Trakia Journal of Sciences, No 3, pp 219-227, 2024

Available online at:
https://trakia-uni.bg

ISSN 1313-3551 (online) doi:10.15547/tjs.2024.03.004

Original Contribution

PREVALENCE OF THE RESPIRATORY SYNCYTIAL VIRUS INFECTION IN THE WINTER AND SPRING MONTHS AMONG BULGARIAN CHILDREN YOUNGER THAN 2 YEARS OF AGE

E. Kostadinova[1*], S. Angelova[2]

[1]Department of Pediatrics, Medical Faculty, Trakia University, Stara Zagora, Bulgaria
[2]Department of Microbiology, Parasitology and Virology, Medical Faculty, Trakia University, Stara Zagora, Bulgaria

ABSRTACT

Lower respiratory tract infections (LRTIs) in children from 2 months to 5 years of age are significant for clinical practice and researchers because of their high incidence, risk of severe clinical course, and development of chronic complications. About 60% of LRTIs are caused by viral infections. **PURPOSE:** To make an etiological diagnosis of viral infections of the lower respiratory tract-acute bronchiolitis and viral pneumonia. To present an epidemiological characterization in relation to the identified viral agents. **METODS:** The subjects of the prospective study were 101 children aged 2 months to 5 years, hospitalized in the Clinic of Pediatrics with clinical manifestations of viral LRTI`s with bronchial obstruction. Clinical material was analyzed according to the main epidemiological parameters: sex, age, place of residence, season, etiological agent of infection, etiological diagnosis according to season, presence of risk factors from family history and comorbidities. Etiological diagnosis of viral LRTIs was made by RT-PCR analysis. **RESULTS:** Epidemiological characteristics of the diagnosed viral LRTIs are presented. The prevalence of male sex was found to be higher (without statistical significance), more frequent among urban children and the highest incidence of viral LRTIs in the age up to 2 years. The etiological diagnosis made shows that respiratory syncytial virus (RSV) is the most common etiological cause of acute bronchiolitis in children under 2 years of age, both alone and in co-infections. **CONCLUSIONS:** RSV is the most common statistically significant etiological cause in winter and spring. Rhinovirus (RV) is the leading etiological cause in summer and autumn. Co-infections involving RSV and RV also have a high incidence during these seasons.

Key words: viral lower respiratory tract infections, RSV, RV, co-infections, age 2 months to 5 years

INTRODUCTION

Lower respiratory tract infections (LRTIs) in children 2 months to 5 years of age are significant for clinical practice and researchers because of their high incidence, risk of severe clinical course and development of chronic complications. They are responsible for approximately 12% of global childhood mortality (1, 2). About 60% of LRTIs are caused by viral infections, the main causative agents are respiratory syncytial virus (RSV), influenza viruses (IV), parainfluenza viruses (PIV), adenoviruses (AdV), rhinoviruses (RV), coronaviruses (HCOV), metapneumoviruses (HMPV) and bocaviruses (BV) (3, 4). The clinical spectrum of these respiratory viruses covers the entire range of respiratory tract infections, from asymptomatic upper respiratory tract infections to severe LRTIs with poor prognosis. The American Academy of Pediatrics (AAP) defines bronchiolitis as a constellation of symptoms including viral prodromal upper respiratory tract manifestations (rhinorrhea, fever, and cough), followed by difficulty breathing and bronchial obstruction in children younger than 2 years of age (5). Although the course is uncomplicated in mild forms, bronchiolitis is the most common terminal respiratory tract infection and a major cause of hospitalizations in children under 2 years of age, clinical manifestations of acute respiratory failure (ARF) require

***Correspondence to:** *Assoc. professor Emilia Kostadinova, PhD, MD, Department of Pediatrics, Medical Faculty, Trakia University, 11Armeyska Str, 6000, Stara Zagora, Bulgaria, e-mail: kostadinovaemilia@abv.bg, +359888260100*

hospitalization in about 3% of cases and intensive care in 2-6% (6).

Among virus-associated LRTIs, RSV plays a leading role (7). A large-scale study showed that RSV-associated LRTIs resulted in a global cost of 33.1 million, resulting in 3.2 million hospitalizations and about 60,000 in-hospital deaths in children under 5 years of age (8). Diagnosed RSV infections of LRTIs are associated with long-term conditions, such as asthma and recurrent chest wheezing (9), which is a prerequisite for an additional financial burden on healthcare (10). Approximately 50-70% of children become infected with RSV in the first year of life and by the age of 2 years, almost all children have at least one RSV infection. RSV is documented in 50-90% of children hospitalized with bronchiolitis, 5-40% with pneumonia and 10-30% with tracheobronchitis (11).

