

*EUROPEAN RESPIRATORY SOCIETY MONOGRAPH –
SARS, MERS and other Viral Lung Infections*

CHAPTER 5

Respiratory Syncytial Virus

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Cite as: Jha, A., Jarvis, H., Fraser, C. & Openshaw, P. J. Respiratory Syncytial Virus. *Eur. Respir. Soc. Monogr.* 72, 84–109 (2016); doi:10.1183/2312508X.10010315

SUMMARY

Respiratory syncytial virus (RSV) infection has an estimated global incidence of 33 million cases in children younger than 5 years, with 10% requiring hospital admission and up to 199,000 dying of the disease. There is growing evidence that severe infantile RSV bronchiolitis, a condition characterised by an inflammatory reaction to the virus, is associated with later childhood wheeze in some vulnerable children; however, a direct causal relationship with asthma has not yet been established. It is also increasingly recognised as a cause of morbidity and mortality in those with underlying airway disease, immunocompromise and frail elderly persons. Novel molecular based diagnostic tools are becoming established but treatment largely remains supportive, with palivizumab being the only licensed agent currently available for passive prophylaxis of selected pre-term infants. Whilst effective treatments remain elusive, there is optimism about the testing of novel antiviral drugs and the development of vaccines that may induce long-lasting immunity without the risk of disease augmentation.

Keywords: viral lung disease; RSV; paediatric infections; bronchiolitis; mucosal immunity

Funding: EU FP7 PREPARE grant 602525; Wellcome Programme: Immune regulation in viral lung disease, 16 November 2009–15 November 2015 (P25092: 087805/Z/08/Z) National Institute of Healthcare Research (NIHR) Imperial College Healthcare Trust Biomedical Research Centre (BRC) grant P45058; NIHR Senior Investigator Award; and NIHR Health Protection Research Unit (NIHR HPRU) in Respiratory Infections at Imperial College London in partnership with Public Health England. The views expressed are those of the author(s) and are not necessarily those of the National Health Service, the NIHR, the Department of Health or Public Health England.

Disclosures: P.J.M.O reports grants from the Wellcome Trust Translational Award, outside the submitted work.

INTRODUCTION

Since its discovery in 1956, respiratory syncytial virus (RSV) has become recognized as a leading global cause of morbidity and mortality, especially amongst infants in the first six months life. RSV is the commonest cause of childhood acute respiratory infection (ARI) and the major single cause of hospitalization during infancy. In recent decades, it has been realized that it also afflicts various at-risk adults, including frail elderly and immunocompromised persons. In resource-poor settings, it is an important cause of death due to lower respiratory tract infection, second only to pneumococcal pneumonia and *H. influenzae* type B. Resource-limited countries have more than twice the incidence severe disease seen in developed countries and, remarkably, 99% of the global deaths caused by RSV infection (1).

Given this appalling disease toll and the fact that current treatment is simply supportive, it is clear that prevention, early diagnosis and the discovery of specific therapies has enormous potential to improve global health amongst the most vulnerable in society. RSV remains one of the last major viruses for which we currently (in early 2016) have no safe and effective vaccine.

Not only does RSV cause acute disease, but those with severe infections can apparently be left with long term sequelae. These include persistent wheeze and in some cases established inflammatory airway disease (2,3), highlighting the importance of understanding which immune responses correlate with protection and what makes some individuals at risk of severe or delayed disease. Molecular techniques such as real time PCR (qPCR) have now become more affordable and offer rapid and accurate diagnostics, with the ability to identify the disease earlier in at-risk patients, at levels of viral burden too low for conventional detection and affording the potential for community-based epidemiology (4,5). Intensive research in recent decades has accelerated progress on many fronts, with several promising new vaccines in advanced stages of development (6,7). In countries with limited access to healthcare, an effective and affordable vaccine would be transformative.

EPIDEMIOLOGY

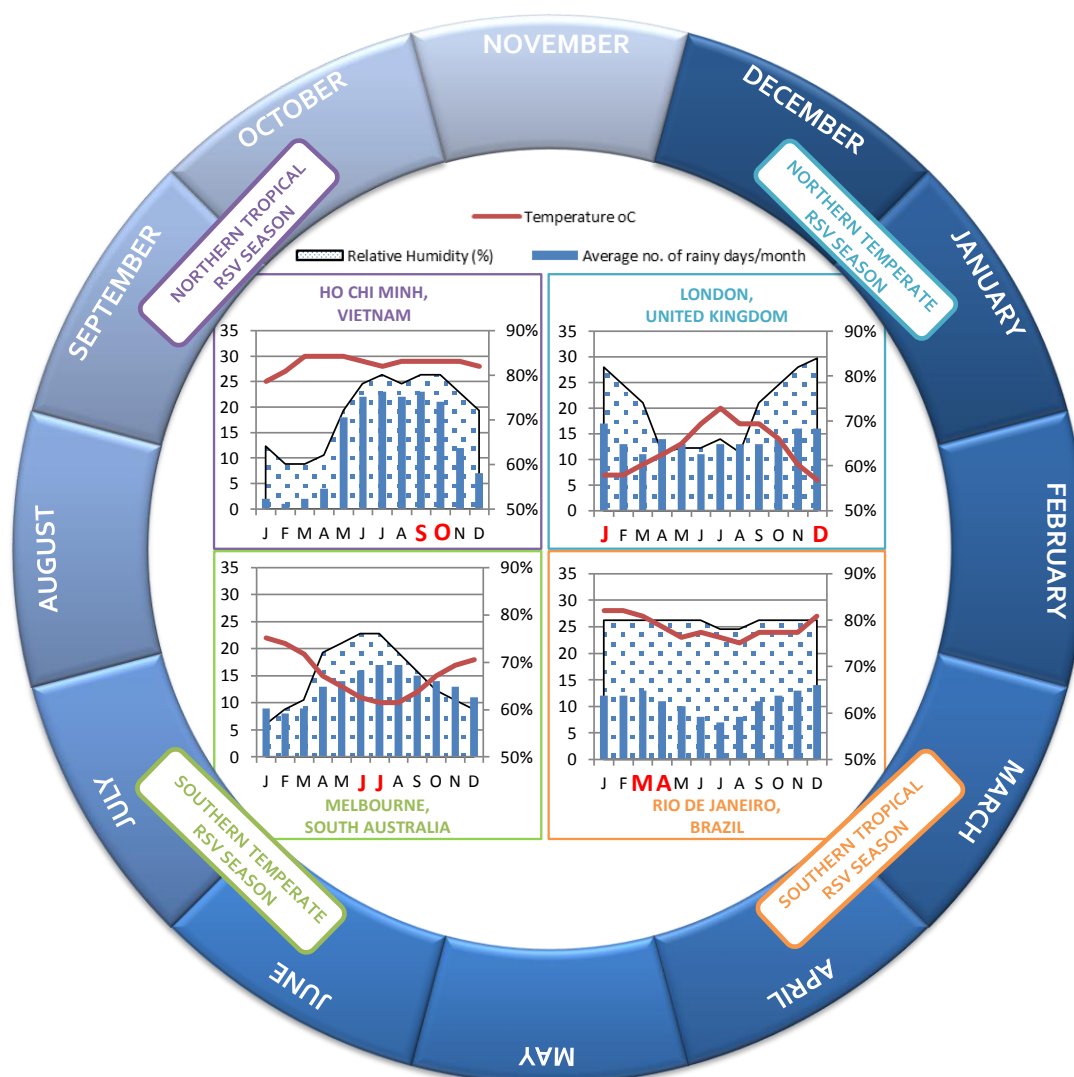
RSV causes significant morbidity and mortality across the globe and is the leading cause of pneumonia and bronchiolitis in infants. In addition to a currently poorly quantified burden in the elderly, it is estimated to cause 30 million acute respiratory infections and more than 60,000 childhood deaths worldwide each year (1,8). The main burden of disease is in the under 5 age group but RSV has considerable impact on various at risk adult populations (9,10). In the USA, RSV is estimated to cause 17,358 deaths per year, 22 % of which are in persons less than 65 years of age (11).

