



Respiratory syncytial virus infection and bronchial hyperreactivity in children up to two years of age in correlation with atopy

Infekcija respiratornim sincicijalnim virusom i bronhijalna hiperreaktivnost kod dece uzrasta do dve godine u odnosu na atopiju

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Abstract

Background/Aim. Bronchiolitis in early childhood caused by respiratory syncytial virus (RSV) is considered to be important risk factor of the recurrent wheezing and asthma development. The aim of this study was to examine the frequency of RSV infection and atopy in children up to two years of age and to determine their correlation with bronchial hyperreactivity. **Methods.** The study included 175 children aged 5–24 months. The presence of RSV infection was identified by serum levels of IgA and IgG determined by ELISA. Bronchial hyperreactivity (BHR) has been defined as the existence of chronic bronchial disease and/or three or more previous suspected diagnosis of acute bronchial disease. Atopy was confirmed by detection of the specific serum IgE using quantitative multitest Phadiatop infant (cut off ≥ 0.35 kUA/L). **Results.** The children with atopy were more frequently infected with RSV (43.3%) than those without atopy (22.8%; $p = 0.02$). The higher frequency of RSV infection was found in children with BHR in comparison with those without it but only in the group who also had atopy (77.8% vs 28.6%, $p = 0.018$). In the female children, BHR and RSV infection were associated in 62.5% of cases, regardless the atopy. In the male children with atopy, RSV infection was associated with BHR in 83.3% of the cases, while in those without atopy, RSV infection with BHR was found in only 17.4% of the cases. **Conclusion.** Children up to two years of age with atopy are more frequently infected with RSV (43.3%) than non-atopic children. Every third child with atopy develops BHR and 77.8% of them also have RSV infection. Atopic children are at higher risk for development of BHR when infected with RSV also.

Key words:

respiratory syncytial viruses; bronchial diseases; hypersensitivity, immediate; comorbidity; child; serbia.

Apstrakt

Uvod/Cilj. Bronholitis u ranom detinjstvu izazvan respiratornim sincicijalnim virusom (RSV), smatra se značajnim faktorom rizika od pojave rekurentnog vizinga i astme. Cilj ovog rada bio je da se ispita učestalost RSV infekcije i atopije kod dece uzrasta do dve godine i da se ustanovi njihova korelacija sa bronhijalnom hiperreaktivnošću (BHR). **Metode.** U istraživanje je bilo uključeno 175 dece uzrasta od 5 do 24 meseca. Prisustvo RSV infekcije je ustanovljeno na osnovu serumske koncentracije anti-RSV IgA i IgG antitela određenih ELISA testom. Hiperreaktivnost bronhijalnog stabla (BHR) je definisana kao postojanje hronične bronhijalne bolesti i/ili tri i više prvih kurativnih pregleda pod dijagnozom akutne bronhijalne bolesti. Postojanje atopije utvrđeno je detekcijom serumskih specifičnih IgE antitela kvalitativnim multitestom *Phadiatop infant* ($\geq 0,35$ kUA/L). **Rezultati.** Deca sa atopijom češće su imala RSV infekciju (43,3%) nego deca bez atopije (22,8%; $p = 0,02$). Veća učestalost RSV infekcije kod dece sa BHR u odnosu na decu bez BHR postojala je samo u grupi atopičara (77,8% vs 28,6%; $p = 0,018$). Ženska deca imala su udruženost BHR i RSV infekcije u 62,5% slučajeva bez obzira na atopiju. Kod muške deca sa atopijom RSV infekcija bila je udružena sa BHR kod 83,3%, dok je kod muške dece bez atopije to bio slučaj kod samo 17,4% slučajeva. **Zaključak.** Deca uzrasta do dve godine sa atopijom češće su imala RSV infekciju (43,3%) nego deca bez atopije. Svako treće dete sa atopijom imalo je BHR, pri čemu je 77,8% imalo i RSV infekciju. Deca sa atopijom koja imaju RSV infekciju su u većem riziku od razvoja BHR.

