

# Update on current views and advances on RSV infection (Review)

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**Abstract.** Respiratory syncytial virus (RSV) infection represents an excellent paradigm of precision medicine in modern paediatrics and several clinical trials are currently performed in the prevention and management of RSV infection. A new taxonomic terminology for RSV was recently adopted, while the diagnostic and omics techniques have revealed new modalities in the early identification of RSV infections and for better understanding of the disease pathogenesis. Coordinated clinical and research efforts constitute an important step in limiting RSV global predominance, improving epidemiological surveillance, and advancing neonatal and paediatric care. This review article presents the key messages of the plenary lectures, oral presentations and posters of the '5th workshop on paediatric virology' (Sparta, Greece, 12th October 2019) organized by the Paediatric Virology Study Group, focusing

on recent advances in the epidemiology, pathogenesis, diagnosis, prognosis, clinical management and prevention of RSV infection in childhood.

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## 1. Introduction

Precision medicine has evolved in recent years allowing the incorporation of novel taxonomies and stratification of patients, and using standardized clinical endpoints, genetic and other biomarker information (1). Its role in paediatric healthcare involves the selection of targeted diagnostic, therapeutic and prevention strategies matched to precise molecular, epidemiological and clinical profile of each patient; the management of respiratory syncytial virus (RSV) infection represents a good paradigm of precision medicine (2). RSV is a single-stranded RNA virus (Figs. 1 and 2), which represents the most frequent viral cause of acute lower respiratory tract infection (ALRTI) in infants, with a worldwide distribution and seasonal occurrence (2-5). It was first isolated in 1956 from nasal secretions of chimpanzees with rhinorrhea and coryza; the novel virus was

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initially named 'Chimpanzee coryza agent' (CCA) (6,7). In the following year, when CCA was also isolated from children with ALRTI, it gained its final name due to the syncytia observed on electron microscopy; syncytia are formed by fusion of infected host cells with neighboring cells leading to the formation of multi-nucleate enlarged cells. Although the formation of syncytia is the hallmark of the cytopathic effect of RSV that is associated with host cellular membrane merging, syncytia are not pathognomonic of RSV (8). Syncytia are also observed in cell culture with several other viruses, such as parainfluenza, HSV-1, HIV and MeV. Recently, the International Committee on Taxonomy of Viruses (ICTV), which authorizes and organizes the classification and naming of viral species, grouped RSV under the genus Orthopneumovirus within the family Pneumoviridae (9,10).

Bronchiolitis is the most common clinical manifestation of RSV infection in infants and although it is usually self-limiting, in infancy it accounts for a significant number of hospitalizations and paediatric intensive care unit (PICU) admissions (3). Despite its association with relatively high morbidity and mortality in premature neonates and in certain paediatric populations with underlying conditions, such as immunodeficiency and congenital heart disease, RSV infection may also lead to hospitalization of previously healthy, full-term infants (11,12). RSV-positive bronchiolitis is characterized by airway inflammation and oedema, mucus production and debris leading to airway obstruction and turbulent gas flow. Even though various therapeutic interventions have been tried, such as bronchodilators, hypertonic saline and corticosteroids, supportive care remains the mainstay in most settings, with gentle suctioning of nasal secretions, prone position, fluid replacement and oxygen or respiratory support, as necessary. Several clinical trials on the management and prevention of RSV-positive bronchiolitis have been recently completed, are underway, or in development (3). Currently, there is rapid expansion of RSV vaccine candidate development and there is hope that one will become available in the near future. Of course, the safety of vaccines proposed for primary immunization in an antigen naïve child remains the top priority.

This review article summarizes the key messages of the plenary lectures, oral presentations and posters of the '5th Workshop on Paediatric Virology' held in Sparta (Greece) on October 12th, 2019, which was focused on RSV (Table I). This workshop was organized by the Paediatric Virology Study Group (PVSG) and was co-chaired by Dr Simon B. Drysdale, Consultant and Honorary Senior Lecturer in Paediatric Infectious Diseases at St. George's University Hospital NHS Foundation Trust and St. George's, University of London (London, UK), Professor Barbara Rath, Co-founder and Chair of the Vienna Vaccine Safety Initiative (Berlin, Germany), Honorary Professor of Nottingham School of Medicine (Nottingham, UK) and Research Director at the University of Bourgogne-Franche-Comté (Besançon, France), Professor Maria Theodoridou, Professor Emerita of Paediatrics at the University of Athens School of Medicine (Athens, Greece), President of the National Immunisation Committee and f. President of the Hellenic Paediatric Infectious Diseases Society, Dr Georgia Papaioannou, Head of the Paediatric Radiology Department at 'Mitera' Children's Hospital in Athens (Greece), Dr Ioannis N. Mammias, Consultant Paediatrician on

the island of Euboea (Greece) and Coordinator of the PVSG and Professor George P. Chrousos, Professor Emeritus of Paediatrics and Endocrinology at the University of Athens School of Medicine (Athens, Greece). The workshop was held under the auspices of the World Academy of Sciences (WAS) and was supported by the Laboratory of Clinical Virology of the University of Crete School of Medicine and the First Department of Paediatrics of the University of Athens School of Medicine.

