Role Of Viral Coinfections In Asthma Development In Children With Severe Bronchiolitis In Early Childhood

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Abstract

BACKGROUND. Severe viral bronchiolitis is associated with a higher risk of developing asthma, but little is known about the medium-term prognosis and the lung function evolution of patients admitted for viral coinfection-associated bronchiolitis. Our main objective was to compare the lung function, the prevalence of asthma and the rate of allergic sensitization at 6-9 years in children hospitalized for bronchiolitis with viral coinfection versus single viral infection.

METHODS. Observational, longitudinal study in children previously hospitalized for viral bronchiolitis with current age between 6-9 years. Clinical and epidemiological data were collected. Fraction of exhaled nitric oxide determination, spirometry and skin prick test for common aeroallergens were performed.

RESULTS. A total of 244 bronchiolitis-admitted children (52 coinfections and 192 single infections), with current age 6-9 years, accepted to participate by telephone answering the clinical questionnaires. Of them, 181 patients agreed for a medical visit. The overall frequency of asthma was 21%, being this prevalence almost twice as high in the viral coinfection group ($p = 0.049$). The coinfection cohort had more than twice as many admissions ($p = 0.04$), was more likely to receive montelukast ($p = 0.06$) and salmeterol/fluticasone treatment ($p = 0.03$) than the single-infection one. No differences regarding lung function values or allergic sensitization rate were observed between both groups.

The variables independently related to current asthma at 6-9 years were: viral coinfection during bronchiolitis ($p = 0.004$), allergic rhinitis ($p = 0.001$), food allergy ($p = 0.05$) and atopic dermatitis ($p = 0.017$).

CONCLUSIONS. Severe bronchiolitis associated with double or multiple viral detection in the first 24 months is an independent risk factor for higher frequency and greater severity of asthma at 6-9 years, being this risk almost three times higher compared to single infection. This fact is suggested by the higher frequency of current asthma, symptoms in intercrisis periods, maintenance anti-asthma treatment and number of hospitalizations for recurrent wheezing in children with coinfection compared to single infection. Early viral etiology identification in severe bronchiolitis might facilitate the prompt prediction and treatment of asthma in 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.

Although multiple studies have analyzed the impact of viral coinfections in acute bronchiolitis outcome, their role remains controversial, as conflicting results have been reported regarding their eventual association with short-term severity (1–5).

Regarding the medium and long-term outcome, 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 (6–13). However, to our knowledge, only one study, previously published by our group, has analyzed the medium-term respiratory outcome in patients with previous severe viral coinfection bronchiolitis. In this study, conducted by telephone interview, children with viral coinfection were 2.5-fold more likely to develop asthma at 6–9 years compared to those with single viral infection (14). The main objective of the present study was to compare respiratory morbidity, lung function and allergic sensitization in children previously admitted for severe bronchiolitis associated with viral coinfection and those with a single viral infection.

Methods

An observational, cohort study was conducted as part of a prospective study of severe viral respiratory infections in children. The methods of this study, as well as the clinical definitions were previously published (14). 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.

Clinical evaluation

All children currently aged 6–9 years, previously admitted to hospital for bronchiolitis at 0–24 months of age, between September 2008 and December 2011, with positive viral detection and whose parents agreed to participate were included.

The presence of serious illnesses preventing lung function tests performance or parents'/guardian's refusal to participate were considered exclusion criteria.

Parents were contacted by telephone and invited to a clinical interview. Respiratory symptoms were assessed using a clinical-epidemiological questionnaire (14) and the ISAAC questionnaire for asthma symptoms for 6-7-year-old children, previously validated and translated to Spanish (15). Current asthma prevalence was estimated by the proportion of patients who responded positively to 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 (16). Recurrent wheezing was defined as the presence of wheezing diagnosed by a doctor in the first 4 years of life (17).

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

FeNO was measured using the NIOX VERO® handheld device, considering normal FeNO values < 25 ppb (18). Spirometry was performed following the Spanish Pneumology and Thoracic Surgery Society recommendations (19). The following variables were collected: FVC (forced vital capacity), FEV1 (forced

expiratory volume in one second), FEV1/CVF and FEF25-75 (mean expiratory flow between 25% and 75% of FVC). The results were expressed as a percentage of Zapletal's reference values (20) and as a z-score of the predicted values according to the reference values of The Global Lung Function Initiative (GLI) (21).

