or ''no''). Each factor was added separately to the model to investigate its influence on the association. We made a distinction between possible confounding factors (sex, season of birth, siblings, breast feeding, daycare, maternal allergy, ethnicity, educational level and study year, as strains of RSV may differ in virulence between years [18–20]) and factors that may be in the causal chain, for example birth weight and maternal smoking [21]. We studied the effect of those intermediates in order to understand whether they explain the association between neonatal lung function and RSV hospitalisation for preventive purposes. Possible confounders, affecting the odds ratio by $\geqslant$5%, were added to the multivariable model for both C_{rs} and R_{rs}.

For our second research question the association between lung function and the development of post-RSV wheeze was investigated. Again, each possible confounding factor was added separately to the model to investigate its influence on the association. In addition to the aforementioned factors, we also added the intermediates pre-RSV wheeze and RSV bronchiolitis hospitalisation to the model separately, to study whether lung function was associated with post-RSV wheeze, independent of the presence of pre-RSV wheeze and RSV bronchiolitis hospitalisation. These factors may explain any association between neonatal lung function and post-RSV wheeze. Poisson regression was used to analyse the difference in number of days with wheeze between hospitalised patients and nonhospitalised RSV-positive infants. Results are presented as odds ratios, 95% confidence intervals and p-values. Statistical analysis was performed using SPSS statistics version 20.0 (IBM Corp., Armonk, NY, USA).

Results

The complete cohort consisted of 2689 infants, from which a successful lung function measurement was obtained in 2133 (79.3%) infants. Of the complete cohort with a successful lung function measurement, 18 (0.84%) infants were hospitalised for RSV bronchiolitis (fig. 1). Of the infants with a successful lung function measurement 417 (19.5%) infants were sampled for viral infections during one of the two sampling periods. In 84 (20.1%) infants a positive RSV sample was detected; 52 (61.9%) of the infants were sampled during an episode with wheeze or cough, and 32 (38.1) were sampled randomly at the start of every month. Of those, 22 (68.8%) infants did have respiratory symptoms at the time of sampling. Baseline characteristics for the three groups are presented in table 1. The virus sampling group was representative for the complete study group (data not shown).

Infants with and without a successful lung function were compared. Except for a higher birth weight in the group without successful lung function, no significant differences between groups were found (table S1). We did not find differences between children with and without a successful lung function measurement in the cohort with swabs (data not shown).

Neonatal lung function in RSV-positive infants

Children who were hospitalised for RSV bronchiolitis had significantly lower median (interquartile range (IQR)) *C*rs (41.2 (33.2–45.8) *versus* 47.4 (40.1–52.6) mL·kPa^{-1}, p=0.03) and higher median (IQR) *R*rs (8.2 (5.8–9.1) *versus* 6.3 (5.2–7.6) kPa·s·L^{-1}, p=0.10) (fig. 2). Table 2 shows the odds ratios for the association between lung function and RSV hospitalisation. In the univariable model, each 10 mL·kPa^{-1} increase in *C*rs was associated with 45% lower odds for hospitalisation. After adjustment for potential confounders this association strengthened: each 10 mL·kPa^{-1} increase in *C*rs was associated with 66% lower odds for hospitalisation. Adding intermediate factors to the model slightly weakened the association between *C*rs and RSV hospitalisation, but it remained statistically significant.

Each kPa·s·L^{-1} increase in *R*rs was associated with 20% higher odds of RSV hospitalisation. After adjustment for study year, this association attenuated (OR 1.30, 95% CI 1.02–1.66).

