

**Rhinovirus bronchiolitis and recurrent wheezing: one year follow-up**

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## **Abstract**

The association between bronchiolitis and recurrent wheezing remains controversial. In this prospective study, we assessed risk factors for recurrent wheezing during a 12-month follow-up in 313 infants younger than 12 months hospitalized for their first episode of bronchiolitis. Demographic, clinical and laboratory data were obtained with a questionnaire and from medical files. A total 14 respiratory viruses were concurrently assayed in nasal washings. Parents were interviewed 12 months after hospitalization to check whether their infants experienced recurrent wheezing.

The rate of recurrent wheezing was higher in infants with bronchiolitis than in controls (52.7% vs 10.3%;  $p < 0.001$ ). Multivariate analysis identified as major independent risk factors for recurrent wheezing, rhinovirus infection (OR = 3.3; 95% CI 1.0-11.1), followed by a positive family history for asthma (OR = 2.5; 95% CI 1.2-4.9).

In conclusion, the virus most likely to be associated with recurrent wheezing at 12 months after initial bronchiolitis is rhinovirus, a viral agent that could predict infants prone to the development of recurrent wheezing.

## Introduction

Bronchiolitis, an airway disease primary located in the small peripheral bronchioles is among the major causes of hospital admission in infants younger than 1 year (1). Bronchiolitis can also cause long-term respiratory sequelae. Despite numerous clinical studies conducted since the 1950s to investigate the association between bronchiolitis and the subsequent development of asthma the link between recurrent wheezing, asthma, atopy and bronchiolitis remains controversial (2-4). The main reason is that bronchiolitis is a clinical diagnosis encompassing various disease entities whose immunopathogenesis differs according to viral aetiology, wheezing phenotype, and preceding inflammatory state. About half the clinical trials conducted over recent years have used 12 months and the remainder 24 months as an upper age limit of bronchiolitis, and half the studies have focused on the first episode of wheezing (5). A study conducted in children with suspected bronchiolitis showed that the two clinical factors linked to inflammatory (atopy-related factors) and virological risk factors for asthma are age and number of previous wheezing episodes (5). To avoid confounding in long-term results, prospective follow-up studies designed to determine how genetic predisposition, demographic characteristics and viral aetiology influence the development of recurrent wheezing and asthma should define bronchiolitis according to strict clinical criteria, excluding asthma-prone children.

Although infants hospitalised for bronchiolitis are frequently infected with other viruses, the primary infection causing bronchiolitis is respiratory syncytial virus (RSV) (6). To date, most studies on recurrent wheezing and asthma after bronchiolitis have focussed nearly exclusively on RSV, but increasing evidence now suggests that other viruses, and principally rhinovirus (RV), play an equally important role (7-9).

In a well-characterized cohort of infants aged < 12 months with acute bronchiolitis, we have already shown that although the major pathogen responsible for bronchiolitis remains RSV, the infection can also be caused by RV and human bocavirus (hBoV) (6). Few data are

available on the relationship between respiratory viruses other than RSV and recurrent wheezing after bronchiolitis in infants younger than one year (10).

Our main purpose in this prospective, single-centre study designed to continue our previous research (6) was to assess the risk factors for recurrent wheezing during a one-year follow-up in a well-characterised homogeneous cohort of infants younger than one year hospitalised for the first episode of acute bronchiolitis caused by RSV and other respiratory viruses. We concurrently assayed a battery of 14 viruses known to cause bronchiolitis. To analyse possible risk factors for recurrent wheezing we obtained demographic, clinical and virological features at hospital admission and compared rates of recurrent wheezing 12 months later.

## Patients and methods

*Patients* We prospectively enrolled 313 consecutive full-term infants (median age 2.0 months, range 7 days–11 months, 170 (54% male) with a diagnosis of acute bronchiolitis hospitalised in the Paediatric Emergency Department, “Sapienza” University Rome, during five consecutive annual epidemic periods from October 2004 through May 2005, October 2005 through May 2006, October 2006 through May 2007, October 2007 through May 2008 and October 2008 through May 2009.

