Lung Function, Allergic Sensitization and Asthma in School-Aged Children After Viral-Coinfection Bronchiolitis

Sara Ruiz
Severo Ochoa University Hospital. Leganés. Madrid
Cristina Calvo
La Paz University Hospital
Francisco Pozo
National Center of Microbiology. ISCIII. Madrid
Inmaculada Casas
National Center of Microbiology. ISCIII. Madrid
María Luz García-García (marialuz.hso@gmail.com)
Severo Ochoa University Hospital. Leganés. Madrid

Research Article

Keywords: Viral coinfection, Bronchiolitis, Asthma, Lung function, Fraction of exhaled nitric oxide, Allergic sensitization, Allergic rhinitis.

Posted Date: October 21st, 2021

DOI: https://doi.org/10.21203/rs.3.rs-969658/v1

Abstract

BACKGROUND. Our main objective was to compare the lung function, the rate of allergic sensitization and the prevalence of asthma at 7-9 years in children hospitalized for bronchiolitis with viral coinfection versus single viral infection.

METHODS. Observational study in children with previous bronchiolitis and current age 7-9 years. Clinical data were collected. Fraction of exhaled nitric oxide (FeNO) determination, spirometry and skin prick test for common aeroallergens were performed.

RESULTS. A total of 181 children hospitalized for bronchiolitis (40 coinfections and 141 single infections), with median age of 8.3 years (IQR:7.5-9.1) were included. Single-HRV-infections showed lower basal FEV1(%) than coinfections (p=0.04) and lower z-score FEV_1 than single-RSV-infections(p=0.04) or coinfections(p=0.02). Also, single-HRV-infections had lower post-bronchodilator FEV1(%) and z-score FEV_1 values than coinfections (p=0.03 and p=0.03). Single-HRV-bronchiolitis was an independent risk factor for FEV_1<80%(p=0.007).

FeNO value >25 ppb was detected in 21(12.5%) cases, without differences between viral groups(p=0.768). The prevalence of allergic sensitization was similar in coinfections (31.4%) *versus* single infections (38.7%), (p=0.428). The highest frequency of allergic rhinitis was observed in single-HRV patients(p=0.004).

The respiratory morbidity at 7-9 years of coinfected patients was similar to the single-HRV ones. In contrast, the likelihood of current asthma was up to 5 times higher in RSV/HRV coinfections than in the single-RSV-infections ones (p=0.012).

CONCLUSIONS. The respiratory morbidity at 7-9 years of age after severe bronchiolitis is significantly higher in single-HRV or viral coinfection patients that in single-RSV ones. Single-HRV-bronchiolitis is independently associated with lower lung function at school-age.

Background

Respiratory syncytial virus (RSV) causes up to 75% of bronchiolitis cases, but other agents also associated with lower respiratory tract infection in this age group include rhinovirus (HRV), human bocavirus (HBoV), human metapneumovirus (hMPV), influenza virus (FLU) or parainfluenza virus (PIV), identified as a single infection or viral coinfection [1–4]. Viral coinfections are relatively common in bronchiolitis, with a frequency that varies from 10 to 40% in hospitalized infants. However, data on clinical severity in coinfections compared with single-infections during acute bronchiolitis are contradictory, with some studies suggesting greater severity in coinfections, whereas others do not find any significant differences between both groups [5–7]. Regarding the medium and long-term respiratory morbidity after bronchiolitis associated to viral coinfection, data are even scarcer. It is well known that infants suffering from severe RSV bronchiolitis, but also HRV and hMPV bronchiolitis, are at increased

risk of asthma development during childhood [8–15]. However, to our knowledge, only one study, previously published by our group, has analysed the medium-term respiratory outcome in patients with previous severe viral coinfection bronchiolitis [16]. In that study, conducted by telephone interview, children with viral coinfection were 2.5-fold more likely to develop asthma at 6-8 years compared to those with single viral infection.

The aim of the current study was to assess the lung function and the occurrence of atopy and asthma, at 7-9 years, in children previously hospitalized for bronchiolitis, with particular focus on the role of viral coinfections.

Methods

Study design and subjects

This was an observational, longitudinal, post-bronchiolitis, hospital-based follow-up study, that is a part of an ongoing prospective investigation of respiratory tract infections in children, approved by the Medical Ethics Committee. Written informed consent was obtained from all the parents/caregivers after full explanation of the study protocol. All methods were carried out in accordance with relevant guidelines and regulations.

Children below 2 years of age, hospitalized for acute bronchiolitis in the Severo Ochoa University Hospital (Spain), between September 2008 and December 2011, were included (N=351). A two-phase follow-up study was conducted. In the first one, whose results were already published, all previously included patients at admission were invited to a telephone follow-up visit, to compare the frequency of asthma in children with severe bronchiolitis associated with viral coinfection *vs.* single infection [16]. A total of 244 children (52 coinfections and 192 single-infections) were located and agreed to participate. In the current phase of the study, the aforementioned 244 children were invited to a face-to-face medical consultation at the hospital, including a clinical questionnaire, lung function assessment and skin prick test (SPT) for allergy. Figure 1.

Clinical evaluation

Respiratory symptoms were assessed using a clinical-epidemiological questionnaire [16 and the ISAAC questionnaire for asthma symptoms for 6-7-year-old children, previously validated and translated to Spanish [17]. Use of asthma medication during the preceding 12 months was also recorded (bronchodilators, inhaled corticosteroid, combination inhaled corticosteroid/long-acting beta-2 agonists (ICS/LABA), leukotriene antagonists).

Current asthma prevalence was estimated by the proportion of patients who responded affirmatively to the question number 2 of the ISAAC questionnaire (*wheezing or whistling in the chest in the past 12 months*), the one which has demonstrated the greatest correlation with current asthma prevalence in validation studies [18]. Recurrent wheezing was defined as the presence of wheezing episodes diagnosed

by a doctor in the first 4 years of life [19]. *Current allergic rhinits* was defined as present if at least two of the following three criteria were fulfilled: sneezing or a runny or blocked nose without having a cold; nasal allergy/ hay fever medication, both in the past 12 months; doctor-diagnosed allergic rhinitis or hay fever ever [20].

Fraction of exhaled nitric oxide (FeNO) measurement and lung function tests

FeNO was measured using the NIOX VERO® handheld device, considering normal FeNO values < 25 ppb [21].

Lung function was evaluated by forced spirometry, performed in accordance with the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) [22]. In order for the spirograms to be accepted, the second highest FEV_1 and FVC had to lie within 0.15 L of the highest value, and the highest FEV_1 and FVC were picked for the analyses. FEV_1 /FVC and the mid-expiratory flow at 50% of FVC (MEF50) were taken from the spirogram with the highest sum of FEV_1 and FVC. The flow-volume spirometry parameters were given as percentages of population-based, sex-specific, height related references, namely percentage of predicted [23]. The variables collected were: FVC (forced vital capacity), FEV_1 (forced expiratory volume in one second), FEV_1/FVC and FEF_{25-75} (mean expiratory flow between 25% and 75% of FVC). The results were values according to the reference values of The Global Lung Function Initiative (GLI) available at https://www.ers-education.org/guidelines/global-lung-function-initiative/about/ [24].