## 2. Epidemiology of RSV infection

*Understanding the burden of RSV infections in real-time.* RSV poses significant disease burden in infants and children worldwide (13), and the international paediatric community is only beginning to appreciate its global impact in both high and low resource settings (4,14,15). The clinical presentation of an RSV infection depends on the patient's age and individual risk factors (16,17). The concept of measuring individual-level differences in disease severity has drawn the attention of both public health stakeholders and regulatory agencies in recent years (18-20). The impact of RSV infections of course should be differentiated from the disease caused by influenza and other viral respiratory infections (21,22). However, a recent extensive literature review and prospective cohort have shown that in infants and children this cannot be done based on clinical symptoms alone; distinguishing RSV from other viral respiratory infections requires laboratory confirmation (23,24). With RSV vaccines and antiviral agents in development, it will be important to: a) diagnose RSV infections in a timely manner; b) differentiate RSV from other forms of acute respiratory infections; and c) communicate the test results back to patients and parents/caregivers along with information on the individual disease risk and severity.

*The PEDSIDEA programme.* The Vienna Vaccine Safety Initiative (ViVI, <https://www.vi-vi.org>) is an international non-profit research organization which, in collaboration with academic institutions and public health agencies in Europe and the United States, has developed digital tools and programs to improve the quality of care for children and adults with ALRTI or influenza-like illnesses (ILI) (13,25,26). Taking a person-centered approach, the ViVI Disease Severity Score ('ViVI Score') is a mobile application enabling healthcare professionals to measure disease severity at the point of care within minutes. It was designed to provide a uniform approach to define *ad hoc* severity at any given time point, based on extensive literature review as well as WHO Criteria for uncomplicated and complicated ILI (27). In collaboration with the Robert Koch Institute, the ViVI Score was validated in a cohort of 6,000 children (age 0-18) in Berlin, Germany and subsequently used in a European pilot project entitled 'Partnering for Enhanced Digital Surveillance of Influenza-like Disease and the Effectiveness of Antivirals and Vaccines' (PEDSIDEA) (27,28). Since then, the PEDSIDEA programme has been implemented in community clinic networks and adult intensive care units in the United States for the real-time digital surveillance of influenza and RSV disease incidence and severity at the point of care (29). The programme is expected to continue monitoring, in great detail, the clinical outcomes

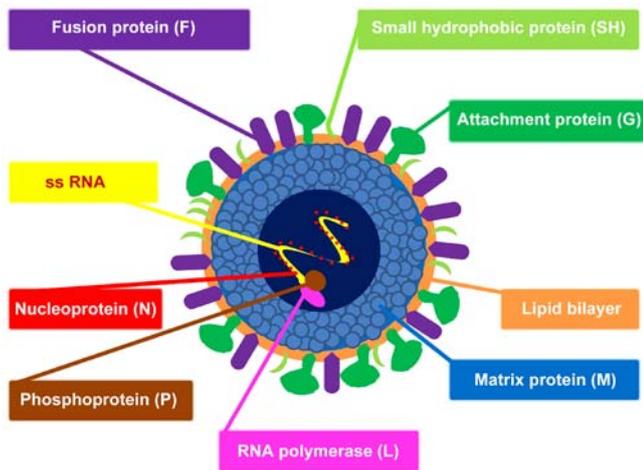


Figure 1. Schematic structure of RSV. The virus envelope contains the fusion protein (F), the small hydrophobic protein (SH) and the attachment protein (G). Underlying the envelope is the matrix protein (M). The nucleocapsid consists of single-stranded RNA (ss RNA) encapsulated by the nucleoprotein (N). Associated with the nucleocapsid are the RNA polymerase (L) and the phosphoprotein (P). RSV, respiratory syncytial virus.



Figure 2. RSV genome organisation. Genes are listed in sense (coding) orientation (3'-to-5') in which each box represents a gene encoding a separate mRNA for each protein. NS1 and NS2, nonstructural proteins; N, nucleoprotein; P, phosphoprotein; M, matrix protein; SH, small hydrophobic protein, G, attachment proteins; F, fusion protein; M2, second matrix protein; L, RNA polymerase; RSV, respiratory syncytial virus.

of 'natural' RSV and other viral respiratory infections in children and adults, including patients at the extremes of age. Understanding the real-world disease burden may help facilitate the study of the effectiveness of novel influenza and RSV antiviral agents and vaccines, once they become available (30).