Bronchiolitis was defined as the first episode of acute lower airways infection characterised by a history of upper respiratory tract infection followed by acute onset of respiratory distress with cough, tachypnoea, retraction and diffuse crackles on auscultation (having wheezing alone was not considered sufficient for inclusion in the study) (11). Exclusion criteria were underlying chronic diseases (including cystic fibrosis, chronic pulmonary disease, congenital heart disease, and immunodeficiency).

*Controls* We prospectively enrolled 39 consecutive infants (median age 4.2 months, range 24 days–12 months, 17 (44% male) with an acute disease unrelated to the respiratory system, hospitalised in our paediatric department during the first annual epidemic period from October 2004 through May 2005. Detailed demographic, clinical and laboratory data were obtained from parents of patients and controls with a structured questionnaire and from patients’ medical files. Studied variables included age at recovery, gender, type of delivery, breast-feeding history, family smoking habits, number of siblings, family history for asthma and atopy, presence of atopic eczema, white blood-cell count, blood eosinophil count, blood C-reactive protein (CRP) concentration, chest radiological findings and number of days hospitalisation. On hospital admission, each infant was assigned a clinical severity score ranging from 0 to 8 according to respiratory rate ( $<45/\text{min} = 0$ ,  $45\text{--}60/\text{min} = 1$ ,  $>60/\text{min} = 2$ ) arterial oxygen saturation in room air ( $>95\% = 0$ ,  $95\text{--}90\% = 1$ ,  $<90\% = 2$ ), presence of

retractions (none = 0, present = 1, present + nasal flare = 2), and ability to feed (normal = 0, reduced = 1, intravenous fluid replacement = 2) (6).

Parents of all infants were asked to participate in the study and gave written informed consent. The study was approved by the hospital institutional review board.

### ***Follow-up***

At 12-months after discharge one of the authors (FM) interviewed parents of all infants using a standardized telephone procedure with a structured questionnaire seeking information on recurrent wheezing. Of the 39 families in the control group all replied, whereas of the 313 families of infants with bronchiolitis contacted, only 262 (83.7%) replied (Figure 1). The only reason for dropout was a change in telephone number. The term “recurrent wheezing” was defined as two or more physician-verified episodes of wheezing during a one year follow-up. None of the control infants had bronchiolitis during the first year of life.

### **Virus detection**

From 1 to 3 days after hospitalisation, all infants underwent nasal washing obtained with 3 ml of sterile saline solution injected into each nostril and collected with a syringe. All samples were delivered on ice within 1-2 hours to the virology laboratory and on arrival, if needed, they were vortexed with beads to dissolve mucous. Two hundred µl of respiratory specimens was subjected to nucleic acid extraction with the total nucleic acid isolation kit (Roche Diagnostics, Mannheim, Germany) eluting into 50 µl of the supplied elution buffer. A panel of reverse transcriptase-polymerase chain reaction (RT-PCR) or nested PCR assays was developed for detecting 14 respiratory viruses including RSV, influenza virus (IV) A and B, human coronavirus (hCoV) OC43, 229E, NL-63, HUK1, adenovirus, RV, parainfluenza virus (PIV) 1-3, human bocavirus (hBoV) and human metapneumovirus (hMPV) as previously described (12). During the course of the study, a novel group of previously unrecognized

human rhinoviruses was recognised, established and named RV species C (13). The RV-PCR primers used throughout our study target highly conserved regions in the 5' untranslated region (UTR) (forward primer position 436-453; reverse primer position 537-555, according to HRV35 genome numbering) corresponding to the P2 and P3 regions reported as conserved among all RV genotypes, including RV group C (13). Hence our RV-PCR primers almost certainly detected RV C as well as RV species A and B. Even though RV genotyping was beyond the purpose of this study, we retrospectively tried to amplify a larger genomic sequence in the 5' UTR (14) from frozen stored samples to prove RV C detection. Owing to the frailty of the respiratory samples kept stored at -80°C for years, we succeeded in sequencing and genotyping only 9 RV-positive samples, 7 were RV A and 2 RV C positive.