Spirometric values were considered to be normal when FEV_1 and FVC were ≥ 80%, FEV_1/FVC > 90% and FEF25-75 ≥ 65% of their values [25].

Post-bronchodilator test was considered positive when an increase of FEV_1 of at least 12% compared to the baseline was observed after administration of 400 µg of inhaled salbutamol.

Allergic sensitization

Allergic sensitization was evaluated by skin prick test (SPT) to common aeroallergens, following the recommendations of the European Academy of Allergy and Clinical Immunology [26]. The following allergens were used: *Dermatophagoides Pteronyssinus, Dermatophagoides Farinae, Alternaria Alternata*, *Cladosporium*, grass, cypress, olive and platanus pollen, dog and cat dander.

Standardized extracts (Abelló®) were used, with a positive control (10 mg/ml histamine) and a negative one (glycerol saline vehicle solution). The test was considered positive when the wheal diameter was greater than or equal to that of histamine and 3 mm larger than the negative control. Atopy was defined as a positive SPT for at least one allergen.

Statistical analysis

The sample size needed to detect a difference in FEV_1(%) of at least 7 percentage points, with an alpha error of 5% and a power of 80%, was calculated. Estimating a rate coinfection/single infection of 1/3, 37 cases in the coinfection group and 111 in the single infection one would be needed.

Categorical variables were described using absolute and relative frequencies. Continuous variables were described using mean and standard deviation (normal distribution) or median and interquartile range (non-normal distribution).

Group comparisons were performed with Student's t-test, Pearson's chi-square test or Fisher's exact test for tables with frequencies <5, as appropriate. Data not normally distributed were analysed by the Mann–Whitney U-test. Logistic regression models were constructed to assess a set of potential risk factors for current asthma. Each variable was entered separately into univariate models, and odds ratios (OR) with 95% CI were calculated. Explanatory factors with p-values < 0.2 in univariate analysis were further analysed in a multiple regression model. P-value of <0.05 was regarded as statistically significant. All analyses were two tailed, and were performed using the Statistical Package for the Social Sciences (SPSS), version 21.0.

Results

Subjects

Of the 244 patients included in the telephone-follow-up study, 181 (40 coinfections and 141 single-infections) could be contacted again and agreed to a medical visit at hospital. The main reason for drop-out was a change in their telephone number. Children who lost follow-up did not differ significantly from others regarding initial hospitalization, gender, type of virus, prematurity, and age at inclusion. Figure 1.

All the 181 included patients completed the questionnaire, while 172 (95%) agreed to take part in SPT, and 177 (97.7%) agreed to perform lung function tests. Five children were not able to cooperate in the spirometry test and 14 were not able to perform the manoeuvre to measure FeNO, so that, 172 spirometries and 167 FeNO measurements were finally obtained.

Clinical characteristics

The median age at follow-up was 8.3 years (IQR: 7.5-9.1). Regarding the clinical features during admission for bronchiolitis, no significant differences were found between coinfections and single-infections (16). The most frequently identified viruses in single-infections were RSV (70, 50%), HRV (29, 16%) and hMPV (8, 6%), whereas the most frequent viruses in coinfections were HRV (26, 65%), RSV (24, 60%) and HBoV (12, 30%). The most common associations were RSV+HRV (15, 29%) and RSV + HBoV (12, 23%).

The frequency of maternal asthma was more than six times higher in single-infections (14.2%% vs. 2.5%; p=0.04; OR:6.44, CI95%:0.84-49.06), who also showed a higher prevalence of atopic mothers (33.6% vs. 15%, p=0.023; OR: 2.86, CI95%:1.12-7.30). Atopic dermatitis (54% vs. 35%, p=0.035; OR:2.17, CI95%:1.05-

4.50) and allergic rhinitis (39% vs. 17.5%, p=0.01; OR:3.01, CI95%:1.25-7.29) were also more frequent in single-infections than in coinfections. No other differences regarding family history of asthma or atopy were found between both groups.

Comparison of respiratory morbidity at 7-9 years of children with single-infections *vs.* viral coinfections

A high proportion of patients in both groups reported some episode of recurrent wheezing, 82.7% in the coinfection group and 69.7% in the single-infection group (p=0.06; OR: 2.07, CI95%:0.95-4.52). Similar number of children in both groups needed rehospitalization for wheezing, 35% in coinfections and 36% in single-infections (p=0.892), but the number of wheezing-related admissions was significantly higher in children with coinfections (3.6±3.5 *vs.* 1.8±1.7, p=0.005), who also reported higher frequency of respiratory symptoms in the intercrisis periods (11.4% *vs.* 3.4%, p=0.037; OR:4.16, CI95%:0.98-17.57).

Both groups required chronic asthma treatment with a similar frequency (52% *vs.* 57.5%, p=0.141). However, the likelihood of receiving the combination inhaled corticosteroid/long-acting β2-agonist (ICS/LABA), indicated for a greater severity step, was 3.9 times higher in the coinfection group (15% *vs.* 4.3%, p=0.017).

In the multivariate analysis viral coinfection (p=0.026), allergic rhinitis (p<0.001), maternal atopy (p=0.02) and passive smoking (p=0.045), were independently associated with chronic asthma treatment requirement. Table 1.

Table 1
Bivariate and multivariate analysis of possible risk factors associated with chronic asthma treatment in the compiled coinfection and single-infection bronchiolitis groups.

	Bivariate Analysis			Multivariate Analysis		
Risk Factor for chronic asthma treatment	**P-value**	**Crude Odds Ratio**	**Confidence interval 95%**	**P-value**	**Adjusted Odds Ratio**	**Confidence interval 95%**
Viral Coinfection	0.09	1.69	0.91-3.13	0.026	2.11	1.1-4.08
Atopic Dermatitis	0.004	2.11	1.26-3.54			
Allergic Rhinitis	<0.001	3.27	1.81-5.89	<0.001	3.23	1.74-6.00
Environmental Tobacco Smoke	0.035	1.89	1.04-3.43	0.046	1.89	1.01-3.55
Maternal Atopy	0.007	2.26	1.24-4.10	0.022	2.10	1.11-3.96
Siblings Atopy	0.08	1.64	0.94-2.86			
Risk Factor for Budesonide treatment	**P-value**	**Crude Odds Ratio**	**Confidence interval 95%**	**P-value**	**Adjusted Odds Ratio**	**Confidence interval 95%**
Atopic Dermatitis	0.008	2.10	1.20-3.65			
Allergic Rhinitis	<0.001	2.92	1.61-5.27	0.002	2.64	1.44-4.86
Mother´s Atopy	0.001	2.69	1.46-4.95	0.01	2.29	1.22-4.31
Siblings´ Atopy	0.09	1.63	0.91-2.93			
Risk Factor for ICS/LABA* treatment	**P-value**	**Crude Odds Ratio**	**Confidence interval 95%**	**P-value**	**Adjusted Odds Ratio**	**Confidence interval 95%**
Viral Coinfection	0.026	3.41	1.09-10.63	0.004	23.41	2.81-194.89
Infiltrate/atelectasis	0.05	3.64	0.88-15.08	0.027	10.01	1.31-74.66
Food Allergy	0.07	3.31	0.84-13.09			
Allergic Rhinitis	0.004	4.71	1.48-14.99	0.006	15.64	2.21-110.37
Siblings´ Asthma	<0.001	8.37	2.62-26.71	0.003	21.55	2.93-158.64
* ICS/LABA: Inhaled corticosteroid/long-acting β2-agonist						

In addition to viral coinfection (p=0.004), the prescription of ICS/LABA was independently associated with allergic rhinitis (p=0.006), siblings´ asthma (p=0.003) and infiltrate/atelectasis during the admission

for bronchiolitis (p=0.027).