RSV was isolated in 1957 (12), and belongs to the family Paramyxovuruses, genus Pneumovirus. It has an outer envelope and a negative single-stranded RNA genome of about 15.2 kb, possessing 10 genes that encode 11 proteins (13). The most significant of these are the transmembrane glycoproteins G, which binds the virus to the respiratory epithelium and F, which penetrates and infects the epithelial cells of the lung (14). RSV-induced inflammation causes significant damage to the lung epithelium due to an excessive airway inflammatory response by recruited immune cells at the site of infection and the altered profile of cytokines. As a result of RSV-induced interactions with airway epithelial cells, mucus hypersecretion, shedding of infected epithelial cells, and collapse of the lower airways occur as a substrate for a severe clinical course (15). In recent years, the role of RV as an etiological agent of acute bronchiolitis has been investigated. According to some authors, RV may be associated with 20-40% of cases of acute bronchiolitis (16). Data from the literature are conflicting regarding the severity of the clinical course of HRV bronchiolitis and its association with long-term sequelae, such as recurrent bronchial obstruction and asthma (17). Human metapneumovirus (HMPV) may also be associated with the etiology of a significant proportion of bronchiolitis (18). PIV-1, 2 and 3 cause acute respiratory infections (ARI) in individuals of all ages. Severe clinical symptomatology is seen mainly in children under 2 years of age: croup (laryngo-tracheo-bronchitis), pneumonia, and bronchiolitis (19). Adenoviruses, rhinoviruses, enteroviruses, coronaviruses and bocaviruses also cause ARI with different severity, spectrum of clinical manifestations and different epidemiological characteristics (4). Sometimes more than one type of respiratory virus is demonstrated, with RSV, AdV, HCOV, and RV being relatively common partners in co-infections.

The clinical manifestations of the different viral agents are similar, so an etiological diagnosis based on clinical parameters alone is uncertain. The potential for etiologic diagnosis of viral LRTI`s by immunofluorescence analysis and molecular assays, such as polymerase chain reaction (PCR) has increased in recent years.

AIM

This study aimed to make an epidemiological characterization of viral infections of the lower respiratory tract in the age group from 2 months to 5 years, diagnosed and treated in the Pediatrics Clinic of the University Hospital "Prof. Dr. St. Kirkovich" Stara Zagora in 2021-2023, by determining the etiological agent.

MATERIAL AND METHODS

The subjects of the prospective study were 101 children aged 2 months to 5 years, hospitalized in the Clinic of Pediatrics with clinical manifestations of viral LRTI`s with bronchial obstruction. Clinical material was analyzed according to the main epidemiological parameters: sex, age, place of residence, season, etiological agent of infection, etiological diagnosis according to season, presence of risk factors from family history and comorbidities. Criteria for hospitalized children with bronchiolitis include impaired general condition, impaired feeding, signs of dehydration, tachypnea/apnea, expiratory-type dyspnea with "wheezing" breathing, retraction, thoraco-abdominal asynchronism, nasal breathing, cyanosis, muscle hypotension, pulmonary auscultatory findings of fine crepitations, and radiographic evidence of pulmonary hyperventilation. Viral pneumonia was proven clinically and radiologically with the objectification of pulmonary interstitial changes. Criteria for bronchial obstruction were the presence of cough, distant chest wheezing with expiratory-type dyspnea, cyanosis, and rales. Depending on the severity, bronchial

obstruction was defined as mild, moderate or severe. Routine blood tests (full and differential blood count, serum biochemistry profile, blood gas analysis, microbiological examination of nasopharyngeal aspirate/throat swab were performed in all patients. Bacterial infection was ruled out by the generally accepted clinical and laboratory indicators. Distribution by nosologic diagnosis was performed: Acute bronchiolitis (n=91), and viral pneumonia (n=10).