At study entry on admission, a total of 195 viruses were detected in the nasal wash specimens from 174 (55.6%) of the 313 infants with bronchiolitis: RSV in 130 (74.7%), hBoV in 25 (14.4%), RV in 24 (13.8%), PIV 1-3 in 6 (3.4%), hMPV in 5 (2.8%), IV A in 2 (1.1%) and hCoV in 1 (0.6%). Of the 130 RSV-positive infants, 15 (11.5%) had co-infection with hBoV, 1 with IV A, 1 with PIV 3 and 1 with RV. One of the hMPV-positive infants had co-infection with hBoV and 1 with RV. No viruses could be detected in nasal wash specimens from 139 infants (44.4%). The prevalence of respiratory viruses in our study sample has been addressed in our previous paper (6).

## **Statistical analysis**

A descriptive analysis included calculating percentages, mean and SDs, medians, and ranges for demographic variables and virological results. Distributions of continuous variables were examined for skewness and kurtosis. Differences between groups (with or without recurrent wheezing) were tested for significance using an unpaired t-test (two-tailed) for normally distributed data or non-parametric Mann-Whitney test for non normally distributed data. Chi-square test was applied to analyse categorical independent variables qualitatively. Multivariate logistic regression models were used to identify clinical and virological variables associated with the main endpoints (to assess the risk factors for recurrent wheezing during a one-year follow-up after the first episode of acute bronchiolitis caused by RSV and other respiratory viruses). Only variables with  $p < 0.15$  in the univariate analysis were included in the multivariate model. Results from logistic models were described by odds ratios (ORs) and 95% confidence intervals (CIs). P values  $< 0.05$  were considered to indicate statistical significance. Data were analysed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).



## Results

### *Infants with bronchiolitis versus controls*

No differences were observed for gender, family history of asthma, family history of atopy and smoking exposure between infants with bronchiolitis and controls. The median age was higher in the control group than in infants with bronchiolitis (4.2 months, range 24 days–12 months vs 2.0 months, range 7 days–11 months;  $p < 0.001$ ). Within the first year after hospital admission for bronchiolitis, a higher percentage of infants with bronchiolitis than controls had episodes of recurrent wheezing (52.7% vs 10.3%;  $p < 0.001$ ).

### *Children hospitalised for acute bronchiolitis with and without recurrent wheezing*

Of the 262 infants whose parents answered the telephone interview at one-year follow-up, 138 infants (52.7%) experienced recurrent wheezing and 124 (47.3%) did not. Several demographic characteristics differed in infants with and without recurrent wheezing. For example, the percentage of male infants, albeit not significant, was higher in the group with recurrent wheezing group than in those without (60.9% vs 50.8%;  $p < 0.09$ ). Infants with recurrent wheezing also had a significantly higher rate of family history for asthma than infants without recurrent wheezing (28.3% vs 14.3%;  $p < 0.001$ ). No significant differences were found in relation to demographic characteristics including type of delivery, birth weight, age at recovery, weight at recovery, breast feeding, exposure to smoking, family history for atopy, atopic eczema, clinical severity score and days of hospitalisation (Table 1).

The total number of blood eosinophils and the percentage of infants with an absolute number of blood eosinophils greater than 400 cells/ $\mu$ L were higher in infants with recurrent wheezing than in those without ( $p < 0.01$  and  $p < 0.02$ ). The number of children with a CRP concentration under 0.8 mg/dl and absence of lung consolidations on chest X-ray differed significantly between children with and without recurrent wheezing ( $p < 0.01$  and  $p < 0.01$ ) (Table 1).

The distribution of viruses differed in infants with and without recurrent wheezing (Table 2). The rates of recurrent wheezing differed also according to the virus causing acute bronchiolitis: 80% for RV, 66.7% for hBoV, 50% for other viruses, 45.5% for RSV+hBoV ( $p < 0.05$  vs RV) and 43.3% for RSV ( $p < 0.005$  vs RV).

Odds ratios assessing possible demographic and clinical factors influencing the development of recurrent wheezing after acute bronchiolitis indicated an increased risk of wheezing during the first year of follow-up for blood eosinophils  $> 400$  cells/ $\mu$ L (OR = 4.9; 95% CI 1.1-22.9), RV bronchiolitis (OR = 4.0; 95% CI 1.3-12.2), family history for asthma (OR = 2.7; 95% CI 1.5-4.9), CRP  $< 0.8$  mg/dl (OR = 1.9; CI 1.1-3.1), absence of lung consolidation (OR = 1.7; CI 1.0-3.1).