Current asthma at 7-9 years (affirmative response to question number 2 of the ISAAC questionnaire) was independently associated to viral coinfection (p=0.004; OR:3.2, 95% CI:1.4-6.9), allergic rhinitis (p=0.001; OR:3.50; 95%CI:1.70-7.20) food allergy (p=0.05; OR: 2.6; 95%CI:1.0-6.80) and atopic dermatitis (p=0.004; OR:2.30; 95%CI:1.20-4.70). Although positive SPT was associated with current asthma in the univariate analysis (p<0.001; OR:4.29, CI95%:1.98-9.27), the association did not hold in the multivariate analysis.

After the global comparison between coinfections and single-infections, single-RSV and single-HRV groups were compared with the coinfection one.

Comparison of respiratory morbidity at 7-9 years of children with single-RSV and single-HRV *vs.* viral coinfections

Compared with single-RSV infections, children with viral coinfections needed significantly more chronic asthma treatment (p=0.05), montelukast (p=0.02) and ICS/LABA (p=0.005), presented more symptoms in the intercrisis periods, had been more frequently diagnosed with asthma ever and required higher number of admissions for asthma. Table 2. When single-RSV infections were specifically compared to RSV/HRV coinfections, the likelihood of current asthma was up to 5 times higher in coinfections (44% *vs.* 13%, p=0.012, OR:5.47, CI95%1.29- 23.25).

Table 2
Comparison of respiratory morbidity at 7-9 years of age in children with a history of bronchiolitis due to viral coinfection, single RSV infection and HRV infection.

	Viral coinfection (N= 40)	Single RSV infection (N = 70)	P-value†	Single-HRV infection (N=23)	P-value¥
Recurrent wheezing	37(92.5%)	59 (84%)	0.214	23(100%)	0.178
Admissions for wheezing	14(35%)	20 (29%)	0.483	14(61%)	**0.04**
Number of admissions for wheezing	3.5 (3.5)	1.28 (1.2)	**0.03**	2.9 (1.9)	0.555
Symptoms in the intercrisis periods	4(11.4%)	1(1.5%)	**0.03**	1(4%)	0.108
Chronic asthma treatment	23(57.5%)	27(38.6%)	**0.05**	17(74%)	0.193
Budesonide	16 (40%)	24(34%)	0.549	13(56.5%)	0.205
Montelukast	19(47.5%)	18(25.7%)	**0.02**	15 (65%)	0.174
Salmeterol/ fluticasone	6 (15%)	1 (1.4%)	**0.005**	1 (4%)	0.195
RSV: respiratory syncytial virus					
HRV: rhinovirus					
† Single- RSV infections compared to viral coinfections					
¥ Single- HRV infections compared to viral coinfections					

In contrast, respiratory morbidity at 7-9 years of coinfection-patients was quite similar to the single-HRV ones, with the exception of higher frequency of asthma admissions in children with coinfection (61% vs. 35%, p=0.045; OR:2.89, CI95%:1.10-8.33). Table 2.

When single-HRV infections were specifically compared to single-RSV ones, higher frequency of current asthma was observed (39% vs. 14.7%, p=0.01, OR:3.72, CI95%:1.27-10.90), as well as more asthma treatment (74% vs. 38%, p=0.003, OR:4.51, CI95%:1.58-12.87) and more admissions for asthma (61% vs. 29%, p=0.005, OR:3.89, CI95%:1.45-10.41). The association of single-HRV infections with current asthma remained statistically significant even in children without allergic sensitization (25% vs. 4%, p=0.028).

Skin prick test

Skin prick tests to common aeroallergens were performed on 172 children, of which 64 (37%) tested positive, mainly to outdoors allergens (grass pollen). Forty-six of them were polysensitized.

The prevalence of allergic sensitization in coinfections (31.4%) was similar to that of single infections (38.7%), (p=0.428). No differences were detected when coinfections were compared with single-HRV or single-RSV infections. On the other hand, atopy was significantly more frequent in children with current asthma (p<0.001, OR:4.29, CI95%:1.99-9.27), atopic dermatitis (p=0.02; OR: 2.10, CI95%:1.2-3.95), food allergy (p=0.001, OR: 6.0, CI95%: 1.84-19.52), allergic rhinitis (p<0.001, OR:9.54, CI95%:4.63-19.66), maternal asthma (p= 0.01; OR:3.33, CI95%:1.23-8.96) and paternal atopy (p=0.05; OR:1.94, CI95%:0.97-3.87).

Overall, allergic rhinitis was diagnosed in 62(34%) cases, with the highest prevalence in children with single-HRV infection (52%), when compared to single-RSV (31%, p=0.07) or to coinfections (17%, p=0.004). We also found that 25 out of 42 children (60%) with current asthma had allergic rhinitis, compared with 37 out of 137 (27%) children without current asthma (p<0.001). On the other hand, 25 (40%) of the 62 children with allergic rhinitis had current asthma, compared with 14% of those without allergic rhinitis (p<0.001, OR:3.97; CI95%:1.93-8.18).

Lung function

A total of 177 spirometries were performed, of which 172 were considered to be valid according to standard quality criteria. FEV_1< 80% was found in 15 cases (9%), 2 in the coinfection group and 13 in the single-infection one (54% of them were single-HRV). FEV_1/FVC<90% was observed in 16 cases (2 coinfections and 14 single-infections).

FEV_1 values, expressed as percentage, tended to be slightly higher in the coinfection group, although without statistical significance (p=0.06). Patients with single-HRV infections showed lower FEV_1 values in comparison to the coinfection ones (p=0.04). In contrast, no differences were found in FEV_1 values between single-RSV infections and coinfections. Table 3.

The z-score FEV_1 values were higher in the coinfection group (p=0.04) compared to the single-infection one as a whole, but no differences were found when compared only with the single-RSV one (p=0.188). Children with HRV-single infection showed lower z-score FEV_1 values (-0.637±1.187) than children with single-RSV (-0.131±0.957, p=0.04) and with coinfection (p=0.03). Bronchopulmonary dysplasia and infiltrate/atelectasis during bronchiolitis were also associated with lower z-score FEV_1 value (p<.001 and p=0.04 respectively).

