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ORIGINAL ARTICLE

Lung function of preterm infants before and after viral infections

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Abstract Our aim was to determine whether viral lower respiratory tract infections (LRTIs) adversely affect prematurely born infants' lung function at follow up. Seventy infants, median gestational age 34 (range, 24–35)weeks were prospectively followed; 32 had an RSV (n=14) or another respiratory viral (n=18) LRTI (viral LRTI group) and 38 had no LRTI (no LRTI group). Six of the viral LRTI and five of the no LRTI group had been hospitalised. Nasopharyngeal aspirates (NPAs) obtained whenever the infants had an LRTI. Lung function (functional residual capacity [FRC_{He}], compliance [Crs] and resistance [Rrs] of the respiratory system) was measured at 36 weeks postmenstrual age (PMA) and 1 year corrected. At 1 year, lung volume (FRCpleth) and airways resistance (Raw) were also assessed. There were no significant differences in the lung function of the two groups at 36 weeks PMA but at 1 year, the viral LRTI compared to the no LRTI group had a higher mean Raw (23 versus 17 cm H_2O/l/s, p=0.0068), the differences remained significant after adjustment. *Conclusion*: These results suggest viral LRTIs, regardless of whether hospitalisation is required, adversely affect prematurely born infants' airway resistance at follow up.

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Abbreviations

BPD	Bronchopulmonary dysplasia
Crs	Compliance of the respiratory system
FRC_{He}	Functional residual capacity (by helium gas)
FRCpleth	Functional residual capacity (by plethysmograph)
LRTI	Lower respiratory tract infection
NPA	Nasopharyngeal aspirate
PCR	Polymerase chain reaction
PMA	Post menstrual age
Raw	Airway resistance
Rrs	Resistance of the respiratory system
RSV	Respiratory syncytial virus

Introduction

Prematurely born children frequently suffer chronic respiratory morbidity at follow up: frequent troublesome symptoms requiring treatment and lung function abnormalities even in adolescence and young adulthood. It is possible that viral infections may contribute to that increased morbidity, as greater health care utilisation and lung function abnormalities at school age have been reported following respiratory syncytial virus (RSV) lower respiratory tract infections (LRTI) [9]. Poorer lung function at 36 weeks postmenstrual age (PMA), however, was found in very prematurely born infants who developed an RSV LRTI [2] and in prematurely born infants with a wide range of gestational ages who required hospitalisation because of a symptomatic RSV LRTI [7]. It may then be that RSV LRTIs occur in those destined to have reduced lung function at follow up because of poorer premorbid lung function. Indeed, term born infants who developed viral bronchiolitis in infancy had reduced lung function soon after birth, that is, before they had had viral bronchiolitis and had similar levels of reduced lung function at 11 years of age [27]. Our aim, then, was to determine whether viral LRTIs do affect prematurely born infants' lung function at follow up, by undertaking lung function measurements at 1 year corrected in a subset of those initially assessed at 36 weeks PMA [7].

Materials and methods

A prospective cohort study had been undertaken in which infants born at less than 36 weeks of gestational age were eligible for entry if they were born prior to the onset of the RSV season in either 2008 or 2009. The RSV season was defined as 1st October to 31st March, consistent with UK experience [5]. Consecutive infants, whose parents gave informed written consent, were recruited. The study was approved by the Research Ethics Committee of King's College Hospital NHS Trust. The lung function results at 36 weeks PMA from the cohort have been previously reported [7]. In addition, the health care utilisation results of the cohort related to rhinovirus LRTIs have also been published [6]. Infants were included in this part of the study if they underwent lung function assessments at 1 year corrected.

The infants had undergone lung function measurements at 36 weeks postmenstrual age (PMA) prior to neonatal or maternity unit discharge as previously described [7]. Lung volume was assessed by measurement of functional residual capacity (FRC_{He}) using a commercially available helium gas dilution system (EBS 2615, Equilibrated Bio Systems, New York). The initial and equilibration helium concentrations were used to calculate FRC, which was corrected for oxygen consumption (7 ml/kg/min) [13] and converted to body temperature and water vapour saturated conditions. FRC was measured at least twice to obtain two results which were within 10 % of each other [19] The paired FRC results were then averaged and related to body weight. The mean intra-subject coefficient of variability of the measurement of FRC was 5 %. Compliance (Crs) and resistance (Rrs) of the respiratory system were measured using the single breath occlusion technique as previously described [7]. The mean Crs and Rrs results were calculated from at least five technically acceptable occlusions. The mean intra-subject coefficients of variability of the Crs and Rrs measurements were 12 and 11 %, respectively.