Seasonality

Seasonality of RSV infection varies across the globe. Temperate zones tend to experience RSV epidemics during late autumn, winter and/or spring (12) whereas tropical and arctic climates see less well defined annual variation with year-round disease described in some settings (13). In northern tropical areas the seasonality is associated with a decrease in temperature and increase in rainfall, whereas in more tropical climates yearly outbreaks can occur during the warm and rainy seasons (see figure 1) (14,15).

In Europe, RSV is predominantly a disease of winter with peaks of illness in December and January in the United Kingdom, Belgium, Germany and the Netherlands with a later peak of March in Mediterranean countries such as Greece (16,17); biennial cycles of RSV activity have been described in northern Europe (15). The association of an increase in RSV cases with declining temperatures has been attributed to increased indoor crowding leading to enhanced viral transmission, to lower temperatures increasing viral stability and to chilling increasing host susceptibility or activation of dormant virus (18).

Figure 1. Annual cycle of global peak RSV incidence and associated climactic factors. Temperate countries typically have peak incidence of RSV during their winter months, which are associated with lower temperatures, higher rainfall and higher humidity. There is greater variability in tropical regions, which are prone to biennial and perennial RSV. Limited information is available for some regions, e.g. Africa. Data from [12–15].



Infants and Children

Pneumonia is the leading cause of mortality of children worldwide with RSV being the commonest single cause of acute respiratory infection (ARI) in young children (8,19). By 18 months of age, 87% of children have antibodies to RSV and by the age of 3 years, virtually all children have been infected (20).

A large meta-analysis estimated there to be 33.8 million cases of RSV associated ARI worldwide in 2005 in children less than 5 years of age but this is likely to be an underestimate; previous studies using active methods for data collection, such as home visits, have shown much higher incidence rates (1). In the USA, RSV has been estimated to account for 20% of acute respiratory infection hospitalisations in children under 5 (17 per 1000 children under 6 months and 3 per 1000 under 5)

but 99% of RSV deaths occur in low-income countries with a lack of access to basic, supportive care being a key factor (1,8). Children are at highest risk of death in the first 6 months of life (11).

The majority of children suffering from RSV ARI have no underlying comorbidities. Low birth weight and prematurity are major risk factors for hospitalisation, as is congenital or acquired immunodeficiency and various cardiopulmonary, chronic lung disease and neurodevelopmental disorders (21,22). Environmental factors contributing to increased susceptibility are side-stream tobacco smoke exposure, lack of breastfeeding and low socioeconomic status (23).

Adults and Elder Persons

There remains no globally accepted definition or reliable classification of acute respiratory infection, making it difficult to compare studies and to make accurate estimates of disease burden. Adding to these problems, variations in sampling methodology and diagnostic testing across studies provides further hurdles. With major differences in healthcare provision and access to resources in different geographical and socioeconomic settings, it is difficult to estimate the true impact of RSV disease in diverse settings. Despite these caveats, we can state with reasonable certainty that it is rare for healthy immunocompetent adults to require hospitalisation due to RSV infection.

However, infection with RSV is so prevalent that it is estimated to cause a disease burden at least comparable to influenza in elderly persons (24). Given the aging population structure in Western countries, adult RSV-associated mortality is becoming an ever greater problem that poses a huge health and economic burden (9,11). A recent north-American study examined the impact of RSV during four consecutive winters amongst healthy elderly patients (n=608), high-risk adults (those with chronic heart or lung disease, n= 540) and patients hospitalised with acute respiratory illness (n=1388). The average age across all groups was more than 70 years; RSV infection developed annually in 3-7% of healthy elderly patients, 4-10% of high-risk adults and was present in 8-13% of patients hospitalised with acute respiratory infection. During the study period, RSV infection accounted for 11 % of hospitalizations for pneumonia, 11 % for chronic obstructive pulmonary disease (COPD), 5 % for congestive heart failure, and 7 % for asthma (10). In the UK, it is estimated that amongst the adult population, those over the age of 65 account for 36 % of GP episodes, 79% of hospitalisations and 93% of deaths due to RSV each season and RSV was responsible for more morbidity than influenza (24). Globally, RSV accounts for 7.4% of elderly (>65 years) individuals presenting with influenza-like-illness (25).

The Immunocompromised

In both children and adults, various immunodeficiency states predispose to RSV infection and disease. In addition to HIV-infected children being liable to develop RSV-ARI, they are 3.5 times more likely to be hospitalized than non HIV infected children (21), are more likely to present with pneumonia, have greater evidence of

bacterial co-infection and higher mortality rates than HIV-uninfected children (26). RSV is a common cause of ARI in haematopoietic stem cell transplant (HSCT) patients, with rates of infection being as high as 2-17% in some settings. As well as increased susceptibility to infection, these patients are also liable to progress to more severe disease. RSV pneumonia in this patient group has a mortality of up to 83% (27). Factors linked to severity and poor outcome include male sex, the type of graft (allogeneic), myeloablative regimen, cytomegalovirus seropositivity, increasing age, unrelated donor transplant, graft-versus-host disease (GVHD) and becoming infected within the first 3 months of transplant (28).