FEV_1 values <80% were observed more frequently in children with single-HRV- bronchiolitis than in single-RSV (p<0.001) or coinfections (p=0.006). The likelihood of having FEV_1 values <80% was 6 times higher in single-non-RSV infections than in single-RSV ones (3% *vs.* 17%, p=0.007, OR:6.60, CI95%:1.40-31.05). Bronchopulmonary dysplasia (p<0.001) and wheezing after exercising in the past 12 months were also associated with FEV_1<80% (p=0.009). Table 4. Children with allergic rhinitis (p=0.663) or allergic sensitization (p=0.136) did not show higher frequency of FEV_1 values <80%. The variables associated with FEV_1 values < 80% in the bivariate analysis, with P-value <0.20, were entered in a stepwise logistic

regression analysis. Single-HRV bronchiolitis was the only risk factor independently associated with FEV_1 values < 80% (p=0.023; OR:7.80, CI95%:1.33-45.63).

Table 4
Risk factors for FEV_1 values < 80% in children 7-9 years of age, with history of severe bronchiolitis, viral coinfections and single-infections.

Risk factor for FEV_1 < 80%	**P- value**	**Odds Ratio**	**Confidence interval 95%**
Viral coinfection	0.392	0.52	0.11-2.40
Single-Rhinovirus *vs.* Single-RSV infection	**<0.001**	15.40	2.90-81.68
Single-Rhinovirus *vs* viral coinfection	**0.006**	8.40	1.56-45.20
Single-RSV *vs* viral coinfection	0.547	0.54	0.07-4.04
Allergic rhinitis	0.663	1.27	0.43-3.76
Atopy	0.136	0.38	0.10-1.41
Bronchopulmonary dysplasia	<0.001	13.08	7.76-22.05
Wheezing after exercise past 12 months	0.009	4.09	1.32-12.62
Prematurity	0.08	2.86	0.81-10.04

The z-score FEV_1/FVC value was also higher in the coinfection group (p=0.05) compared to the single infection one. Lower z-score FEV1/FVC values were more frequently found in children with bronchopulmonary dysplasia (p<.001), chronic asthma treatment (p=0.07), current asthma (p=0.016) and asthma ever (p=0.03). Children with premature birth (p=0.06), and hypoxia during the admission for bronchiolitis (p=0.09) tended to have lower z-score FEV1/FVC values, although without statistical significance.

FEV_1/FVC values ≤ 90% of predicted were observed more frequently in children with allergic rhinitis (p=0.015), chronic asthma treatment (p=0.025), treatment with budesonide (p=0.05), montelukast (p=0.009) and salmeterol/fluticasone (p=0.05), current asthma (p=0.01), wheezing after exercise (p=0.002) and night cough in the past 12 months (p=0.02). With respect to viral etiology, no differences between coinfection and single-infections were identified. Table 5.

Table 5

Risk factors for FEV_1/FVC values <90% in children 7-9 years of age, with history of severe bronchiolitis, viral coinfections and single-infections.

Risk factor for FEV_1 /FVC ≤90%	P- value	Odds Ratio	Confidence interval 95%
Viral coinfection	0.331	0.47	0.10-2.19
Single-HRV *vs.* Single RSV infection	0.660	1.60	0.27-9.39
Single-HRV *vs.* Single non-HRV infection	0.820	0.83	0.17-4.01
Single-HRV *vs* viral coinfection	0.567	1.80	0.23-13.77
Single-RSV *vs* viral coinfection	0.895	1.12	0.19-6.45
Allergic rhinitis	0.015	3.53	1.21-10.26
Chronic asthma treatment	0.025	4.01	1.01-14.63
Budesonide	0.050	2.74	0.95-7.93
Montelukast	0.009	4.31	1.33-13.97
Salmeterol/fluticasone	0.050	3.77	0.91-15.66
Current asthma	0.010	3.67	1.28-10.50
Wheezing after exercise past 12 months	0.002	5.00	1.68-14.86
Night cough past 12 months	0.020	3.47	1.14-10.47
HRV: Rhinovirus			
RSV: Respiratory syncytial virus			

Regarding the post-bronchodilator test, although no difference was found in the proportion of patients with positive test between coinfections and single infections (21.6% *vs.* 16.7%, p=0.520), FEV_1(%) values were higher in the coinfection group than in the single-infection one (107.1%±14.1 *vs.* 101.6 ±14.7, p=0.04), as well as FEV_1/FVC values (106.5%± 5.3 *vs.* 103.8±8.5, p=0.02). Children with single-HRV infections had lower post-bronchodilator FEV_1 values than those with coinfections (98.2 ± 16.1 *vs.* 107.15 ±1 4.11, p=0.03), as well as lower z-score FEV_1 values (-0.28±1.25 *vs.* 0.43±1.18, p=0.03). Conversely, no differences could be found between post bronchodilators values of children with single-RSV infections and those with coinfections.

The probability of positive bronchodilator tests was higher in children with current asthma (48% *vs.* 19.5%, p=0.001).

FeNO

FeNO could be measured in 167 patients who were able to perform a valid test. Overall, 21 (12.5%) cases presented values >25 ppb, without significant differences between children with coinfection (9.4%) and children with single infection (12.6%) (p=0.768).

The proportion of children with FeNO >25 ppb was significantly higher in those with atopy (p<0.001), atopic dermatitis (p=0.019), allergic rhinitis (p=0.003), history of admission for asthma (p=0,02) and ever asthma (p=0.004). Children with current asthma (p=0.09) and wheezing after exercise in the past 12 months (p=0.09) also showed a tendence to present higher levels of FeNO. Table 6.

Table 6
Clinical features associated with fraction of exhaled nitric oxide (FeNO) > 25 ppb, at 7-9 years of age, in children with a history of bronchiolitis with single-viral infection and viral coinfection.

	FeNO > 25 ppb (N= 20)	**FeNO ≤ 25 ppb (N = 147)**	**P- value**
Viral Coinfection	3(15%)	29 (20%)	0.614
Single-HRV infections vs **coinfections**	2(40%)	20 (41%)	0.483
Single-RSV infections vs **coinfections**	8(73%)	58(67%)	0.686
Atopic dermatitis	15 (75%)	69(47%)	**0.02**
Allergic rhinitis	13(65%)	46(31%)	**0.003**
Positive prick test	16(84%)	45(32%)	**<0.001**
Asthma admissions	12(60%)	50(34%)	**0.024**
Current asthma	8 (40%)	33(23%)	0.09
Asthma ever	8(40%)	21(40%)	**0.004**
Wheezing after exercise in the past 12 months	6 (30%)	22 (15%)	0.09
RSV: respiratory syncytial virus			
HRV: rhinovirus			

Discussion

To our knowledge, this is the first study to compare the lung function of school-age children with a history of severe bronchiolitis associated with viral coinfection *vs.* single-viral infection. Our results show that the coinfection group had higher lung function values (FEV_1, z-score FEV_1 and z-score FEV1/FVC values) than the single-infection one at 7-9 years, considered as a whole. However, when analyzing the viral subgroups, this difference holded only in cases of single-HRV infection, but not in the single-RSV ones. In

fact, when lung function of patients with viral coinfections were compared with those with single-RSV infections, no significant differences could be found. In contrast, almost all pulmonary function values of the single-HRV group, both pre and post bronchodilator, were significantly lower than those of coinfections. Also, the likelihood of having FEV_1< 80% was up to 15 times higher in the single-HRV group compared to the single-RSV one and up to 8 times higher than in the coinfection group.