Infants underwent lung function assessment at 1 year of corrected age in the Amanda Smith lung function laboratory. Measurements were not made if the infant had been symptomatic with a respiratory tract infection during the previous 3 weeks as per the ERS/ATS recommendations [8]. Infants were sedated with chloral hydrate (80 mg/kg). Infants were monitored using pulse oximetry (Datex-Ohmeda 3800, Hatfield, UK) throughout pulmonary function testing and until they had woken. Once asleep, the infant was placed supine inside a plethysmograph (Department of Medical Engineering, Hammersmith Hospital, London, UK) as previously described [3]. The infant breathed through an appropriately sized Rendell-Baker face mask, sealed around the nose and mouth with silicone putty. Lung volume (FRCpleth) was calculated from a minimum of three end inspiratory occlusions [28] and related to body weight. Airway resistance (Raw) was calculated electronically during initial inspiration and between 0 and 50 % of maximal inspiratory flow [26]. On completion of the plethysmographic measurements, FRC_{He} and then Crs and Rrs (as described above) were measured.

Following neonatal unit discharge, infants were followed prospectively until 1 year corrected age. The parents were asked

to contact the research team when their infant was symptomatic with signs consistent with an LRTI, that is cough, wheeze and/or shortness of breath. Infants were considered to have a viral LRTI if they had cough/runny nose with lower respiratory tract signs (crackles, wheeze or signs of respiratory distress such as tachypnoea, shortness of breath). In addition, parents were telephoned every 2 weeks by researchers to ascertain whether their infant had been or was symptomatic. A researcher visited the home on every occasion that an infant had an and a nasopharyngeal aspirate (NPA) was obtained. NPAs were also obtained from all infants admitted to hospital with an LRTI. Real time reverse transcription polymerase chain reaction (PCR) was performed on the NPAs for nine viruses (rhinovirus, human metapneumovirus, influenza A and B, parainfluenza 1–3 and RSV A and B) in three multiplexes with an RNA internal control as previously described [7]. Adenovirus (DNA virus) was tested by a duplex real time PCR assay incorporating a DNA internal control [7]. In addition, another multiplex, including an MS2 phage internal control, was developed using previously published primers and probes [1, 17, 20, 21] which tested for enterovirus, parechovirus and human bocavirus. The neonatal notes were examined to determine if the infant had been exposed to antenatal steroids and/or antenatal infection (maternal positive blood culture, histologically proven chorioamnionitis, maternal temperature with a positive culture from a high vaginal swab or rupture of the membranes of duration greater than 24 h). Data were also collected regarding administration of surfactant, whether the infant had had a postnatal infection (positive blood culture or suspected clinical infection with a raised C reactive protein, increased or decreased neutrophil count, and/or decreased platelet count), developed bronchopulmonary dysplasia (BPD, oxygen dependency beyond 28 days), the length of their neonatal unit/maternity unit hospitalisation and whether they had received palivizumab. Only infants with BPD who had required supplementary oxygen until at least 1 week before neonatal intensive care unit discharge and were being discharged during the RSV season were given palivizumab.

Analysis

Infants who had an LRTI, but no virus was detected were excluded from the analysis.

The remaining infants were divided into two groups:

(i) Infants who never had a symptomatic LRTI (no LRTI group)
(ii) Infants who had at least one symptomatic LRTI from which either RSV or another respiratory virus was detected from the NPA (viral LRTI group).

For continuous variables, the *t* test and Mann–Whitney *U* test were used as appropriate to explore any differences between the two groups. Chi-square and Fisher's exact test were used as appropriate for binary variables. Multivariable regression models were used to examine the association between viral status and lung function at 1 year of age after adjusting for possible confounders after checking that the model residuals were approximately normally distributed. Principal Component Analysis (PCA) was used to summarise the confounder variables and provide parsimonious regression models. Neonatal factors only were adjusted for in the first stage and both infant and neonatal factors were adjusted for in the second stage. For details regarding the variables used, see footnote under Table 4. All statistical analyses were performed using Stata (v.12.1 TX, USA).

