

REGULAR ARTICLE

Recurrent wheezing 36 months after bronchiolitis is associated with rhinovirus infections and blood eosinophilia

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ABSTRACT

Aim: Links between respiratory syncytial virus bronchiolitis and asthma are well known, but few studies have dealt with wheezing following bronchiolitis induced by other viruses. We assessed the risk factors for recurrent wheezing in infants hospitalised for acute viral bronchiolitis.

Methods: We followed 313 infants for three years after they were hospitalised for bronchiolitis, caused by 14 different viruses, to identify risk factors for recurrent wheezing. Parents provided feedback on wheezing episodes during telephone interviews 12 (n = 266), 24 (n = 242) and 36 (n = 230) months after hospitalisation.

Results: The frequency of wheezing episodes diminished during the follow-up period: 137 children (51.7%) at 12 months, 117 (48.3%) at 24 months and 93 (40.4%) at 36 months. The risk of wheeze after three years was OR = 7.2 (95% CI 3.9–13.3) if they had episodes of wheezing during the first year after bronchiolitis, 16.8 (8.7–32.7) if they had episodes of wheezing during the second year and 55.0 (22.7–133.2) if they wheezed during both years. Blood eosinophils >400 cells/ μ L (OR 7.7; CI 1.4–41.8) and rhinovirus infections (3.1; 1.0–9.4) were the major risk factors for recurrent wheezing.

Conclusion: Recurrent wheezing 36 months after infant bronchiolitis was associated with rhinoviruses and blood eosinophilia.

INTRODUCTION

Bronchiolitis is a clinically diagnosed condition that is often associated with long-lasting sequelae in children. Around 40–50% of infants requiring hospital admission for bronchiolitis will have recurrent wheezing episodes in the first years of life (1,2).

As viral diagnostic procedures have advanced, there is increasing evidence about how rhinoviruses contribute to bronchiolitis. Rhinoviruses are now the major aetiological agents for acute respiratory infections in nonhospitalised infants with, and without, risk factors for atopic sensitisation (3,4) and for acute wheezing and asthma exacerbation in children. Furthermore, rhinovirus infections during the first year of life increase the risk of recurrent wheezing and asthma in unselected infants and in infants with risk factors for atopic sensitisation (5–10).

Most of the studies published to date have analysed the relationship between respiratory syncytial virus (RSV) bronchiolitis and wheezing and asthma. Few studies have

addressed the relationship between bronchiolitis induced by viruses other than RSV and wheezing and asthma (11–13).

In 2012, we published a study that prospectively followed 313 infants for 12 months after they had been hospitalised for bronchiolitis caused by different respiratory viruses when they were less than one year of age (14). We found that, 12 months after hospitalisation, infants in the recurrent wheezing groups were more likely to have a family history of asthma, blood eosinophilia (>400 cells/mL) and a

Key notes

- Links between respiratory syncytial virus bronchiolitis and asthma are well known, but few studies have dealt with wheezing following bronchiolitis induced by other viruses.
- This study evaluated risk factors for recurrent wheezing three years after 313 infants were hospitalised for bronchiolitis caused by 14 respiratory viruses.
- Blood eosinophilia and rhinoviruses detected during bronchiolitis were significant risk factors for the development of recurrent wheezing in the three years after hospitalisation.

Abbreviation

CI, Confidence interval; OR, Odds ratio; RSV, Respiratory syncytial virus.

rhinovirus infection than children who did not have wheezing. Multivariate analysis showed that having a rhinovirus infection and a family history of asthma were major independent risk factors for recurrent wheezing 12 months after hospitalisation.

This prospective, single-centre study builds on that research. It was primarily designed to assess the risk factors for recurrent wheezing during a three-year follow-up in a well-characterised homogeneous cohort of infants hospitalised for acute bronchiolitis. Our latest study focused specifically on RSV and rhinoviruses.