Nasopharyngeal swabs were collected from all 101 children included in the study in an appropriate viral transport medium satisfying the requirements for PCR analysis. Viral nucleic acids were extracted from the samples as a first step using an automated Insta NX® system from Himedia, India. Commercial Insta NX™ Viral RNA Purification Kit was used, and the manufacturer's instructions were followed. Real Time RT-PCR method was used for the detection, typing and subtyping of, respiratory syncytial virus (RSV), metapneumovirus (HMPV), parainfluenza viruses type 1/3 (PIV1/3), rhinoviruses (RV), adenoviruses (AdV) and human coronaviruses-HCOV (HKU1, OC43, 229E, NL43). Amplification was performed using specific primers and Taqman® probes (FAM/BHQ) for IV, RSV, HMPV, PIV 1/3, RV, AdV, HCOV(HKU1, OC43, 229E, NL43) and commercial AgPath-ID One Step RT-PCR kits (ThermoFisher Scientific) and 1copy™ COVID-19/FluA/FluB/RSV qPCR Kit (Clinomics Inc, Korea). Primer sequences, probes and temperature protocols for RT-PCR analysis were adopted from previously published assays from Kodani et al. 2011; Zlateva et al. 2007; Dare et al. 2007(20,21,22). Primers, probes and positive controls for detection/typing and subtyping of influenza viruses are part of the commercial 1copy™ COVID-19/FluA/FluB/RSV qPCR Kit.

RESULTS

Demographic data

1.Distribution of hospitalized patients with LRTI`s by sex.

The group consists of 101 cases with an average are 13.12±13.3 months (range 1 – 60 months). There are no significant differences in age between boys and girls (p=0.789) **(Table1).**

Table 1. *Distribution by sex*

	Sex	N	Mean	Std. Deviation	Std. Error Mean
age_months	Girls	40	14.05	15.002	2.372
	Boys	61	13.28	13.471	1.725

P=0.789

2.Histogram of the distribution by age is presented in **Figure 1.**

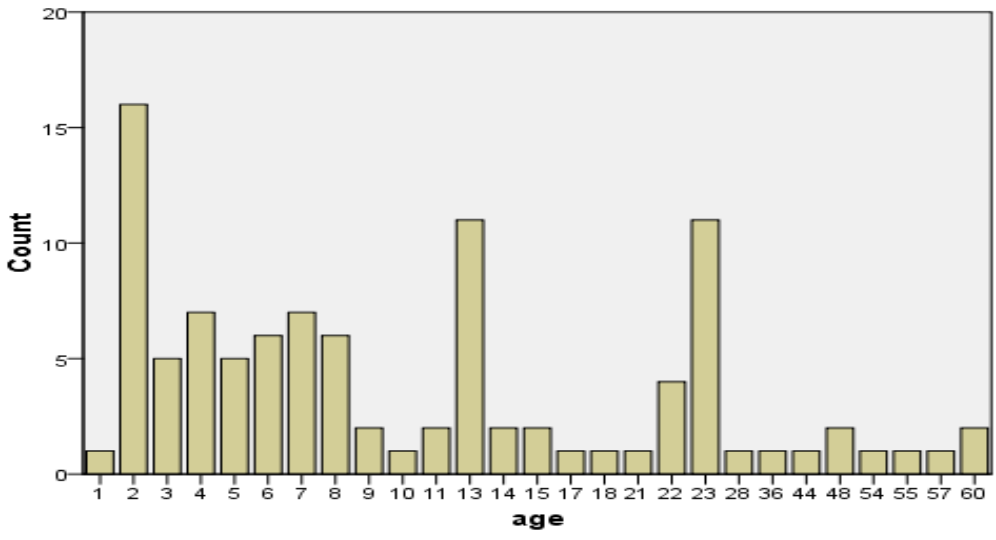

Figure 1. Distribution by age.

There was a significant prevalence of the LRTIs among children aged less than 2 years (24 months). Only 10 (9.9%) children were aged between 28 to 60 months and all of them were diagnosed with pneumonia. Incidence of the LRTIs was highest among children aged 2 months (15.8%; 16 of 101).

3.Distribution by place of residence

The distribution of patients by place of residence, presented in **Figure 2** shows a 2:1 higher incidence among urban children compared to those raised in rural areas.