The multivariate logistic regression model identified as the most important independent risk factor for recurrent wheezing, RV infection (OR = 3.3; 95% CI 1.0-11.1), followed by a positive family history for asthma (OR = 2.5; 95% CI 1.2-4.9). Another independent risk for recurrent wheezing was the absence of lung consolidation on chest X ray (OR = 2.6; 95% CI 1.1-6.1) (Table 3).

## Discussion

In this prospective, single-centre study conducted in a university paediatric department we found that among infants younger than one year hospitalised for their first episode of acute bronchiolitis, those with bronchiolitis caused by RV and a positive family history of asthma have a substantially higher risk for recurrent wheezing during one-year follow-up after their initial episode than those with bronchiolitis caused by RSV and other respiratory viruses.

A distinctive point in our study is that unlike previous studies, we prospectively investigated recurrent wheezing in a clinically well-characterised cohort of infants aged less than 12 months admitted to hospital for their first episode of acute bronchiolitis, already studied in our previous research (6), and also assayed their nasal washes simultaneously for a large battery of 14 different respiratory viruses. Conversely, other studies defined bronchiolitis differently (infants aged less than 24 months and with wheezing) and enrolled only those with RSV bronchiolitis (15-17).

Our decision to define bronchiolitis according to strict clinical criteria ensured that we provide homogeneous data suitable for future prospective long-term follow-up and excluded patients with various differing clinical entities, for example wheezing bronchitis, and asthma-prone children. Despite its high frequency, bronchiolitis remains a clinical diagnosis without a common international definition. Whereas some investigators define bronchiolitis as an episode of viral wheezing in infants younger than 24 months (18), others define it as the first episode of a lower respiratory-tract infection with diffuse bilateral crackles in infants younger than 12 months of age (11). Our study, selecting infants under 12 months, with their first acute respiratory infection and crackles at chest auscultation, therefore avoids factors that might confound clinical trials and long-term follow-up of bronchiolitis. It also provides new evidence underlining how risk factors such as genetic predisposition, demographic characteristics and viral aetiology can influence the development of recurrent wheezing.

When we analysed the number of infants with episodes of recurrent wheezing during a one-year follow-up, as expected we found a considerably higher rate of recurrent wheezing in infants with bronchiolitis than in the control group (52% vs 10.3%). Notwithstanding these findings, like other studies, we found similar hereditary factors in infants with and without bronchiolitis (4, 15-17). In their longitudinal study, Sigurs et al also found that despite similar hereditary factors in infants with and without bronchiolitis, a significantly larger percentage of the infants with RSV bronchiolitis had episodes of recurrent wheezing after a one-year follow-up (17). Collectively these findings confirm that having bronchiolitis during the first year of life is a risk factor for the subsequent development of recurrent wheezing (3-4, 17). The mechanisms underlying this increased incidence of wheezing after bronchiolitis are still unclear (19-20).

When we compared demographic characteristics and clinical findings in infants with and without recurrent wheezing after bronchiolitis, we found several differences. First of all, we confirmed the tendency towards male predominance in infants with recurrent wheezing (17). The slightly higher frequency of wheezing in boys seems to fit in well with current knowledge that high airway resistance in male infants and low functional residual capacity in female infants predispose male infants to wheezing in association with lower respiratory tract infection (21).

Two demographically and clinically important differences we observed between the two groups were the significantly higher percentage of infants with a positive family history for asthma and the significantly higher number of blood eosinophils in infants with recurrent wheezing. Other studies of bronchiolitis also found heredity for asthma and blood eosinophilia risk factors for the development of recurrent wheezing and asthma (22-23). Hence infants with bronchiolitis in whom wheezing develops might be genetically predetermined to do so, and severe respiratory disease might ultimately develop whatever respiratory virus they encounter early in life. This finding accords with Ehlenfield et al's

conclusion that eosinophilia at the time of bronchiolitis predicts the development of wheezing persisting into later childhood (24). Whether eosinophils play important roles in the onset of recurrent wheezing or are just a marker of allergic symptoms remains uncertain.

