Chapter

One of the Main Problems of Infants: Bronchiolitis

Şule Gökçe

Abstract

Acute bronchiolitis, which is the most common acute lower respiratory system disease, is resulting in significant morbidity and mortality in children less than 2 years. Respiratory syncytial virus (RSV) is the most common causative pathogen for over 30 million new acute lower respiratory infection episodes in children under 5 years of age. Rhinovirus, adenovirus, influenza virus, parainfluenza, and other respiratory viruses also cause acute bronchiolitis as the sole pathogen or as coinfection with or without RSV. Cardiovascular disease, chronic pulmonary disease, immunodeficiency, and premature birth are important risk factors for hospitalization and increase the risk of acute bronchiolitis-associated respiratory failure or even death. Bronchiolitis is a clinical diagnosis that varies from mild illness to severe respiratory failure. The severity of bronchiolitis is evaluated with several parameters including wheezing, retraction, respiratory rate, and general situation. However, the most important clinical finding is the presence or absence of hypoxemia and whether the patient can tolerate respiratory distress. Fluid support and oxygen supplementation by nasal cannula, face mask, or head box are critical for the treatment of bronchiolitis. Commonly used bronchodilators, corticosteroids, ribavirin, and antibiotics have not been shown to be effective in improving the clinical course of the bronchiolitis.

Keywords: acute bronchiolitis, infant, RSV

1. Introduction

Acute bronchiolitis is the most common lower lung disease that causes substantial morbidity and hospitalization in young infants under 6 months of age [1, 2]. In the first year of life, approximately 20–30% of children suffer from acute bronchiolitis that is frequently seen during the winter season, and infants are hospitalized with bronchiolitis [3]. During the epidemic season (late-autumn and winter months), the rate of bronchiolitis requiring hospitalization in all infants smaller than 12 months have been reported as 3% of in the US and Europe [4]. Prematurity and being born during the RSV season are risks factors for hospitalization [5]. A recent study has stated that the rate of hospitalization for bronchiolitis is 5.4% in preterm infants in the first 12 months of life [6]. A few studies particularly focused on the costs of bronchiolitis hospitalization reported that bronchiolitis admissions cost more than 500 million dollars annually and a co-diagnosis of bronchiolitis and pneumonia almost doubles the cost of the hospitalization [7].

Bronchiolitis is a viral disease in the infant period. Respiratory syncytial virus (RSV) is the most common agent that causes 50–80% of the cases. Its peak clinical

severity is seen between third and fifth days. Respiratory syncytial virus bronchiolitis is responsible for a short history of low-grade fever, cough, coryza, and difficulty in breathing and feeding. Infants who are under 6 weeks of age might be present with apnea alone without other clinical symptoms [8]. There are several predispositions to developing RSV infection in infants. A few of them are presence of an older sibling, birth in the RSV season, low birth weight, male sex, young age (<6 months), exposure to smoking, young maternal age, and suburban residence. Having congenital heart disease, chronic lung disease, immunodeficiency, cystic fibrosis, Down syndrome, or cerebral palsy increases the severity of RSV bronchiolitis [9]. In the pathogenesis of RSV bronchiolitis, there are a defective inflammatory response and cellular damage related to viral replication. Annually, RSV associated with lower respiratory tract infection in infants and young children leads to around 3.2 million hospitalizations and 59,000 deaths worldwide [10]. To date, there is no modality to prevent RSV infection. RSV vaccines, that named a formalin-inactivated RSV vaccine, have been improved in the mid-1960s. Due to the fact that the vaccines had caused "vaccine enhanced disease", the subunit vaccines schedule were not recommended. Afterward, RSV immune globulin (RespiGamTM) that reduced RSV-mediated hospitalizations had been prophylactically developed for infants with several risks for severe RSV disease. Currently, SynagisTM (palivizumab) is used to decrease RSV related hospitalizations by >55%. SynagisTM is implemented for newborns with a birth age of 35 weeks or less and infants under 6 months in RSV season. Additionally, for under 2 years, children with chronic lung disease treatment and hemodynamically serious congenital heart disease in the last 6 months are proposed to prevent severe RSV infection with the monoclonal antibody. SynagisTM should be given once a month at a dose of 15 mg/kg as long as the risk of RSV infection persists [11]. No matter what virus, the main treatment of bronchiolitis is liquid and oxygen therapy. However, Alansari et al. tested the efficacy of the anti-RSV monoclonal antibody palivizumab in infants <3 months of age with RSV bronchiolitis. Results of clinical trials have shown that intravenous palivizumab did not appear to help young infants with acute RSV-positive bronchiolitis [12]. Not only RSV has been reported as the most common cause of acute bronchiolitis in children younger than 1 year, but also the global annual rate of RSV hospitalization among children <5 years is 4.4 per 1000 lower respiratory tract infection in a systematic review and meta-analysis [8, 9, 13, 14]. History of prematurity is also reported to be a leading cause of mortality in acute bronchiolitis [15]. Due to the fact that the RSV vaccine is not available, prophylaxis with the monoclonal antibodies, palivizumab and motavizumab, has been developed to prevent RSV associated with mortality-morbidity in premature infants [16].