Ključne reči:

respiratorni sincicijalni virusi; bronhusi, bolesti; hipersenzibilnost, rana; komorbiditet; deca; srbija.

Introduction

Respiratory syncytial virus (RSV) is considered to be the most important causative agent of respiratory diseases in children in their earliest age^{1,2}. The incidence of RSV infection in early age in countries of European Union ranges from 5.4% to 40.8%³⁻⁵. In our population, positive RSV IgG was found in 24% of children within their first two years of life (in the age of 5–12 months – 12.9% and in the second year – 47%)⁵.

RSV belongs to the genus of Pneumovirus and its genome is presented as a single-stranded RNA in the form of the negative chain. It is transmitted by aerosol or by the direct contact with the infected person⁶. Virus contagiousity degree is high and it has been confirmed that if only one RSV positive child comes to the kindergarten, 90% of healthy children will also be affected⁷. RSV infection most frequently remains in ciliary cells where the movements of cilia are disordered and infected cells peel off⁷. Epithelial cells damage initiates development of edema with the serum proteins excretion in the airways lumen. This results in respiratory obstruction and the initiation of immune response in the airways serosa. All these contribute to development of the respiratory disease symptoms and bronchial hyperreactivity⁸.

RSV bronchiolitis in early childhood is considered to be important risk factor of the recurrent wheezing and asthma development⁹, but a rather large number both of retrospective and prospective studies suggest that RSV infection itself could be determinant of bronchial hyperreactivity (BHR)^{10,11}. The previous study from Serbia reported that 20.3% of children with expressed bronchitis and/or bronchiolitis had anti-RSV IgG, while these antibodies were found in 33.3% of children diagnosed with asthma (J45) or chronic bronchial obstruction (J44.9)⁵.

More than one half of all asthma cases develop before the third year of age and an early occurrence of asthma in the infant age is most frequently manifested by wheezing during viral respiratory infections^{12,13}. It has been confirmed that allergic sensitization is an important risk factor for development of wheezing during and after RSV infection¹⁴. In children with atopy, the defect of respiratory epithelium due to the previous exposure to allergens, represents the risk factor for development of asthma after recurrent viral infections¹⁵. On the contrary, epithelium impaired by frequent infections may become the spot of more intensive absorption of aeroallergens, intensifying in this way the effect of allergens upon exacerbation of asthma¹⁵. It has been suggested that the second year of age is the risky period for the remodeling of airways, which is important as the pathologic basis of asthma¹⁶. Also, this age is the risky period for the synergic effect of both infection and atopy in asthma development¹⁷. On the other hand, some authors suggest that development of bronchial hyperreactivity is rather related to individual characteristics of children (such as male sex) than to RSV bronchiolitis¹⁸.

In Serbia, there are no so far published data on RSV infection as determinant of BHR development in correlation with atopy. The aim of this study was: to examine the frequency of RSV infection and atopy in children up to two years of age and to determine the correlation of RSV infection and atopy with bronchial hyperreactivity.

Methods

The study included 175 children 5–24 months of age from the territory of the city of Kragujevac. Data on individual and sociodemographic characteristics were obtained from the questionnaire. The poll was conducted in the Outpatient Clinic in Kragujevac and in the Center for Allergic Diseases and Asthma Prevention of the Institute of Public Health in Kragujevac. Data on respiratory diseases were obtained from the data base of the Medical Center of Kragujevac.

The diagnosis of RSV infection was based on serum anti-RSV IgA and IgG levels above the cutoff value. Venous blood was taken from children in the morning; after 2 hours it was centrifuged and serum was stored at -75°C before analysis. Serum IgA and IgG antibodies were determined by the quantitative ELISA (SERION ELISA classic, Institute Virion/Serion GmbH, Würzburg, Germany). Serum levels of the specific anti-RSV antibodies were determined on the basis of optic values density, 4PL method (Single-point quantification using the 4PL method) by using the SERION software program (SERION easy base 4PL-Software evaluate). Positive anti-RSV IgG result was defined as ≥ 15 U/mL concentration and for IgA ≥ 10 U/mL. Finding of just one specific anti-RSV antibody (IgA or IgG class) was considered an evidence of RSV infection.