Sample size

A sample size of at least 30 in each of the two groups allowed the detection of a difference in the means of the lung function test results of 0.85 standard deviations (SDs), with 90 % power and two-sided 5 % significance. In a previous study [3], a difference of one SD was detected between the lung function results of infants who had or had not had an RSV LRTI.

Results

Two hundred and fifty one infants were eligible for inclusion into the overarching study. Seventy infants were included in this part of the study (Fig. 1). Three (4 %) infants received palivizumab, none of whom were admitted to hospital because of an RSV LRTI.

Thirty-eight infants did not have a symptomatic LRTI (no LRTI group). Thirty-two infants had a viral LRTI (Table 1). Fourteen infants had at least one RSV LRTI but the majority of them also had another viral LRTI (rhinovirus, adenovirus, human metapneumovirus or enterovirus). Twelve infants had a dual viral infection LRTI and three a triple viral infection (LRTI). Five infants in the no LRTI group (ophthalmology operation, patent ductus ligation, inguinal hernia repair, gastroenteritis, head injury) and six infants in the viral LRTI group (RSV $n=5$) were admitted to hospital. There were significant differences between the two groups with regard to their birth weight ($p=0.006$), exposure to antenatal steroids ($p=0.036$), duration of supplemental oxygen ($p=0.023$) and the duration of neonatal unit stay ($p=0.009$) (Table 2). Only two infants attended daycare.

There were no significant differences in the lung function results of the two groups at 36 weeks PMA (Table 3). At 1 year corrected age, the Rrs ($p=0.024$) and the Raw results ($p=0.0068$) differed significantly between the two groups (Table 3). After full adjustment, the difference for Rrs of 6.9 was reduced to 5.2 cm H_2O/l/s and was no longer statistically significant (Table 4). The difference in the Raw results between the groups was reduced from 6.0 to 4.69 cm H_2O/l/s, but remained statistically significant ($p=0.021$) (Table 4). After