**RSV infection: not for children only.** RSV was not recognized as a potentially serious problem in older adults until the 1970s, when outbreaks of the virus occurred in long-term care facilities for the elderly (31-33). Since then, additional studies in hospitalized adults have suggested that RSV may be an important cause of illness in adults. Molecular diagnostics suggest that RSV positive specimens are commonly identified in elderly and high-risk adults, in a frequency similar to that of seasonal influenza (31,34). Even though a positive RSV respiratory panel does not equate pathogenesis, it has been suggested that RSV may account for as much as 10,000 deaths annually in the United States among individuals above the age of 65 years (31-33,35). This, in addition to the morbidity in infants, has stimulated interest in RSV vaccines and antiviral agents. Additional natural history studies are needed to better understand the actual burden of RSV infection among the elderly and high-risk adults.

### 3. Pathogenesis, diagnosis and prognosis of RSV infection

**The immune response to RSV infection.** Maternal antibodies may be able to mitigate RSV disease severity in young

infants (17,36-38). It is assumed that the transplacental passage of RSV-specific antibodies occurs predominately during the third trimester of pregnancy. High titers can potentially protect term infants up to four months of age (38). In premature infants, passive immunity may be 'compromised' (39), but still has a role to play. The degree to which breast feeding may also contribute to passive immunity and to priming of immune system is currently under investigation (40). Once infection is established, the innate immune system plays a dual role in lowering the viral load and in mounting a secondary immune response. Prematurity and other conditions that compromise the immune response may lead to reduced levels of antiviral cytokines, such as the interferons (41). In infants, reduced signaling by TLRs and altered antigen-presenting cell functions, including low interleukin (IL)-12 and enhanced IL-6 and IL-23 production, coupled with reduced activation of regulatory T cells, may result in an adaptive response that is skewed toward Th2 and Th17 and away from protective Th1 and CTL responses (42). The potential specific role(s) of certain pattern recognition receptors in humans has/ve been suggested by the fact that certain TLR missense mutations are associated to a phenotype with propensity to wheezing (43). Apart from the individual genetics (44), the stage of lung maturation among term and premature infants also impacts on the Th1 to Th2 switch. In the case of prematurity, the mucosa prior to alveolarization is being deluged with Th2 inflammatory responses, even in the earlier stages of bronchopulmonary dysplasia (45). Impaired Th1 activation, coupled with little or no B cell memory, and inhibition of antibody production by IFN $\gamma$ , produces low-titer, low-affinity antibody (46). The result may be a poorly protective and dysregulated defense mechanism that leads to bronchiolitis in susceptible infants (43). Later, the host immune response is permanently oriented to the direction of wheezing exacerbations, specifically triggered by RSV (47).

**MicroRNAs as potential bio-markers in RSV infection.** MicroRNAs (miRNAs) are involved in post-transcriptional gene regulation and play significant roles in the maintenance of the airway epithelial barrier of the respiratory tract (48-56). miRNAs have been implicated in the modulation of antiviral defense mounted by host innate and adaptive immunity, involving not only immune effector and inflammatory cells, but also parenchymal cells (50). Respiratory viruses, including RSV, attack, as a primary target, the epithelial cells of the respiratory tract causing an altered expression of distinct miRNAs in the airway cells. The human innate immune response inhibits RSV replication early after inoculation, mainly through the action of interferons (53). Multiple miRNAs are induced by infection in a cell-type-specific fashion (51). RSV appears to alter host cell gene expression also through regulation of expression of miRNAs related to the interferon response (50,53). Abnormal expression of miRNAs has been detected in both peripheral blood cells and airway epithelial cells in RSV-infected infants (54). Understanding alterations in miRNA expression profiles and identifying miRNA target genes in relation to the pathogenesis of RSV may help clarify the mechanisms of virus-host interactions, and immune dysfunction leading to airway hyper-reactivity and chronic respiratory diseases, such as asthma (49,50,53,54). There are several methods for the purification, quantification and

Table I. The top key messages of the '5th workshop on paediatric virology' on RSV infection in children.