Previous studies have analysed the association between RSV bronchiolitis and pulmonary function sequelae, although there has been little standardization between studies, with contradictory results [8–10, 27–30]. A recent systematic review [31] including 31 studies, whose primary outcome was the evaluation of long-term pulmonary sequelae measured by pulmonary function test, in children with previously RSV respiratory infection during the first 3 years of life, found no association between RSV infection and abnormal pulmonary function in 13 studies, while 16 did reported this association. Many of the studies included in that review enrolled small numbers of participants, only five studies included more than 100 patients, many of them used different pulmonary function techniques and reported different pulmonary function indices. But nevertheless, their results reflect varying abnormal pulmonary function when compared with infants without bronchiolitis, being the most commonly described, obstruction to airflow or obstructive airways disease with or without bronchodilator reversibility. Most studies included in that systematic review compared pulmonary function in children with previously RSV bronchiolitis with a control group of healthy infants. This is not the case in our study, in which the aim was to compare lung function in children admitted for bronchiolitis with single *versus* doble or multiple viral infection and not *versus* a control group of healthy children. We report here, for the first time, that children hospitalized for single-HRV bronchiolitis have significant lower lung function values, at 7-9 years, than those admitted for bronchiolitis with viral coinfection or with single RSV-infection. In fact, single-HRV-bronchiolitis was the only risk factor independently associated with FEV_1 < 80% The sample size of our study was calculated to compare the lung function of viral-coinfection bronchiolitis with single-infections, but not to compare the different single-infections with each other. For this reason, other single respiratory infections could not be compared and larger studies are needed.

Guilbert et al. [32] in a longitudinal cohort of children at risk of asthma, prospectively explored the relationships among early life virus-specific wheezing, childhood lung function, and asthma from 4 to 8 years of age. They provided novel evidence that early HRV-wheezing illnesses in high-atopy risk infants are related to lower lung function in childhood, with significantly lower FEV1%, FEV0.5, FEF25-75, FEV1/FVC and FEV0.5/FVC compared with children without HRV-wheezing illnesses. In contrast, children with RSV-wheezing illnesses did not have significant differences in any of the measured spirometric indices when compared with children who did not wheeze with RSV. These relationships were less pronounced but were still significantly different after administration of bronchodilator and thus, are not likely explained by increased airway tone alone. Our results also evidence that children with early single-HRV bronchiolitis have significantly lower FEV_1 (%), lower z-score FEV_1, both pre- and post-bronchodilator compared with single-RSV and with viral coinfections. However, whereas children included in Gilbert´s study where part of a birth cohort of high-risk atopic children followed-up on an outpatient basis, with

mostly mild respiratory infections in the first 3 years of age, all of our patients were hospitalized infants < 24 months of age and therefore, with more severe bronchiolitis. As Guilbert et al. [26] argued, although they did not find evidence that RSV-wheezing illnesses were associated with reduced lung function, few of their study participants had severe illness requiring hospitalization. It is possible that more severe RSV illnesses could lead to significant reductions in lung function. In our case, as previously published, similar severity regarding the acute episode of bronchiolitis was found between coinfections and single infections, with no significant differences in oxygen therapy requirement or length of hospital stay. Therefore, the different respiratory morbidity observed at 7-9 years of age cannot be attributed to greater or lesser severity of acute bronchiolitis and according to our results, could be more related to the different viral etiology of the acute episode.

With respect to atopy, in the prospective multicentre observational EuroPrevall-iFAAM birth cohort study, the median prevalence of allergic rhinitis in 10 563 school-age children was 13.3%. Studies on the prevalence of atopy among children with history of lower respiratory viral infection (LRTI) have shown contradictory results. Some studies have found an increased risk of allergic sensitization after LRTI in childhood [33], other reported protection against allergic sensitization through the stimulation of Th-1 cytokine production [34], whereas other studies did not find any influence of childhood viral LRTI on the risk of subsequent atopy [35]. The results of a recent systematic review and meta-analysis suggest that there is no association between LRTI at < 5 years and positivity of skin prick test or atopic dermatitis. In contrast, the overall analyses showed that there was a higher frequency of allergic rhinoconjunctivitis in children with history of LRTI in the first 5 years of age (OR = 1.7 [95%CI = 1.1–2.9]) [36]. Ho et al. [37] also found that bronchiolitis before the age of two years, was independently associated with an increased risk of allergic rhinitis in 6–8-year-old children in Taipei. Our results show a global prevalence of allergic rhinitis of 34% in children previously hospitalized for bronchiolitis, considerably higher than the observed in 9623 6-7-year-old Spanish children in the GAN Phase I survey, with figures ranging from 12.5 to 18.8% [38], suggesting that there is undoubtedly an association between the two entities, which should be further studied. On the other hand, we also describe different prevalence of allergic rhinitis depending on the viral etiology of the acute bronchiolitis, with single-HRV infections having the highest rate of allergic rhinitis (52%) at 7-9 years of age, compared with single-RSV (31%, p=0.07) or with coinfections (17%, p=0.004). It is worth noting that single-HRV patients had the highest rate of allergic rhinitis, and that the likelihood of current asthma at 7-9 years in our cohort was 4 times higher in children with allergic rhinitis. Also, the lowest values of pulmonary function at 7-9 years of age were also found in single-HRV infected children. All these data strongly suggest that children with severe single-HRV-bronchiolitis have the highest risk of presenting allergic rhinitis and lower lung function values at 7-9 years of age when compared with single-RSV infections or with coinfections.

The mechanism of the association of bronchiolitis, mainly that associated with single-HRV infection, with allergic rhinitis is not yet well known. Korppi et al. [39] in a prospective post-bronchiolitis follow-up, showed that IL33 rs1342326 polymorphism was independently associated with allergic rhinitis and could also be associated with severe childhood asthma at school age. Our group, in a previous study [40], detected nasal IL-33 cytokine secretion in infants < 2 years of age hospitalized with bronchiolitis, with

significantly higher detection rate in patients with HRV-infection, who also showed higher nasal concentrations of IL-33 than non-HRV-infants. In other recent study by our group, we also found high type 2 innate lymphoid cells (ILC2), and higher nasal levels of IL-33, TLR3, IFNG, IL10 and FLG mRNA in infants hospitalized with bronchiolitis [41]. IL-33 plays an important role in T helper (Th) type 2 immunity activating, for example, Th2-type lymphocytes, mast cells and eosinophils leading finally to allergic diseases such as allergic rhinitis [42]. IL-33 have been constantly found in nasal and lung epithelium and in serum, sputum and bronchoalveolar lavage of adults with asthma [43]. In view of all these data, it could be hypothesized that early viral lower respiratory tract infections, mainly HRV-infections, could enhance the release of different cytokines, such as IL-33, that activates the innate type-2 cells (ILC2) and type-2 helper (Th2) cells through IL-1RL1, leading to amplified production of cornerstone type-2 cytokines and could partly explain the higher rate of allergic rhinitis and current asthma found in our single-HRV-bronchiolitis patients.