Lower respiratory tract diseases comprised acute and chronic bronchial diseases and pneumonia. The group of children with acute bronchial diseases included those with the diagnosis J20 – acute bronchitis (*Bronchitis acuta*) and J21 – acute bronchiolitis (*Bronchiolitis acuta*). The group of children with chronic bronchial diseases included those with the diagnosis J44.9 – chronic obstructive pulmonary disease (*Morbus pulmonis obstructivus chronicus alius*) and J45 – asthma (*Asthma bronchiale*). The group with pneumonia included children with the diagnoses J12–18, that is, viral, bacterial and non-classified pneumonias (*Pneumonia viralis, bacterialis and non specificatus*).

Bronchial hyperreactivity is defined as the presence of chronic bronchial disease (J44 or J45) and/or as three or more previous suspected diagnosis of acute bronchial disease (J20 or J21).

The presence of atopy was confirmed when the serum specific IgE was detected by the quantitative multitest Phadiatop infant (cut off ≥ 0.35 kUA/L). Allergens used as antigens in this test were proteins from the egg white, cow milk, peanuts, shrimps, cat's and dog's hair, mites, pollen of the silver birch, Timothy grass, ambrosia and nettle¹⁹. Phadiatop infant test was carried out *in vitro* by the fluorescent immunoassay using Immunocap-100 device (Phadia AB Upsala, Sweden). Children with atopy included those with positive Phadiatop infant test, namely, children with the serum level of specific IgE ≥ 0.35 kUA/L. Non-atopic children had serum specific IgE antibodies below 0.35 kUA/L level, that is, their Phadiatop infant test was negative.

Statistical analysis data was performed using the SPSS software package 20.0 (IBM SPSS Statistic for Windows, Amonk, NY, USA). For analysis of statistical differences in frequencies of the dependent variable in comparison with categorical variables, χ^2 test was used. The table of contin-

gence was used to determine differences in frequencies among variables with more than 2 categories (3×2 , 4×2 ...).

Examination was carried out in accordance with ethical standards of the Helsinki Declaration from 1975, revised in 1983. The study was performed within the plan for allergic diseases prevention in children treated at the Institute of Public Health in Kragujevac and approved by the Ethical Board of the mentioned institute, as well as by the Faculty of Medical Sciences in Kragujevac. Biological samples (children's blood) were taken in the Medical Center under pediatric control and in accordance with their age and pediatric protocol for standard control of hematologic parameters for each child's blood count. The parents were informed about the purpose, aim and methods of this study and they gave informed consent for their children's inclusion into the study.

Results

The results on RSV infection and atopy in the different groups of children are presented in Table 1. A statistically significant difference in frequency of RSV infection was

proven among the different age groups, where RSV was most frequent in the group of children 7–12 months old ($p = 0.000$). Also, the children born in autumn were more often infected with RSV ($p = 0.059$).

The children up to two years of age had RSV infection in 26.3% (46/175) and atopy in 17.1% (30/175). The children with atopy had more often RSV infection (43.3%; 13/17) in comparison with those without atopy (22.8%, 33/145; $p = 0.020$), (Figure 1).

No statistically significant difference in BHR frequency was found between the children with atopy (30%; 9/30) and those without atopy (19.3%, 28/145; $p = 0.192$). In the group of children with BHR a higher frequency of RSV infection (37.8%; 14/37) was observed in comparison with those without BHR where RSV infection was confirmed in 23.2% of the cases, (32/138; $p = 0.072$). A higher frequency of RSV infection in children with BHR in comparison with those without BHR, was found only in the group of children with atopy (77.8%, 7/9 vs 28.6%; 6/21; $p = 0.018$), (Figure 2).

