RESEARCH ARTICLE

RSV-hRV co-infection is a risk factor for recurrent bronchial obstruction and early sensitization 3 years after bronchiolitis

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To assess risk factors of recurrent bronchial obstruction and allergic sensitization 3 years after an episode of acute bronchiolitis, whether after ambulatory care treatment or hospitalization. A monocentric prospective longitudinal study including infants aged under 1 year with acute bronchiolitis was performed, with clinical (severity score), biological (serum Krebs von den Lungen 6 antigen), and viral (14 virus by naso-pharyngeal suction detection) assessments. Follow-up included a quaterly telephone interview, and a final clinical examination at 3 years. Biological markers of atopy were also measured in peripheral blood, including specific IgEs towards aero- and food allergens. Complete data were available for 154 children. 46.8% of them had recurrent wheezing (RW). No difference was found according to initial severity, care at home or in the hospital, respiratory virus involved, or existence of co-infection. A familial history of atopy was identified as a risk factor for recurrent bronchial obstruction (60% for RW infants versus 39%, $P = 0.02$), as living in an apartment (35% versus 15%, $P = 0.002$). 18.6% of the infants were sensitized, with 48.1% of them sensitized to aeroallergens and 81.5% to food allergens. Multivariate analysis confirmed that a familial history of atopy ($P = 0.02$) and initial co-infection RSV-hRV ($P = 0.02$) were correlated with the risk of sensitization to aeroallergens at 3 years. Familial history of atopy and RSV-hRV co-infection are risk factors for recurrent bronchial obstruction and sensitization.

KEYWORDS
bronchiolitis, prospective study, recurrent bronchial obstruction, sensitization, virus

1 | INTRODUCTION

Bronchiolitis is the most common cause of lower respiratory infection in the first year of life. Although most children with bronchiolitis have mild disease and are managed at home, infants admitted to hospital for the condition almost always have severe symptoms.[1,2] The clinical relevance of the identification of the specific pathogens or combination of pathogens infecting a child remains unclear. However, children with human rhinovirus (hRV) may have different short- and long-term outcomes[3-8] compared to children with respiratory syncytial virus (RSV). We showed that clinical severity was not correlated with the level of serum Krebs von den Lungen 6 antigen (KL-6), a biomarker of epithelial dysfunction, in previously healthy children,[9] which indicates that the various clinical outcomes depend more on the adaptive capacities of the host rather than the intensity of epithelial dysfunction. Prediction of the risk of recurrent wheezing or long-term asthma from the first year of life can be challenging.[10] Against this background, we decided to prospectively follow a cohort of infants with an early episode of acute bronchiolitis, either hospitalized or cared for at home. Our main objective was to assess the risk factors for developing symptoms of recurrent bronchial obstruction during a 3-year follow-up after an episode of bronchiolitis in the first year of

life. The secondary objective was to identify in this cohort the risk factors associated with allergic sensitization at the age of 3.

2 | PATIENTS AND METHODS

Consecutive infants with acute bronchiolitis, aged under 1 year, and examined in the Pediatric Emergency Department during one epidemic season, from October 2011 to May 2012, were considered for inclusion.

The clinical, biological, and radiological parameters collected at inclusion have been described elsewhere.[9] Briefly, bronchiolitis was diagnosed as recommended in the international guidelines on the diagnosis and management of acute bronchiolitis.[11].

Detailed demographic data were obtained from the parents by a structured questionnaire. Studied variables comprised age, gender, exposure to tobacco smoke, familial (parents or sister or brother), or personal history of atopy, and nursery attendance.

Each infant's condition was classified as mild, moderate or severe according to a severity score calculated from SpO2, respiratory rate, and respiratory effort on admission.[12]

Patients were admitted to hospital (inpatient group) on the basis of French national guidelines.[13] Infants who did not require hospitalization (outpatient group) were cared for at home.