TABLE 1 General characteristics of the study population

	Total cohort	Hospitalised RSV patients	Nonhospitalised RSV-positive infants	p-value[#]
Subjects n	2133	18	84	
Males	1033 (48.4)	10 (55.6)	48 (57.1)	0.903
Birth weight g	3519±509	3538±620	3596±529	0.688
Birth length cm	51 (49–52)	51 (49–53)	51 (50–52)	0.263
Gestational age weeks	39.9 (39.0–40.9)	40.2 (39.1–40.5)	40.0 (39.0–41.0)	0.696
Age at bronchiolitis weeks	NA	29.9 (18.1–48.8)	30.4 (20.7–38.9)	0.719
Siblings	1133/2103 (53.9)	10/17 (58.8)	43/83 (51.8)	0.602
Breastfed in first 3 months of life	1395/1994 (70.0)	13/17 (76.5)	63 (75.0)	0.899
Daycare in first 3 months of life	895/2000 (44.8)	11/17 (64.7)	44 (52.4)	0.357
Maternal allergy	147/1797 (8.2)	1/17 (5.9)	3/79 (3.8)	0.548
Maternal smoking during pregnancy	130/2126 (6.1)	1 (5.6)	3/83 (3.6)	0.546
Western mother	1852 (79.7)	15/17 (88.2)	66/80 (82.5)	0.568
Highly educated mother	1221/1782 (68.5)	15/17 (88.2)	74/80 (92.5)	0.566

Data are presented as n (%), n/N (%), mean±SD or median (interquartile range), unless otherwise stated. RSV: respiratory syncytial virus; NA: not applicable. [#]: p-value for differences between the two RSV groups.

DOI: 10.1183/09031936.00009314

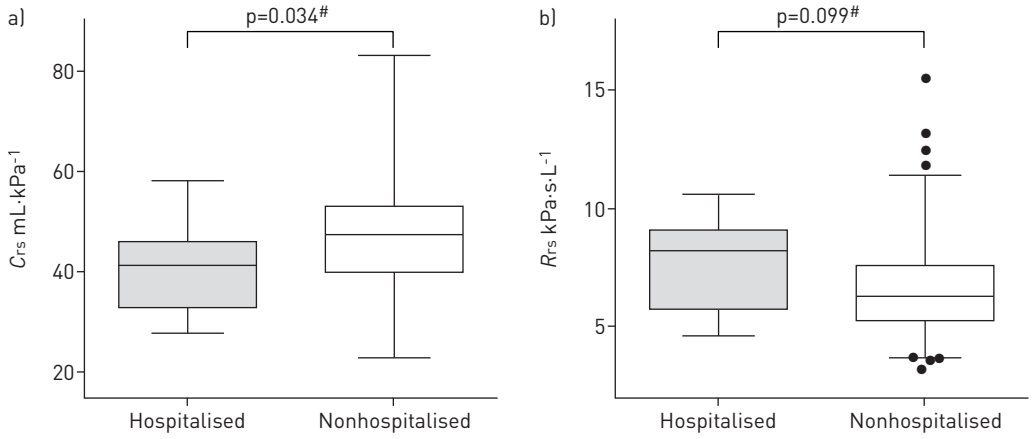

FIGURE 2 Crude median a) compliance (*C*rs) and b) resistance (*R*rs) of the respiratory system for 18 hospitalised respiratory syncytial virus (RSV) patients and 84 nonhospitalised RSV-positive infants. #: p-values based on logistic regression analysis.

Neonatal lung function and post-RSV wheeze

Data about days with wheeze prior to the RSV infection were available in 15 hospitalised infants and 80 nonhospitalised infants. As diaries were filled in up to the age of 12 months, information about post-RSV wheeze was not available for four infants that were hospitalised at or after the age of 12 months, and for five nonhospitalised infants with a RSV-positive swab at the age of 12 months. In two hospitalised infants and one nonhospitalised infant this information was missing. No differences were found between children who were and were not included in the analysis (data not shown). A total of 34 out of 90 children experienced post-RSV wheeze for $\geqslant$1 day: 10 (83.3%) out of 12 hospitalised RSV patients *versus* 24 (30.8%) out of 78 RSV-positive nonhospitalised infants, $p<0.001$.

Furthermore, the median (IQR) number of days with post-RSV wheeze per month was higher in the hospitalised group as compared with the RSV-positive nonhospitalised infants (0.7 (0.2–4.1) *versus* 0.0 (0.0–0.4), $p<0.001$). Prior to the RSV infection hospitalised infants experienced more days with wheeze per month compared with nonhospitalised infants (median (IQR) 0.5 (0.0–1.4) *versus* 0.0 (0.0–0.0), $p=0.002$).