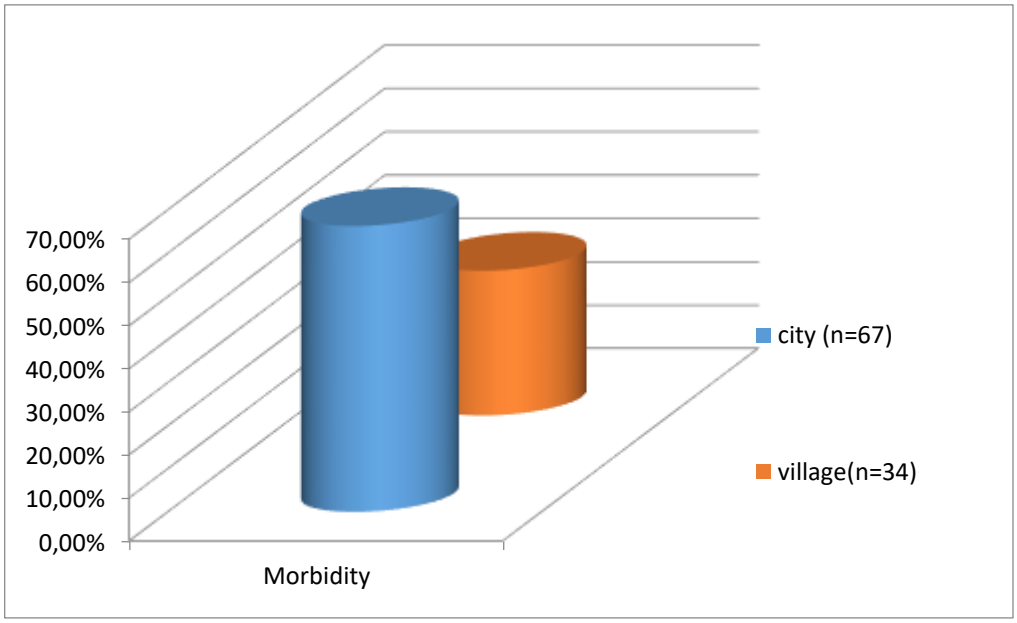

Figure 2. Distribution by place of residence

4. Distribution of LRTIs clinical manifestation by the season is presented in **Table 2.**

Table 2. *Distribution of LRTIs* clinical manifestation *by season*

			Season				Total
			spring	summer	autumn	Winter	
Dg_code	Bronchiolitis	Count	10_a	35_a	5_a	41_a	91
		% within Dg_code	11.0%	38.5%	5.5%	45.1%	100.0%
	Pneumonia	Count	3_a	2_a	0_a	5_a	10
		% within Dg_code	30.0%	20.0%	0.0%	50.0%	100.0%
Total		Count	13	37	5	46	101
		% within Dg_code	12.9%	36.6%	5.0%	45.5%	100.0%

Each subscript letter denotes a subset of season categories whose column proportions do not differ significantly from each other at the .05 level.

There is no significant difference between cases with bronchiolitis (n=91) and pneumonia (n=10) with respect to disease seasonality (chi2=3.966; p=0.265).

5. The etiological diagnosis of viral LRTIs

The etiological diagnosis of viral LRTIs was found in 77.22% (n=78) of the clinical cases presented in **Figure 3**. In 22.54% (n=23) no etiological agent was identified. RSV infection was the leading etiologic cause for the LRTIs, found in a total of 46,15% of all with proven etiology (n=36), respectively bronchiolitis (n=34) and pneumonia (n=2). The second most

common etiological cause was RV infection, found in 25% (n=19), bronchiolitis(n=18) and viral pneumonia(n=1), respectively. The third cause was co-infections, proven in 18.42% (n=14), RSV/RV (n=3), RSV/HMPV (n=2), RSV/AdV (n=2), RSV/RV/OC43 (n=1), RSV/AdV/HKU1 (n=1), RV/AdV (n=2), RV/HKU1 (n=1), IV/HMPV(n=2), respectively. The fact that in the higher percentage 64% (9/14) there was the presence of RSV was notable. Single cases with HMPV(n=2); HCOV(n=3), HKU1(n=2), OC43(n=1); IV(n=3); PIV(n=1) were detected.