PATIENTS AND METHODS

Patients

We prospectively followed up 313 infants hospitalised for bronchiolitis, without concomitant chronic diseases, from October 2004 to May 2008 by the Paediatric Emergency Department, Sapienza University, Rome, for three consecutive years. The infants had a median age of two months, with a range of seven to 11 months, and 170 were (54%) males. At the third year of follow-up, the children's median age was 3.2 years with a range of 36.2 to 47 months. Bronchiolitis was defined as the first episode of acute lower respiratory 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 (15).

Methods

During the infants' hospitalisation, we obtained data on clinical symptoms and demographic factors, using a structured questionnaire and patients' medical files. When they were admitted to hospital, each infant was assigned a

clinical severity score as previously described (15). A total of 14 respiratory viruses were detected through the nasal wash specimens by real-time polymerase chain reaction and nested polymerase chain reaction. These were: RSV, influenza virus A and B, human coronavirus OC43, 229E, NL-63, HUK1, adenovirus, rhinovirus, parainfluenza virus 1–3, human bocavirus and human metapneumovirus (16).

The parents were interviewed 12, 24 and 36 months after their child was discharged, with the aid of a questionnaire, to gather information on possible wheezing episodes (14). Wheezing episodes were classified according to the Ly et al. (17) classification. Recurrent was defined as two or more physician verified episodes of wheezing a year for three consecutive years, and occasional was defined as two or more physician verified episodes of wheezing during the three-year follow-up period. The main outcome was the established risk factors for recurrent wheezing during the three-year follow-up.

Statistical analysis

The main outcomes in this post hoc analysis were two or more episodes of wheezing during the follow-up period after bronchiolitis versus the demographic and clinical variables studied. Data are presented as mean, standard deviation, median, range and as percentages with categorical variables. Data for clinical and demographic characteristics (Table 1) were tested with a three-way analysis of variance (ANOVA), followed by a Bonferroni correction post hoc test when applicable. The chi-square test was used to analyse categorical independent variables. Multivariate logistic regression models were used to assess the risk factors for recurrent wheezing during the three-year follow-up after the first episode of acute bronchiolitis. Only variables with $p < 0.15$ in the univariate analysis

Table 1 Clinical and demographic characteristics of infants with, and without, wheezing during a three-year follow-up after bronchiolitis

Variables studied	No wheezing	Occasional wheezing	Recurrent wheezing	p-value
Subjects n	85	74	71	
Males*	49.4	55.4	62	0.29
Caesarean section*	51.8	56.2	54.9	0.85
Birth weight in kg	3.2 ± 0.5	3.0 ± 0.5	3.2 ± 0.5	0.04
Age at recovery in months	2.4 (0.23–11)	2.0 (0.37–11)	2.6 (0.37–10.2)	0.14
Weight at recovery in kg	5.0 ± 1.5	4.9 ± 1.4	5.4 ± 1.7	0.10
Breast feeding at admission*	34.9	31.0	34.1	0.48
Eczema*	5.9	2.7	5.7	0.60
Family history of asthma*	14.1	27	19.7	0.13
Family history of atopy*	29.4	35.1	32.4	0.74
Exposure to smoking*	41.7	38.4	47.8	0.51
Clinical severity score	4.2 ± 2.4	4.6 ± 2.3	3.7 ± 2.5	0.08
Days of hospitalisation	5.3 ± 2.2	5.7 ± 2.4	4.7 ± 2.0	0.03
Eosinophils >400 cells/μL*	1.2	1.4	11.3	0.003
CRP <0.8 mg/dL*	53.6	58.3	66.2	0.28
Absence of lung consolidations*	6.9	14.5	23.3	0.03
Radiological air trapping*	40.3	40.6	30	0.38

Data are expressed as % of positive cases (*), mean ± SD or median (range), unless otherwise stated. CRP, C-reactive protein. p-values are not corrected for multiple testing. Data were tested by ANOVA.

were included in the multivariate model. Results from the logistic models were described by odds ratios (OR) and 95% confidence intervals (CI). p -values < 0.05 were considered to indicate statistical significance. Data were analysed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

The parents of all infants consented to their enrolment in the follow-up study and gave written informed consent. The Ethics Committee at the Policlinico Umberto I hospital, Sapienza University, Rome, approved the study.