VIROLOGY

RSV is an enveloped virus that belongs to the genus Pneumovirus, family Paramyxoviridae. It is a negative sense, single stranded RNA virus consisting of 11 proteins encoded by a 15.2-kb RSV genome. It has a non-segmented genome so, unlike influenza, it does not have the capacity for re-assortment of genome segments and thus to undergo antigenic shifts that have the potential to cause large pandemics.

RSV was originally characterized in 1956 from a colony of chimpanzees with coryza and termed chimpanzee coryza agent (29). It was later isolated from a number of infants and recognized as being a human virus that caused mild illness in a susceptible group of chimpanzees (30). It was re-termed respiratory syncytial virus, as it causes cells to fuse with neighboring cells, creating large multinucleated syncytia.

RSV has been classified into two distinct subgroups (A and B), based on antigenic variability (31). These two groups have been described based on the reactions of two major surface proteins to monoclonal antibodies; the G protein facilitates viral attachment to host ciliated cells of the airway (32) while F mediates virion and host cell fusion (33).

Additional antigenic variability occurs within the A and B subgroups with the most extensive antigenic and genetic diversity being found in the attachment glycoprotein G, therefore playing a significant role in RSV pathogenicity and immune evasion. RSV subtype A viruses may be marginally more virulent and may replicate to higher titers than the group B viruses *in vivo* and *in vitro* (34). New RSV genotypes appear periodically and tend

to predominate and replace the circulating strain of RSV. However the original circulating strain does not become extinct, with new and old genotypes occurring alongside each other for relatively long periods (35).

Viral Structure and Proteins

RSV particles are pleomorphic with both spherical and filamentous particles of different sizes (36). The virions display three surface proteins: F, G and SH (small hydrophobic) (figure 2) (37). Proteins G and F are integral for virion attachment and fusion; binding to disaccharide subunits called GAGs and RhoA respectively (38,39). Following fusion the virion releases the nucleocapsid into the cytosol, enabling the RNA to enter the host cell. The M2 mRNA has two overlapping ORFs encoding M2-1

and M2-2. The M2-2 gene governs the transition from transcription to production of genomic RNA (40). The large (L) protein is a viral RNA-dependent RNA polymerase that contains multiple enzyme functions required for RSV replication, it enters the genome and mRNA is transcribed. Replication generates a complete positive-sense RNA complement of the genome – the antigenome that acts as a template for replication. Throughout replication the N protein encapsulates the RNA, which prevents degradation. M protein co-ordinates the assembly of the envelope proteins with the nucleocapsid proteins (N, P, and M2-1) and facilitates budding of new immature virions using the host cell membrane. Filamentous virions show a helical assembly of M (matrix) proteins that play a critical role in formation of infectious filamentous particles (41).

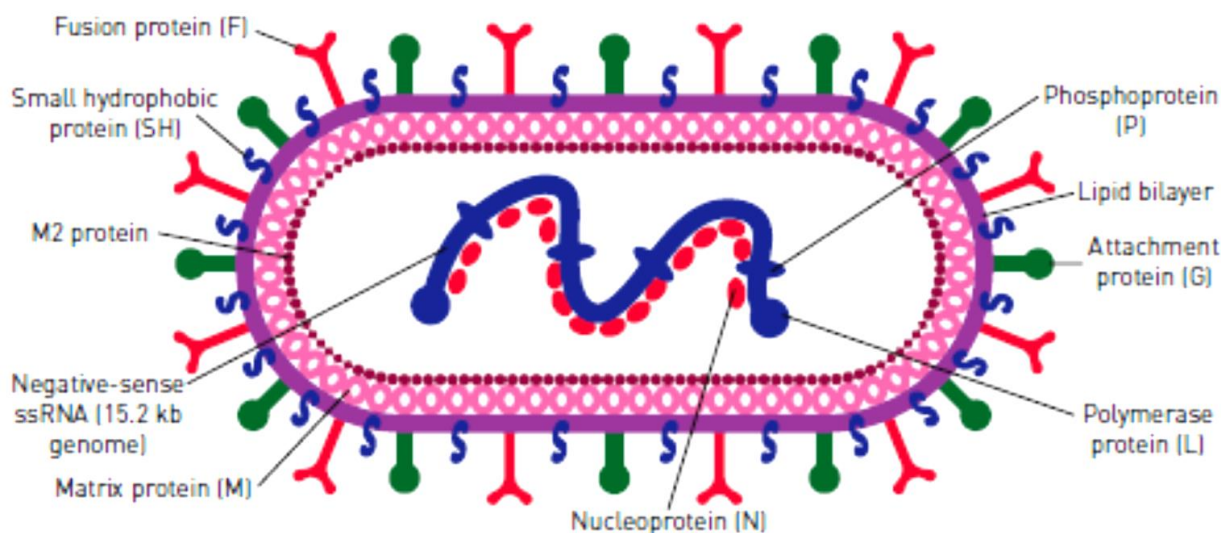


Figure 2. Diagrammatic cross-section through an infectious RSV filament. ss: single-stranded.

PATHOGENESIS

RSV is a highly contagious virus and can cause outbreaks both in the community and hospital setting (42,43). Infection begins via inoculation of the nose or eyes *via* large particle or direct contact, with an incubation period of 2-8 days. RSV can survive for prolonged periods on skin, cloth, and other objects thereby facilitating its spread (44). How it survives so well is puzzling, since lab-grown virus is notoriously unstable at room temperature (45).

RSV infects the ciliated epithelial cells of the upper and lower respiratory tract. In the lower airways the virus specifically targets the ciliated cell of the

bronchioles, but can also be found in type 1 pneumocytes and intraepithelial dendritic cells. More recently it has been demonstrated that RSV can infect basal cells (the progenitors for the conducting airway epithelium) where it has the potential to influence the morphogenesis of the airway epithelium(46).

Infection causes widespread inflammation with peribronchial monocyte and T-cell infiltration, epithelial necrosis, sub mucosal edema and mucus-overproduction (47). Infection and tissue damage tends to be patchy rather than diffuse. Airway obstruction occurs in the small airways secondary to sloughing of epithelial cells, mucus plugs and accumulated luminal and intraluminal immune cells. Additionally, syncytia are occasionally observed, more commonly in immunocompromised patients (48). The degree to which this depends on viral or host variation is currently poorly understood. Possibly, the development of severe disease results from an

unfortunate collision of a particular viral strain with a host that responds adversely to that specific strain.