Another finding that may help to characterise children with recurrent wheezing was the significant difference between the two groups in the blood CRP level and in the percentage of infants with radiological lung consolidations. Infants with recurrent wheezing had a lower blood CRP concentration and a fewer radiologically-documented lung consolidations than non-wheezing infants. These radiological findings suggest that the two groups of infants (with and without recurrent wheezing) differ not only in having risk factors for atopy and asthma (family history of asthma and eosinophilia), but also in their pathogenetic response to the different viruses.

Finally, in the group of infants with bronchiolitis, when we analysed the rate of recurrent wheezing in relation to specific viruses detected during the illness, we found a larger number of infants with recurrent wheezing among infants with RV than among those with hBoV, RSV, dual infection RSV+hBoV, and other respiratory viruses. Multivariate logistic regression analysis of possible risk factors for the development of recurrent wheezing during one year after the acute episode of bronchiolitis in the 138 infants identified RV bronchiolitis as by far the most important risk factor, whereas bronchiolitis from RSV and other respiratory viruses was not a risk factor. These so far unreported findings obtained by strictly defining bronchiolitis and simultaneously assaying 14 respiratory viruses provide reliable data that may confirm previous reports in various clinical settings that infants in whom RV is detected are those predisposed to asthma and atopy. Several studies conducted over the past 5 years have shown the relationship between RV infection in the first years of life and the development of recurrent wheezing and asthma. In two community-based cohort studies Regamey et al. and Kusel et al., have shown that RV is the most frequent virological agent in upper and lower respiratory tract infections in both unselected infants and in infants with risk

factors for atopic sensitisation followed for one year (25-26). In their study evaluating how RV infection contributed to the subsequent development of wheezing during infancy in a high-risk birth cohort, Jackson et al. found that moderate-to-severe RV wheezing illness in infancy was the most significant risk factor not only for the development of preschool wheezing at the age of 3, but also for the development of asthma at age 6 years (8). Similar results came from a follow-up study of 82 infants below 2 years of age, hospitalised for infection associated with wheezing and evaluated three years later, conducted by Kotaniemi-Syrjänen and colleagues, who found that RV infection leading to hospitalisation during infancy was an early predictor of the subsequent development of asthma (9). Finally, in a retrospective study with a 3-year follow-up, Valkonen et al. showed that recurrent wheezing develops at substantially higher rates in infants hospitalised with bronchiolitis (less than 2 years of age) caused by viruses other than RSV than in children with RSV-induced bronchiolitis (27). Unfortunately, in this early study the investigators were unable to look for other respiratory viruses in infants with non-RSV bronchiolitis. Overall, the various reported results suggest that the risk of wheezing developing after an initial episode of bronchiolitis in infancy differs according to the type of viral infection. The aforementioned studies all differ from our study in several important ways. First, in defining bronchiolitis they included infants older than 12 months. Second, because they failed to specify whether they studied the first episode of bronchiolitis they could have included infants with other clinical entities (wheezing bronchitis). Third, none of the studies simultaneously sought 14 different respiratory viruses likely to cause bronchiolitis.

Current knowledge therefore clearly links RV bronchiolitis and recurrent wheezing during the first year of life. It therefore raises the question does RV bronchiolitis actually cause recurrent wheezing? Alternately, RV bronchiolitis might serve to disclose infants who are already predisposed to this disease owing to abnormal lung physiology or immune

response, or both. Indeed, RV seems to preferentially affects the lower airways causing bronchiolitis in atopic children prone to wheezing (28-30).

Our study has limitations. First, we studied fewer infants with RV than with bronchiolitis from RSV and other respiratory viruses. Second, we could not confirm whether parents gave reliable information in the phone interview. Third, as we discussed in our previous paper (6) our virus detection rate was poor. Fourth, we did not test for RV subtypes, in particular species C.

In conclusion, the major risk factors for wheezing episodes after acute bronchiolitis are RV infection and a positive heredity for asthma. A major goal for future research efforts should therefore be to define genetic, environmental and viral factors that promote wheezing after RV bronchiolitis. A longer serial follow-up will show whether RV infection identifies those infants predisposed to persistent wheezing.