Post-bronchodilator test was considered positive when an increase of FEV1 of at least 12% compared to the baseline was observed after administration of 400 µg of salbutamol.

Allergic sensitization

Allergic sensitization was evaluated by skin prick test (SPT) testing against common pneumo-allergens, following the recommendations of the European Academy of Allergy and Clinical Immunology (22–24). 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 diameters of the papule were the same or greater than those obtained with histamine.

Statistical analysis

The sample size needed to detect a difference in FEV1 of at least 7 percentage points, with an alpha error of 5% and a power of 80%, was calculated, estimating 37 cases in the coinfection group and 111 in the single viral infection one.

Categorical variables were described used absolute and relative frequencies. Continuous variables were described using mean and standard deviation (normal distribution) or median and interquartile range (non-normal distribution). To compare qualitative variables, Chi2 test or Fisher’s exact test was used, while for quantitative variables we used the Student's T test or Mann Whitney's U test. In order to calculate the independent association between the concerning variable (viral coinfection) with the other variables included in the study, a multivariate analysis was performed using logistic regression. Multivariate stepwise logistic regression analysis was used to calculate the adjusted odds ratios (OR) with 95% confidence intervals (CI 95%) for estimating the association between different factors and asthma. All analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 21.0.

Results

Among the 351 patients admitted for bronchiolitis with positive viral detection and current age 6–9 years, 244 (52 coinfections and 192 single infections) were located and accepted to participate by telephone answering the clinical questionnaires. A total of 181 patients agreed to a medical visit. 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.

The most common virus detected during admission for bronchiolitis was RSV (133/ 55%), followed by HRV (63/ 26%). Viral coinfection was detected in 21% of patients, mainly RSV-HRV (15/6%) followed by RSV-HBoV (7/3%).

Clinical characteristics

Personal and family background, as well as clinical characteristics during the admission for bronchiolitis are shown in Table 1.

The rate of maternal asthma was more than three times higher in children with single infection ($p = 0.05$), who also showed a trend to have atopic mothers more frequently, although it did not reach statistical significance ($p = 0.07$). No differences were found in remaining clinical variables studied during the episode of bronchiolitis, with the exception of single or multiple viral identification.

The medium age at the time of the follow-up visit was 7 years. After admission for bronchiolitis, a high proportion of patients in both groups reported some episode of wheezing, almost a third of them requiring hospital admission by this reason. The average number of admissions for a wheezing episode was twice as high in children with viral coinfection ($p = 0.04$), who also reported a higher frequency of respiratory symptoms in intercrisis, being the difference close to the statistical significance ($p = 0.06$).

Both groups required chronic asthma treatment with a similar frequency. However, the likelihood of receiving the combination salmeterol/fluticasone, indicated for a greater severity step, was 3.4 times higher in the coinfection group ($p = 0.03$). Also, the prescription of montelukast showed a trend to be more frequently used in coinfections, but nearly reaching statistical significance ($p = 0.06$). Table 2.

The variables associated with therapy prescription for chronic asthma in the bivariate analysis were analyzed using logistic regression. We observed that viral coinfection, allergic rhinitis, and maternal atopy and smoking, were independently associated with chronic asthma treatment requirement. Table 3.

The ISAAC questionnaire for asthma symptoms for 6-7-year-old children was answered by the parents. The responses obtained are shown in Table 4. Two hundred and two (83%) patients reported wheezing and 43 (17.6%) had been diagnosed with asthma at any time. The overall prevalence of current asthma, assessed by the affirmative response to question 2 (wheezing in the last 12 months), was 20%, with a 2-fold increase in probability for coinfections (31%) compared to single infections (18%) ($p = 0.04$).

We performed a bivariate analysis of the variables possibly associated with current asthma, both related to the acute episode of bronchiolitis and to family and personal history. The results are shown in Table 5. Current asthma was significantly associated with the presence of atopic dermatitis ($p = 0.001$), allergic rhinitis ($p < 0.001$), food allergy ($p = 0.012$), maternal asthma ($p = 0.04$) and viral coinfection during admission for bronchiolitis ($p = 0.04$).