Table 3 shows the association between lung function and the presence of any days with post-RSV wheeze. Each 10 mL·kPa^{-1} increase in *C*rs was associated with 44% lower odds for post-RSV wheeze. After adjustment for potential confounders this association strengthened: each 10 mL·kPa^{-1} increase in *C*rs was associated with 55% lower odds for post-RSV wheeze. Each kPa·s·L^{-1} increase in *R*rs was associated with 34% higher odds of post-RSV wheeze. After adjustment for study year, this association attenuated (OR 1.42, 95% CI 1.11–1.82).

Adding pre-RSV wheeze to the model did slightly weaken the association between both *C*rs and *R*rs and post-RSV wheeze. Similar results were seen after addition of RSV hospitalisation to the model.

Discussion

This study showed that in term infants impaired neonatal lung function precedes a severe course of RSV infection.

There was no significant association between *R*rs and hospitalisation, except after correction for study year. However, increased *R*rs and lower *C*rs were both associated with post-RSV wheeze, independent of the severity of the disease and the presence of pre-RSV wheeze. Although the role of premorbid lung function in the severity of RSV disease has been suggested in preterm and high risk infants, to our knowledge, this is the first study confirming this effect in an unselected birth cohort of healthy term infants, which account for the majority of hospitalisations for RSV bronchiolitis.

Several risk factors for a severe course of disease during RSV infection, including premature birth [1, 4], have been identified. Two studies assessed the role of neonatal lung function in RSV bronchiolitis in prematurely born infants [4, 10]. DRYSDALE *et al.* [10] prospectively studied 159 premature born infants that were sampled at each episode of wheeze, cough or shortness of breath. They found impaired neonatal lung function in patients that were hospitalised for a viral lower respiratory tract illness, including RSV, compared with nonhospitalised patients. BROUGHTON *et al.* [4] prospectively studied 39 premature infants and described significantly higher premorbid *R*rs in symptomatic RSV patients compared with patients with no lower respiratory tract illness. They concluded that in preterm infants abnormal airway function is

TABLE 2 Association between lung function and respiratory syncytial virus (RSV) hospitalisation in 102 RSV-positive infants

	C_{rs} 10 mL·kPa^{-1}		R_{rs} kPa·s·L^{-1}	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Crude	0.55 (0.31–0.96)	0.03	1.20 (0.97–1.49)	0.10
Adjusted for potential confounders				
Males	0.55 (0.31–0.96)	0.04	1.20 (0.97–1.49)	0.10
Birth length cm	0.56 (0.31–1.02)	0.06	1.18 (0.94–1.47)	0.15
Gestational age weeks	0.54 (0.30–0.96)	0.04	1.20 (0.96–1.49)	0.11
Season of birth April to September	0.53 (0.30–0.94)	0.03	1.20 (0.97–1.49)	0.10
Siblings	**0.60 (0.34–1.05)**	0.07	1.17 (0.93–1.46)	0.18
Breastfed in first 3 months of life	**0.49 (0.27–0.89)**	0.02	1.18 (0.95–1.47)	0.14
Daycare in first 3 months of life	**0.45 (0.24–0.84)**	0.01	1.22 (0.97–1.53)	0.09
Maternal allergy	0.55 (0.31–0.97)	0.04	1.19 (0.95–1.48)	0.13
Western mother	0.53 (0.30–0.96)	0.04	1.19 (0.95–1.49)	0.13
Highly educated mother	0.55 (0.31–0.98)	0.04	1.18 (0.95–1.47)	0.14
Study year	**0.50 (0.27–0.92)**	0.03	**1.30 (1.02–1.66)**	0.03
Adjusted for intermediates				
Maternal smoking during pregnancy	0.55 (0.31–0.97)	0.04	1.20 (0.97–1.49)	0.10
Birth weight g	**0.50 (0.26–0.94)**	0.03	1.20 (0.96–1.50)	0.11
Adjusted multivariable model[#]	0.34 (0.15–0.73)	<0.01	1.30 (1.02–1.66)[¶]	0.03