Environmental Factors

Environmental factors include exposure to tobacco smoke, air pollution and indoor crowding (day care attendance, nursing home or hospital stay); the presence of an older sibling is an independent risk factor for the development of RSV disease in infancy (16). There have been many studies that have documented the link between tobacco smoke and RSV infection. A 2012 systematic review of 30 studies reported that tobacco smoke exposure places infants and young children at increased risk of hospitalization for RSV-attributable lower respiratory tract infection, in addition to increasing the severity of illness (49). The association between air pollution and severe RSV disease is also well documented. It has been shown in vivo that diesel engine emissions cause a more pronounced peribronchial/bronchiolar inflammation with increased mucous cell metaplasia in the context of RSV infection (50).

Viral Factors

There is a well-documented correlation between the level of virus replication and severity of infection (51,52). Unlike other viruses, immunity to RSV infection is incomplete and short-lived, and reinfection is common throughout life. This is not largely attributable to viral evolution in response to host immunity; instead, RSV seems to be able to modulate and evade the immune system. It does this in several ways. The G protein is key to this; being present in membrane-bound and secreted forms. Secretory protein G acts as a decoy, aiding with virus evasion of antibody-mediated neutralization(53). It also exhibits a CX3C fractalkine like motif which binds to chemokine receptor CX3CR1 inducing leucocyte chemotaxis (54). Additionally the cysteine rich portion of G protein is directly able to inhibit signaling from toll like receptors (2, 4, and 9) all of which reduce the host immune response.

Furthermore two nonstructural (NS) proteins NS1 and NS2 which are highly expressed during early RSV infection, down-regulate interferon production and apoptosis leading to enhanced viral replication (55). The small hydrophobic (SH) transmembrane protein increases membrane permeability and to a lesser extent has also been linked to decreased apoptosis(56). Within the virion, RNA is tightly bound by N protein and this is thought to prevent degradation and also aid with

concealment from pattern recognition receptors, promoting infectivity in host cells.

Host factors

Multiple factors can increase susceptibility to RSV infection and are associated with a more severe disease course. These include premature birth, comorbidities (cardiac and respiratory), immunodeficiency and age. There is increasing evidence that there may be some genetic associations of RSV susceptibility and severity. For example, it has been shown that identical twins are often concordant for severe RSV disease. The host genetic contribution for this propensity is estimated to account for approximately 20% of the variance (57).

The genetic polymorphisms that affect RSV pathogenesis often lie in genes involved in innate defences, surfactant proteins, host cell receptors, neutrophil responses, Th1/Th2 responses and other components of adaptive immunity (58). Ongoing large genome-wide approaches are likely to further improve our knowledge in this area.

IMMUNE RESPONSE

As described previously, virtually all children have been infected by the age of 2; most immunocompetent children clear the infection within 3 weeks (59). Some infants are predisposed to more severe infection and those infected in the first 6 months are subsequently prone to wheeze until at least 6 years of age (3), and possibly through to adulthood (2).

Early Immune Responses

The recognition of pathogen-associated molecular patterns (PAMPs) present on viruses by pattern recognition receptors (PRRS) such as Toll-like receptors (TLRs) and RNA-sensing RIG-I-like receptors (RLRs) leads to the release of antimicrobial mediators by the host mucosal immune system and serves to form an effective first line of resistance against infection. Type 1 responses are characterized by release of type I IFNs (IFN- α and IFN- β) and Type III IFNs (IFN- λ 1, IFN- λ 2, IFN- λ 3(60)). These are potent antiviral mediators induced early in infection from epithelial cells and resident immune cells with alveolar macrophages recently being discovered in a murine model to be major contributors to IFN production leading to the subsequent recruitment of inflammatory monocytes to the lungs (61).

Dendritic cells are also key to bridging innate and adaptive responses by transporting viral antigens to cervical and pulmonary lymph nodes and presenting them to CD4⁺ T-cells, which in turn activates CD8⁺ T-cells and B-lymphocytes. They return to the site of infection to further promote local induction of pro-inflammatory mediators and cellular efflux (62).

There is debate about whether defective or exuberant immune responses are responsible for the infection spreading from a limited mucosal infection (in most people) to more extensive lung immunopathology or even extrapulmonary manifestations (in susceptible individuals) (63–65). An insufficient type 1 response as well as exaggerated type 2 responses (characterised by IL-4, IL-5 and IL-13) have been implicated in causing susceptibility to RSV bronchiolitis (66,67) and an inability to produce sufficient IFN- γ early in life may increase risk of developing severe disease (68). Gene polymorphisms of TLR4, IL-4 receptor α and IL-8 have also been associated with severe RSV bronchiolitis, highlighting the important role of innate immunity, although their biological role in active disease is yet to be fully understood (69–71).

The naive immune system of infants is characterized by hyporesponsiveness to infections and viral mimetic stimuli *in vitro* when compared to adults (64,72) with premature infants of less than 35 weeks gestational age being at the highest risk due to compromised pulmonary development and deficient serum immunoglobulins (73). In term infants, maternal specific RSV-neutralizing antibodies are present from the third trimester and are stable until birth, with higher cord blood levels being protective against RSV infection although only protective for the first 6 months of life (74).

Established Infection, Reinfection and Resolution

Once infection is established, cellular immunity and immunoregulation plays a critical role in promoting viral clearance and limiting pathology, which is clearly demonstrated in paediatric patients with primary or acquired immunodeficiency who have a much longer duration of infection and can shed virus for several months (75,76). Classically, helper T cells that make interferon gamma (Th1 Cells) are important in antiviral defense, but it is possible that other helper T cell subsets contribute towards eliminating the virus (77). In particular, IL-10 producing cells inhibit disease and inflammation in

mice infected with RSV, especially during recovery (78). Cytotoxic CD8⁺ lymphocytes (CTLs) may cause neutrophil efflux (79,80) whilst more abundant airway resident memory T cells (Trm) at baseline correlate with reduced viral load and symptoms during experimental human adult infection (81). Interestingly, virus and T cell inflammation may remain present in asymptomatic convalescent volunteers in this model (81). Certain macrophage subtypes (M2) and pDCs likely play an important role in modulating local immune responses and restricting T cell-mediated inflammation (82,83). The ability of RSV to re-infect may also be explained by defective RSV-specific IgA memory B cell responses (84,85). Some individuals suffer dual infection with RSV and other viruses, which can lead to greater severity of disease and is associated with decreased IFN- γ responses (86).