Rhinovirus is the second most common pathogen in acute bronchiolitis. Epidemiologic studies have stated that rhinoviruses-A and -C are to be the more common subtype acute respiratory infections and wheezing illnesses, and have reported that rhinovirus is related to moderate and severe bronchiolitis and in childhood [17]. In COAST cohort study, rhinovirus-A and -C species were associated with a higher risk of moderate-to-severe acute respiratory infection compared with those with rhinovirus-B infection [18]. The prevalence of rhinovirus causing acute lower respiratory infection varies between 17 and 35% among young children. A study from Turkey conducted by Gökçe et al. showed that the respiratory viral agent exhibited seasonal patterns with the number of RSV and rhinovirus cases peaking in the winter season [19]. Data on long-term outcomes report that the rate of recurrent wheezing is significantly higher in rhinovirus infections. Additionally, several cohorts confirmed that rhinovirus causing wheezing illness in early life is a significant predictor of asthma. Teeratakulpisarn et al. showed that the children

diagnosed with rhinovirus bronchiolitis could be possessed of recurrent wheezing. However, these symptoms mostly disappeared before the age of 6, and nearly half of the study patients subsequently had asthma [20, 21]. Another study from Italy also showed that recurrent wheezing 36 months after infant bronchiolitis was associated with rhinoviruses and blood eosinophilia [22].

Studies have reported that influenza, coronavirus, adenovirus, human bocavirus, human metapneumovirus, and parainfluenza viruses cause acute bronchiolitis as the sole pathogen or as coinfection with a similar seasonal pattern. Miron et al. aimed a study to assess the prevalence of sole and mixed respiratory organisms infection/detection in young children diagnosed with acute bronchiolitis. In this study, 590 respiratory organisms were detected in 423 (91%) children, and the two most commonly detected agents were RSV and rhinovirus [23]. In bronchiolitis, the most common dual infection was between RSV and rhinovirus, and the second was between RSV and human bocavirus. Adenovirus coinfections were also reported as the third most frequent. A study conducted in the United States asserted that dual infections had more ratios of hospitalizations than single infections [24]. Contrary, Calvo et al. stated that coinfections do not increase the severity [25].

The risk of acute bronchiolitis-associated respiratory failure or death is more seen in children previously diagnosed with cardiovascular disease, chronic pulmonary disease, and immunodeficiency. Persistently increased respiratory effort, hypoxemia, apnea, and acute respiratory failure define severe bronchiolitis that requires intensive monitoring and repeated examinations. Risks of severe bronchiolitis generally increase in infants with chronic lung disease, congenital heart disease, anatomic defects of the airways, immunodeficiency, and neurologic disease. It has also been reported that male gender, indigenous status, exposure to tobacco smoke, and poor socioeconomic factors were to be associated with severe bronchiolitis. Various polymerase chain reaction (PCR) techniques provide us to diagnose the etiology of acute bronchiolitis.