RESULTS

We contacted 313 families and asked them to take part in a telephone interview. Of these, 266 (85%) agreed in the first year, 242 (77%) agreed in the second year, and 230 agreed (73%) in the third year (Appendix S1). The only reason for the dropout during the three-year follow-up was a change in telephone numbers. Of the 230 families who answered the questionnaire during the third year of follow-up, 71 (30.9%) children had recurrent wheezing, 74 (32.2%) had occasional wheezing, and 85 (36.9%) had no wheezing. No differences were found in the clinical and demographic variables between the 83 infants who dropped out at follow-up and the 230 whose parents answered the phone calls when the three-year follow-up ended. Of these 230 children, 99 (42.9%) were infected with RSV, 20 (8.6%) with a rhinovirus, 19 (8.2%) with human bocavirus, four (1.7%) with human metapneumovirus, three (1.3%) with parainfluenza virus 3, one (0.4%) with parainfluenza virus 1, one (0.4%) with influenza virus A and one (0.4) with human coronavirus. Ten (4.3%) infants had RSV and were co-infected with the human bocavirus, one had RSV and the parainfluenza virus 3, and one had RSV and a rhinovirus. One of the infants with human metapneumovirus positive was co-infected with the human bocavirus, and another was co-infected with a rhinovirus. No viruses were detected in the nasal wash specimens from 96 infants (41.7%). We were only able to sequence and genotype nine rhinovirus-positive samples, and this showed that seven were positive for rhinovirus A and two for rhinovirus C.

When we assessed the OR for wheezing three years after acute bronchiolitis, this indicated an increasing risk of the 140 infants who only wheezed in the first year (OR 3.9; 95% CI 7.2–13.3), the 115 who only wheezed in the second year (OR 16.8; 95% CI 8.7–32.7) and the 112 infants who wheezed in both the first and second year (OR 55; 95% CI 22.7–133.2). When we analysed the percentage of children with recurrent wheezing, occasional wheezing and no wheezing, according to the virus identified during acute bronchiolitis, we found that most of the infants with rhinovirus bronchiolitis had recurrent wheezing, followed by infants with occasional wheezing, and without wheezing ($p = 0.008$). In contrast, most of the children who had RSV bronchiolitis as infants never had wheezing episodes, followed by children who had occasional wheezing and recurrent wheezing ($p = 0.02$). No

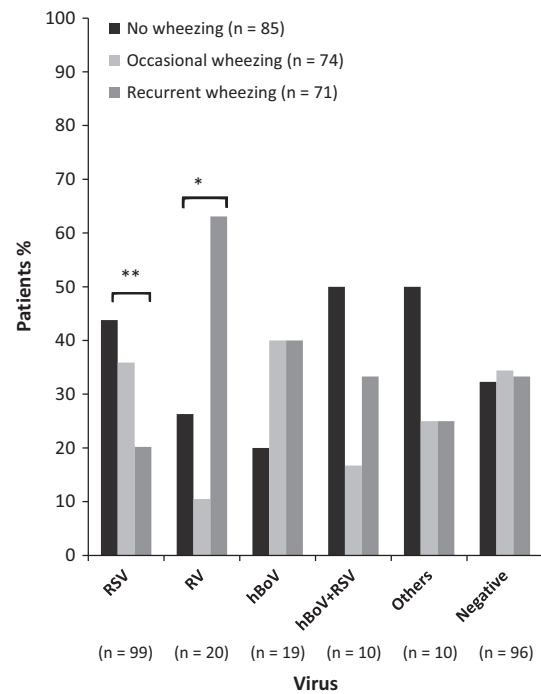


Figure 1 Percentage of children with no wheezing, occasional wheezing and recurrent wheezing at three years of follow-up, according to the virus identified during bronchiolitis. RSV: respiratory syncytial virus; RV: rhinovirus; hBoV: human bocavirus; * $p < 0.008$ ** $p < 0.02$.

significant differences were observed when other viruses were studied (Fig. 1).