Epidemiology of RSV infection	<p>Being able to compare severity over time and/or across cohorts is useful in hospital-based QI programmes but also in multi-centre networks, such as PEDSIDEA</p> <p>Understanding the real-world disease burden caused by RSV will facilitate the study of the effectiveness of antivirals and vaccines, once they become available</p> <p>Recent epidemiological data indicate that RSV infection is an important illness in elderly and high-risk adults, with a disease burden similar to that of non-pandemic influenza</p>
RSV and immune response	Maternal RSV-specific antibodies transmitted transplacentally during the third trimester of pregnancy are related to RSV disease severity in young infants
RSV and miRNAs	A greater understanding of miRNAs may enable them to be used as biomarkers of severe RSV infection and as novel targets for treatment or prophylaxis of RSV infection
RSV and thrombocytosis	Thrombocytosis in RSV-positive bronchiolitis does not require routine prophylactic anti-platelet treatment or further investigations
RSV and asthma	<p>There is compelling evidence that severe respiratory infection induced by RSV is associated with subsequent development of asthma later in childhood</p> <p>Further understanding of the role of RSV in asthma pathogenesis will enable our understanding of the impact of future vaccines against RSV in asthma prevention</p>
RSV as a cause of PIBO	<p>There are only few reports in the literature of children with PIBO secondary to RSV as a single infection</p> <p>Further research is required in order to investigate the potential impact of RSV co-infection in the severity and worse outcome in children with PIBO</p>
Imaging of RSV infection	<p>Although imaging cannot diagnose RSV infection, it is important to identify the possible pattern of viral disease, in order to avoid unnecessary administration of antibiotic therapy and predict possible late effects</p> <p>Standard radiological techniques, including CT, are unable to distinguish between acute bronchiolitis caused by RSV versus that caused by other respiratory viruses</p> <p>HRCT of the lungs may be required to assess possible bronchial thickening and remodeling, the development of bronchiectasis and air-trapping</p>
Antivirals against RSV	<p>Ribavirin is currently the only licensed antiviral medication used to treat RSV infection; it has very limited efficacy and multiple toxicities, which means its use is usually reserved for severely immunocompromised children</p> <p>Due to ethical and technical constraints human challenge models are only undertaken in adults, but if a product is shown to be efficacious in this setting it allows a faster move to trials in children than traditional trials which often take much longer to do</p> <p>A greater understanding of individual data in newly developed pharmaceutical agents against RSV will potentially lead to future personalized treatment regimens</p>
RSV and PICU	<p>HFNC might have a role as a rescue therapy for children with RSV-positive bronchiolitis admitted to PICU to reduce their requirement for high-cost intensive care</p> <p>Heliox could be useful in addition to standard medical care in the management of children with RSV-positive bronchiolitis admitted to PICU</p>
Prevention of RSV infection	<p>To date, there is only one product available for prevention of RSV infection, palivizumab, the mAb that has been shown to reduce hospital admission due to RSV infection in some high risk infants by up to 80%</p> <p>There are currently 43 RSV vaccines in development; of these, 21 are in clinical trials in humans; 14 in Phase 1, five in Phase 2 and two in Phase 3, 12 vaccines are in trials in children, 4 in pregnant women and 10 in older adults</p> <p>A possible route to licensure is currently being sought with the US FDA and European licensing agencies for a maternal RSV vaccine, bringing hope of a vaccine becoming available that could save the lives of countless young infants worldwide</p>

RSV, respiratory syncytial virus; mAb, monoclonal antibody; FDA, Food and Drug Administration; QI, quality improvement, PEDSIDEA, Partnering for Enhanced Digital Surveillance of Influenza-like Disease and the Effectiveness of Antivirals and Vaccines; ALRTI, acute lower respiratory tract infection; ILI, influenza-like illness; CT, computed tomography; HRCT, high-resolution computed tomography; ICTV, International Committee on Taxonomy of Viruses; miRNAs, microRNAs; PIBO, post infectious bronchiolitis obliterans; PICU, Paediatric Intensive Care Unit; HFNC, high-flow nasal cannula.

characterization of miRNA expression profiles in biofluids, whole blood samples and tissue samples obtained in *in vivo* studies (55). Further research on miRNAs is expected to clarify their value as biomarkers of RSV infections and their sequelae (i.e., recurrent wheezing and asthma).

*Thrombocytosis and RSV infection.* The most frequent causes of secondary thrombocytosis in childhood are acute respiratory tract infections (57-61). To date, several authors have reported significantly higher mean platelet counts in patients with RSV than in patients with other acute respiratory tract infections (61-64). Thrombocytosis is more likely to occur in younger patients, who have clinical manifestations of wheezing and dyspnoea (62,64). Moreover, thrombocytosis has been suggested as an early marker of RSV infection (62). Excessive thrombocytosis has also been detected at an early stage in cases of RSV-positive bronchiolitis (65). It has been proposed that thrombocytotic patients have a more severe clinical course and longer duration of hospitalization and that the platelet count may be a useful clinical marker associated with ALRTI severity (61,64,66,67). Conversely, other authors have found that platelet counts do not correlate with disease severity and clinical outcome (68,69). Routine prophylactic anti-platelet treatment or further investigations are not necessary in children with RSV-positive bronchiolitis and thrombocytosis (61,68,70).