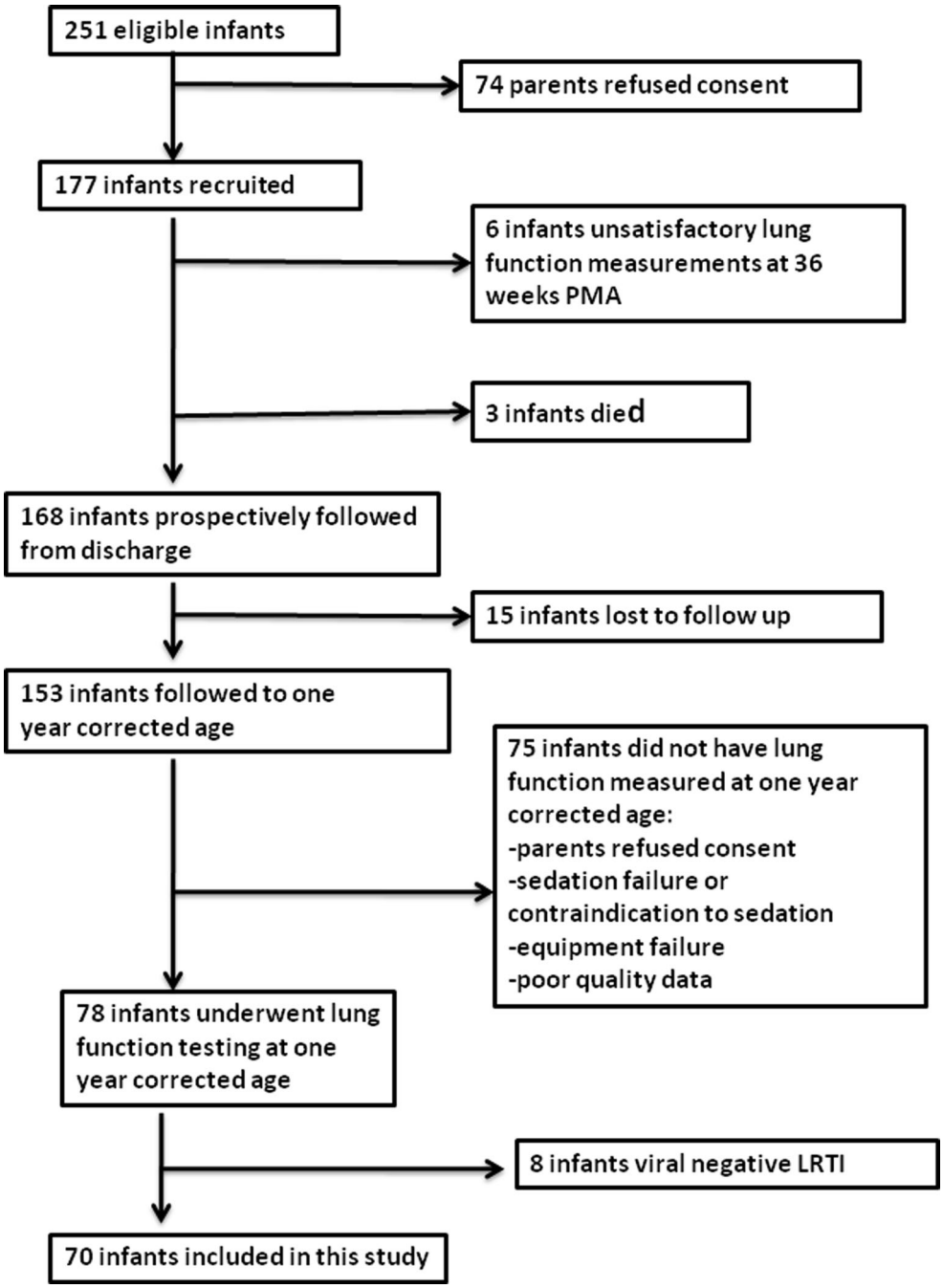

Fig. 1 Consort diagram of recruitment

adjusting additionally for baseline lung function results, for those lung function measures for which we had both baseline and 1 year results, the *p* values changed not all or marginally (FRC 0.79 to 0.79, Rrs 0.091 to 0.08 and Crs 0.84 to 0.82)

Discussion

We have demonstrated that, even after adjustment for neonatal and infant factors, the viral LRTI compared to the no LRTI group had significantly worse airway resistance at 1 year corrected, that is, after they had had viral LRTIs. Yet, there were no significant differences in the lung function at 36 weeks PMA, that is, before the viral LRTIs. Those results then suggest the viral LRTIs were responsible for the deterioration in lung function. We have previously reported higher airway resistance results at 1 year corrected following RSV LRTIs in a very prematurely born cohort born at a median of 28 weeks of gestation [3]. In this study, we have not reported the RSV LRTI results separately, as the majority of the infants who had had an RSV LRTI also had another viral LRTI. The current infants who had suffered viral LRTIs were born at a

Table 1 Number of viruses detected by real time PCR

	Viruses detected
RSV A	5
RSV B	11
Rhinovirus	9
Adenovirus	7
Human metapneumovirus	4
Influenza A	3
Influenza B	2
Parainfluenza 1	3
Parainfluenza 2	1
Parainfluenza 3	5
Enterovirus	8
Parechovirus	0
Bocavirus	1

Data are displayed as the number of occasions a virus was detected. Some infants had more than one viral LRTI

median gestational age of 32 weeks and only six of them had been hospitalised. Hence, our results highlight that even mild viral LRTIs in moderately, prematurely born infants are associated with reduced lung function at follow up.

Most studies demonstrating lung function abnormalities at follow up have reported only infants who were hospitalised because of their LRTI [4, 11, 12, 22, 24]. There have, however, been some studies of term born infants which have included nonhospitalised infants [18, 23, 25]. In one study, infants who had mild bronchiolitis were demonstrated not to be at increased risk between the ages of 8 and 12 years for airway hyperreactivity or abnormalities in pulmonary function [18]. Similarly, in another study, although RSV LRTIs were associated with an increased risk of wheeze at age six, the risk decreased markedly with age and was no longer significant at age 13 [18]. Reduced maximal flow at FRC (Vmax FRC) and increased airway responsiveness to metacholine challenge, however, were demonstrated in 18 infants during infancy, nine of whom were not hospitalised, 10 months after an episode of bronchiolitis [25]. In none of those studies were there attempts to identify respiratory viruses. Those results [18, 23, 25] may mean that viral LRTIs cause chronic morbidity only in early childhood. Further longitudinal studies are required to test that hypothesis, as it is possible that rhinovirus LRTIs may have longer term adverse effects. In the COAST study [10], infants at high risk of developing wheeze (at least one atopic parent) were followed up. Infants who wheezed with a rhinovirus infection in the first 3 years after birth were demonstrated to have reduced lung function as assessed by spirometry at 5 to 8 years of age compared to those infants without a rhinovirus "wheezy" illness. In another study [16], hospitalisation for "wheezy" rhinovirus infections in the first 2 years after birth was a risk factor for reduced lung function (increased bronchial responsiveness to exercise) at 8 years of age. We have previously demonstrated that rhinovirus LRTIs were associated with increased healthcare utilisation in infancy in