Analysis of RSV infection and atopy according to age showed that the children with BHR tended to be more often

Table 1
Results of respiratory syncytial virus (RSV) infection and the presence of atopy in different groups of children

Group	Total n* = 175 [n* by category (%)]	RSV infection (%) (n* RSV pos./ n* in category)	<i>p</i>	Atopy (%) (n* with atopy /n* by category)	<i>p</i>
Age (months)					
5–6	59 (33.7)	20.3 (12/59)	0.000	11.9 (7/59)	0.163
7–12	74 (42.3)	14.9 (11/74)		16.2 (12/74)	
13–24	42 (24.0)	54.8 (23/42)		26.2 (11/42)	
Sex					
male	101 (57.7)	27.7 (28/101)	0.614	18.8 (19/101)	0.494
female	74 (42.3)	24.3 (18/74)		14.9 (11/74)	
Natural feeding					
non breastfed	114 (65.1)	28.1 (32/114)	0.463	15.8 (18/114)	0.516
breastfed	61 (34.1)	23 (14/61)		19.7 (12/61)	
Season of birth					
spring	39 (22.3)	38.5 (15/39)	0.059	25.6 (10/39)	0.162
summer	40 (22.9)	32.5 (13/40)		12.5 (5/40)	
autumn	59 (33.7)	22 (13/59)		20.3 (12/59)	
winter	37 (21.1)	13.5 (5/37)		8.1 (3/37)	

* The number of patients.

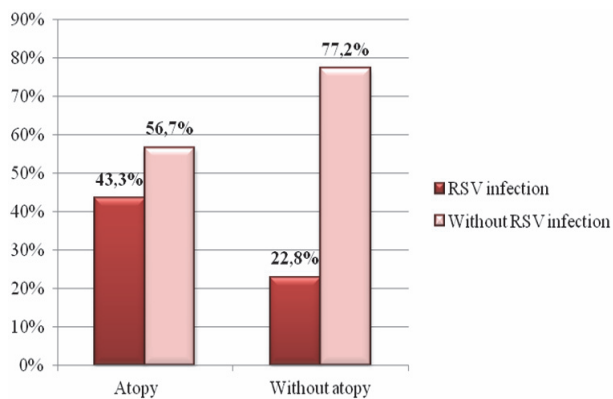


Fig. 1 – Frequency of atopy in children with or without respiratory syncytial virus (RSV) infection.

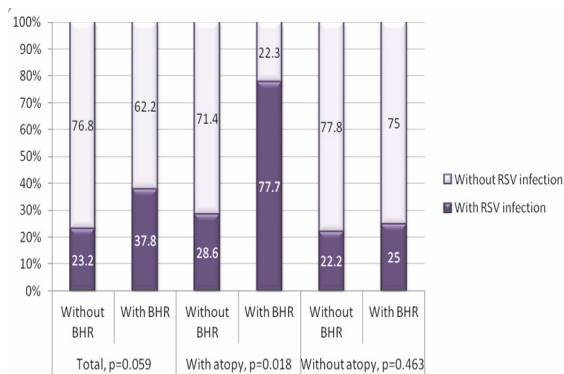


Fig. 2 – Frequency of respiratory syncytial virus (RSV) infection in children with and without bronchial hyperreactivity (BHR), regarding the presence or absence of atopy.

non-atopic in older groups. On the other hand, children with atopy tended to develop BHR more often as age advanced. In the non-atopic children, the frequency of RSV infection as a causative agent of BHR was 30–33% of those older than 7 months, while in the children with atopy and BHR, RSV infection was present in 85.7% of children in the second year of age (Figures 3 and 4).

Analysis of the children with BHR ($n = 37$) confirmed no statistically significant difference in the presence of atopy between male (20.7%, 6/29) and female sex (37.5%, 3/8; $p = 0.292$). The presence of BHR together with RSV infec-

tion was found in 62.5% of female children and was equally frequent in girls with and without atopy. In male children with atopy, RSV infection was associated with BHR in 83.3%, but in male children without atopy BHR was associated with RSV infection in only 17.4% (Figure 5).