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**Table 1** Demographic, clinical and radiological factors in the 262 infants without and with recurrent wheezing after a 12-month follow-up. Data are expressed as a percentage of positive cases and as \*mean  $\pm$  SD or \*\*median and range.

Demographic characteristics	Without recurrent wheezing (n = 124)	With recurrent wheezing (n = 138)	p value
Gender (male) (%)	50.8	60.9	0.09
Cesarian section (%)	51.2	53.3	0.74
Birth weight (kg)*	3.1 $\pm$ 0.5	3.1 $\pm$ 0.5	0.73
Age at recovery (months)**	2.1 (1.0 – 4.2)	2.0 (1.1 – 3.1)	0.15
Weight at recovery (kg)*	5.0 $\pm$ 1.5	5.3 $\pm$ 1.5	0.15
Breast feeding (months)**	1.0 (0.1 – 2.0)	1.1 (0.2 – 2.1)	0.12
Eczema (%)	5.6	4.4	0.64
Family history for asthma (%)	14.3	28.3	<0.001
Family history for atopy (%)	33.3	31.2	0.70
Exposure to smoking (%)	46.0	43.7	0.71
Clinical severity score**	4.0 (2.7 – 6.2)	4.0 (2.0 – 5.0)	0.12
Days of hospitalization**	5.0 (4.0 – 6.0)	5.0 (3.0 – 6.0)	0.19
Leucocytes (x 10 <sup>3</sup> cells/ $\mu$ L)**	10.0 (7.5 – 14.3)	11.4 (8.8 – 15.0)	0.11
Eosinophil count (cells/ $\mu$ L)**	57.7 (13.0 – 150.0)	86.2 (27.0 – 210.0)	<0.01
Eosinophils > 400 cells/ $\mu$ L (%)	1.6	7.4	<0.02
CRP < 0.8 mg/dl (%)	50.8	65.9	<0.01
Absence of lung consolidation (%)	8	20.3	<0.01
Radiological air trapping (%)	36.9	32.5	0.48

**Table 2** Virus distribution in the 262 infants with and without recurrent wheezing.

<b>Viruses</b>	<b>Without recurrent wheezing (n = 124)</b>	<b>With recurrent wheezing (n = 138)</b>
<b>RSV</b>	45.6	31.9
<b>RV (*,**)</b>	3.2	10.9
<b>RSV + hBoV</b>	4.8	3.6
<b>hBoV</b>	2.4	3.6
<b>hMPV</b>	0.8	0.7
<b>PIV type 1</b>	0.8	0
<b>PIV type 3</b>	0.8	1.4
<b>IV A</b>	0.8	0
<b>hCoV</b>	0.7	0
<b>RSV +PIV type 3</b>	0.8	0
<b>RSV + RV</b>	0.8	0
<b>hBoV + hMPV</b>	0	0.7
<b>RV + hMPV</b>	0	0.7

RSV= respiratory syncytial virus; RV= rhinovirus; hBoV= human bocavirus; hMPV= human metapneumovirus; PIV = parainfluenza virus; hCoV= human coronavirus; IV A=influenza A virus

\*= RV vs RSV + hBoV,  $p < 0.05$

\*\* = RV vs RSV,  $p < 0.005$

**Table 3** Multivariate logistic regression analysis testing possible risk factors for recurrent wheezing in the 138 infants hospitalised for bronchiolitis.

<b>Variables</b>	<b>p value &lt;</b>	<b>Odds ratios</b>	<b>95% confidence intervals</b>
<b>Gender (male)</b>	0.10	1.6	0.9-2.7
<b>Family history for asthma</b>	0.01	2.5	1.2-4.9
<b>Clinical severity score <math>\geq 5</math></b>	0.90	1.0	0.5-1.8
<b>C reactive protein &lt; 0.8 mg/dl</b>	0.08	1.7	0.9-2.9
<b>Eosinophils &gt; 400 cells/<math>\mu</math>L</b>	0.30	2.2	0.4-11.6
<b>Absence of lung consolidation</b>	0.04	2.6	1.1-6.1
<b>Rhinovirus</b>	0.05	3.3	1.0-11.1