Variables with a value $p < 0.20$ were introduced into a logistic regression model in order to avoid confounding factors. After multivariate analysis, the three variables related to atopy remained

independently associated with current asthma at 6–9 years: allergic rhinitis (p = 0.001), food allergy (p = 0.05) and atopic dermatitis (p = 0.017). In addition, the history of admission for bronchiolitis with viral co-infection was also an independent risk factor for the development of asthma at 6–9 years (p = 0.004; OR 3.2, 95% CI 1.4–6.9). Table 6.

Skin prick test

Skin prick tests against common aeroallergens were performed on 172 children, where 64 (37%) of them tested positive, 46 of which were polysensitized. The most common were outdoor allergens, mainly grass pollen.

The prevalence of allergic sensitization in coinfections (31.4%) was similar to that of single infections (38.7%), (p = 0. 428). In contrast, the rate of positive prick test was significantly higher in children with current asthma (67.6%) than in children without current asthma (31.6%), p < 0.001.

Lung function

A total of 177 spirometries were performed, of which 172 were valid. Both groups showed normal lung function values. No statistically significant differences were found between the two groups in the basal FEV1 value, but the z score was slightly higher in the coinfection group (0.1 ± 1.1 *vs*. -0.3 ± 1.1, p = 0.04) compared with the single infection one.

No difference was found between both groups in the proportion of patients with positive bronchodilator test (21.6% in coinfections *vs.* 16.7% in single infections, p = 0.520).

When analyzing lung function values of children with current asthma we did observe a significant decrease in the FEV1/FVC ratio (p = 0.01) and the MEF50 (p = 0.024) compared to children without current asthma. Also, the proportion of positive bronchodilator tests was more frequent in children with current asthma (48% *vs*. 19.5%, p = 0.001). Table 7.

FeNO

FeNO was 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 simple infection (12.6%) (p = 0.768).

In contrast, the proportion of children with FeNO > 25 ppb was significantly higher in children with current asthma (23%) compared to children without current asthma (10%), p = 0.04.

Discussion

Our results show that the respiratory morbidity of children with a previous history of severe bronchiolitis associated with viral coinfection is, in the first 6–9 years of life, significantly higher than that of children with a simple viral infection. This is suggested by the higher frequency of recurrent wheezing, symptoms in intercrisis periods, chronic asthma treatment requirement, number of hospitalizations due to a

respiratory cause, and prevalence of current asthma in children with viral coinfection compared to simple infections.

Although the pathogenic mechanism is not well known and the possible causal relationship between infant bronchiolitis and the subsequent development of asthma is not yet well defined, there is unquestionable scientific evidence of the existence of an association between both entities. Many studies have reported this relationship, initially with RSV and later with other viruses such as hMPV, HBoV and especially HRV (8–11, 25), which, when identified in bronchiolitis, is associated with up to ten times the risk of developing asthma at 6 years of age (10, 26).

However, most of the large prospective studies have focused their analysis on single viral infections, and very few have analyzed the role of viral coinfections in the later development of recurrent wheezing and asthma.

Amat et al., (27), in a 3-year follow-up study of 154 children with a previous history of bronchiolitis (inpatient and outpatient), observed that 46.8% of them had been diagnosed with recurrent wheezing at age 3, identifying as risk factors only a family background of atopy and living in an apartment. In contrast, neither the type of virus identified, nor the single or multiple viral infections showed association with recurrent wheezing development. In contrast, RSV-HRV viral coinfection was independently associated with allergic sensitization at 3 years. In previous studies of atopy phenotypes, allergic sensitization was associated with an increased risk of later development of asthma in children (28–32). Lee et al. found that sensitization to outdoor allergens is associated with an increased risk of new-onset asthma and bronchial hyperresponsiveness (33). In addition, allergic sensitization is one of the major criteria of the modified Asthma Predictive Index (mAPI), used to predict asthma at 6, 8 and 11 years in children < 3 years with recurrent wheezing (34). Our own results show a 4-fold increased risk of asthma in children with allergic sensitization. Therefore, although Amat et al., (27) did not find association between viral coinfection and asthma at 3 years, the association of coinfections with allergic sensitization could be a predictor of asthma development in these coinfected children later in life.

The other study that evaluated the medium-term respiratory morbidity of viral coinfections is that of Petrarca et al., who, in a retrospective, follow-up telephone study at 36 months after admission for bronchiolitis, found no association between coinfection and recurrent wheezing, despite the higher family history of asthma in patients infected with a mixed infection (35). As in Amat´s study, the length of the follow-up period was shorter than ours and no data regarding asthmatic treatment or admissions for asthma were provided.