The fraction of exhaled nitric oxide has been suggested as a non-invasive biomarker of eosinophilic inflammation [44]. Although some authors found that FeNO measurements in infants with recurrent wheezing episodes were associated with persistence of wheezing through age 3 years [45], others like Mikalsen et al. [46] found no differences between 11-year-old children hospitalized for bronchiolitis and the control group. FeNO, in the study of Mikalsen et al. was associated with atopy, but not with asthma in both groups. Our results also showed no differences regarding FeNO levels between coinfection and single infection groups. By contrast, children with allergic sensitization had significantly higher FeNO values than not sensitized ones. No child with asthma but without allergic sensitization had elevated FeNO. These results, in line with those obtained by Mikalsen et al. [45] suggest that only children who develop atopic asthma have eosinophilic airway inflammation, translated by elevated levels of FeNO.

According to our results, the probability of developing asthma at age 7-9 years was almost twice as high in children with coinfection (31%) than in those with single-infection considered as a whole (18%), being coinfection an independent risk factor for current asthma at school age. In addition, patients with viral coinfection showed greater respiratory morbidity, with higher rate of admissions for asthma, higher rate of treatment with the combination inhaled corticosteroid/long-acting β2-agonist (ICS/LABA), usually prescribed for moderate/severe asthma, and persistence of symptoms in the intercrisis periods. All these data strongly suggest that coinfections seem to be associated with increased respiratory morbidity, at least until the age of 7-9 years, when compared with single-infections. However, as was observed for pulmonary function, when coinfections were compared only with single-HRV infections, no significant differences were found in terms of frequency of current asthma or chronic anti-asthma treatment, although the likelihood of been admitted for asthma were almost 3 times higher in the coinfection group. In contrast, the frequency of current asthma in single-RSV children was 5 times lower than in children with coinfection and 4 times lower than in children with single-HRV infection. Our data suggest that bronchiolitis associated with single-HRV or viral coinfection pose a significantly higher risk, not only for the development of asthma, but also for more severe asthma, than single-RSV bronchiolitis. Hyvärinen et al. [47] reported in 2005 for the first time, the link from HRV- induced wheezing in infancy to emergence of childhood asthma. Since then, the role of HRV in bronchiolitis and the subsequent development of

recurrent wheezing and asthma has been described [48, 49] and this association still remained significant in children ≥10 years [50]. Recently, Hasegawa et al. [51] found that the risk of developing recurrent wheeze differed by the causative virus of the bronchiolitis, with higher risk for children with rhinovirus C infection compared to RSV. As previously mentioned, we could not compare the role of different types of HRV because our sample size was not calculated to achieve this goal. On the other hand, Lukkarinen et al. [52], in a follow up study in 127 children, found that HRV-induced first wheezing episode predicts atopic but not nonatopic asthma at school age. In contrast, in our cohort, single-HRV bronchiolitis remained associated with asthma even in children without allergic sensitization.

Our study has potential limitations. Firstly, the study did not have information from healthy controls because our objective was to compare the role of different viral etiologies in early infancy on the development of asthma among infants with severe bronchiolitis. Also, the sample size was calculated to compare the lung function at 7-9 years in children previously admitted for bronchiolitis with single infection or co-infection and therefore, some of our findings, not related with this goal, should be confirmed in larger studies with enough statistical power. Finally, the number of single infections with viruses other than RSV or HRV is not very large, due to the high rate of coinfections among some viruses like HBoV.

The strengths of the study are the prospective design and longitudinal follow-up. Only hospitalized patients with bronchiolitis younger than 2 years were included. At admission, RT-PCR to identify 16 respiratory viruses were performed in nasopharyngeal aspirate and clinical data were prospectively recorded. At follow-up, a standardized and validated clinical questionnaire (ISAAC questionnaire) was used and lung function as well as FeNO measurement could be performed in a high percentage of patients. On the other hand, specific tests (SPT) were done to confirm allergic sensitization.

In summary, according to our results, the respiratory morbidity at 7-9 years after severe bronchiolitis associated with viral coinfection or single-HRV infection is significantly higher than single-RSV infections. Single-HRV bronchiolitis is independently associated with lower lung function values. Clinical studies attempting to identify the young children at increased risk for HRV- bronchiolitis are critical to the development of therapeutic and prevention strategies.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Severo Ochoa Hospital. Written informed consent was obtained from all the parents/caregivers after full explanation of the study protocol. All methods were carried out in accordance with relevant guidelines and regulations.

Consent for publication

Non applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This study has been partially supported by Fondo de Investigaciones Sanitarias –Spanish Health Research Fund Grant PI12/0129. There was no additional external funding received for this study. The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author's contributions

Conceptualization: María Luz García-García., Cristina Calvo, Sara Ruiz, Francisco Pozo, Inmaculada Casas.

Data curation: María Luz García-García, Cristina Calvo, Sara Ruiz, Francisco Pozo, Inmaculada Casas.

Formal analysis: María Luz García-García.

Funding acquisition: María Luz García-García, Francisco Pozo, Inmaculada Casas.

Investigation: María Luz García-García, Cristina Calvo, Sara Ruiz, Francisco Pozo, Inmaculada Casas.

Methodology: Cristina Calvo, Sara Ruiz, Francisco Pozo, Inmaculada Casas.

Project administration: Inmaculada Casas.
Resources: Inmaculada Casas.
Software: María Luz García-García.
Supervision: María Luz García-García., Cristina Calvo

Validation: María Luz García-García.

Visualization: María Luz García-García, Sara Ruiz, Cristina Calvo, Francisco Pozo, Inmaculada Casas.

Writing – original draft: María Luz García-García, Sara Ruiz, Cristina Calvo.

All authors read and approved the final manuscript.