It is thought that the increased susceptibility and severity of RSV in the elderly is secondary to immunosenescence, but how this occurs has not been fully defined. It has been shown that elderly and younger individuals have comparable RSV neutralization antibody titres, but there may be an impairment of T cell mediated immunity (87,88). Studies of RSV disease in mice indicate the importance of regulatory T cells (Treg) in maintaining an effective and non-pathogenic response to RSV infection, and have highlighted the dysregulation of immune responses that are characteristic of bronchiolitis (89).

CLINICAL MANIFESTATIONS

Acute Presentation in Children

RSV infection in children can result in a range of disease phenotypes from mild upper respiratory tract symptoms to life-threatening lower airway involvement necessitating hospital admission and mechanical ventilation. Infants are at greater risk from RSV infection than older children with an increased likelihood of lower airway involvement in the form of both bronchiolitis and pneumonia (1).

Clinical presentation of RSV often begins with typical upper respiratory tract signs and symptoms including coryza, cough and a febrile illness (90). On examination, a rhinitis and pharyngitis may be seen in association with conjunctival signs and erythema to the tympanic membrane (64). Less frequently bulging of the tympanic membrane may be seen if otitis media is also present. Nasal

congestion and generalised malaise can lead to poor feeding and subsequently dehydration (64).

In approximately one third of infants with viral respiratory infections, progression to lower respiratory tract involvement is observed (64), most commonly in the form of bronchiolitis although pneumonia and laryngotracheitis (croup) can also develop (91). Bronchiolitis presents as worsening respiratory function with tachypnoea, wheeze and noisy breathing, as well as systemic viral features such as fever and lethargy (90,91). Some infants develop vomiting and poor feeding with dehydration (64). In young infants apnoea has also been described (92) and indeed may be the presenting symptom early in the disease. Signs of respiratory distress including nasal flaring, costal and intercostal recession and cyanosis, should be assessed for by clinicians in determining the need for admission to hospital in addition to other markers of disease severity including hydration status and level of alertness. Polyphonic expiratory wheeze and fine crepitations made be heard on auscultation of the chest (91). According to current

guidelines, chest radiographs are not routinely performed in children with simple bronchiolitis but may show features of hyper-expansion including increased anterior-posterior diameter and diaphragmatic flattening as well as patchy bilateral infiltrates and atelectasis (figure 3) (90,93). Pneumonia with consolidation seen on chest x-ray can be due to the virus itself or a secondary bacterial infection. Extra-pulmonary manifestations of RSV are uncommon but have been described and include seizures, hyponatraemia, cardiac arrhythmia and failure and hepatitis (65).

The differential diagnosis for children presenting with signs and symptoms of lower and upper respiratory infection includes other viral infections such as rhinovirus, metapneumovirus and influenza, primary bacterial pneumonia, congenital conditions such as cystic fibrosis, asthma and inhaled foreign bodies.

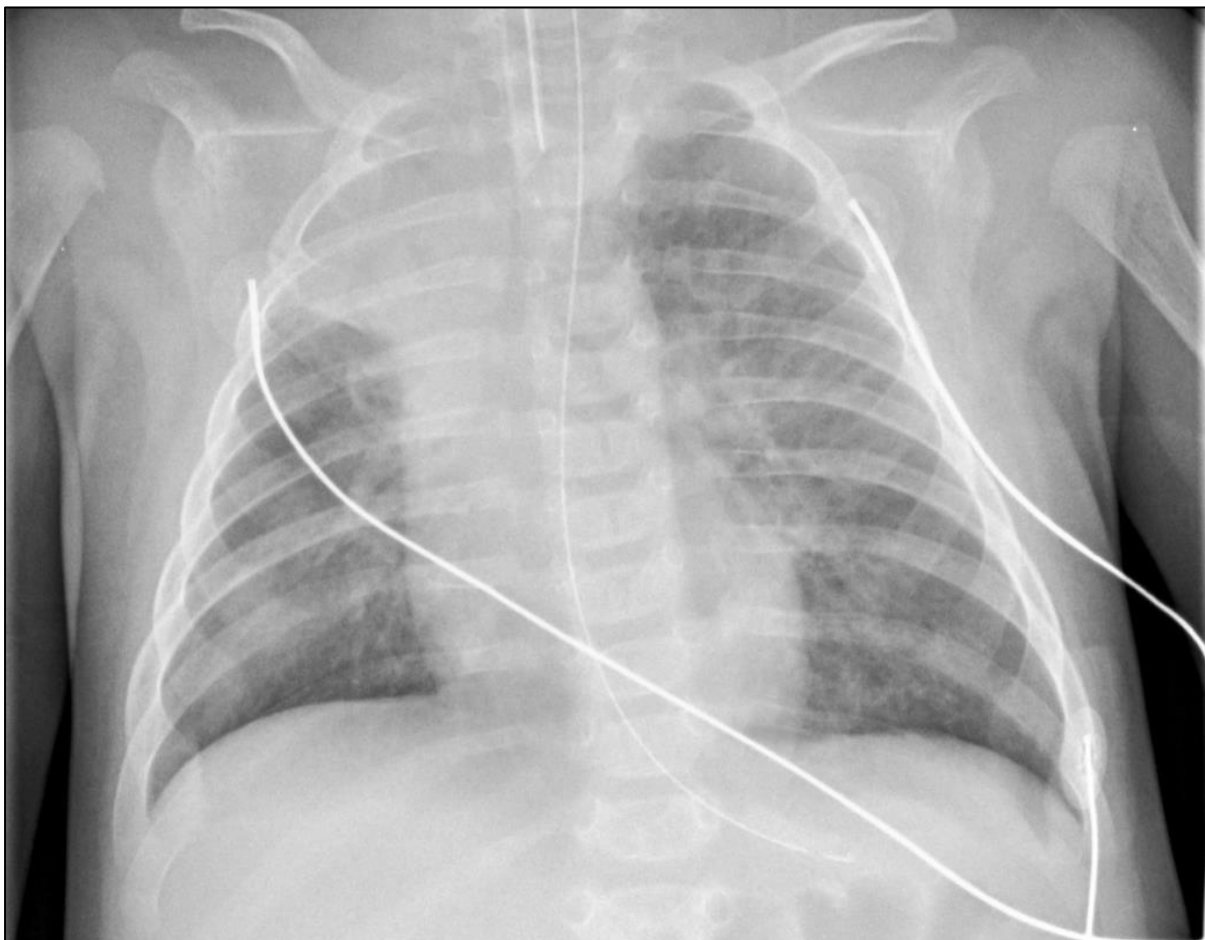


Figure 3. Chest radiograph of a 3-month-old infant with severe RSV bronchiolitis: demonstrating a collapsed right upper lobe, hyperinflated left lung and bilateral perihilar, peribronchovascular thickening. The patient is intubated and has a nasogastric tube in situ.