C_{rs}: compliance of the respiratory system; R_{rs}: resistance of the respiratory system. Each model consists of the crude model plus adjustment for one single variable. Bold indicates odds ratios that are affected ⩾5% by adjustment. [#]: adjusted for siblings, breastfeeding, daycare and study year; [¶]: adjusted for study year.

associated with subsequent symptomatic RSV lower respiratory tract illness. Recently, CHAWES *et al.* [11] described airway hyperresponsiveness as a risk factor of acute severe bronchiolitis, including RSV bronchiolitis, in children of atopic mothers.

The largest number of RSV infections occurs in otherwise healthy infants without any known risk factors. Several studies have described the association between a pre-existent decreased lung function and the development of wheezing illnesses during unspecified viral respiratory tract infections in term infants [5, 7, 22]. Until now, RSV specific data on unselected healthy children were lacking. Several prediction models for RSV hospitalisation in infants have been published [18, 23, 24], but none incorporated neonatal lung function measurements.

Both C_{rs} and R_{rs} were associated with RSV, for R_{rs} this was after adjustment for study year. Although the virulence of the RSV strain is not associated with neonatal lung function, we have previously shown that lung functions have improved over the years in our cohort [25]. One important explanation for this phenomenom may the smoke-free legislation. Previous studies have found an association between a reduced airway calibre (reflected by maximal flow at functional residual capacity or R_{rs}) and wheezing symptoms in the first years of life [5, 7, 8, 22]. Our results, showing an independent association between R_{rs} and post-RSV wheeze, are in line with these findings.

C_{rs} and R_{rs} possibly reflect distinct tissue properties that are differently associated with wheezing phenotypes later in life; impaired R_{rs} is associated with wheezing early in childhood and impaired C_{rs} with phenotypes that persist into later childhood [26, 27]. As hospitalised RSV patients have lower C_{rs}, they are likely to be more prone to a severe course of RSV, as well as to persistent respiratory complaints up until school age. Although the underlying mechanisms whereby reduced C_{rs} leads to RSV hospitalisation are unknown, it is possible that reduced C_{rs} reflects differences in lung characteristics leading to both a severe RSV infection and childhood asthma.

Several prospective studies have described the association between RSV bronchiolitis and the development of post-bronchiolitis wheeze [28–30]. Our data suggest that besides the severity of the RSV infection, premorbid increased R_{rs} and decreased C_{rs} also are independent determinants of post-RSV wheeze. Up to 40% of all children experience wheezing after an RSV infection [31]. Whether wheezing is also present in these children before the occurrence of the RSV infection is unknown. Lower neonatal lung function is associated with an increased risk of wheeze in the first years of life [26]. We have shown that the association between both C_{rs} and R_{rs}, and post-RSV wheeze is not dependent on the presence of pre-RSV wheeze or the

DOI: 10.1183/09031936.00009314

TABLE 3 Association between lung function and the presence of post-respiratory syncytial virus (RSV) wheeze in 90 RSV-positive infants