*RSV-positive bronchiolitis and asthma.* There is compelling evidence that infants with severe RSV infection in the early months of life have a subsequent increased risk of developing recurrent wheezing and/or asthma, with a prevalence of up to 30% compared with non-RSV groups (71-75). Whether this association is causal has been the subject of considerable debate on the potential role of RSV infection in the pathogenesis of asthma as well as the impact of asthma predisposition (genetic, environmental exposure, etc.) on the clinical course of RSV infection. A recent large retrospective cohort analysis of Australian children born between 2000 and 2010 suggested that different subgroups of high risk children, who developed RSV disease within the first 2 years of life, continued to be at elevated risk of having a first asthma hospitalization beyond the age of 7 years (76). On the other hand, large epidemiological observational studies demonstrate that the vast majority of infants hospitalized for RSV bronchiolitis do not fit into an 'at-risk' group (atopy, family history, etc.), suggesting that viral or host factors not thought of as classical risk factors, may play a role in disease severity (74,77). Prospective studies with RSV-immunoprophylaxis (e.g., palivizumab) suggest that long-term effects of RSV prophylaxis appear less efficacious in infants with a family history of atopy. In addition, palivizumab decreases parent-reported recurrent wheeze, however the incidence of physician-diagnosed asthma is similar (78). A recent single-blind, randomized, placebo-controlled trial showed that RSV prevention in otherwise healthy preterm infants, did not have a major effect on asthma or lung function at the age of 6 years (79).

Considering the above findings, perhaps a more appropriate conclusion would be that RSV infection is important in the mechanism of wheezing development, at least in the first few years of life (80). RSV possesses the ability to counteract host

defense systems through complex mechanisms that facilitate viral replication. This significant increase in asthma frequency seems to be predominantly related to long-term changes in neuroimmune control of airway tone rather than to allergic sensitization. In contrast to RSV bronchiolitis, atopy has been clearly associated with childhood asthma development after RSV-induced early wheezing (81,82). High-risk (parental atopy or asthma) birth cohort studies from Wisconsin, United States, and Australia have shown that young children suffering from RSV-induced wheezing episodes are at high risk of developing school-age asthma (81,82). Further prospective, follow-up studies are needed to clarify individual and environmental factors that promote more severe viral illnesses and long-term adverse respiratory outcome of children hospitalized for severe RSV infection. Developing a greater understanding of the pathophysiological mechanisms through which RSV causes recurrent wheezing/asthma, will lead to an evidence-based prevention strategy and perhaps reduce the subsequent risk for asthma (71).

*Predicting asthma following RSV-positive bronchiolitis.* The biomarker CCL5 (previously known as RANTES, a  $\beta$ -chemoattractant for inflammatory cells including T-lymphocyte subsets), in the nasal epithelium during RSV bronchiolitis, is strongly predictive of physician-diagnosed asthma (83,87). Furthermore, it has been suggested that prematurely born infants have a predisposition to RSV infection-related respiratory morbidity, including subsequent respiratory dysfunction (44,85). Single-nucleotide polymorphisms in genes coding for IL-8, IL-19, IL-20, IL-13, mannose-binding lectin, IFNG and RANTES, have been associated with wheezing following RSV LRTI in term-born infants (85). The site of infection might be another important factor related to asthma risk, thus viral ALRTI in infancy indicates an increased risk of subsequent asthma, while gastrointestinal infections might be protective (86). Asthma after severe RSV bronchiolitis is positively correlated with maternal asthma, exposure to high levels of dog allergen, aeroallergen sensitization and recurrent wheezing; day care attendance and white race have been associated with decreased asthma risk (87). Several host factors, including respiratory allergy and virus-induced interferon responses, viral virulence factors, individual risk factors (e.g., young age, especially the first 6 months of life, small lung size and genetics), and environmental exposures (e.g., exposure to tobacco smoke, airway microbiome) modify the risk of virus-induced wheezing and promote more severe wheezing illnesses and the risk for progression to asthma (86,88). The anti-RSV mAb palivizumab decreases the risk of severe RSV-induced illness and subsequent recurrent wheeze in prematurely born infants (89). Further understanding of the role of RSV in asthma pathogenesis may help develop vaccines against RSV as a way of asthma prevention.

*Post infectious bronchiolitis obliterans caused by RSV.* Bronchiolitis obliterans (BO) is a chronic and irreversible lung disease leading to the obstruction and/or obliteration of the small airways (90). Most cases of BO in children are post infectious (PIBO) and are mainly associated with adenovirus infections, although other viruses may also be



Figure 3. Chest radiograph of a 14-month-old girl admitted to hospital with RSV-positive ALRTI. There is hyperinflation of the lungs and presence of bronchial wall cuffing (arrows), without evidence of consolidation or effusion. RSV, respiratory syncytial virus; ALRTI, acute lower respiratory tract infection.



Figure 5. Same child; expiratory transverse thin-section CT scan through the same level as Fig. 4 reveals hyperlucent areas of air-trapping bilaterally (arrows). CT, computed tomography.