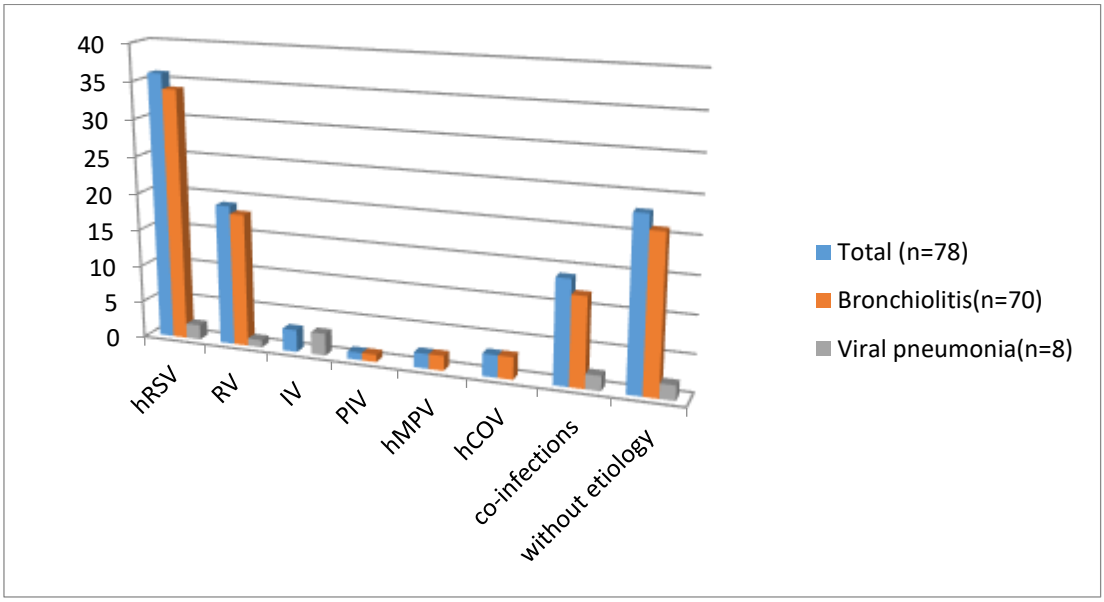

Figure 3. Etiological characteristics of the viral LRTIs according to the clinical diagnosis

6. The distribution of patients with bronchiolitis, less than 2 years of age, by the season is presented in **Table 3 and Figure 4.** The significant seasonality of the viral infection resulted in bronchiolitis was observed (chi2=27.101; df=12; p=0.007). RSV was the most common viral agent causing bronchiolitis during spring and winter, while RV infection was more frequent in summer and autumn.

Table 3. *Distribution of patients with bronchiolitis, less than 2 years of age, by the season*

Viral_Code * season Crosstabulation

			Season				Total
			Spring	summer	autumn	Winter	
Viral_Code	Negative	Count	2_a	14_a	1_a	6_a	23
		% within season	20.0%	40.0%	20.0%	14.6%	25.3%
	RSV	Count	7_a	3_b	$1_{a,b}$	23_a	34
		% within season	**70.0%**	8.6%	20.0%	**56.1%**	37.4%
	RV	Count	0_a	9_a	2_a	7_a	18
		% within season	0.0%	**25.7%**	**40.0%**	17.1%	19.8%
	co-infection	Count	1_a	6_a	1_a	4_a	12
		% within season	10.0%	17.1%	20.0%	9.8%	13.2%
	Other	Count	0_a	3_a	0_a	1_a	4
		% within season	0.0%	8.6%	0.0%	2.4%	4.4%
Total		Count	10	35	5	41	91
		% within season	100.0%	100.0%	100.0%	100.0%	100.0%

Each subscript letter denotes a subset of season categories whose column proportions do not differ significantly from each other at the .05 level.