	C_{rs} 10 mL·kPa^{-1}		R_{rs} kPa·s·L^{-1}	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Crude	0.56 (0.35–0.90)	0.02	1.34 (1.07–1.68)	0.01
Adjusted for potential confounders				
Males	0.55 (0.34–0.89)	0.02	1.35 (1.07–1.69)	0.01
Birth length cm	0.58 (0.35–0.97)	0.04	1.33 (1.05–1.67)	0.02
Gestational age weeks	0.57 (0.35–0.93)	0.02	1.33 (1.05–1.67)	0.02
Season of birth April to September	0.57 (0.35–0.93)	0.02	1.34 (1.07–1.69)	0.01
Siblings	0.56 (0.35–0.90)	0.02	1.33 (1.06–1.67)	0.01
Breastfed in first 3 months of life	0.54 (0.33–0.88)	0.01	1.36 (1.07–1.71)	0.01
Daycare in first 3 months of life	0.57 (0.36–0.91)	0.02	1.33 (1.06–1.67)	0.01
Maternal allergy	**0.53 (0.33–0.87)**	0.01	1.30 (1.03–1.63)	0.03
Western mother	**0.52 (0.32–0.85)**	0.01	1.34 (1.06–1.68)	0.01
Highly educated mother	0.54 (0.33–0.88)	0.02	1.32 (1.05–1.66)	0.02
Study year	**0.52 (0.31–0.85)**	0.01	**1.42 (1.11–1.82)**	0.01
Adjusted for intermediates				
Maternal smoking during pregnancy	0.56 (0.35–0.90)	0.02	1.37 (1.08–1.73)	0.01
Birth weight g	**0.59 (0.35–0.98)**	0.04	1.32 (1.05–1.66)	0.02
Pre-RSV wheeze	**0.63 (0.38–1.04)**	0.07	1.31 (1.03–1.66)	0.03
RSV bronchiolitis hospitalisation	**0.66 (0.41–1.07)**	0.09	1.30 (1.03–1.63)	0.03
Adjusted multivariable model	0.45 (0.26–0.77)[#]	<0.01	1.42 (1.11–1.82)[¶]	<0.01

C_{rs}: compliance of the respiratory system; R_{rs}: resistance of the respiratory system. Bold indicates odds ratios that are affected ⩾5% by adjustment. [#]: adjusted for maternal allergy, Western mother and study year; [¶]: adjusted for study year.

severity of the RSV infection, as the association between lung function and pre-RSV wheeze was only marginally affected by pre-bronchiolitis wheeze or by hospitalisation for RSV itself.

Our findings, therefore, provide evidence for the statement that lower lung function in school-aged children that were previously hospitalised for RSV bronchiolitis can not only be attributed to the RSV infection itself, but might be partially pre-existent. The Tucson birth cohort study followed the natural course of lung functions in children up until the age of 22 years and showed that lower lung function in childhood persists into adulthood [32]. Whether RSV adds to this lung function impairment is unknown. Future research, focusing on the prevention of RSV and long-term follow-up will provide more insight into this question.

The major strength of this study is the design and the techniques used. In an unselected birth cohort, lung function was performed before the second month of life, before the occurrence of any respiratory symptoms. Infants were prospectively followed up for respiratory symptoms, and swabs were collected on the second day of each episode of wheeze or cough. Due to the large size of our birth cohort, we were able to detect 18 confirmed RSV bronchiolitis patients within our cohort and to compare these patients with nonhospitalised RSV-positive infants. All RSV diagnoses were confirmed using molecular techniques.

However, some limitations need to be discussed. First, for this study we only selected RSV-positive infants with a successful lung function measurement, as lung function was the main focus of study. A successful lung function measurement is dependent on natural sleep. The measurement was cancelled if the infant woke up before or during the measurement. Because this is likely to occur randomly, it is unlikely that this resulted in bias. However, we did find a higher birth weight in the group without successful lung function measurements; no other significant differences between groups were found (table S1).

Secondly, for our control group, we used two different sampling cohorts within the WHISTLER cohort. These cohorts have been recruited in two different time periods, and were sampled in different ways (during an episode with lower respiratory tract symptoms or regardless of symptoms at the beginning of the month). Correction for the study year did not affect the association between C_{rs} and RSV hospitalisation, but the association between R_{rs} and RSV hospitalisation became stronger, and statistically significant. We might have missed controls with upper respiratory tract infections only, or those that carried RSV along between two sample points.

Thirdly, as the first symptoms of a RSV infection may start before detection of the virus, this may have influenced our definition of pre-RSV wheeze. Therefore, we have not included the days with wheeze during the month in which the RSV infection occurred in our analysis, which in our view excludes this explanation.