Longer Term Complications in Children

In addition to the significant health burden of acute RSV infection, there is evidence of longer term consequences to respiratory function with the development of persistent airway disease. Although a causal relationship remains unclear, an association between RSV infection as an infant and subsequent wheezing, allergy and asthma, sometimes persisting into adulthood, has been demonstrated (2,90,94).

Several longitudinal cohort studies have tracked the incidence of respiratory illness in children with RSV infection in infancy. The Tucson Children's Respiratory Study examined outcomes in children with clinician confirmed RSV lower respiratory tract infection (as defined by the presence of cough, wheeze, breathlessness, hoarseness or stridor, in the presence of an RSV positive diagnostic test) contracted up to the age of three years. They reported an increased prevalence of wheeze in these children up to the age of 11 and persistently impaired lung function until age 13 (95). Sigurs and colleagues investigated a more severely affected group of children who had been hospitalized with RSV lower respiratory tract infection in the first year of life. Their findings correlated with those seen in the less severely affected children; with an increased prevalence of asthma/recurrent wheeze as well as atopy and clinical allergy which persisted throughout childhood (2). Indeed the data collected from these participants at age 18 continued to show differences between healthy controls and affected individuals both in the relative rates of allergy, asthma and recurrent wheeze as well as abnormalities in lung function. They postulated that persistent clinical and lung function abnormalities represent the consequences of airway remodelling stimulated by early RSV infection. The potential pathogenic mechanisms for this remain unclear although impaired T-regulatory function, persistent activation of innate immunity and an early Th2 helper switch have been implicated (96).

Debate remains as to the extent which early RSV infection is causal of later airway disease, by altering immune responses and subsequent airway maturation, or whether children with a predisposition to wheeze and asthma are more likely to develop severe RSV infection. To address this question, a double-blind placebo controlled trial was designed to investigate if prevention of RSV, using the monoclonal antibody palivizumab during RSV season, would impact on subsequent

rates of wheeze. The results demonstrated a substantial reduction in episodes wheeze for up to one year in healthy preterm babies in the treatment group compared to those in the placebo group (97). Of note, the effect of palivizumab on wheeze reduction persisted even after treatment had been stopped and RSV season was complete, suggesting that wheeze reduction was not simply attributable to fewer acute RSV episodes. The authors thus argue their findings support the concept of RSV as a pathogenic stimulus in the development of recurrent wheeze. They postulate that epithelial damage from RSV infection alters local pulmonary immune responses resulting in airway hyper-responsiveness. However, the testing of a high-potency derivative of palivizumab (motavizumab) showed no such effect in term babies, despite a substantial reduction in hospital admissions and non-inferiority compared to palivizumab (98,99). The reasons for the disparity between these two monoclonal drugs are unclear and may simply be evidence against a direct causal relationship between RSV and subsequent wheeze. Another possible explanation lies in the differences between the study populations; with the palivizumab study investigating outcomes in preterm babies and based on parent-reported episodes of wheeze whereas the motavizumab study examined healthy term infants and relied on medically attended wheezing episodes (99).

Presentation in Adults

For the majority of adults infected with RSV the extent of symptoms is minimal, with non-specific features of a viral upper respiratory tract infection including coryza, sore throat, fever and malaise as well as lower respiratory tract symptoms such as cough (100). Those with underlying immune compromise, cardiorespiratory disease or the elderly can experience significantly more morbidity and mortality (9,10). The presentation of RSV in elderly patients is similar to that seen in younger adults, although with a greater severity and increased likelihood of lower respiratory tract involvement (100). Typically, an RSV illness begins with nasal congestion before progressing to cough in 90-97% (9,101). Compared to influenza, systemic symptoms including fever, myalgia and gastrointestinal complaints are less common (102). In contrast to other respiratory tract viral infections, wheeze is frequently noted, even in those without underlying airway disease. Audible crackles on chest examination and infiltrates on chest radiograph occur in 30-50% of elderly patients (9).

In addition to these common respiratory symptoms, immunocompromised patients are predisposed to otalgia and clinical or radiological evidence of sinusitis in these individuals can be useful in distinguishing RSV from other viral causes of infection including cytomegalovirus (CMV) (100,103). Chest radiographs in these patients are frequently abnormal, ranging from interstitial infiltrates to alveolar shadowing and pleural effusions. The severity of infection is closely linked to the degree of immune compromise with bone marrow transplant recipients being at greatest risk. Such patients have pneumonia rates of up to 79% and mortality of 78% post-transplant following infection with RSV (104).

RSV can also cause a deterioration of underlying cardiorespiratory disease. Estimating the percentage of acute exacerbations of COPD attributable to RSV infection is difficult as the appropriate viral diagnostic tests are not routinely employed in clinical practice, but estimates range up to 22% (105). Whilst asthma exacerbations are primarily triggered by rhinovirus, RSV and influenza infection are important contributors to morbidity (106,107). In patients with lung transplants, infection with RSV can result in an acute pneumonitis presenting with increasing breathlessness, cough, wheeze and a fall in lung function (108).

As with children, longer term complications can be seen in adults following recovery from acute RSV infection. In patients with COPD, a permanent decline in lung function following the clinical recovery from an exacerbation is well documented (109). Likewise, in lung transplant recipients RSV infection has been implicated in the development of chronic graft dysfunction characterised by obliterative bronchiolitis (110) with small airway remodelling and air trapping. Evidence for the persistence of RSV infection with the development of a latent state, has been reported in a variety of animal models and observational human studies (111). Although not all studies have supported this hypothesis (112), the potential impact of persistent infection not only as a reservoir for ongoing viral transmission, but also in perpetuating pulmonary immunopathology could be significant.