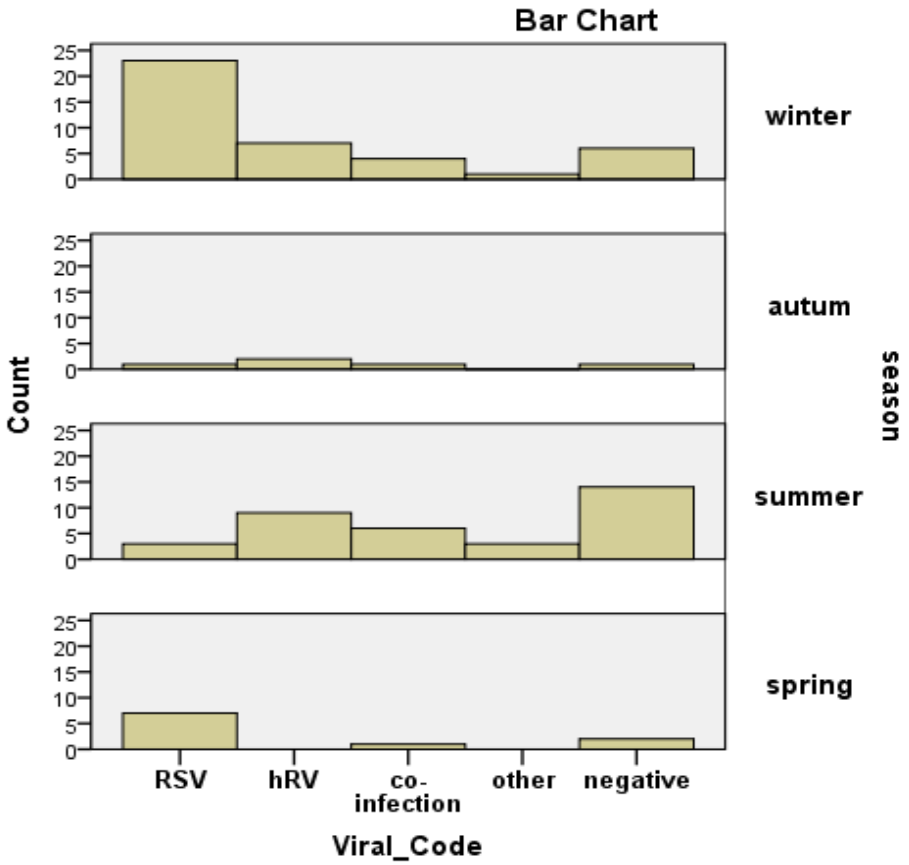

Figure 4. Distribution of patients with bronchiolitis, *less than 2 years of age, by the season*

7. Comorbidities

In 25,75% (n=26) with viral LRTI`s, we found underlying diseases, which included: Bronchopulmonary dysplasia (BPD) (n=8), Central nervous system abnormalities (n=2), Nephrotic syndrome (n=1), Down syndrome (n=1), Cystic fibrosis (n=1), Bronchial asthma (n=2), Urticaria (n=2), Atopic dermatitis/eczema (n=3), Cow's milk protein allergy (CMA) (n=3). The etiology of LRTIs in children with BPD (n=8) was RSV (n=4), RV(n=2), co-infection involving RSV(n=1), etiologic agent not identified (n=1).

8. Risk factors for the development of viral LRTI`s

Family history data showed: contact with acute respiratory diseases in the family (n=28), low socio-economic level of the family and low health culture (n=32), evidence of passive smoking (n=32), lack of breastfeeding (n=36), familial atopy (n=3).

DISCUSSION

Research of respiratory viruses in Bulgaria by modern molecular diagnostic methods, including Real-Time RT-PCR, is mainly carried out in the National Reference Laboratory "Influenza and Acute Respiratory Infections" at the National Center for Influenza and Respiratory Diseases in Sofia. It plays a leading role in the identification and characterization of the features of seasonal and pandemic influenza viruses, as well as in the determination of the spectrum of respiratory viruses involved in the development of acute respiratory diseases of different severity in infant and young childhood patients. Until now, modern molecular techniques and methods based on Real-Time RT-PCR and Multiplex PCR analyses have not been introduced into routine diagnostics at the level of university clinics in Bulgaria. These types of analyses are accurate and rapid and allow reliable diagnosis of a wide range of viral causative agents, which contributes to the

improvement of the diagnostic algorithm in pediatric practice given the frequent infection and severity of viral bronchopulmonary infections in children up to 5 years of age. To date, studies on RSV and HMPV in children under 5 years of age have been conducted in this country mainly by traditional virological techniques and conventional PCR. A significant (39%) prevalence of RSV in children <1 year of age hospitalized with bronchiolitis or pneumonia has been found in 4 regions of Bulgaria (23, 24). The involvement of other respiratory viruses (parainfluenza, adeno-, rhino, etc.) in cases of ARI in our country is not yet well studied.

Our results in hospitalized children with viral LTRI`s showed male preponderance, without significance, higher prevalence among urban children and confirmed the literature data of highest prevalence in the age below 2 years in 90% (25).

Our data show RSV as the most common etiologic agent of acute bronchiolitis, found in winter and spring, and a high incidence of co-infections involving RSV and RV during these seasons. The results of the study indicated RV as the leading etiological agent of acute bronchiolitis in summer and autumn, and a high incidence of co-infections in summer involving RSV and RV.

Etiologically, RSV infection is the leading cause of the development of viral LTRI`s, found in a total of 46,15% of all cases with a proven etiology. The second most frequent etiological cause was RV infection found in 25%, the third cause was co-infections proven in 18.42%. Our results are similar to the results obtained from molecular studies (PCR) by other authors, who found RSV in 70-80% of bronchiolitis cases (26), followed by RV in 20-40% of cases (27) and the frequency of co-infections in more than 30% of hospitalized patients with bronchiolitis (28).