We found no significant differences in the demographic variables between the groups (Table 1). However, when we compared the clinical characteristics, the percentage of infants with an absolute number of blood eosinophils >400 cells/ μ L was significantly higher in infants with recurrent wheezing than in those with occasional wheezing and with no wheezing ($p < 0.003$).

Other important differences we observed were the clinical severity score at hospital admission and the number of days of hospitalisation (Table 1). In particular, children with recurrent wheezing had a lower clinical severity score ($p = 0.08$) and shorter hospitalisation ($p = 0.03$) than children with occasional wheezing and without wheezing. Another significant difference was the chest X-ray findings, as the number of children without lung consolidations differed significantly between children with recurrent wheezing, occasional wheezing and without wheezing ($p = 0.03$). Chest X-ray consolidations were more frequent in children without wheezing than in those with occasional and recurrent wheezing (Table 1).

The multivariate logistic model that we used to investigate the risk factors for infants with recurrent wheezing versus infants with no wheezing and occasional wheezing identified blood eosinophils >400 cells/ μ L as the major independent risk factor, followed by rhinovirus infections (Table 2).

Table 2 Univariate (a) and multivariate (b) model for risk factors associated with recurrent wheezing during a three-year follow-up after bronchiolitis

(a) Univariate analysis

Variables	No wheezing + occasional wheezing	Recurrent wheezing	p-value
Eosinophils >400 cells/ μ L*	1.3	11.3	0.001
Family history of asthma*	14.1	19.7	0.12
Rhinovirus*	5.0	16.9	0.003
Absence of lung consolidations*	10.6	23.3	0.02
Age at recovery in months	2.2 \pm 2.0	2.6 \pm 2.1	0.15

(b) Multivariate analysis

Variables	p-value	OR	95% CI
Eosinophils >400 cells/ μ L	0.01	8.4	1.6–44.5
Family history of asthma	0.85	1.1	0.5–2.4
Rhinovirus	0.03	3.2	1.1–9.6
Absence of lung consolidations	0.14	1.9	0.8–4.6
Age at recovery in days	0.15	1.1	0.9–1.3

Variables in the equation

B	S.E.	Wald	Df	Sign	Exp(B)
0.85	0.15	30.15	1	0.001	1.54

Data are expressed as % of positive cases (*), mean \pm SD, unless otherwise stated. No wheezing plus occasional wheezing in 159 infants and recurrent wheezing in 71 infants.

DISCUSSION

In our previous study, the one-year follow-up showed that infants with bronchiolitis induced by rhinoviruses had a higher risk of developing recurrent wheezing than children with RSV bronchiolitis and other respiratory viruses. The current three-year follow-up study confirms our previous findings, showing that rhinovirus infections are the major risk factor for developing recurrent wheezing three years after bronchiolitis. We were also able to show that more children with recurrent wheezing have blood eosinophil concentrations >400 cells/mm³, fewer lung consolidations on chest X-rays and less severe bronchiolitis than children with occasional wheezing or no wheezing.

When we planned this study, we took special care to avoid the design drawbacks that had made the results of other authors hard to interpret. First, we prospectively monitored a cohort of infants with strictly defined bronchiolitis who were all <12 months of age, with diffuse crackles at chest auscultation. Previous studies that evaluated the correlation between bronchiolitis and asthma in infants defined bronchiolitis as a wheezing episode in infants younger than 24 months (18–20). Our decision to study a homogeneous group of infants with a restricted clinical diagnosis of bronchiolitis allowed us to exclude infants with various clinical entities, such as the first wheezing episode. In addition, we simultaneously studied the presence of 14 respiratory viruses, whereas most of the previous studies on the correlation between bronchiolitis and wheezing only enrolled infants with RSV bronchiolitis, during acute bronchiolitis (11,21). The simultaneous evaluation of 14 different respiratory viruses allowed us to

compare, for the first time, the influence of each virus on the development of recurrent wheezing after bronchiolitis.

A distinctive finding of this study was that 40.4% of children aged 3.2 years had recurrent wheezing, confirming Sigur et al.'s findings that 40% of infants had wheezing three years after RSV bronchiolitis (22). Although the association between bronchiolitis and wheezing is well established, some evidence demonstrates that RSV bronchiolitis not only identifies infants at risk for subsequent wheezing, it can also contribute directly to wheezing causation (23).