Analysis of the children with BHR also showed that 78.4% (29/37) were not breastfed. In the non-breastfed children, detected RSV infection frequently developed in BHR (37.5%) in comparison with RSV non-infected children (20.7%; $p = 0.056$). In the breastfed children this association was not observed ($p = 0.594$).

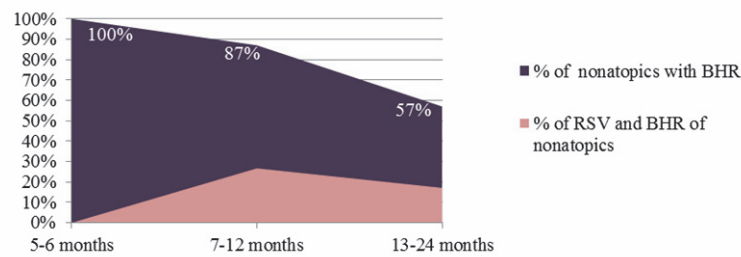


Fig. 3 – Percentage of non-atopic children with bronchial hyperreactivity (BHR) in comparison those with BHR and with syncytial virus (RSV) infection, regarding the age of children.

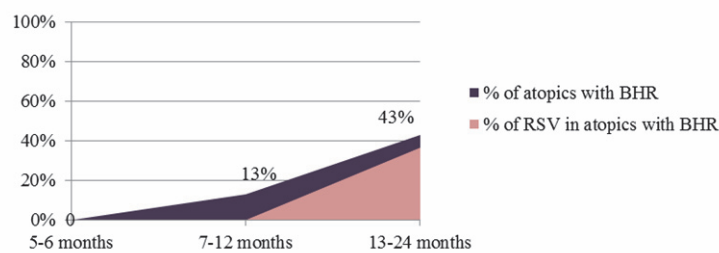


Fig. 4 – Frequency of syncytial virus (RSV) infection in atopics with bronchial hyperreactivity (BHR) regarding the age of children.

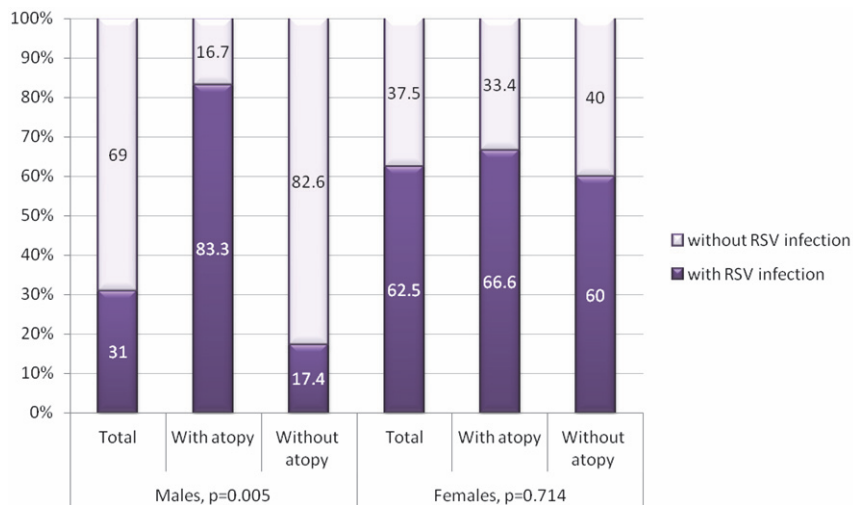


Fig. 5 – Frequency of respiratory syncytial virus (RSV) infection in male and female children with bronchial hyperreactivity (BHR) regarding the presence or absence of atopy.

In the non-breastfed children with atopy, the association of BHR and RSV infection was found in 75% (6/8), while in the non-breastfed children without atopy, this association was discovered in 28.6% (6/21; $p = 0.033$).

The children with atopy born in spring and summer had BHR in 53.3% (8/15), and those born in autumn and winter had BHR in only 6.7% (1/15; $p = 0.007$). In the children without atopy, the difference in BHR frequency between seasons of birth was not observed ($p = 0.487$). The children born in spring or summer who had atopy and who developed BHR, were also positive for RSV infection in 75% (6/8) of the cases.