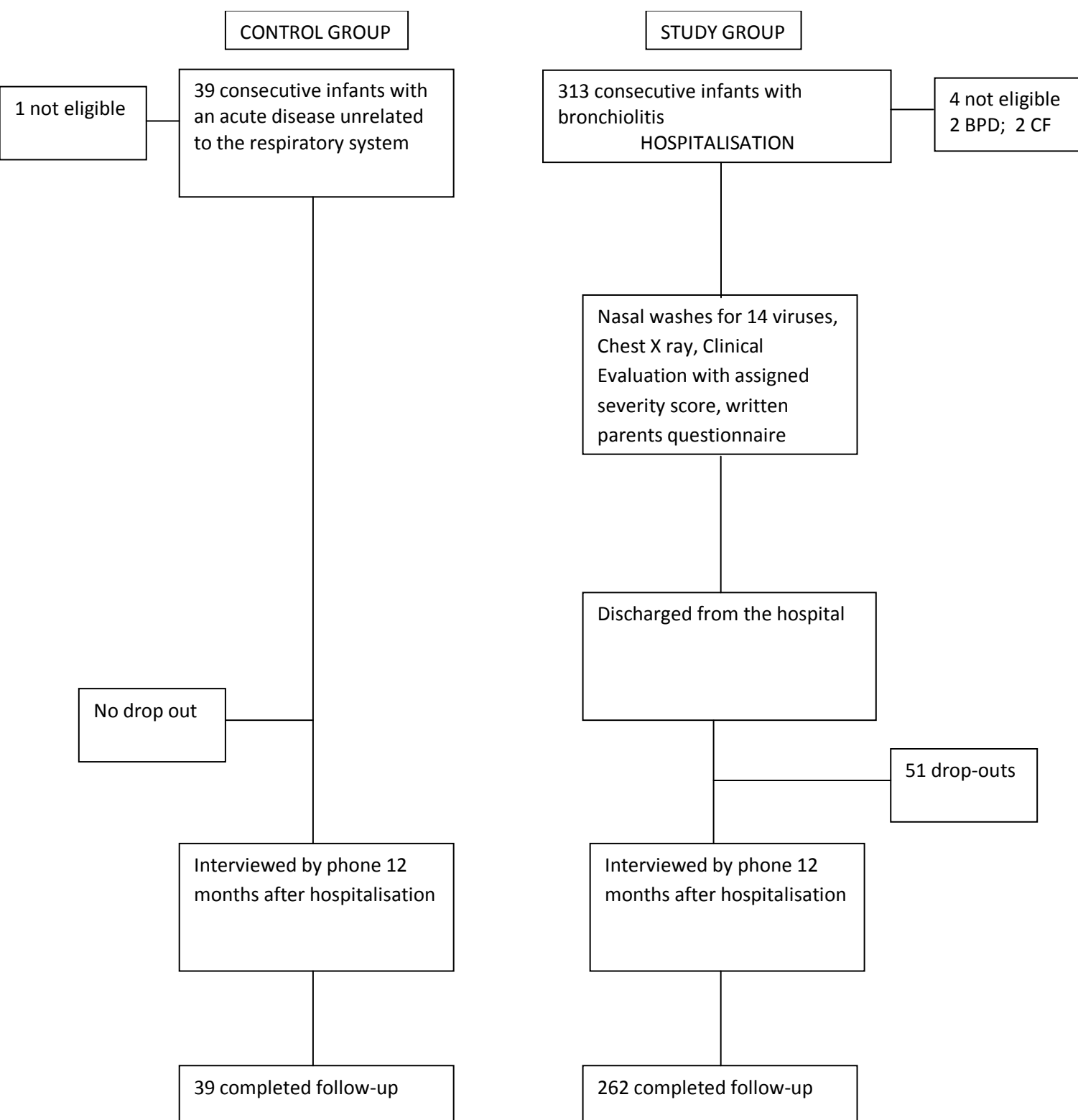


Figure 1. Study Flow Chart

BPD = Bronchopulmonary dysplasia; CF=Cystic fibrosis