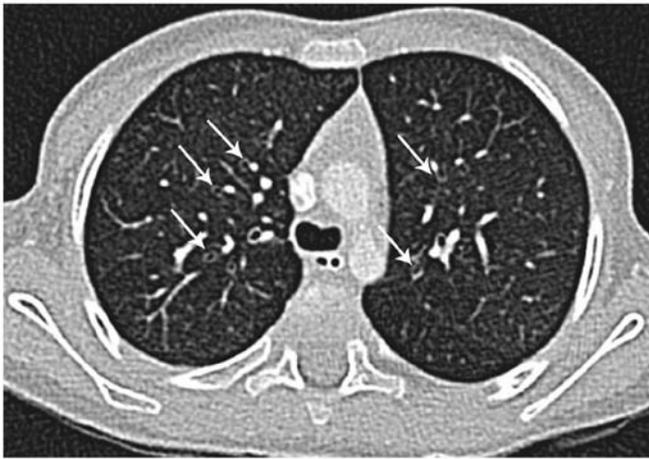


Figure 4. HRCT of the lungs of the same child as Fig. 3 using controlled ventilation technique, performed 4 months later due to recurrent bronchiolitis. Transverse thin-section CT scan through the upper lobes shows bilateral bronchial wall thickening (arrows) without evidence of bronchiectasis. HRCT, high-resolution computed tomography; CT, computed tomography.

implicated, including measles, influenza, parainfluenza and RSV (91-93). An extensive search of the current literature in the context of the workshop demonstrated that RSV is detected in children with PIBO with an incidence ranging from 4.3 to 30% (94-103); however, there are only a few reports of children with PIBO secondary to RSV as a single infection. This creates skepticism about the aetiological role of RSV in PIBO. Further research is required to investigate the potential impact of RSV co-infection in the severity and outcome of children with PIBO.

#### 4. Imaging in children with RSV infection

*Chest radiography and RSV-positive bronchiolitis.* Although imaging cannot confirm the diagnosis of RSV infection, it

is important to identify the possible pattern of viral disease, in order to avoid unnecessary administration of antibiotic therapy and to predict possible late effects (Figs. 3-5) (104). The clinical syndrome of bronchiolitis is commonly diagnosed based on the patient's history and physical examination; chest radiography is not routinely recommended to reach the diagnosis due to recommended restriction of radiation exposure in the paediatric age group (104-106). Chest imaging, however, may be considered when a child with RSV infection and severe ALRTI is admitted to intensive care to better understand the extent of lung involvement and atelectasis, which is common in acute RSV infection (106). It is important to note that chest radiographs in children with RSV infection may be entirely normal or reveal non-specific findings, which are also encountered in other viral infections: most commonly, perihilar opacities and hyperinflation, atelectasis and rarely consolidation and bronchial cuffing or air-leak (107). Radiography is commonly obtained to rule out atelectasis and foreign body aspiration. Guidelines suggest performing a chest radiograph in the presence of significant respiratory distress or hospitalization (108). In newborns with RSV infection, the radiological pattern on chest radiography may be a predictor of clinical outcome (109). However, it is highlighted that chest radiographs should not be routinely performed in children with bronchiolitis to avoid radiation exposure (106). It is also important to emphasize that chest radiograph is not the right way to rule out bacterial infection (106); the correct diagnostic approach for bacterial or ventilator-associated pneumonia in children in the PICU is to perform respiratory culture or Matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry from sputum/aspirate or bronchoalveolar lavage (BAL) specimens.

*Chest CT and RSV-positive bronchiolitis.* Several studies have revealed that standard radiological techniques, including computed tomography (CT), are frequently unable to distinguish between acute bronchiolitis changes caused by RSV vs. those caused by other respiratory viruses (104,108). It is interesting that the radiographic findings, especially in

high-resolution computed tomography (HRCT), reflect the histopathologic changes that RSV infection provokes: plugging or occlusion of the bronchiolar airway lumens by sloughed necrotic and irregular epithelium and exudate, combined with peri-bronchiolar infiltration and reaction with inflammatory cells and submucosal oedema. The infiltration is a combination of neutrophils entering the airway submucosa and epithelial cell debris in the airway lumens. These cellular accumulations are likely to result in acute obstruction of the distal airways, an outcome much more likely to occur in the extremely narrow bronchioles of infants. Because this is combined with the inherent loss of mechanical clearance of these small airways, it likely leads to increased spread of infection, augmented inflammation and clinical signs of wheezing/obstruction (110). Consistent with obstruction, the most common CT findings in RSV pneumonia include centrilobular nodules, ground-glass opacities, air-space consolidation, and peribronchial thickening (111). These findings have a bilateral, usually asymmetric, central and peripheral distribution. Up to 40% of children with bronchiolitis will develop further wheezing episodes in the first five years of life. In very severe or atypical cases, HRCT of the lungs may be required to assess the extent of bronchial thickening and remodeling, the development of bronchiectases and air-trapping (105).

*CNS imaging and RSV infection.* RSV infection is not always restricted to the airways. Case reports have also described clinical pictures resembling viral encephalitis and/or encephalopathic syndromes with severe sequelae in isolated cases (112). The mechanism of the spread of the RSV infection to the CNS compartment remains unclear (112). Brain magnetic resonance imaging (MRI) in infants with CNS involvement has shown predominantly non-specific findings similar to those also encountered in other viral and/or limbic system encephalitides (113). In very rare instances, extra-pulmonary findings in RSV infection have also included acute necrotizing encephalopathy (ANE) and acute hepatic failure with encephalopathy (114). Clinicians should have a high suspicion of ANE in cases of children with a respiratory infection and acute neurological manifestations.