Children were excluded if they had suspected or confirmed underlying chronic diseases (ie, cystic fibrosis, chronic pulmonary disease, congenital heart disease, bronchopulmonary disease, prematurity), or had already had more than one wheezing episode.

A nasopharyngeal aspirate was collected either in the emergency room or within the first 24 h following admission to detect respiratory viruses. The methodology of detection of viruses and dosage of KL-6 are detailed in the study by Amat et al[9]

2.1 | Follow-up

After discharge, one of the authors conducted a telephone interview of the parents of each child, every 3 months until the age of 3 years. A standardized questionnaire designed to elicit information on respiratory symptoms was used for the interview. A complete medical check-up by a pediatric pulmonologist was then performed at the Outpatients Department of CHU-Estaing at the age of 3 years. Recurrent bronchial obstruction was defined according to the frequency of the following symptoms: tachypnoea, wheezing, expiratory stridor, respiratory chest retractions, and doctor-diagnosed wheezes. If ≥3 respiratory symptoms were documented ≥2 times, or if such an episode lasted ≥4 weeks, the subject was classified as suffering from recurrent bronchial obstruction.[14]

2.2 | IgE-sensitization assessment

2.2.1 | In vitro tests

Immunological profile was performed at the age of 3 years at the same time as the medical check-up.

2.3 | Screening of IGE-sensitized patients

Sensitive diagnostic orientation tests (DOTs), based on a fluorescence immuno-enzymatic assay (FEIA) method, were used according to the manufacturer's guidelines to discriminate IgE-sensitized from non-IgE-sensitized children Phadiatop infant® and fx5® (ImmunoCAP®, Phadia 250®, Thermo Fisher Scientific®, Phadia AB, Uppsala, Sweden). The tests provide a semi-quantitative result, negative or positive, associated with a PAU/L value for Phadiatop infant®, and kU/L value for fx5®. The quantitative range for IgEs spans from 0.1 to 100 PAU/L (or kU/L). In this study, a 0.35 cut-off PAU/L, or kU/L was retained as the threshold of positivity of the DOTs. On the basis of this positive or negative result, children were classified into a sensitized patient group or a non-sensitized patient group.

2.4 | Determination of sensitization profile

All sensitized patients underwent an additional analysis with the ImmunoCAP ISAC 112® (Phadia AB) extensive molecular profile test. The measuring range spans from 0.3 to 100 ISU, with 0.3 ISU taken as the threshold of positivity.

Sensitization to food allergens was also specifically studied with IgE ImmunoCAP (Phadia 250®, Phadia AB) against the constituent allergens of the fx5® mix (hen's egg, cow's milk, fish, wheat, peanut, soybean), with the addition of hazelnut). For unitary allergens, ImmunoCap Phadia 250® provides a quantitative result ranging from 0.1 to 100 kUA/L. In this study, 0.1 kUA/L was considered as the threshold of positivity.

Multisensitization was defined if tests were positive for at least two allergens.

2.5 | Statistical analysis

The primary endpoint was the risk of recurrent bronchial obstruction at the age of 3 years after an early episode of acute bronchiolitis.

The secondary endpoint was the risk of sensitization to aeroallergens and/or to food allergens at the age of 3 years.

All analyses were performed with Stata software (version 12, StataCorp, College Station, TX). All tests were two-sided and a P-value <5% was considered statistically significant. The study population was described by frequencies and associated percentages for categorical data, and by mean ± standard-deviation (SD) or median and inter-quartile ranges (IQR) for continuous data, according to the distribution from normality. Univariate analysis of recurrent bronchial obstruction at the age of 3 years was carried out with Chi-squared test for categorical data (or Fisher's exact test when appropriate), Student's t-test for continuous data, or with the Mann-Whitney-test when normality was rejected (using distribution plots and Shapiro-Wilk's test). Multivariable logistic regression was then performed adjusted for clinically relevant and statistically significant factors. Results are shown as adjusted odds ratio (OR) and their 95% interval (95%CI). Sensitization to aeroallergens was analyzed with the same methods as for recurrent bronchial obstruction.