Acknowledgements

Non applicable

References

1. Florin, T.A., Plint, A.C., Zorc, J.J. Viral bronchiolitis. *Lancet* **389,** 211-224 (2017).
2. Calvo, C. et al. Detection of new respiratory viruses in hospitalized infants with bronchiolitis: a three-year prospective study. *Acta. Paediatr.* **99,** 883-7 (2010).
3. Chen, Y.W. et al. Viral etiology of bronchiolitis among pediatric inpatients in northern Taiwan with emphasis on newly identified respiratory viruses. *J. Microbiol. Immunol. Infect.* **47,** 116–21 (2014).
4. Miller, E.K. al. Viral etiologies of infant bronchiolitis, croup and upper respiratory illness during 4 consecutive years. *Pediatr. Infect. Dis. J.* **32**, 950-5 (2013).
5. Richard, N. et al. The impact of dual viral infection in infants admitted to a pediatric intensive care unit associated with severe bronchiolitis. *Pediatr. Infect. Dis. J.* **27,** 213–7 (2008).
6. Petrarca, L. et al. Acute bronchiolitis: Influence of viral co-infection in infants hospitalized over 12 consecutive epidemic seasons. *J. Med. Virol*. **90,** 631-638 (2018).
7. Rodrigues da Silva, E. et al. Severe lower respiratory tract infection in infants and toddlers from a non-affluent population: viral etiology and co-detection as risk factors. *BMC Infect Dis.* **13,** 41 (2013).
8. Sigurs, N., Bjarnason, R., Sigurbergsson, F., Kjellman, B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am. J. Respir. Cri.t Care. Med*. **161,** 1501-7 (2000).
9. Sigurs, N. et al. Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am. J. Respir. Cri.t Care. Med*. **171,** 137-41 (2005).
10. Sigurs, N. et al. Asthma and allergy patterns over 18 years after severe RSV bronchiolitis in the first year of life. *Thorax* **65,** 1045-52 (2010).
11. [1]. Stein, R.T. et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet.* **354,** 541-5 (1999).
12. Jackson, D.J. et al. Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am. J. Respir. Cri.t Care. Med*. **178,** 667-72 (2008).
13. García-García, M.L. et al. Human metapneumovirus bronchiolitis in infancy is an important risk factor for asthma at age 5*. Pediatr. Pulmonol*. **42,** 458-64 (2007).
14. Kotaniemi-Syrjänen, A. et al. Rhinovirus-induced wheezing in infancy - The first sign of childhood asthma? *J. Allergy Clin. Immunol*. **111,** 66-71 (2003).
15. Rossi, G.A., Colin, A.A. Infantile respiratory syncytial virus and human rhinovirus infections: Respective role in inception and persistence of wheezing. *Eur. Respir. J*. **45,** 774-89 (2015).
16. García-García, M.L., et al. Role of viral coinfections in asthma development*. PLoS One*. **12,** e0189083 (2017).

17. Mata Fernández, C., Fernández-Benítez, M., Pérez Miranda, M., Guillén Grima, F. Validation of the Spanish version of the phase III ISAAC questionnaire on asthma. *J. Investig. Allergol. Clin. Immunol*. **15,** 201-10. (2005).
18. Jenkins, M.A. et al. Validation of questionnaire and bronchial hyperresponsiveness against respiratory physician assessment in the diagnosis of asthma. *Int. J. Epidemiol*. **25,** 609-16. (1996).
19. Sonnenschein-Van Der Voort, A.M.M. et al. Preterm birth, infant weight gain, and childhood asthma risk: a meta-analysis of 147,000 European children. *J. Allergy Clin. Immunol.* **133,** 1317-29 (2014).
20. Sigurdardottir, S.T. et al. Prevalence and early-life risk factors of school-age allergic multimorbidity: The EuroPrevall-iFAAM birth cohort. *Allergy* **76,** 2855- 65. (2021).
21. [1]. Buchvald, F. et al. Measurements of exhaled nitric oxide in healthy subjects age 4 to 17 years. *J. Allergy Clin. Immunol.* **115,** 1130-6. (2005).
22. Galdiz, J.B. et al. Espirometría. *Arch Bronconeumol*. **49,** 388-401 (2013).
23. Zapletal, A., Paul, T., Samanek, M. Die Bedeutung heutiger Methoden der Lungenfunktionsdiagnostik zur Feststellung einer Obstruktion der Atemwege bei Kindern und Jugendlichen. *Zeitschrift für Erkrankungen der Atmungsorgane.* **149,** 343-71. (1977).
24. Quanjer, P.H. et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J.* **40,** 1324-43 (2012).
25. Global Initiative for Asthma. Global Strategy for Asthma management and Prevention. Updated 2018. [Date of access 30 nov 2019]. Available in: https://ginasthma.org/2018-gina-report-globalstrategy-for-asthma-management-and prevention/.
26. Bousquet, J. et al. Practical guide to skin prick tests in allergy to aeroallergens. *Allergy.* **67,** 18–24. (2012).
27. Fjaerli, H.O. et al. Acute bronchiolitis in infancy as risk factor for wheezing and reduced pulmonary function by seven years in Akershus County, Norway. *B.M.C. Pediatr*. 2005 **5,** 31 (2005).
28. Korppi, M., Piippo-Savolainen, E., Korhonen, K., Remes, S. Respiratory morbidity 20 years after RSV infection in infancy. *Pediatr. Pulmonol*. **38,** 155–60. (2004).
29. Piippo-Savolainen, E., Remes, S., Kannisto, S., Korhonen, K., Korppi, M. Asthma and lung function 20 years after wheezing in infancy. *Arch. Pediatr. Adolesc. Med*. **158,** 1070. (2004).
30. Hyvärinen, M.K., Kotaniemi-Syrjänen, A., Reijonen, T.M., Korhonen, K., Korppi, M.O. Teenage asthma after severe early childhood wheezing: an 11-year prospective follow-up. *Pediatr. Pulmonol*. **40,** 316–23. (2005).
31. Verwey, C., Nunes, M.C., Dangor, Z., Madhi, S.A. Pulmonary function sequelae after respiratory syncytial virus lower respiratory tract infection in children: A systematic review. *Pediatr. Pulmonol.* **55,** 1567-83. (2020).
32. Guilbert, T. et al. Decreased lung function after preschool wheezing rhinovirus illnesses in children at risk to develop asthma. *J. Allergy Clin. Immunol.* **128,** 532-8. (2011).