## 5. Therapeutics of RSV infection

*Antivirals against RSV.* Thus far, ribavirin is the only antiviral agent that has ever been licensed for the treatment of RSV infection (115). However, its efficacy is not proven and due to significant toxicity its use has been primarily restricted to severe cases in immunocompromised patients with severe RSV-positive ALRTI (116). Several other antiviral candidates have been developed since, but none have been licensed as yet. Types of molecules being tested include influenza antivirals, such as baloxavir, CC-42344, VIS410, immunoglobulin, hyperimmune plasma, MHAA4549A, pimodivir (JNJ-63623872), umifenovir, and HA minibinders, RSV antivirals including presatovir (GS-5806), ziresovir (AK0529), lumicitabine (ALS-008176), JNJ-53718678, JNJ-64417184, and EDP-938, broad spectrum antivirals such as favipiravir, VH244, remdesivir, and EIDD-1931/EIDD-2801, as well as host directed strategies including nitazoxanide, eritoran, and diltiazem (117-119). Novel molecules disrupt various stages

of the virus life cycle, including cell entry, viral replication, and polymerization as well as after virus release through RSV neutralizing anti- or nanobodies (120).

*The human challenge models.* One method used occasionally in phase 2 clinical testing is human challenge models (115). This method was used in Phase 2a clinical testing of the non-fusion inhibitor EDP-938 (ClinicalTrials.gov Identifier: NCT03691623). All participants were inoculated with a known strain of RSV and were then randomized to receive the medication or placebo. The advantage of this methodology is the removal of the variability in exposure with natural infection and the collection of samples at precise, known times after infection, which can aid with the understanding of the biological mechanisms of the infection and development of antiviral agents or vaccines (121,122). Due to ethical and technical constraints, experimental studies are only undertaken in adults, but if a product is shown to be efficacious at this setting, a faster move to trials in paediatrics takes place than in traditional childhood trials.

*RSV therapeutics and personalized medicine.* The current clinical data indicate that RSV disease dynamics may not be identical in all patients (2,3,115,119). More research is needed to identify uniform clinical endpoints reflecting how patients function, thrive, and survive (US Food and Drug Administration) and to understand inter-individual differences in disease presentation, with the goal of ultimately selecting the right treatment for the right patient. A greater understanding of individual differences may ultimately lead to future personalized treatment strategies. Individualized approaches and a well-standardized methodology to assess disease severity at the time of enrolment, as well as during follow-up visits, will require integration of diagnostic, clinical and laboratory markers at the point of care (115). Individualized targeted treatment will constitute an important step in improving outcomes in patients with RSV infection while minimizing toxicity. A greater understanding of individual data in newly developed pharmaceutical agents against RSV will potentially lead to future personalized treatment regimens. Applying such co-ordinated diagnostic, clinical and research efforts constitutes an important step in advancing paediatric care, improving outcomes and limiting global RSV morbidity and mortality.

*High-flow nasal cannula and RSV-positive bronchiolitis.* Over the last decade, high-flow nasal cannula (HFNC) therapy has emerged as a new method to provide respiratory support in children with RSV-positive bronchiolitis (12,123-126). Its main advantages include its ease to set up and the fact that it is well tolerated, leading to better compliance, especially in comparison to other devices of non-invasive ventilation (125,127). Initially, HFNC was trialed in infants with moderate to severe bronchiolitis admitted to PICUs, but nowadays its application has expanded to paediatric wards, even to emergency departments, in order to avoid a PICU admission (127,128). Recent data have shown that it does not significantly reduce time on oxygen compared with standard therapy, suggesting that early use of HFNC does not modify the underlying disease process (129). However, the proportion of children who experi-

ence treatment failure is lower in HFNC and many of those who experience treatment failure on standard therapy can be rescued by HFNC. Additional studies comparing HFNC with continuous positive airway pressure (CPAP) in the PICU setting led to the same conclusion (130,131). Consequently, HFNC may reduce the need for intubation and invasive respiratory support, thus potentially lowering costs and adverse effects of mechanical ventilation, such as ventilator-induced lung injury, infections and exposure to sedatives. In addition to effectiveness, most studies have shown no adverse events with HFNC and have concluded that it is a relative safe method for use even in general wards or emergency departments (123). Few cases of pneumothorax have been reported, abdominal distension has been less significant compared with CPAP, and the majority of infants have been able to be fed orally or by nasogastric tube (123,127).