Fourthly, viral strains may have been less virulent outside the years that the controls were sampled, but in which some of the hospitalisations took place. Correction for study year did strengthen the association between both *C*rs and RSV hospitalisation and *R*rs and RSV hospitalisation, and the association between *R*rs and RSV hospitalisation also became statistically significant.

Finally, for this study we assessed patients that were RSV positive only. We were not able to analyse single virus infections with RSV as this information was not present in the hospitalised patients. Therefore, we cannot exclude the possibility that other viral agents may have contributed to the severity of the infection leading to hospitalisation, or leading to post-RSV wheezing.

We conclude that both decreased *C*rs and increased *R*rs in otherwise healthy infants are risk factors for development of a severe course of RSV bronchiolitis, requiring hospitalisation during RSV infection. Furthermore, decreased *C*rs and increased *R*rs are both independent risk factors for post-RSV wheeze.

Acknowledgements

We thank all the parents and children who participated in this study. Rolien Bekkema and Liesbeth van der Feltz-Minkema (Dept of Paediatric Pulmonology, University Medical Centre Utrecht, Utrecht, the Netherlands) assisted in recruiting the subjects and collecting the data and Myriam Olling-de Kok (Dept of Paediatric Pulmonology, University Medical Centre Utrecht) assisted in secretarial work.

References

1 Hall CB, Weinberg GA, Iwane MK, *et al.* The burden of respiratory syncytial virus infection in young children. *N Engl J Med* 2009; 360: 588–598.
2 Smyth RL, Openshaw PJM. Bronchiolitis. *Lancet* 2006; 368: 312–322.
3 Glezen WP, Taber LH, Frank AL, *et al.* Risk of primary infection and reinfection with respiratory syncytial virus. *Am J Dis Child* 1986; 140: 543–546.
4 Broughton S, Bhat R, Roberts A, *et al.* Diminished lung function, RSV infection, and respiratory morbidity in prematurely born infants. *Arch Dis Child* 2006; 91: 26–30.
5 Martinez FD, Morgan WJ, Wright AL, *et al.* Diminished lung function as a predisposing factor for wheezing respiratory illness in infants. *N Engl J Med* 1988; 319: 1112–1117.
6 Martinez FD, Wright AL, Taussig LM, *et al.* Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 1995; 332: 133–138.
7 Turner SW, Young S, Landau LI, *et al.* Reduced lung function both before bronchiolitis and at 11 years. *Arch Dis Child* 2002; 87: 417–420.
8 van der Zalm MM, Uiterwaal CSPM, Wilbrink B, *et al.* The influence of neonatal lung function on rhinovirus-associated wheeze. *Am J Respir Crit Care Med* 2011; 183: 262–267.
9 Stein RT, Sherrill D, Morgan WJ, *et al.* Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet* 1999; 354: 541–545.
10 Drysdale SB, Wilson T, Alcazar M, *et al.* Lung function prior to viral lower respiratory tract infections in prematurely born infants. *Thorax* 2011; 66: 468–473.
11 Chawes BLK, Poorisrisak P, Johnston SL, *et al.* Neonatal bronchial hyperresponsiveness precedes acute severe viral bronchiolitis in infants. *J Allergy Clin Immunol* 2012; 130: 354–361.
12 Blanken MO, Rovers MM, Molenaar JM, *et al.* Respiratory syncytial virus and recurrent wheeze in healthy preterm infants. *N Engl J Med* 2013; 368: 1791–1799.
13 Katier N, Uiterwaal CSPM, de Jong BM, *et al.* The Wheezing Illnesses Study Leidsche Rijn (WHISTLER): rationale and design. *Eur J Epidemiol* 2004; 19: 895–903.
14 Katier N, Uiterwaal CSPM, de Jong BM, *et al.* Feasibility and variability of neonatal and infant lung function measurement using the single occlusion technique. *Chest* 2005; 128: 1822–1829.
15 van der Zalm MM, Uiterwaal CSPM, Wilbrink B, *et al.* Respiratory pathogens in respiratory tract illnesses during the first year of life: a birth cohort study. *Pediatr Infect Dis J* 2009; 28: 472–476.
16 van der Gugten AC, van der Zalm MM, Uiterwaal CSPM, *et al.* Human rhinovirus and wheezing: short and long-term associations in children. *Pediatr Infect Dis J* 2013; 32: 827–833.
17 Belderbos ME, Houben ML, Wilbrink B, *et al.* Cord blood vitamin D deficiency is associated with respiratory syncytial virus bronchiolitis. *Pediatrics* 2011; 127: e1513–e1520.
18 Rietveld E, Vergouwe Y, Steyerberg EW, *et al.* Hospitalization for respiratory syncytial virus infection in young children: development of a clinical prediction rule. *Pediatr Infect Dis J* 2006; 25: 201–207.
19 Rossi GA, Medici MC, Arcangeletti MC, *et al.* Risk factors for severe RSV-induced lower respiratory tract infection over four consecutive epidemics. *Eur J Pediatr* 2007; 166: 1267–1272.
20 Simoes EAF. Environmental and demographic risk factors for respiratory syncytial virus lower respiratory tract disease. *J Pediatr* 2003; 143: Suppl., S118–S126.
21 Bisgaard H, Loland L, Holst KK, *et al.* Prenatal determinants of neonatal lung function in high-risk newborns. *J Allergy Clin Immunol* 2009; 123: 651–657.
22 Young S, O'Keeffe PT, Arnott J, *et al.* Lung function, airway responsiveness, and respiratory symptoms before and after bronchiolitis. *Arch Dis Child* 1995; 72: 16–24.
23 Houben ML, Bont L, Wilbrink B, *et al.* Clinical prediction rule for RSV bronchiolitis in healthy newborns: prognostic birth cohort study. *Pediatrics* 2011; 127: 35–41.