Table 2 Infant characteristics by viral LRTI status

	No LRTI group	Viral LRTI group	*p* value
n	38	32	
Gestational age (weeks)	34 [28–35]	32 [24–35]	0.17
Born <32 weeks of gestational age	8 (21 %)	13 (41 %)	0.075
Birth weight (g)	2,098 [900–3,174]	1,667 [670–3,154]	0.006
Small for gestational age	4 (11 %)	7 (22 %)	0.32
Male	22 (58 %)	17 (53 %)	0.69
Antenatal smoking	5 (13 %)	9 (28 %)	0.14
Antenatal steroids	22 (58 %)	26 (81 %)	0.036
Surfactant	6 (16 %)	11 (34 %)	0.071
Duration of ventilation (days)	0 [0–15]	2 [0–90]	0.036
Duration of supplemental oxygen (days)	0 [0–58]	2 [0–377]	0.023
Bronchopulmonary dysplasia	3 (8 %)	7 (22 %)	0.17
Family history of atopy[a]	26 (68 %)	21 (60 %)	0.80
Family history of asthma	5 (16 %)	9 (24 %)	0.78
Number of siblings	1 [0–5]	1 [0–5]	0.39
Breastfed	35 (92 %)	25 (78 %)	0.17
Palivizumab given	0 (0 %)	3 (9 %)	0.091
Duration of neonatal unit stay (days)	18 [2–117]	29 [3–188]	0.009

Data expressed as median [range] or *n* (%)

[a] atopy=asthma, eczema and/or hayfever

Table 3 Lung function results at 36 weeks PMA and at 1 year corrected age by virus status

	No LRTI group	Viral LRTI group	*p* value
n	38	32	
At 36 weeks PMA			
Postmenstrual age (weeks)	35 (1.2) [34–41]	36 (1.6) [34–41]	0.018
Weight (g)	2,176 (373) [1,598–3,274]	2,067 (377) [1,200–3,154]	0.23
FRC (ml/kg)	25.5 (3.5) [19–34]	23.7 (6.2) [7.9–35]	0.12
Crs (ml/cmH_2O/kg)	1.48 (0.36) [0.72–2.3]	1.39 (0.39) [0.72–2.4]	0.23
Rrs (cmH_2O/l/s)	72.5 (20) [48–122]	79.4 (21) [43–133]	0.11
At 1 year corrected age			
Corrected age (months)	12.7 (2.1) [5.9–16]	13.2 (2.3) [5.4–19]	0.70
Days between assessments	416 (64) [215–514]	424 (72) [199–618]	0.93
Weight (kg)	9.9 (1.7) [6.4–16]	9.5 (1.3) [7.2–12]	0.48
Length (cm)	78 (3.3) [72–85]	78 (4.1) [71–87]	0.75
FRC (ml/kg)	24.6 (3.9) [17–32]	24.9 (3.7) [18–33]	0.81
Crs (ml/cmH_2O/kg)	1.87 (0.72) [0.25–4.0]	1.78 (0.45) [1.2–2.7]	0.71
Rrs (cmH_2O/l/s)	43.4 (9.9) [28–67]	50.3 (13.9) [34–89]	0.024
FRC_{pleth} (ml/kg)	26.1 (4.3) [19–36]	27.9 (6.5) [20–48]	0.41
R_{aw} (cm H_2O/l/s)	17.2 (5.5) [9.7–33]	23.2 (9.5) [11–45]	0.0068
$FRC_{HE:pleth}$ ratio	0.88 (0.07) [0.74–1.0]	0.87 (0.09) [0.68–1.0]	0.73

Data are displayed as mean (sd) [range]

prematurely born infants [6]. We have not presently analysed the rhinovirus results separately but, of note, the viral LRTI group had had rhinovirus LRTIs on nineteen occasions.