Discussion

Bronchial hyperreactivity, defined as frequent and excessive bronchial narrowing, may be a consequence of genetic factors associated with intensified bronchial reactivity during inflammation, anatomy – small bronchial lumen, disorder of bronchial smooth muscles associated with altered function of the autonomous nerve system, damaged bronchial epithelium²⁰. BHR, that was clinically defined as the presence of chronic bronchial disease and/or 3 or more of any lower respiratory disease, was found in 21.1% of the children up to two years of age. In this study, the groups identified to be at risk for BHR development were the children older than 7 months, male, non-breastfed and positive for RSV infection.

It is known that RSV infection can cause BHR in the critical moment of the pulmonary immune system development in genetically susceptible babies^{21,22}, where age has great importance for innate and adoptive immune response against RSV infection²³. The differentiation of progenitor cells into epithelial alveolar cells that produce immunomodulating factors (surfactant and clara cell protein) plays an important role during alveolar phase of the postnatal lung development within the first several years of life. The main target for viral infection in the lower respiratory tract are ciliary cells of bronchioles and pneumocytes type-1 in alveoli²⁴.

This study confirms that the presence of BHR at the age of 7–12 months is often associated with RSV infection, especially in children without atopy. It is possible that dysfunction of progenitor cells that occurs during RSV infection is responsible for the reduced presence of immunosuppressive factors important for development of chronic inflammation.

In this study, the children without RSV infection most frequently developed BHR at the age of 13–24 months. In those with BHR developed in older age, wheezing and BHR might be the result of rhinovirus (RV) infection, since it was known that children with RV infection were usually older and with the personal and family history of asthma^{25, 26}. A study²⁷, including 198 children at great risk for atopy, confirms that RSV and RV are causative agents of all respiratory diseases. These diseases were associated with persistent wheezing in the first year of life, and in some of the following years developed into asthma.

It has been suggested that children with atopy had different susceptibility to viral infections in comparison with

those without atopy during early childhood, where atopic children are far more susceptible²⁷. In this research, children with atopy were more often infected with RSV (43.3%), than those without atopy (22.8%).

There is a dilemma whether genetic predisposition for atopy creates *per se* susceptibility for asthma after viral infection, or for development of asthma it is necessary to have active expression of the atopic phenotype with sensibilization to environmental allergens in early childhood. In the international study of asthma and allergies in childhood (ISAAC study) various wheezing phenotypes within the “asthma syndrome” are defined. One group includes children who, after being repeatedly exposed to infection in their early childhood, develop “infective asthma” and the other includes children with “atopic asthma”^{27, 28}. However, the present study defined the unique phenotype of BHR in the age up to two years in which RSV infection and atopy were associated. Namely, every third child with atopy had BHR and 77.8% also had RSV infection. In comparison, in the group of children without atopy, BHR was diagnosed in every fifth child, and association with RSV infection was in only 25%. Some recent studies support standpoint, that viral infections of the lower respiratory tract represent the marker of atopic predisposition²⁹. Prospective studies including high risk cohorts of infants whose mothers had asthma, suggested that serious bronchiolitis was a result of already present respiratory susceptibility to viral infection^{29, 30}. Thomsen et al.³¹ studied 8,280 pairs of twins and concluded that RSV probably represented genetic predisposition for asthma. RSV infection followed by BHR is characteristic for the first three years of life and is attributed to the virus tropism for developing pulmonary tissue⁷. In this study, occurrence of BHR in children with atopy was characteristic for the second year of life in which RSV became the most important causative agent of BHR (85.7%). It is possible that the dynamics of pulmonary development in children with atopy, in the second year of life, enables synergistic effect of RSV infection and atopy in BHR development.

In the first year of life, BHR was dominantly observed in children without atopy where RSV infection was discovered in 1/3 of the cases. The frequency of RSV infection in this group of children remained the same in the second year of life. It is possible that unknown defect in pulmonary development may attribute to early occurrence of BHR after RSV infection in children without atopy.