According to our results, the probability of developing asthma at age 6–9 years was almost twice as high in children with coinfection, who had an asthma prevalence of 31% compared to 18% in the group with single viral infections. Moreover, viral coinfection was an independent risk factor for the diagnosis of current asthma at school-age. In addition, patients with viral coinfection showed greater respiratory morbidity than patients with single infection, since they not only developed asthma more frequently, but the course was more severe, as demonstrated by the higher rate of admission for asthma, double that of

children with single infection. Patients in the coinfection group also reported more symptoms in the intercrisis periods, the difference being almost significant (p = 0.06) and needed more frequent maintenance treatment corresponding to a higher level of asthma severity. All these data strongly suggest that coinfections are associated with increased respiratory morbidity, at least until 6–9 years of age.

Regarding maintenance treatment for asthma, Bergroth et al., (36) found that 45% of children previously admitted for bronchiolitis reported prescription of asthma control medication, mainly inhaled corticosteroids, within 48 months after hospitalization. The proportion of treated children was higher in patients with HRV (47%), than in those with RSV (15%) or with non-RSV/HRV bronchiolitis (26%). In our series, 45% of children required treatment coinfection being an independent risk factor that doubled the probability of receiving asthma maintenance treatment and tripled the probability of receiving the combination inhaled glucocorticoid/long-acting beta2 agonist (ICS/LABA). It should be noted that the combination ICS/LABA is usually prescribed as preferred initial treatment in step 4 of asthma treatment (37). Again, both, increased prescription of ICS and ICS/LABA suggest greater severity of asthma in children with a history of bronchiolitis associated with viral coinfection.

In a previous study by Bergroth et al., (38) HRV etiology was associated with more courses of systemic corticosteroids during the follow-up, implying a greater number of asthmatic exacerbations. Although the use systemic steroids was not among the variables in our study, we found a significantly higher rate of recurrent wheezing admissions in the coinfection group, whose treatment usually involves the administration of systemic steroids. Therefore, indirectly, we can also state that patients with viral coinfection (mainly RSV-HRV) require more admissions and receive more treatments with systemic steroids. These data strongly support once again that, in our study, viral coinfections were associated with greater clinical severity in the medium term.

Previous studies have analyzed the association between RSV bronchiolitis and pulmonary function sequelae, mainly obstructive airways disease with varying degrees of bronchodilator reversibility (6–10, 36, 39–41). However, there has been very little standardization between studies (42). A recent systematic review by Verwey et al. (42), 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 reported this association (42). Although abnormal measurements varied across studies, the most commonly described was an obstruction to airflow with or without bronchodilator reversibility. Most of our patients had normal lung function values at 6–9 years and no significant differences were found between the coinfection and the simple viral infection groups. Since in our study lung function was compared among children with severe bronchiolitis with coinfection and with simple infection rather than with a healthy control group, as is usual in other studies, it is not surprising that no difference in lung function was observed between the two groups.

The fraction of exhaled nitric oxide has been suggested as a non-invasive biomarker of eosinophilic inflammation (43). Although some authors found that FeNO measurements in infants with recurrent wheezing episodes were associated with persistence of wheezing through age 3 years (44), others like Mikalsen et al., (45) 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.

All our data suggest that bronchiolitis with viral coinfection is associated with increased respiratory morbidity in the medium term. The pathogenic mechanism is unknown but probably the different immune response triggered by double or multiple infections may play a role. Our group, in a previous study in 213 hospitalized infants with bronchiolitis, showed that RSV-HRV coinfected infants exhibited the highest levels of thymic stromal lymphopoietin (TSLP), that has been identified as a master switch for allergic inflammation and is an important cytokine in the development of allergic asthma. We also found that infants with dual RSV + HRV infection were 9 times more likely to have detectable nasal TSLP and this association was independent of other factors such as age or illness severity. These findings suggest that the immunological response in acute bronchiolitis is partly dependent on virus-specific factors and could partly explain the worse mid-term evolution of coinfections (46).