LABORATORY DIAGNOSIS

The American Academy of Pediatrics (AAP) do not recommend routine use of laboratory techniques

to diagnose RSV bronchiolitis, which should primarily be based on clinical assessment (113). However, due to the many acute viral and bacterial respiratory infections that cause symptoms similar to RSV, an accurate laboratory diagnosis is important and will in the future permit the delivery of specific therapy. Broad-spectrum intravenous antibiotics should be avoided in patients with RSV bronchiolitis, since serious bacterial co-infections are uncommon (114). In addition, accurate diagnosis helps in institution of appropriate infection control measures and in facilitation of discharge planning (115). RSV detection can be performed using culture and non-culture methods, the latter consisting of either antigen or nucleic acid detection.

Viral Culture

The use of cell lines to propagate virus is a long-established technique to diagnose RSV infection. Traditional tube culture is efficient and comprehensive, allows for strain typing and genetic characterization as well as antiviral susceptibility testing (116)(117). The 3-7 day turnaround time however is more suitable for research studies or the investigation of complex cases and during outbreaks.

The clinical sample can also be centrifuged on to a single layer of cells using the shell vial culture method or by the use of a cluster tray of vials, which can reduce processing time to 1-2 days making it more useful for patient management. Due to the labor-intensive nature and technical expertise required for the technique, it is largely restricted to reference laboratories and large teaching hospitals [6, 7]. The labile nature of RSV also means it can lose viral infectivity rapidly unless there is proper storage and rapid transport of specimens for laboratory testing.

Antigen Detection

This technique relies on identifying RSV antigenic fragments by viewing fluorescently labelled proteins under a microscope (direct immunofluorescence assay - DFA) or using a commercially available rapid antigen detection test (RADT)(119). DFA has the advantage of ensuring a higher degree of accuracy in identifying the organism and can be performed in less than an hour but requires expertise in reviewing specimens (117). It has a reported sensitivity and specificity of up to 94% and 96% respectively when compared to real-time PCR (120).

Rapid antigen detection tests (RADT) include enzyme immunoassays (EIA) and chromatographic immunoassays (CIA) and have broad appeal due to their ease of use and applicability as a point of care test, with turnaround times as little as 10 minutes, allowing for early delivery of appropriate therapy (119). A Cochrane review of RCTs, which included 759 children presenting to emergency departments with febrile illness and respiratory symptoms concluded however that whilst the use of rapid viral testing reduced radiological investigations it did not have any impact on length of their visit to the emergency department (121). Overall, antigen detection tests have a range of 80-90% sensitivity in comparison with culture but are prone to higher false positive rates outside of the peak RSV season and are considerably less reliable in older children and adults, who have reduced viral load compared to young children (122–124). This group may benefit from use of either culture or nucleic acid amplification testing (NAAT) to detect RSV.

Nucleic Acid Detection

Molecular tools such as NAAT have transformed the ability to identify causative organisms in ARI and have the distinct advantages of both speed and accuracy over viral culture and RADT respectively (4,117). Despite this only 15% of laboratories in the United States use the technique, which costs \$35-125 for each sample tested (vs. \$2-7 for DFA and \$4 for culture (116)) as well as the equipment and specialist skills required (124).

Reverse Transcriptase PCR (RT-PCR) has been proven to be as sensitive as culture in detecting RSV in hospital (125,126) and community settings (5,8) in both adults and children. This technique has also been used to highlight minimal

asymptomatic carriage of RSV in the community making it a more useful method of accurately diagnosing the disease in this setting (127). Further automation of such techniques will enable even more rapid, high throughput handling of clinical specimens.

In the field of microbiology, molecular detection methods (rather than slower culture based methods which often fail to detect a significant number of organisms) have revolutionized the detection of bacterial pathogens and helped to identify distinct anatomical microbiomes. Similarly, the development of rapid multiplex PCR based technology that can be deployed as a point of care test is likely to institute a paradigm shift in the diagnosis of viral pathogens at an earlier stage of illness. This can be of particular benefit in those at-risk groups (pre-term infants, elderly and those with multiple co-morbidities), who need early intervention or admission (119). A study of more than 430 clinical specimens validated the usefulness of this approach with the use of a 19-valent multiplex PCR test, which included RSV and demonstrated a mean sensitivity of 93% and specificity of 99% (128). With the costs of multiplexing declining it could also be a promising approach for community based epidemiological studies (129). Nonetheless, issues remain about the correlation between detection of the viral nucleic acids and clinical symptoms (130) and multiple respiratory virus detection from the same patient may be indicative of the presence of chronic lung disease rather than severity of the infection (131).

A comparison of the available laboratory techniques for RSV diagnosis in terms of speed, expertise required and relative cost is shown in figure 4.

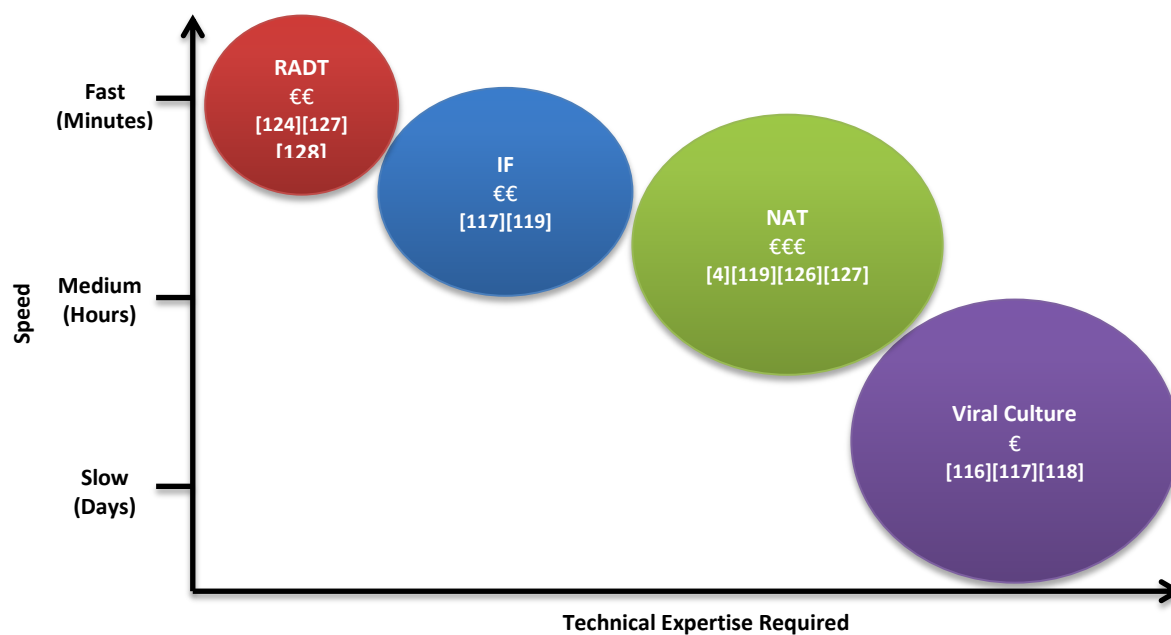


Figure 4. Comparison of laboratory techniques to diagnose RSV. The figure represents estimates and is not to scale. The size of the circles represents the relative sensitivity of each test in detecting RSV. Relative approximate costs are represented as € and are for reagents [116]. Numbers in square brackets indicate the relevant references. RADT: rapid antigen detection test; IF: immunofluorescence; NAT: nucleic acid testing (PCR).