In the Tucson Children's Respiratory Study³² male gender was marked as the risk factor for RSV infection of the lower respiratory tract and Hibbert et al.³³ found that in boys, lower respiratory tract is less developed than in girls. Small lumen of airways is described as a contributing factor in expression of lower respiratory tract diseases in viral infections⁷. In the present study, boys with atopy and expressed BHR had RSV infection in more than 80% of cases, but BHR in male children without atopy was not associated with RSV infection. BHR developed after RSV infection in boys only in case of the present atopy, which implied the importance of genetic factors associated with atopy. On the other hand, it is known that small respiratory lumen may be

the cause of BHR development in male children without atopy and that it could be in the form of transitory wheezing in the infant period not associated with later asthma development¹². In girls, BHR was in 62.5% of cases associated with RSV infection, but equally in both groups, with and without atopy. It is possible that female gender is a determinant of BHR associated with RSV infection, regardless of the presence of atopy.

More expressed Th2 immune response is also associated with BHR after RSV infection⁷. Firstly, RSV infection favors Th2 immune response because it blocks the production of interferon (IFN) from the plasmacytoid cells and reduces interleukin (IL-12) production^{7,34}. Then, during the infant period, immune response is underdeveloped and physiologic Th2 immune response is present. Also, Th2 immune response is the dominant cellular response in the developed bronchus-associated lymphoid tissue (BALT)³⁵. There is a question whether respiratory defect caused by immune response during viral infection is the result of Th2 immune response or a consequence of reduced mechanism of immunosuppression. Children with atopy, beside being genetically prone for development of Th2 response, also have reduced function of the immune response regulation through transforming growth factor beta 1 (TGFβ1) and IL-10. Somewhat insufficient regulation of the immune response with lower TGFβ1 is particularly present in the initial phase of infection³⁶, which may be the reason for chronic inflammation in children with atopy. In this study, non-breastfed children most often had BHR associated with RSV infection in comparison with breastfed children. Mother's milk is not only the source of nutritive elements for infants, but it also has potent protective effect, owing to present immunoglobulins and immunomodulating effect, owing to immunosuppressive cytokines such as TGFβ1 and IL-10³⁷.

The results obtained in animal models clarify interaction between viral infection and exposition to inhalation allergens: infection can create a pro-allergic milieu and if an experimental animal was exposed to inhalation of these allergens for certain period of time, allergen-specific respiratory inflammation could be developed. Also, previous epi-

demiological data support direct causative role of RSV infection in development of sensibilization in the early phases of asthma³⁴. The present study confirmed that children with atopy and born either in spring or summer often have RSV infection associated with BHR (more than 50%). This was not the case with children born in autumn or winter, so it is possible that there is a synergy between RSV infection and exposure to pollen resulting in BHR development.

It is known that the correlation between respiratory infections and asthma/persistent wheezing is complicated and seemingly based upon interaction of the host factors (such as age and the stage of development of innate and adoptive immune response at the moment of infection) and infectious agents. Resistance to viral infection and atopic sensitization depends on the number of immune mechanisms that are strongly regulated in early life, particularly among children with high risk of atopy³⁸. Additional studies on asthma pathogenesis are necessary, especially those that will include the following characteristics: primary cause of defect in the respiratory barrier of children with atopy; RSV tropism for respiratory epithelium during lung development; allergens as reactive molecules having chemical and enzymatic effect upon respiratory epithelium; disorders of the immunoregulatory mechanisms in BALT of children; disbalance of tissue factors during infection such as M2 muscarine receptors on smooth muscles in respiratory tract.

Conclusion

The results of this study suggest that the presence of bronchial hyperreactivity associated with respiratory syncytial virus infection can be early risk marker for asthma development, in all female children and in male children with atopy within the first two years of life. Therefore, early asthma prevention should include routine detection of respiratory syncytial virus infection and atopy in all children in primary health care. Also, very helpful would be education of parents on how to reduce children's exposure to infection and inhalation of allergens during critical periods of early life.

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