The main limitation of our study is the small number of bronchiolitis with single infections other than RSV or HRV, given the high prevalence of coinfections among some viruses like HBoV. The main strength is to be able to include a cohort of patients, included on admission for bronchiolitis, with clinical and virological data prospectively collected on admission.

In summary, having severe bronchiolitis with double or multiple positive viral detections is an independent risk factor for higher frequency and greater severity of asthma at 6–9 years. The early identification of viral etiology of severe bronchiolitis might facilitate the early prediction and treatment of asthma in school age.

List Of Abbreviations

CI 95%: 95% confidence interval

FEF25-75: mean expiratory flow between 25% and 75% of FVC

FeNO: fraction of exhaled nitric oxide

FEV1: forced expiratory volume in one second

FLU: Influenza virus

FVC: forced vital capacity

GLI: global lung function initiative

HBoV: Human bocavirus

HMPV: Human metapneumovirus

HRV: Rhinovirus

ICS/LABA: inhaled glucocorticoid/long-acting beta2 agonist

mAPI: modified Asthma Predictive Index

OR: odds ratio

PICU: Pediatric Intensive Care Unit

PIV: Parainfluenza virus

RSV: Respiratory syncytial virus

SPSS: statistical Package for the Social Sciences

SPT: skin prick test

TSLP: thymic stromal lymphopoietin

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.

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Author's contributions

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Tables

Table 1
Background and clinical characteristics at admission of infants with bronchiolitis associated with viral coinfection *versus* single infection

	Viral coinfection (n = 52)	Single infection (n = 192)	P value	OR (CI95%)
Current age* (years)	7.5 (0.9)	7.4 (0.9)	0.778	-
Breastfeeding	43 (82.7%)	160 (83.3%)	0.913	0.9 (0.4–2.2)
Daycare attendance	43 (82.7%)	121 (63%)	0.007	2.8 (1.3–6.1)
Siblings < 5 years-old	34 (85%)	109 (80.1%)	0.489	1.4 (0.5–3.7)
Atopic dermatitis	19 (36.5%)	89 (46.4%)	0.206	0.7 (0.4–1.3)
Food allergy	5 (9.5%)	18 (9.5%)	0.958	1 (0.4–2.9)
Allergic rhinitis	8 (15.4%)	60 (31.3%)	0.024	0.4 (0.2–0.9)
Asthma	2 (3.8%)	26 (13.5%)	0.05	0.3 (0.1–1.1)
Mother	4 (7.7%)	26 (13.5%)	0.255	0.5 (0.2–1.6)
Father	8 (15.4%)	27 (14.1%)	0.809	1.1 (0.5–2.6)
Siblings				
Atopy	8 (15.4%)	52 (27.2%)	0.07	0.5 (0.2–1.1)
Mother	11 (21.2%)	42 (21.9%)	0.911	0.9 (0.5-2)
Father	15 (28.8%)	56 (29.2%)	0.964	0.9 (0.5–1.9)
Siblings				
Smoking	14 (26.9%)	44 (22.9%)	0.547	1.2 (0.6–2.5)
Mother	15 (28.8%)	65 (34%)	0.481	0.8 (0.4–1.5)
Father				
Smoking during pregnancy	9 (17.3%)	36 (18.8%)	0.8	0.9 (0.4-2)
Age at broncjhiolitis* (days)	163.5 (124.9)	161.7 (171.3)	0.942	-
Male sex	29 (55.8%)	87 (45.5%)	0.191	1.5 (0.8–2.8)
Prematurity	6 (11.8%)	23 (12.2%)	0.937	0.9 (0.4–2.5)
Fever ≥ 38,5ºC	30 (57.7%)	91 (47.6%)	0.199	1.5 (0.8–2.8)
Maximum Tª*	38.6 (0.6)	38.7 (0.6)	0.428	-
SatO2 < 95%	26 (50%)	120 (63.2%)	0.086	0.6 (0.3–1.1)

	Viral coinfection (n = 52)	Single infection (n = 192)	P value	OR (CI95%)
Abnormal X-ray	13 (34.2%)	55 (37.7%)	0.694	0.9 (0.4–1.8)
PICU	2 (4%)	8 (4%)	0.918	0.9 (0.2–4.5)
Antibiotics	10 (19.2%)	26 (13.5%)	0.305	1.5 (0.7–3.4)
Leukocyte* (cells/mcl)	13638 (5656)	14038 (13840)	0.892	-
C-reactive protein* (mg/L)	22.8 (26.4)	25.9 (31.2)	0.662	-
Fever duration*	3.2 (2.1)	2.6 (1.9)	0.187	-
Duration of hypoxemia*	3.7 (2.7)	3.3 (2.7)	0.507	-
Days of admission*	4.2 (2.8)	4.5 (2.8)	0.447	-

*Mean (standard deviation)

OR (Odds Ratio)

CI (Confidence interval)

PICU: Pediatric Intensive Care Unit

Table 2
Respiratory evolution in children with viral coinfection versus single infection bronchiolitis.