2.6 | Ethics

All the infants' parents gave written informed consent for their child to participate. The study was approved by the local Ethics Committee (Comité de Protection des Personnes Sud-Est I, Saint-Etienne, France) and was posted on Clinical Trials (www.clinicaltrials.gov) under the ID number NCT01437956.

3 | RESULTS

Two hundred and twenty-two children were initially recruited. Complete data after the 3-year follow-up were available for 154 children, aged 3.4 ± 0.3 years. The main reason for drop-out was a change in telephone number. Children lost to follow-up did not differ significantly from others regarding initial hospitalization, gender, type of virus, birth term, serum KL6 level, initial score of severity, and age at inclusion.

3.1 | Results on recurrent wheezing (Table 1)

A total of 46.8% children had a diagnosis of recurrent bronchial obstruction at the age of 3. According to univariate analysis, they did not significantly differ from children who had not, except for familial history of atopy (60% versus 39%, respectively, P = 0.02), and living in an apartment (35% versus 15%, respectively, P = 0.002). No difference was found according to the kind of respiratory virus involved, nor to the existence or not of co-infection. Table 1

According to multivariate analysis, we did not either find any relationship between initial severity (clinical score, level of KL-6, hospitalization or not), the type of viruses and the occurrence of recurrent bronchial obstruction.

A familial history of atopy was identified as a risk factor for recurrent bronchial obstruction (OR 2.34, 95%CI [1.09-5.03], P = 0.03). Living in a house seemed to play a protective role compared to living in an apartment (OR 0.22, 95%CI [0.08-0.59], P = 0.003).

3.2 | Sensitization profiles

Nine parents objected to the blood test. DOTs identified 18.6%[27] sensitized patients at the age of 3:13 were sensitized to aeroallergens, 22 to food allergens, and 3 to both. The median rate of IgEs was higher for patients sensitized to aeroallergens (0.83, IQR [0.2-5.85]) than for patients sensitized to food allergens (0.21, IQR [0.13-0.52]).

TABLE 1 Baseline characteristics of children according to the recurrent bronchial obstruction outcome at the age of 3

	Recurrent bronchial obstruction (n = 72)	No recurrent bronchial obstruction (n = 82)	P-value
Male gender	44 (61)	42 (51)	0.22
Birth weight (kg)	3.2 ± 0.5	3.2 ± 0.5	0.45
Initial severity score			0.31
Mild	27 (38)	23 (28)	
Moderate	29 (40)	33 (40)	
Severe	16 (22)	26 (32)	
Inpatients group	55 (76)	64 (78)	0.82
Serum KL6 level (IU/mL)	255 ± 109	227 ± 162	0.12
Familial history of atopy	**37 (60)**	**29 (39)**	**0.02**
Exposure to tobacco smoke at home	32 (49)	33 (51)	0.60
Exposure to tobacco smoke in utero	18 (25)	16 (20)	0.41
Atopic dermatitis at 3years examination	7 (10)	8 (10)	0.99
Living in an apartment	**25 (35)**	**12 (15)**	**0.002**
RSV	51 (72)	66 (80)	0.21
hRV	20 (28)	24 (29)	0.88
Influenzae virus	0 (0)	1 (1)	1
Human metapneumovirus	6 (8)	6 (7)	0.80
Adenovirus	8 (11)	6 (7)	0.40
Bocavirus	5 (7)	9 (11)	0.40
Coronavirus	7 (10)	8 (10)	0.98
Parainfluenzae virus	5 (7)	1 (1)	0.10
Picornaviridae	20 (28)	26 (32)	0.63
Co-infection	24 (34)	30 (37)	0.72

Categorical variables expressed as n and % and continuous variables as mean ± standard deviation. Boldfaced text indicates significance (P < 0.05). RSV = respiratory syncytial virus, hRV = human rhinovirus, hMP = human metapneumovirus.