The clinical studies for new diagnostic measurements have been brought forth by clinicians in order to predict severe bronchiolitis, because severe bronchiolitis might be associated with morbidity and mortality in infants. It has been found that children with RSV had a more severe initial clinical presentation. Bamberger et al. stated that infants with RSV bronchiolitis, especially young infants, had high clinical severity score on admission when compared to those with other respiratory viruses [26]. In this respect, it has been aimed to evaluate the accuracy of virologic testing for RSV in detecting patients at risk for more severe disease. Hasegawa et al. have reported that the major viruses (RSV-A, RSV-B, rhinovirus, adenovirus, and hMPV) had different temporal patterns in a study which was multicenter-multiyear prospective cohorts of the US infants with severe bronchiolitis. Their data provide guidance for optimal timing of RSV immunoprophylaxis, effective prophylactic (e.g., immunoprophylaxis), and treatment (e.g., antiviral agents) strategies in infants at higher risk for severe bronchiolitis [27]. Another study stated that infants attended daycare, had older siblings, had high parental educational levels, had birth weights of >4 kg, and were born between April and September had a 10-fold higher risk for severe RSV infection than those who had no these factors [28]. These features provide us to differentiate between infants with high risks of RSV bronchiolitis and to target preventive and monitoring approach.

2. Pathophysiology and pathogenesis

Generally, acute bronchiolitis is characterized by upper respiratory symptoms (e.g., rhinorrhea) followed by lower respiratory infection with inflammation

which is commenced by a pathogen and leads to epithelial necrosis in the bronchial epithelium. Classical symptoms of bronchiolitis including wheezing, crackles, and bronchospasm consist of partial obstruction in the lumen through the accumulation of degenerated squamous epithelium secretions [29]. Bronchiolar obstruction with edema and accumulation of mucus and cellular debris in the airways can persist for many weeks or months following acute bronchiolitis [30]. The reconstruction process may result in complete recovery. However, it can also be characterized by exaggerated proliferation of granulation tissue. There is an exaggerated inflammatory response mediated by cytokines especially T-helper 1 in the pathogenesis of acute bronchiolitis with a variable cytokine profile, according to the infective virus. Due to the fact that exaggerated proliferation causes narrowing or obliteration of the airway lumen, severe clinical findings can be seen in some cases [31–33].

The mucosal innate immune system procures a strong barrier to respiratory infections. In particular, RSV and/or rhinovirus can trigger/induce the concomitant production of type I (IFNα/β) and type III (IFNλs). A study was designed to investigate the airway type III IFN receptor (IFNLR1/IL10RB) expression during respiratory syncytial virus or human rhinovirus bronchiolitis. The results of this study showed that the association of IFNLR1 with rhinovirus infection could cause more severe bronchiolitis and blood eosinophilia. The type III IFN receptor also dictates an important role in the host immune response during bronchiolitis [34].

2.1 Etiology

Respiratory syncytial virus is the most common etiologic pathogen in acute bronchiolitis with a rate of 50–80%. Various studies have shown that other viruses, including adenovirus, coronavirus, parainfluenza, influenza, rhinovirus, human bocavirus, and human metapneumovirus, are associated with acute bronchiolitis [35]. Rhinovirus (RV), which is the most common human respiratory pathogens and are responsible for most upper respiratory infections (e.g., the common cold), is the second most commonly associated viral bronchiolitis [36–38]. In recent years, new human respiratory viruses like human metapneumovirus, human bocavirus, and new human coronaviruses have also been reported as possible pathogens causing acute bronchiolitis [38]. Respiratory viruses could occur as coinfection with other respiratory viruses: dual, triple, or more [37, 39, 40]. Today, various viral diagnostic tests provide us to determine the epidemiological differences/clinical characteristics of respiratory viruses. One of the diagnostic methods is multiplex polymerase chain reaction which has been the most commonly used method [41]. Rarely, several atypical infections, for instance, *Bordetella pertussis*, *Mycoplasma pneumonia*, *Simkania negevensis*, and a Chlamydia-like intracellular organism have also shown in bronchiolitis [42–44].