As found in our patients, RSV bronchiolitis is usually more severe with a history of low birth weight and preterm delivery, congenital cardiopathy, and cystic fibrosis. Other authors have also indicated a higher risk of severe course and mortality in patients with severe prematurity, and congenital pulmonary anomaly (pulmonary hypoplasia), chronic heart disease, congenital or acquired immunodeficiency syndrome (29).

We have found a severe clinical course of RSV bronchiolitis in infants up to 12 months of age who are not breastfed, live in overcrowded conditions with poor social and living conditions, with the presence of smokers in the family and markedly young mothers. Similar risk factors have been identified by other authors, who have also associated them with disease severity, particularly in boys under 6 months of age (25).

Despite advances in neonatal care over the past decade, there has been no decrease in the incidence of clinical cases with Bronchopulmonary dysplasia (BPD). In children with BPD, respiratory pathology is common during the first two years of life, with the development of viral broncho-pulmonary infections requiring intensive treatment and prolonged oxygen therapy. In our patients with BPD, deterioration of respiratory status occurs slowly and progressively, with the observed acute exacerbations almost always associated with RSV infection. Despite the introduction of prevention in high-risk groups with Palivizumab (Synagis), which is a monoclonal antibody targeting RSV F protein II, treatment in these children remains challenging.

The morbidity of viral LRTIs in the age of 2 months to 5 years is multifactorial. It is the result of an increased exposure to viruses, and an imperfect immune system response as a result of genetic predisposition, with underlying premorbid pathology influencing the severity of the clinical course.

Why is it important to know who has the virus?

With early detection of RSV, specific antiviral therapy can be administered to prevent complications such as severe bronchiolitis and respiratory failure responsible for lethality, especially in high-risk children. Several approaches are used in current global practice (30). Evidence-based recommendations on the diagnosis, treatment, and prevention have been proposed with a high degree of consensus. Although supportive care remains the cornerstone of treatment for RSV infections, new therapeutic approaches such as novel monoclonal antibodies, vaccines, drug therapies, and viral surveillance techniques are being introduced (31). With early detection of RSV, specific therapy (antiviral with Ribavirin or others) can be administered to prevent complications such as severe bronchiolitis and

severe respiratory failure responsible for lethality, especially in high-risk children.

CONCLUSIONS

Detection of viral pathogens based on polymerase chain reaction, namely RT-PCR analysis, is an option for the rapid and sensitive diagnosis, preferred for clinical purposes.

Acute bronchiolitis presents male predominance, higher prevalence among urban children and significant incidence in the age group below 2 years.

RSV is the most common etiologic agent of acute bronchiolitis in children younger than 2 years of age, both alone and in coinfections.
RSV is the most common etiologic cause of bronchiolitis in winter and spring. RV is the leading etiologic cause in summer and autumn. Co-infections involving RSV and RV are also high in frequency during these seasons.

With early detection of RSV infection, specific therapy can be administered to prevent complications in high-risk children.

ACKNOWLEDGMENTS

This work was supported by Grant: № 2/2021 from the Fund for Scientific and Mobile Project from the Faculty of Medicine at Trakia University, Stara Zagora, Bulgaria.