3.3 | Sensitization to aeroallergens

Additional analysis for patients with positive Phadiatop® using the ISAC extensive molecular profile test showed that four were mono-sensitized and nine were sensitized to two or more aeroallergens. The most frequently involved allergens were house dust mites (8/16), followed by pollens (4/16), and animal danders (3/16).

The type of virus involved in the first episode of bronchiolitis was significantly associated with sensitization to aeroallergens at the age of 3 (P = 0.021). The frequency of sensitization to aeroallergens was lower in case of initial infection by hRV (P = 0.02) whereas RSV-hRV co-infection was associated with a higher frequency of sensitization to aeroallergens compared to any mono-infection (18% of sensitization with co-infection versus 5.3% without, P = 0.019).

Nine children with positive ISAC test had a familial history of atopy, which was significantly associated with a higher risk of sensitization to aeroallergens at the age of 3 (16% with a history versus 4.3% without, P = 0.021).

Multivariate analysis confirmed that familial history of atopy (OR = 5.12, 95%CI [1.33-19.65], P = 0.02) and initial RSV-hRV co-infection (OR = 3.88, 95%CI [1.24-12.1], P = 0.02) were correlated with the risk of sensitization to aeroallergens at 3 years.

3.4 | Sensitization to food allergens

Additional tests for the 25 patients with positive fx5® using IgE ImmunoCAP showed that 14 (56%) were multisensitized to food allergens. All of them were sensitized to hen's egg and cow's milk, six were sensitized to wheat, two to peanut or hazelnut, and one child was sensitized to fish. However, these sensitizations were quantitatively low, most of them being inferior to 1 kU/L.

4 | DISCUSSION

We feel that our study sample is more closely representative of whole patients population since most infants suffering from acute bronchiolitis are cared for at home and only 1-10% need to be hospitalized. Yet, most other studies have involved infants admitted to hospital, which introduces significant selection bias that we considered important to avoid. This distinction between infants hospitalized or managed at home can partly explain why our results differ from those of other similar studies.

The first point to emerge from the results of this prospective study is the high frequency of recurrent bronchial obstruction 3 years after the initial episode, about 50%, which is in line with that in other documented reports.[4-7]

In contrast, unlike in many publications, we did not find any correlation between the risk of recurrent bronchial obstruction, initial severity, virus type, and serum KL-6 level. In the study of Nenna et al[15] higher RSV-RNA load and higher interferon λ2/3 levels were found in children with recurrent wheezing at 36 months of follow-up. However, Koponen et al[16] evaluated the outcome of infants hospitalized for bronchiolitis under 6 months who underwent a control visit at 6.5 years. The risk for asthma was lower after RSV bronchiolitis than after bronchiolitis caused by other viruses.

4.1 | Risk of recurrent bronchial obstruction at 3 years according to initial viral infection

Falkenstein-Hagander et al[17] have evaluated the virological features of children hospitalized for acute respiratory distress during a winter season in Sweden. RSVA/B was the most common (50.9%), followed by entero/rhinovirus (21.6%). RSV dominated as the viral cause of respiratory distress. Since now, the role of RSV-hRV co-infection in bronchiolitis severity is not so clear, may be due to discrepancies in the detection of hRV. For instance, in a study from Miller et al[18] most HRV-C co-detections are with RSV. But finally in a larger study HRVs were statistically the least likely virus of 17 examined to be associated with co-infections.[19] However, Costa et al[20] showed that severe disease in hRV infections were caused mainly by presence of RSV in co-infections, in accordance with other results from Yoshida et al.[21] These results could suggest indirect evidence of a higher risk of subsequent asthma in infants RSV-hRV coinfected, as children with severe bronchiolitis during infancy have an increased early childhood asthma morbidity.[22] Our results, including outpatients and hospitalized children with both documented viral infections, may bring new insights about the role of RSV-hRV coinfection regardless of initial severity of bronchiolitis.