2.2 Diagnosis

American Academy of Pediatrics Clinical Practice Guidelines has reported the definition of acute bronchiolitis in 2006. According to the guideline, bronchiolitis has been described as the first episode of wheezing in children under 24 months of age who have respiratory findings during the viral infection episode. Tachypnea, nasal flaring, chest retractions, and wheezing and/or rales are clinical characteristic features of acute bronchiolitis. Rhinorrhea, cough, tachypnea, wheezing, rales, and increased respiratory effort manifested as grunting, nasal flaring, and intercostal and/or subcostal retractions are clinical signs and symptoms of bronchiolitis [3]. Early presentations of asthma or wheeze with viral infections may potentially overlap with the diagnosis of bronchiolitis. Chest radiographs and laboratory studies

may be thought of on clinical suspicion after evaluating the differential diagnosis for secondary or comorbid bacterial infection, complications, or other conditions.

Viral diagnosis methods that are not routinely suggested for testing, including antigen detection or immunofluorescence of nasal secretion wash or nasal aspiration, rapid antigen tests, and PCR, are only suggested for identifying specific viral agents in children with bronchiolitis if the results will determine discontinuation of palivizumab prophylaxis, initiation, or continuation/discontinuation of antibiotic therapy [45–48].

Bronchiolitis must be distinguished from a variety of acute and chronic diseases including asthma, pneumonia, airway lesions, congenital lung disease or diaphragmatic hernia, cystic fibrosis, congenital heart disease, sepsis, and severe metabolic acidosis that might present with similar presentation. Atypical clinical findings like lack of preceding upper respiratory tract symptoms, witnessed an episode of choking, and poor growth may be useful to discriminate from acute bronchiolitis. It should be considered further investigation for the differential diagnosis.

2.3 Hospital admission and investigations

Though bronchiolitis is usually a self-limiting entity, several infants have severe bronchiolitis and should be safely managed at hospital. Severity score of bronchiolitis is described with several clinical parameters including wheezing, retraction, respiratory rate, and general situation (Wang respiratory score) [49]. However, the severity score has not been shown to be useful in a clinical setting. Therefore, the scoring system is not generally used in hospitalization decision. If the infants have any of features, such as apnea, difficulty in feeding, severe respiratory distress with accessory muscle use or grunting, respiratory rate greater than 60/min, diagnostic uncertainty, and cyanosis/hemoglobin saturation < 92%, they are should be referred for hospital admission. Infants with specific risk factors, such as poor socioeconomic circumstances, a history of prematurity, congenital heart disease, or chronic lung disease, also need to be hospitalized. Around 1–5% of infants might need pediatric intensive care support in bronchiolitis [50]. If infants under 6 months of age or with comorbidities, they are more likely to require intensive care unit admission. After hospitalization, the infants with severe respiratory distress, exhaustion, failure to maintain hemoglobin saturation above 92–94% with supplemental oxygen or with recurrent apnea should be followed-up in intensive care units. Since the diagnosis of acute bronchiolitis is done clinically, infants with bronchiolitis require no further investigations. During the hospitalization, hemoglobin saturation should be measured using pulse oximetry to determine the requirement for supplemental oxygen.

2.4 Assessment

Initially, the management of bronchiolitis is assessed by clinical features. Persistently increased respiratory effort, hypoxemia, apnea, and acute respiratory failure show severe bronchiolitis. Therefore, basic airway management and emergency endotracheal intubation should be considered in a child with deterioration and respiratory failure. In nonsevere bronchiolitis, supportive care including adequate hydration and relief of nasal congestion/obstruction are the mainstays of management for infants with bronchiolitis. Disease progression should also be monitored. Although bronchodilators (inhaled or oral), glucocorticoids, nebulized hypertonic saline, or leukotriene inhibitors have been used in some situations, randomized trials do not recommend pharmacologic interventions in nonsevere bronchiolitis.

Fluid support is absolutely necessary for infants with any level bronchiolitis. Because bronchiolitis causes difficulty in maintaining adequate hydration and children with bronchiolitis may also decrease intake the fluid due to tachypnea and respiratory distress. Related to clinical features, fluid replacement treatment may have been provided by parenteral or small frequent feedings or orogastric or nasogastric feedings in children who can tolerate enteral feedings strategies.

The second most important support treatment is oxygen supplementation by nasal cannula, face mask, or head box to provide the SpO_2 > 90–92% for infants. If there is insufficient oxygen therapy during support treatment, it means that a progression to respiratory failure. In order to reduce the work of breathing, improve gas exchange, and avoid the need for endotracheal intubation, heated humidified high-flow nasal cannula (HFNC, also called high-flow warm humidified oxygen) therapy and/or continuous positive airway pressure (CPAP) are recently used mostly.