REFERENCES

1. Levels & Trends in Child Mortality: Report 2020. Estimates developed by the United Nations Inter-agency Group for Child Mortality Estimation. New York: United Nations Children's Fund; 2020.
2. World Health Statistics 2019. Monitoring health for the SDGs, sustainable development goals. Geneva: World Health Organization; 2019.
3. Nair H. et al. Global burden of acute lower respiratory infections due to respiratory syncytial virus in young children: a systematic review and meta-analysis. *Lancet*. 2010 May 1;375(9725):1545-55.
4. Ali A,et al. Role of human metapneumovirus, influenza A virus and respiratory syncytial virus in causing WHO-defined severe pneumonia in children in a developing country. *PLoS One*. 2013 Sep 18;8(9):e74756.
5. Ralston, S.L.et al. Clinical Practice Guideline: The Diagnosis, Management, and Prevention of Bronchiolitis. *Pediatrics* 2014, 134, e1474–e1502.
6. Hasegawa, K.; Tsugawa, Y.; Brown, D.F.; Mansbach, J.M.; Camargo, C.A. Trends in Bronchiolitis Hospitalizations in the United States, 2000–2009. *Pediatrics* 2013, 132, 28–36.
7. Borchers AT, et al. Respiratory syncytial virus–a comprehensive review. *Clin Rev Allergy Immunol.* 2013;45(3):331–79.
8. Ting Shi et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet*, 2017 Sep 2;390(10098):946-958.
9. Xavier Carbonell-Estrany et al Long-Term Burden and Respiratory Effects of Respiratory Syncytial Virus Hospitalization in Preterm Infants—The SPRING Study PLoSONE. (2015)
10. Brigitte Fauroux et al.The Burden and Long-term Respiratory Morbidity Associated with Respiratory Syncytial Virus Infection in Early Childhood. *Infect Dis Ther*.2017 Jun;6(2):173-197.
11. Papenburg J, BoivinG. The distinguishing features of humanmetapneumovirus and respiratorysyncytial virus. *Rev Med Virol*,2010, 20:245–260.
12. R Chanock, L Finberg Recovery from infants with respiratory illness of a virus related to chimpanzee coryza agent (CCA). II. *Epidemiologic aspects of infection in infants and young children*.,1957 Nov;66(3):291-300.
13. Collins PL, Melero JA. Progress in understanding and controlling respiratory syncytial virus: still crazy after all these years. *VirusRes*. 2011;162(1–2):80–99.
14. McLellan JS, Ray WC, Peeples ME. Structure and function of respiratory syncytial virus surface glycoproteins. *Curr Top Microbiol Immunol.* 2013;372:83–104.
15. Zhivaki D et al. Respiratory syncytial virus infects regulatory B cells in human neonates via chemokine receptor CX3CR1 and promotes lung disease severity. *Immunity.* 2017 02 21; 46(2):301-314
16. Jartti, T.et al. Bronchiolitis. *Pediatr. Infect. Dis. J.* 2009, 28, 311–317.
17. Carlotta Biagi et al. Rhinovirus Infection in Children with Acute Bronchiolitis and Its Impact on Recurrent Wheezing and Asthma

Development.MDP; Published: 21 October 2020
18. Walker CLF, RudanI, LiuL, et al. Global burden of childhood pneumonia and diarrhoea.*The Lancet*, 2013, 381(9875): 1405 -1416
19. Hall CB. Respiratory syncytial virus and parainfluenza virus. *N Engl J Med*, 2001, 344(25).
20. Kodani, M.et al. „Application of TaqMan Low-Density Arrays for Simultaneous Detection of Multiple Respiratory Pathogens“, *J Clin Microbiol*., 49:2175-82,2011
21. Dare RK. et al. Human Coronavirus Infections in Rural Thailand: A Comprehensive Study Using Real-Time ReverseTranscription Polymerase Chain Reaction Assays HCoV Infections in Thailand • JID 2007:196.
22. Zlateva KT, et al. Subgroup prevalence and genotype circulation patterns of human respiratory syncytial virus in Belgium during ten successive epidemic seasons. *J Clin Microbiol.* 2007;45(9):3022-30.
23. Korsun N, et al. Predominance of ON1 and BA9 Genotypes of Respiratory Syncytial Virus (RSV) in Bulgaria, 2016-2018. *J Med Virol*. 2020
24. Korsun N, et al. Viral pathogens associated with acute lower respiratory tract infections in children younger than 5 years of age in Bulgaria. *Braz J Microbiol*, 2019 Jan;50(1):117-125.
25. Xiao-Bo Zhang et al. Clinical characteristics and risk factors of severe respiratory syncytial virus-associated acute lower respiratory tract infections in hospitalized infants. *World J Pediatr*, 2014 Nov;10(4):360-4.
26. Vandini, S.et al. Respiratory Syncytial Virus: The Influence of Serotype and Genotype Variability on Clinical Course of Infection. Int. J. Mol. Sci. 2017, 18, 1717.
27. Jartti, T. et al Bronchiolitis. *Pediatr. Infect. Dis. J.* 2009, 28, 311–317.
28. Bosis, S.et al. Role of respiratory pathogens in infants hospitalized for a first episode of wheezing and their impact on recurrences. Clin. Microbiol. *Infect*. 2008, 14, 677–684.
29. Mohapat Shyam, Richard F Lockey Respiratory Syncytial Virus Infection: From Biology to Therapy A Perspective February 2008 *World Allergy Organization Journal* 1(2):21-8
30. Dvir Gatt et al. Prevention and Treatment Strategies for Respiratory Syncytial Virus (RSV) MDPI. 2023
31. Charl Verwey , Ziyaad Dangor , Shabir A Madhi Approaches to the Prevention and Treatment of Respiratory Syncytial Virus Infection in Children: Rationale and Progress to Date. *Paediatr Drugs* 2024 Mar;26(2):101-112.