Some studies have shown a decline in lung function during infancy in prematurely born infants [14, 15]. In one [15], there was a decline in small airway function as assessed by Vmax FRC over the first few months after birth in infants born between 32 and 35 weeks of gestation who had no initial respiratory problems. In another, which included very prematurely born infants who had BPD, there was a decline in Vmax FRC between 6 and 12 months in those who had initially been supported by conventional ventilation [14]. In this study, Rrs and Crs results improved between 36 weeks PMA and 1 year corrected in both groups.

Our study has a number of strengths and some limitations. Consecutive infants were recruited whose parents gave informed consent. The infants tended to be relatively "mature" prematurely born infants and hence, not surprisingly, only a small proportion was admitted to hospital because of the LRTI. There were significant differences in the infant characteristics between the two groups. The infants in the viral LRTI group had significantly longer durations of both ventilation and supplementary oxygen. The median values, however, were zero and 2 days which would seem unlikely to have a major influence on lung function at 36 weeks PMA. In addition, any adverse influence may have been offset by a greater proportion of the viral LRTI group having received steroids. The viral LRTI group was of significantly lower birth weight

Table 4 Difference in lung function between groups (viral LRTI—no LRTI) after regression modelling to adjust for confounding neonatal and infant factors

	Difference in lung function between viral groups after adjusting for neonatal factors[a]	*p* value	Difference in lung function between viral groups after adjusting for neonatal and infant factors[a]	*p* value
FRC (ml/kg)	0.52 (−1.52, 2.55)	0.61	0.28 (−1.81, 2.36)	0.79
Rrs (cmH_2O/l/s)	5.84 (−0.11, 11.8)	0.054	5.21 (−0.86, 11.3)	0.091
Crs (ml/cmH_2O/kg)	−0.026 (−0.32, 0.27)	0.86	−0.031 (−0.34, 0.28)	0.84
FRC_{pleth} (ml/kg)	1.59 (−1.36, 4.54)	0.29	1.43 (−1.49, 4.34)	0.33
R_{aw} (cm H_2O/l/s)	5.50 (1.31, 9.68)	0.011	4.69 (0.74, 8.63)	0.021
$FRC_{HE:pleth}$ ratio	−0.01 (−0.05, 0.03)	0.58	−0.01 (−0.05, 0.03)	0.69

The results are expressed as the mean (95 % CI)

[a] Neonatal factors were gender, gestational age, birth weight, antenatal steroids and use of surfactant. Principal component analysis reduced these five factors to three components. Infant factors were days on ventilation, BPD status, breastfed, palivizumab given and length of stay in hospital. Principal component analysis reduced the six factors to two components

which likely explains their longer neonatal unit stay, the timing of discharge being decided using a predetermined weight. Infants were followed prospectively and hence we "captured" LRTIs regardless of whether the infants required hospitalisation or the infants remained in the community. Only 51 % of the 153 infants followed to 1 year corrected age had lung function measurements, but this is similar to a previous study in which sedation was required for lung function measurements at 1 year corrected [3]. We did not include the infants with viral negative LRTIs in the analysis, as we already had the control group of the infants who had no LRTIs. In addition, the viral negative LRTI group could include those with viral LRTIs, but with a viral load below the limit of detection. We were able to test for a wide variety of respiratory viruses, which highlighted that the majority of infants who had RSV LRTIs also had other viral LRTIs. Hence, we cannot report whether there was any impact of RSV LRTIs alone on lung function at follow up.

In conclusion, our results suggest viral LRTIs, regardless of hospitalisation, adversely affect prematurely born infants' lung function at follow up. This may make them at greater risk of poorer outcomes with subsequent viral LRTIs.

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Conflict of interest Abbott Laboratories, who supported Mrs Wilson and Mrs Alcazar, market palivizumab a monoclonal antibody against RSV.

Contributorship list AG and SLJ designed the study. SBD, MA and TW collected the data, SBD, MS and MZ undertook the viral studies. JL and JLP undertook the statistical analysis. SBD, MP, SB and GFR were involved in the lung function studies. All authors were involved in producing the manuscript.

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