2.5 Clinical course

Bronchiolitis, which is a self-limited disease, often resolves without complications. Generally, the respiratory status improves over 2–5 days in bronchiolitis. Standard strategies include hand hygiene to reduce the risk of bronchiolitis to minimize the transmission of infectious agents. Additionally, avoiding passive exposure to cigarette smoke and contact with individuals with respiratory tract infections might reduce the risk of bronchiolitis.

Author details

Şule Gökçe
Faculty of Medicine, Department of Pediatrics, Ege Üniversity, Izmir, Turkey

*Address all correspondence to: sule.gokce@ege.edu.tr

References

[1] Shay DK, Holman RC, Newman RD, Liu LL, Stout JW, Anderson LJ. Bronchiolitis-associated hospitalizations among US children, 1980-1996. JAMA. 1999;**282**:1440-1446

[2] Boyce TG, Mellen BG, Mitchel EF Jr, Wright PF, Griffin MR. Rates of hospitalization for respiratory syncytial virus infection among children in Medicaid. The Journal of Pediatrics. 2000;**137**:865-870

[3] American Academy of Pediatrics Subcommittee on Diagnosis and Management of Bronchiolitis. Diagnosis and management of bronchiolitis. Pediatrics. 2006;**118**:1774-1793

[4] Smyth RL, Openshaw PJ. Bronchiolitis. Lancet. 2006;**368**:312-322

[5] Walton RP, Johnston SL. Role of respiratory viral infections in the development of atopic conditions. Current Opinion in Allergy and Clinical Immunology. 2008;**8**:150-153

[6] Lanari M, Prinelli F, Adorni F, et al. Risk factors for bronchiolitis hospitalization during the first year of life in a multicenter Italian birth cohort. Italian Journal of Pediatrics. 2015;**41**:40

[7] Pelletier AJ, Mansbach JM, Camargo CA Jr. Direct medical costs of bronchiolitis hospitalizations in the United States. Pediatrics. 2006;**118**:2418-2423

[8] Ricci V, Delgado Nunes V, Murphy MS, Cunningham S, Guideline Development Group and Technical Team. Bronchiolitis in children: Summary of NICE guidance. BMJ. 2015;**350**:h2305

[9] Murray J, Bottle A, Sharland M, et al. Risk factors for hospital admission with RSV bronchiolitis in England: A population-based birth cohort study. PLoS One. 2014;**9**:89186

[10] Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, et al. Mortality associated with influenza and respiratory syncytial virus in the United States. Journal of the American Medical Association. 2003;**289**:179-186

[11] The Impact-RSV Study Group. Palivizumab, a humanized respiratory syncytial virus monoclonal antibody, reduces hospitalization from respiratory syncytial virus infection in high-risk infants. Pediatrics. 1998;**102**:531-537

[12] Alansari K, Toaimah FH, Almatar DH, El Tatawy LA, Davidson BL, Qusad MIM. Monoclonal antibody treatment of RSV bronchiolitis in young infants: A randomized trial. Pediatrics. 2019;**143**:1-7

[13] Center for Disease Prevention and Control. Acute bronchiolitis-associated outpatient visits and hospitalizations among American Indian and Alaska native children—United States, 1990-2000. MMWR. Morbidity and Mortality Weekly Report. 2003;**52**:707-710

[14] Miller EK, Gebretsadik T, Carroll KN, et al. Viral etiologies of infant bronchiolitis, croup and upper respiratory illness during 4 consecutive years. The Pediatric Infectious Disease Journal. 2013;**32**:950-955

[15] Wainwright C. Acute viral bronchiolitis in children—A very common condition with few therapeutic options. Paediatric Respiratory Reviews. 2010;**11**:39-45

[16] Resch B, Kurath-Koller S, Eibisberger M, Zenz W. Prematurity and the burden of influenza and respiratory syncytial virus disease. World Journal of Pediatrics. 2016;**12**:8-18