4.2 | Role of atopic background

Backman et al[23] evaluated the association of early-childhood risk or protective factors for asthma and lung function reduction in adults 30 years after bronchiolitis (47 cases) in infancy. Low blood eosinophil count on admission was a significant protective factor and high blood eosinophil count during convalescence was a significant risk factor for asthma in adulthood. Recent studies have often reported increased frequency of asthma in cases of acute bronchiolitis due to rhinoviruses.[4-7,24,25] Balekian et al[26] observed that the frequency of severe bronchiolitis and childhood asthma was similar to that in other documented reports. Among the children with severe bronchiolitis, 27.6% developed asthma by the age of 5 years.

We observed a relationship between the occurrence of recurrent bronchial obstruction and the existence of an atopic or asthmatic family, as often reported in the literature. Midulla et al[4] identified rhinovirus infection and a positive familial history for asthma as major independent risk factors for recurrent wheezing. The same team published results at 3 years.[27] Blood eosinophils >400 cells/μL and rhinovirus infections were the major risk factors for recurrent wheezing. Holt and Sly[28] identified viral infections occurring against a background of allergic sensitization to aeroallergens as a potent risk factor for the development of asthma that can persist through childhood and adulthood.

4.3 | Role of urban and rural environment

The risk of recurrent bronchial obstruction is greater for children living in an apartment than for those living in a house. Those were not living in apartment reside in rural area or in farm. This finding can be explained by protective factors related to the type of farm housing common in our region and by more common concentrations of pollutants of all types in children living in apartments.[29–31] Parsons et al[32] examined the effect of living in a farm environment on asthma incidence in children. A total of 10 941 children aged 0-11 years who were free of asthma and wheeze at the baseline (1994-1995) were included. The 14-year cumulative incidence of asthma among children living in farming environments was 10.18%, which was significantly lower than that observed for children living in rural non-farming (13.12%) and non-rural environments (16.50%). This cohort study provides further evidence that living in a farming environment during childhood protects against asthma in adolescence and adulthood and provides further support for the hygiene hypothesis.[33,34] However, we need to analyze our results on the environment carefully, as we analyzed respiratory outcome only after 3 years and throughout childhood or adulthood.

4.4 | Risk or allergic sensitization

Allergic sensitization is found in 16% of patients and 21.6% of recurrent wheezers are sensitized, which is unsurprising given the young age of the population concerned and the lack of sensitivity of ISAC technique against food allergens. Most patients are not sensitized to food or aero-allergens. However, sensitized patients tend to be multisensitized. It is difficult to compare our results regarding allergic sensitization with those of other published works. For Nuolirvita et al,[35] current atopy is considered if the child has either allergic rhinitis or atopic dermatitis. Other authors base the diagnosis of atopy on skin prick tests with or without association with physician diagnosis of allergy.[16,36,37]) A co-infection of RSV and rhinovirus associated with an atopic predisposition promotes awareness of sensitization to aeroallergens. This finding is in agreement with a study suggesting that hRV plays a major role in triggering allergy. Two immunologic factors, low interferon responses and indicators of atopy (eosinophilia, allergen-specific IgEs), are closely associated with susceptibility to hRV-bronchiolitis.[38] Perez et al[39] have discussed the evidence for the presence of abnormal innate antiviral immunity, exaggerated production of the master Th2 molecule thymic stromal lymphopoietin (TSLP), and altered antimicrobial host defense in the airways of asthmatic individuals with acute hRV infection. It is possible that the epithelial damage induced by the RSV promotes replication of the hRV by allowing access to the deep receptors of this virus.[5]

5 | CONCLUSION

Our study shows that there is significant frequency of recurrent bronchial obstruction 3 years after the initial episode irrespective of its severity or of the type of virus involved. Familial atopy and environmental factors play an important role in the incidence of recurrent episodes. Allergic sensitization is a certain risk factor for asthma in the longer term. This specific infant population should therefore be closely monitored, especially those with RSV-hRV co-infection.

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