DOI: 10.1183/09031936.00009314

24 Simões EAF, Carbonell-Estrany X, Fullarton JR, *et al.* A predictive model for respiratory syncytial virus (RSV) hospitalisation of premature infants born at 33–35 weeks of gestational age, based on data from the Spanish FLIP Study. *Respir Res* 2008; 9: 78.

25 Koopman M, van der Gugten AC, Uiterwaal CS, *et al.* Smoke-free legislation results in better neonatal lung function what leads to less wheezing illnesses and asthma in early childhood. [E-letter], *Pediatrics* (February 25, 2013). http://pediatrics.aappublications.org/content/131/2/e495.abstract/reply Date last accessed: March 10, 2014.

26 van der Gugten AC, Uiterwaal CSPM, van Putte-Katier N, *et al.* Reduced neonatal lung function and wheezing illnesses during the first five years of life. *Eur Respir J* 2013; 42: 107–115.

27 Håland G, Carlsen KCL, Sandvik L, *et al.* Reduced lung function at birth and the risk of asthma at 10 years of age. *N Engl J Med* 2006; 355: 1682–1689.

28 Korppi M, Piippo-Savolainen E, Korhonen K, *et al.* Respiratory morbidity 20 years after RSV infection in infancy. *Pediatr Pulmonol* 2004; 38: 155–160.

29 Henderson J, Hilliard TN, Sherriff A, *et al.* Hospitalization for RSV bronchiolitis before 12 months of age and subsequent asthma, atopy and wheeze: a longitudinal birth cohort study. *Pediatr Allergy Immunol* 2005; 16: 386–392.

30 Bont L, Van Aalderen WM, Versteegh J, *et al.* Airflow limitation during respiratory syncytial virus lower respiratory tract infection predicts recurrent wheezing. *Pediatr Infect Dis J* 2001; 20: 277–282.

31 Bont L, Steijn M, Van Aalderen WM, *et al.* Seasonality of long term wheezing following respiratory syncytial virus lower respiratory tract infection. *Thorax* 2004; 59: 512–516.

32 Stern DA, Morgan WJ, Wright AL, *et al.* Poor airway function in early infancy and lung function by age 22 years: a non-selective longitudinal cohort study. *Lancet* 2007; 370: 758–764.