[17] Bochkov YA, Gern JE. Rhinoviruses and their receptors: Implications for allergic disease. Current Allergy and Asthma Reports. 2016;**16**:30

[18] Lee WM, Lemanske RF Jr, Evans MD, et al. Human rhinovirus species and season of infection determine illness severity. American Journal of Respiratory and Critical Care Medicine. 2012;**186**:886-891

[19] Gökçe Ş, Kurugöl Z, Koturoğlu G, Çiçek C, Aslan A. Etiology, seasonality, and clinical features of viral respiratory tract infections in children hospitalized with acute bronchiolitis: A single-Center study. Global Pediatric Health. 2017;**4**:1-7

[20] Teeratakulpisarn J, Pientong C, Ekalaksananan T, Ruangsiripiyakul H, Uppala R. Rhinovirus infection in children hospitalized with acute bronchiolitis and its impact on subsequent wheezing or asthma: A comparison of etiologies. Asian Pacific Journal of Allergy and Immunology. 2014;**32**:226-234

[21] Jackson DJ, Gangnon RE, Evans MD, Roberg KA, Anderson EL, Pappas TE, et al. Wheezing rhinovirus illness in early life predicts asthma development in high-risk children. American Journal of Respiratory and Critical Care Medicine. 2008;**178**:667-672

[22] Midulla F, Nicolai A, Ferrara M, Gentile F, Pierangeli A, Bonci E, et al. Recurrent wheezing 36 months after bronchiolitis is associated with rhinovirus infections and blood eosinophilia. Acta Paediatrica. 2014;**103**:1094-1099

[23] Miron D, Srugo I, Kra-Oz Z, Keness Y, Wolf D, Amirav I, et al. Sole pathogen in acute bronchiolitis: Is there a role for other organisms apart from respiratory syncytial virus. The Pediatric Infectious Disease Journal. 2010;**29**:7-10

[24] Drews AL, Atmar RL, Glezen WP, et al. Dual respiratory virus infections. Clinical Infectious Diseases. 1997;**25**:1421-1429

[25] Calvo C, García-García M'a L, Pozo F, Paula G, Molinero M, Calderón A, et al. Respiratory syncytial virus coinfections with rhinovirus and human bocavirus in hospitalized children. Medicine. 2015;**94**:1-7

[26] Bamberger E, Srugo I, Abu Raya B, Segal E, Chaim B, Kassis I, et al. What is the clinical relevance of respiratory syncytial virus bronchiolitis? Findings from a multicenter, prospective study. European Journal of Clinical Microbiology & Infectious Diseases. 2012;**31**:3323-3330

[27] Hasegawa K, Goto T, Hirayama A, Laham F, Mansbach J, Piedra P, et al. Respiratory virus epidemiology among US infants with severe bronchiolitis: Analysis of 2 multicenter, multiyear cohort studies. The Pediatric Infectious Disease Journal. 2019;**38**:180-183

[28] Houben ML, Bont L, Wilbrink B, Belderbos ME, Jan LLK, Visser GHA, et al. Clinical prediction rule for RSV bronchiolitis in healthy newborns: Prognostic birth cohort study. Pediatrics. 2011;**127**:35-41

[29] Colby TV. Bronchiolitis. Pathologic considerations. American Journal of Clinical Pathology. 1998;**109**:101-109

[30] Hall CB. Respiratory syncytial virus and parainfluenza virus. The New England Journal of Medicine. 2001;**344**:1917-1928

[31] Garcia C, Soriano-Fallas A, Lozano J, Leos N, Gomez AM, Ramilo O, et al. Decreased innate immune cytokine responses correlate with disease severity in children with respiratory syncytial virus and human rhinovirus bronchiolitis. Pediatric Infectious Disease. 2012;**31**:86-89

[32] McNamara PS, Flanagan BF, Hart CA, Smyth RL. Production of chemokines in the lungs of infants with severe respiratory syncytial virus bronchiolitis. The Journal of Infectious Diseases. 2005;**191**:1225-1232

[33] Van Schaik SM, Tristram DA, Nagpal IS, Hintz KM, Welliver RC, Welliver RC. Increased production of IFN-gamma and cysteinyl leukotrienes in virus-induced wheezing. The Journal of Allergy and Clinical Immunology. 1999;**103**:630-636