	Viral coinfection (n = 52)	Single infection (n = 192)	P value	OR (CI 95%)
Recurrent wheezing	48 (92.3%)	159 (82.8%)	0.09	2.5 (0.8–7.4)
Numbrer of wheezing episodes/year*	3.8 (2.9)	3 (2.3)	0.113	-
Wheezing-related admissions	15 (28.8%)	59 (31.1%)	0.76	0.9 (0.5–1.8)
Number of wheezing-related admissions*	3.4 (3.4)	1.4 (1.6)	0.04	-
Intercrisis symptoms	4 (11.4%)	4 (3%)	0.06	4.2 (0.9–17.6)
Asthma treatment	28 (53.8%)	81 (42.4%)	0.141	1.6 (0.9–2.9)
Budesonide	16 (30.8%)	58 (30.4%)	0.955	1 (0.5–1.9)
Montelukast	24 (46.2%)	62 (32.5%)	0.06	1.8 (0.9–3.3)
Salmeterol/fluticasone	6 (11.5%)	7 (3.7%)	0.03	3.4 (1.1–10.6)

*Mean (standard deviation)

OR (Odds Ratio)

CI (Confidence interval)

Table 3
Multivariate analysis of risk factors associated with the prescription of asthma treatment

	P value	OR (CI 95%)
Viral coinfection	0.026	2.1 (1.1–4.1)
Allergic rhinitis	< 0.001	3.2 (1.7-6)
Maternal atopy	0.022	2.1 (1.1–3.9)
Maternal smoking	0.046	1.9 (1-3.5)

OR (Odds Ratio)

CI (Confidence interval)

Table 4
Asthma symptoms according to the ISAAC questionnaire in 6-9-year-old children previously admitted with bronchiolitis, single *versus* multiple viral infection

	Viral coinfection (n = 52)	Single infection (n = 192)	P value	OR (CI 95%)
1. Ever wheezing or whistling in the chest at any time in the past	45 (86.5%)	157 (81.8%)	0.419	1.4 (0.6–3.4)
2. Wheezing or whistling in the chest in the last 12 months	16 (30.8%)	35 (18.2%)	0.044	1.9 (1-3.9)
3. Number of attacks of wheezing in the last 12 months*	2.2 (0.4)	2.1 (0.2)	0.211	-
4. Number of sleep disturbance due to wheezing in the last 12 months*	0.9 (0.8)	1.4 (0.7)	0.03	-
5. Wheezing severe enough to limit child's speech to only one or two words at a time between breaths in the last 12 months	5 (35.7%)	9 (25.7%)	0.503	1.6 (0.4–6.1)
6. Ever had asthma	8 (15.4%)	35 (18.2%)	0.633	0.8 (0.4–1.9)
7. Wheezing during or after exercise in the last 12 months	6 (11.5%)	24 (12.5%)	0.851	0.9 (0.4–2.4)
8. Dry cough at night apart from a cough associated with a cold or chest infection in the last 12 months	9 (17.3%)	23 (12%)	0.313	1.5 (0.7–3.6)

*Mean (standard deviation)

OR (Odds Ratio)

CI (Confidence interval)

Table 5
Background and clinical characteristics during admission for bronchiolitis in asthmatic *versus* non-asthmatic patients