33. Nagayama, Y. et al. Allergic predisposition among infants with bronchiolitis. *J. Asthma*. **24,** 9–17 (1987).
34. Holt, P.G., Sly, P.D., Bjorkstén, B. Atopic versus infectious diseases in childhood: a question of balance? *Pediatr. Allergy Immunol*. **8,** 53–58. (1997).
35. Stein, R.T. et al. Respiratory syncytial virus in early life and risk of wheeze and allergy by age 13 years. *Lancet.* **354,** 541–545. (1999).
36. Kenmoe, S., Kengne-Nde, C., Fatawou Modiyinji, A., Bigna, J.J., Njouom, R. Association of early viral lower respiratory infections and subsequent development of atopy, a systematic review and meta-analysis of cohort studies. *PLoS One* **15,** e0231816 (2020).
37. Ho, C., Wu, W. Risk factor analysis of allergic rhinitis in 6–8 year-old children in Taipei. *PLoS One* **16,** e0249572. (2021).
38. Strachan, D.P. et al. Worldwide time trends in prevalence of symptoms of rhinoconjunctivitis in children: Global Asthma Network Phase I. *Pediatr. Allergy Immunol.* **28,** doi: 10.1111/pai.13656 (2021). Online ahead of print.
39. Korppi, M. et al. IL33 rs1342326 gene variation is associated with allergic rhinitis at school age after infant bronchiolitis. *Acta Paediatr* **109,** 2112-6. (2020).
40. García-García, M.L. et al. Thymic stromal lymphopoietin, IL-33, and periostin in hospitalized infants with viral bronchiolitis. Med (United States). **96,** e6787. (2017).
41. Sastre B, et al. Bronchiolitis and recurrent wheezing are distinguished by type 2 innate lymphoid cells and immune response. *Pediatr. Allergy Immunol.* **32,** 51-59. (2021).
42. Johansson, K., McSorley, H.J. Interleukin-33 in the developing lung. Roles in asthma and infection. *Pediatr. Allergy Immunol.* **30,** 503-510. (2019).
43. Drake, L.Y., Kita, H. IL-33: biological properties, functions, and roles in airway disease. Immunol Rev. **278,** 173- 184 (20179.
44. Pijnenburg, M.W.H., De Jongste, J.C. Exhaled nitric oxide in childhood asthma: A review. *Clin. Exp. Allergy*. **38,** 246–59 (2008)
45. Elliott, M. et al. Exhaled nitric oxide predicts persistence of wheezing, exacerbations, and decline in lung function in wheezy infants and toddlers. *Clin. Exp. Allergy*. **43,** 1351–61. (2013).
46. Mikalsen, I.B., Halvorsen, T., Øymar, K. Exhaled nitric oxide is related to atopy, but not asthma in adolescents with bronchiolitis in infancy. *BMC Pulm Med*. **13**, 66 (2013).
47. Hyvärinen, M.K., Kotaniemi- Syrjänen, A., Reijonen, T.M., Korhonen, K., Korppi, M.O. Teenage asthma after severe early childhood wheezing: an 11- year prospective follow- up. *Pediatr. Pulmonol*. **40,** 316-22 (2005).
48. Biagi, C., Rocca, A., Poletti, G., Fabi, M., Lanari, M. Rhinovirus infection in children with acute bronchiolitis and its impact on recurrent wheezing and asthma development. *Microorganisms*. **8,** 1620 (2020)

49. Bergroth, E., et al. Rhinovirus Type in Severe Bronchiolitis and the Development of Asthma. *J. Allergy Clin. Immunol. Pract*. **8,** 588-595.e4. 2020
50. Liu, L. et al. Association between rhinovirus wheezing illness and the development of childhood asthma: a meta-analysis. *B.M.J. Open* **7,** e013034. (2017).
51. Hasegawa, K. et al. Association of Rhinovirus C Bronchiolitis and Immunoglobulin E Sensitization During Infancy With Development of Recurrent Wheeze. *JAMA Pediatr.* **173**, 544-552 (2019).
52. Lukkarinen, M. et al. Rhinovirus-induced first wheezing episode predicts atopic but not nonatopic asthma at school age. *J. Allergy Clin. Immunol.* 2017 **140,** 988-95. (2017).

Figures

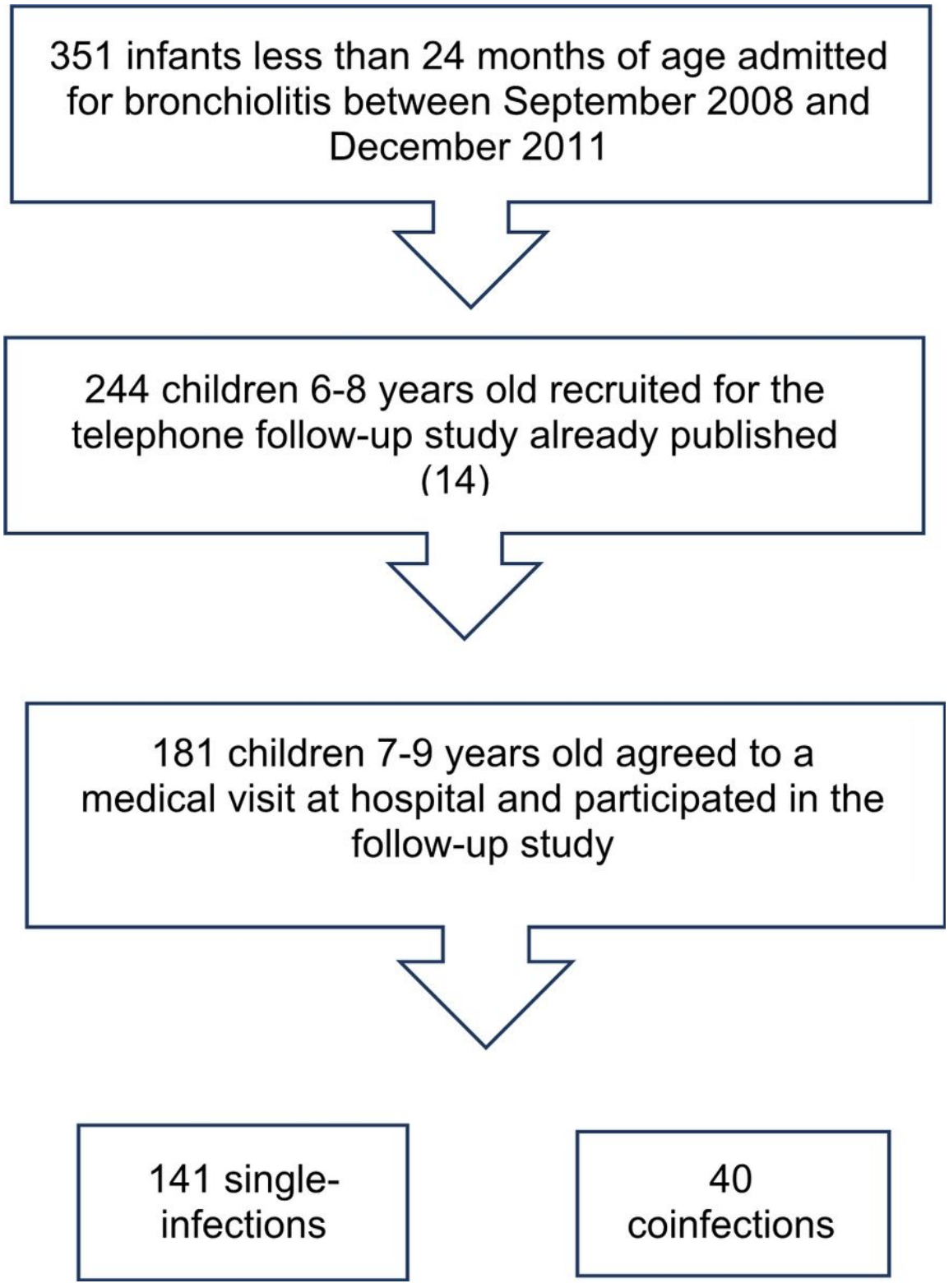

Figure 1

Flow chart of the subjects included in the study from the initial cohort to the follow-up cohort.