As reported in our previous study (14), we can confirm that the rhinovirus was the only one of the 14 respiratory viruses tested that was recognised as a risk factor for recurrent wheezing three years after hospitalisation for bronchiolitis (24,25). We observed no correlation between RSV bronchiolitis and bronchiolitis caused by other respiratory viruses and subsequent wheezing episodes. A major study by Sigurs et al. followed 47 infants hospitalised for RSV bronchiolitis and 93 control children for 18 years (24). Their definition of bronchiolitis was similar to ours, but it only included infants with RSV infections. It found a strong correlation between RSV infection and wheezing after a three-year follow-up. However, if their study was appropriately designed, the main result of the correlation between RSV bronchiolitis and wheezing might be expected, because acute bronchiolitis on its own is a well-known risk factor to develop wheezing, regardless of the viral aetiology.

In a more recent study, Valkonen et al. showed that children younger than 24 months, who were hospitalised for bronchiolitis caused by viruses other than RSV,

experienced substantially higher rates of recurrent wheezing during a three-year follow-up than children with RSV bronchiolitis (25). By defining bronchiolitis as a wheezing episode in infants younger than 24 months, they might have included infants with wheezing, but they were unable to detect other respiratory viruses in infants with non-RSV bronchiolitis. In contrast, when Koponen et al. (26) looked at a cohort of infants aged less than six months hospitalised for bronchiolitis, they found that the risk of asthma was lower after RSV bronchiolitis than after bronchiolitis caused by other respiratory viruses. The study retrospectively analysed the presence of nine viruses during acute bronchiolitis, but no single virus was predominant in the non-RSV group with current asthma. To our knowledge, our study is the first to simultaneously compare 14 respiratory viruses and only show a correlation between rhinovirus bronchiolitis and recurrent wheezing at the age of three years. These findings confirm previous reports that rhinovirus infections in the first year of life are associated with wheezing and asthma (27). If other studies confirm our latest findings, the major viruses that contribute to the development of recurrent wheezing after bronchiolitis might be rhinoviruses rather than RSV. Further studies are needed to clarify this important finding.

One clinically important difference that we observed between the three groups in our study was the significantly higher numbers of blood eosinophil in infants with recurrent wheezing. Other studies on bronchiolitis also found that blood eosinophilia was a risk factor for the development of persistent wheezing and asthma (28,29). This finding agrees with Ehlenfield et al.'s conclusion that eosinophilia during bronchiolitis predicts the development of persistent wheezing later in childhood (30).

When we analysed other clinical characteristics in our children, we identified two important differences between the groups. First, children with recurrent wheezing had fewer chest X-ray consolidations than children with occasional wheezing and without wheezing, and this finding confirmed our previous study on recurrent wheezing one year after bronchiolitis (14). Second, children with recurrent wheezing had less severe bronchiolitis than infants with occasional wheezing and without wheezing. Unfortunately, our findings in this study prevent us from determining whether the severity of the disease really influences the development of wheezing, because we only studied infants who had been hospitalised for severe bronchiolitis and we have no data about the outcome of mild bronchiolitis in infants who were not hospitalised.

Possible limitations of our study include the fact that it included a much smaller number of infants with rhinovirus bronchiolitis and other respiratory viruses than infants with RSV bronchiolitis. In addition, we could not confirm whether parents provided reliable information during the telephone interview, and we only asked whether the child presented more than two wheezing episodes in the past year without specifying the exact number of episodes. Another limitation was the poor viral detection rate already discussed in our previous paper. Finally, we could not test all

the rhinoviruses detected for rhinovirus subtypes, in particular type C.

In conclusion, our results showed that rhinovirus infections significantly predicted recurrent wheezing development in a cohort of infants with bronchiolitis followed for three years. A major goal for future research efforts should, therefore, be to define host and viral factors that promote wheezing after bronchiolitis in infants.

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CONFLICT OF INTEREST

None declared.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Study flow chart