	Asthmatic (n = 49)	Non asthmatic (n = 195)	P value	OR (CI 95%)
Breastfeeding	43 (84.3%)	160 (82.9%)	0.81	1.1 (0.5–2.6)
Daycare attendance	30 (58.8%)	134 (69.4%)	0.151	0.6 (0.3–1.2)
Siblings < 5 years-old	33 (80.5%)	110 (81.5%)	0.886	0.9 (0.4–2.3)
Atopic dermatitis	33 (64.7%)	75 (38.9%)	0.001	2.9 (1.5–5.5)
Food allergy	10 (19.6%)	13 (6.7%)	0.012	3.4 (1.4–8.2)
Allergic rhinitis	26 (51%)	42 (21.8%)	< 0.001	3.7 (1.9–7.1)
Asthma	10 (19.6%)	18 (9.3%)	0.04	2.4 (1-5.5)
Mother	10 (19.6%)	20 (10.4%)	0.074	2.1 (0.9–4.8)
Father	9 (17.6%)	26 (13.5%)	0.449	1.4 (0.6–3.2)
Siblings				
Atopy	17 (33.3%)	43 (22.4%)	0.107	1.7 (0.9–3.4)
Mother	12 (23.5%)	41 (21.2%)	0.725	1.1 (0.5-2-4)
Father	20 (39.2%)	51 (26.4%)	0.074	1.8 (0.9–3.4)
Siblings				
Smoking	16 (31.4%)	42 (21.8%)	0.152	1.6 (0.8-3-3)
Mother	18 (35.5%)	62 (32.3%)	0.685	1.1 (0.6–2.2)
Father				
Smoking during pregnancy	10 (19.6%)	35 (18.2%)	0.822	1.1 (0.5-2-4)
Age at bronchiolitis* (days)	175.4 (186.6)	158.5 (155.6)	0.510	-
Male sex	29 (56.9%)	87 (45.3%)	0.142	1.6 (0.8–2.9)
Prematurity	9 (17.6%)	20 (10.6%)	0.170	1.8 (0.8–4.3)
SatO2 < 95%	31 (60.8%)	115 (60.2%)	0.941	1 (0.5–1.9)
Abnormal X-ray	12 (34.3%)	56 (37.6%)	0.716	0.8 (0.4–1.9)
Antibiotic treatment	5 (9.8%)	31 (16.1%)	0.262	0.6 (0.2–1.5)
Viral coinfection	16 (31.4%)	36 (18.7%)	0.049	1.9 (0.9–3.9)

	Asthmatic (n = 49)	Non asthmatic (n = 195)	P value	OR (CI 95%)
Days of hypoxemia*	2.8 (2.2)	3.5 (2.8)	0.204	-
Days of hospital stay*	4.1 (2.5)	4.5 (2.9)	0354	-

*Mean (standard deviation)

OR (Odds Ratio)

CI (Confidence interval)

Table 6
Risk factors involved in asthma development at 6–9 years

	P value	OR (CI 95%)
Allergic rhinitis	0.001	3.5 (1.7–7.2)
Food allergy	0.05	2.6 (1-6.8)
Atopic dermatitis	0.017	2.3 (1.2–4.7)
Viral coinfection	0.004	3.2 (1.4–6.9)

OR (Odds Ratio)

CI (Confidence interval)

Table 7
Lung function in children with coinfection *versus* single viral infection bronchiolitis and asthmatic *versus* non-asmathic patients.

	Viral coinfection (n = 52)	Single infection (n = 192)	P value	Asthmatic	Non asthmatic	P value
FEV_1 (% predicted)	102.2 (14.4)	97.6 (13.2)	0.066	97.3 (14.3)	99.1 (13.4)	0.462
FEV_1 z score	0.1 (1.1)	-0.3 (1.1)	0.04	-0.4 (1.1)	-0.1 (1.1)	0.179
FVC (% predicted)	98.2 (12.2)	96.6 (11.7)	0.456	98.6 (12.2)	96.4 (11.7)	0.308
FVC z score	0.12 (1)	-0.04 (0.9)	0.357	0.03 (1)	-0.02 (0.9)	0.772
FEV_1/FVC (% predicted)	104.3 (7,9)	101.9 (8.8)	0.148	99.3 (9.7)	103.5 (7.9)	0.01
FEV_1/FVC z score	0.1 (1.1)	-0.3 (1.2)	0.05	-0.6 (1.3)	-0.1 (1.1)	0.02
FEF_{25-75} (% predicted)	90.5 (23.1)	85.7 (25)	0.289	79.4 (25.7)	89.3 (23.9)	0.024
FEF_{25-75} z score	-0.2 (1.1)	-0.5 (1.2)	0.108	-0.8 (1.2)	-0.3 (1.1)	0.01