[34] Pierangelia A, Statzua M, Nennab R, Santinellia L, Petrarcab L, Frassanitob A, et al. Interferon lambda receptor 1 (IFNL1R) transcript is highly expressed in rhinovirus bronchiolitis and correlates with disease severity. Journal of Clinical Virology. 2018;**102**:101-109

[35] Bezerra PG, Britto MC, Correia JB, et al. Viral and atypical bacterial detection in acute respiratory infection in children under five years. PLoS One. 2011;**6**:18928

[36] Mansbach JM, Piedra PA, Teach SJ, et al. Prospective, multicenter study of viral etiology and hospital length-of-stay in children with severe bronchiolitis. Archives of Pediatrics and Adolescent Medicine. 2012;**166**:700-706

[37] Papadopoulos NG, Moustaki M, Tsolia M, et al. Association of rhinovirus infection with increased disease severity in acute bronchiolitis. American Journal of Respiratory and Critical Care Medicine. 2002;**165**:1285-1289

[38] Berry M, Gamieldien J, Fielding BC. Identification of new respiratory viruses in the millennium. Viruses. 2015;**7**:996-1019

[39] Stempel HE, Martin ET, Kuypers J, Englund JA, Zerr DM. Multiple viral respiratory pathogens in children with bronchiolitis. Acta Paediatrica. 2009;**98**:123-126

[40] Ray CG, Minnich LL, Holberg CJ, et al. Respiratory syncytial virus-associated lower respiratory illnesses: Possible influence of other agents. The group health medical associates. The Pediatric Infectious Disease Journal. 1993;**12**:15-24

[41] Liolios L, Jenney A, Spelman D, Kotsimbos T, Catton M, Wesselingh S. Comparison of a multiplex reverse transcription-PCR-enzyme hybridization assay with conventional viral culture and immunofluorescence techniques for the detection of seven viral respiratory pathogens. Journal of Clinical Microbiology. 2001;**39**:2779-2783

[42] Nakamura A, Sakano T, Nakayama T, et al. Neonatal pertussis presenting as acute bronchiolitis: Direct detection of the *Bordetella pertussis* genome using loop-mediated isothermal amplification. European Journal of Pediatrics. 2009;**168**:347-349

[43] Sung RY, Chan RC, Tam JS, et al. Epidemiology and aetiology of acute bronchiolitis in Hong Kong infants. Epidemiology and Infection. 1992;**108**:147-154

[44] Kahane S, Greenberg D, Friedman MG, et al. High prevalence of "Simkania Z" a novel Chlamydia-like bacterium, in infants with acute bronchiolitis. The Journal of Infectious Diseases. 1998;**177**:1425-1429

[45] Ahluwalia G, Embree J, McNicol P, Law B, Hammond GW. Comparison of nasopharyngeal aspirate and nasopharyngeal swab specimens for respiratory syncytial virus diagnosis by cell culture, indirect immunofluorescence assay, and enzyme-linked immunosorbent assay. Journal of Clinical Microbiology. 1987;**25**:763-767

[46] Macfarlane P, Denham J, Assous J, Hughes C. RSV testing in bronchiolitis: Which nasal sampling method is best? Archives of Disease in Childhood. 2005;**90**:634-635

[47] Bordley WC, Viswanathan M, King VJ, et al. Diagnosis and testing in bronchiolitis: A systematic review. Archives of Pediatrics and Adolescent Medicine. 2004;**158**:119-126

[48] Harris JA, Huskins WC, Langley JM, Siegel JD. Pediatric special interest Group of the Society for Healthcare Epidemiology of America. Health care epidemiology perspective on the October 2006 recommendations of the subcommittee on diagnosis and management of bronchiolitis. Pediatrics. 2007;**120**:890-892

[49] Wang EE, Milner RA, Navas L, Maj H. Observer agreement for respiratory signs and oximetry in infants hospitalized with lower respiratory infections. The American Review of Respiratory Disease. 1992;**145**:106-115

[50] Parker MJ, Allen U, Stephens D, et al. Predictors of major intervention in infants with bronchiolitis. Pediatric Pulmonology. 2009;**44**:358-363