*Heliox and RSV-positive bronchiolitis.* Since RSV-positive bronchiolitis is associated with airway obstruction and turbulent gas flow, its clinical course can be improved by Heliox, which facilitates gas flow through high-resistance airways (132-138). Heliox is a mixture of helium-oxygen, which can be administered by all modes of ventilation in spontaneously breathing patients by face mask, HFNC or CPAP, and can be adjusted to specific ventilators in intubated children. Current evidence suggests that the addition of Heliox may significantly reduce clinical scores evaluating respiratory distress and the respiratory rate, and may enhance CO<sub>2</sub> elimination in the first hour after starting treatment in infants with acute refractory RSV bronchiolitis (134,139). Recently, Seliem and Sultan (140) reported that Heliox results in improvement of oxygenation when used with high flow nasal cannula in infants with acute RSV bronchiolitis, during the initial phase of therapy. The combination of Heliox with CPAP also seems to be beneficial, as the application of CPAP may reduce the fiO<sub>2</sub> needed in these infants. However, no benefit has been observed in terms of need for intubation and mechanical ventilation, length of treatment or PICU stay. In addition, its application in the emergency department does not change the discharge rate (138,139). More clinical trials are needed to define the population that may respond to Heliox and its place in the therapeutic regimens of RSV bronchiolitis.

## 6. Prevention of RSV infection

*Passive immunization and Palivizumab.* The prevention of RSV morbidity and mortality remains a global healthcare priority (115,141). According to the World Health Organization (WHO), the strategic focus for the prevention of RSV infection in children and adults includes the passive administration of immunoglobulins, as well as active immunization. Passive immunization is currently the only option available to infants less than 6 months of age, which can be achieved through administration of antibodies to the infant or through active immunization of the mother during pregnancy. Passive immunity wanes fast over time, thus, active immunization is the preferred approach for infants above six months of age, as well as older children and adults, including the elderly (141). To date, there is only one product available for prevention of

RSV infection, palivizumab, the monoclonal antibody (mAb) that has been shown to reduce hospital admission due to RSV infection in some high-risk infants by up to 80% (142). It is expensive and, thus, reserved for high risk infants, mainly in high income countries.

*Active immunization against RSV: looking back to the past.* While antibodies are costly and transitory in their effect, active vaccination would represent the most cost-effective approach for the prevention of RSV infections and their transmission to high-risk individuals (115,141). Up to date, several vaccine candidates are in development, but none have reached licensure yet (143,144). One of the main barriers for the development of RSV vaccines has been the fact that the majority of severe cases in infants occur within the first three months of life, i.e. at a time when active immunization is not really possible (145). Additional caution has been employed during vaccine design because of the failure of a historical vaccine [formalin inactivated RSV (FI-RSV)], which triggered a severe adverse effect, enhanced respiratory disease (ERD) (146). For more than 50 years live-attenuated vaccine approaches have been unsuccessful because of the difficulty in balancing immunogenicity and vaccine safety. It is worth noting that only live-attenuated vaccines have been tested for active infant immunization.

*Active immunization against RSV: perspectives.* Recent breakthroughs in determining the structure and antigenic content of the RSV fusion (F) glycoprotein has enhanced interest in vaccine development research (115,141,147,148). The general approaches to vaccine development include engineered viruses that use knowledge of RSV gene function, naturally attenuated chimeric virus combining genes from RSV-related viruses, viral vectors encoding RSV surface antigens, and nucleic acid vaccines using plasmid DNA or messenger RNA encoding RSV antigens (149,150). As of August 2019, 43 RSV vaccines were in development (151). Of these, 21 are in clinical trials in humans; 14 in Phase 1, five in Phase 2 and two (one just completed) in Phase 3. Twelve vaccines are in trials in children, four in pregnant women and 10 in older adults (some products are undergoing trials in more than one target population). Vaccine types under investigation include live-attenuated/chimeric, particle-based, subunit and recombinant vector vaccines. This highlights the variety and breadth of immunization types and different populations that are being investigated to find an answer to the 60-year-old problem of producing a safe and effective RSV prophylactic agent.

*The ResVax.* The most advanced candidate vaccine, ResVax, is an RSV fusion protein recombinant nanoparticle with aluminum phosphate as an adjuvant (115,152,153). The new approach to develop this vaccine is based on engineering small particles that carry altered RSV proteins. The nanoparticles sensitize the immune system to the virus so that when a person comes in contact with it the immune system delivers a robust response. The Phase 3 clinical trial included more than 4,600 pregnant women examining the efficacy of prevention of RSV disease in infants through maternal immunization. Although the trial narrowly missed its primary end point

of a reduction in medically attended RSV-positive ALRTI, it showed a 44% vaccine efficacy against RSV hospitalization, 25% efficacy against all respiratory hospitalizations and 39% efficacy against all-cause severe hypoxaemia (152). A possible route to licensure is currently being sought with the US Food and Drug Administration (FDA) and European licensing agencies, bringing hope of a vaccine that could save the lives of countless young infants worldwide.

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### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

### Competing interests

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