Anatomical Sampling

Nasopharyngeal aspirates (NPA) have traditionally been the gold standard in diagnosing RSV infection (132) but head to head comparisons of nasal swab sampling versus aspirate have shown equivalent results for most respiratory viruses (133,134). Swabs are prone to lower detection rates, which may be due to the lower volume of sample being present on them.

Flocked nasal swabs have better ability than NPAs to detect RSV due to their effect of dislodging virus-infected epithelial cells from the mucosa (4). They may be more suited to the outpatient setting, as they appear to be better tolerated than NPAs (135) and are also superior to nasal washes (136). Swabs can be considered for routine testing in the community but for hospitalized patients or if strain typing is required, an NPA is more appropriate.

In immunocompromised patients, nasal wash has very poor sensitivity in detecting RSV, likely due to lower viral load, with these patients benefiting from lower airway sampling (137). The use of serology in diagnosing RSV in children is limited by the passive transfer of maternal antibodies and is

not useful in adults due to the persistence of antibodies from repeat infections.

ANTIVIRAL THERAPY AND OTHER POTENTIAL TREATMENTS

Despite significant interest in disease-modifying therapies for RSV, the mainstay of treatment remains supportive. For the majority of affected children and adults illness is self-limiting and simple measures such as hydration and antipyretics are sufficient. In those in whom symptoms are more severe intensive supportive treatment in a supervised hospital environment may be required (96).

Supportive

The most recent guidelines from the American Academy of Pediatrics (AAP) (113) and The National Institute for Health and Care Excellence (NICE) (138) in the UK primarily focus on appropriate delivery of supplemental oxygen and hydration (table 1). Both guidelines advise delivery of supplemental oxygen using non-invasive and positive pressure ventilation strategies, as well as intravenous or nasogastric fluids if oral hydration is insufficient. In addition, NICE recommends consideration of

upper airway suctioning in children with respiratory distress, feeding difficulties or apnoea. Unlike NICE guidance, the AAP recommend nebulized saline as an optional treatment in children with an expected length of stay greater than 72 hours. This strategy has generated some controversy as the evidence base for its benefit has been conflicting. Despite their frequent

empirical use, no recommendation is made regarding antipyretics (such as acetaminophen and ibuprofen), antitussives or decongestants by any of the most recent guidelines. Chest physiotherapy has not been recommended, although NICE adds the caveat that it can be employed if children have relevant co-morbidities (138).

Table 1. Current evidence for therapeutic options in managing RSV bronchiolitis

Supportive	Inconclusive	Not supportive
Oxygen	Ribavirin	Nebulised bronchodilators
Ventilation (invasive and non-invasive)	Nebulised hypertonic saline	Nebulised adrenaline
Nasogastric/i.v. fluids if unable to maintain oral intake	Upper airway suctioning	Steroids (oral/inhaled)
Palivizumab for prevention in high-risk babies	Azithromycin	Antibiotics without evidence of bacterial infection
	IVIg	Montelukast

Based on guidelines published by the American Academy of Pediatrics [113], The National Institute of Health and Care Excellence [138] and reference [96]. IVIG: i.v. immunoglobulin.

Antivirals and other Pharmacotherapy

A variety of additional therapeutic agents have been used empirically for the treatment of bronchiolitis including systemic and inhaled corticosteroids, nebulized bronchodilators and nebulized adrenaline. Guidelines now recommend that clinicians do not use these therapies in the treatment of children with bronchiolitis (96). Antibiotic use is still permitted by the AAP guidelines if there is evidence of bacterial co-infection but this recommendation is not made elsewhere (138,139). In adults with an RSV-associated exacerbation of underlying lung disease the use of nebulized bronchodilators and oral corticosteroids remains commonplace. Some commentators argue that there is a theoretical risk that immunosuppression in this group of patients may potentiate the severity of viral infection via defects in humoral immunity (140), but the evidence is poor.

Ribavirin is currently the only licensed antiviral for the treatment of RSV in children, but the evidence is poor (141). Consequently, most current guidelines make either no recommendation or do not recommend its routine use, that is also limited by its toxicity, including to staff (64,141). In adults, ribavirin is used off-license and is usually reserved for those with severe immunocompromise such as hematopoietic stem cell transplants recipients (142) although the evidence base for its use is weak. In studies examining the effect of ribavirin

(nebulized or systemic) in immunocompromised patients with RSV infection, there were trends towards improved outcomes with reduced progression to lower respiratory tract involvement and less all-cause mortality (27). However, none of these studies was sufficiently powered or robustly designed for definite conclusions to be drawn. The value of concomitant intravenous immunoglobulin administration alongside ribavirin also remains unproven (141).

Immunoglobulins, both RSV specific and non-specific, have also been used in the treatment of RSV related bronchiolitis in children. Like many other therapeutic interventions, the evidence base for this remains weak and a Cochrane review in 2006 concluded that there was no statistically significant benefit of such treatment (143).

Montelukast, a leukotriene inhibitor used in the treatment of asthma, has also been tried in the treatment of bronchiolitis. A 2015 Cochrane review considered five randomized control trials comparing montelukast treatment with placebo in children less than 2 years of age with a diagnosis of bronchiolitis. Two of the five studies examined the impact of montelukast acutely during the hospital admission whereas the other 3 studies focused on longer term use over several weeks following a diagnosis of bronchiolitis to prevent ongoing episodes of wheeze. The authors concluded that whilst none of the studies demonstrated a difference between montelukast and placebo, no

definite conclusions could be drawn due to statistical inconsistencies and weaknesses of the studies (144).

Azithromycin, a macrolide antibiotic with immunomodulatory properties, was shown to have no impact on clinical outcome measures such as duration of hospital stay, oxygen supplementation or symptom scores when given to infants during acute RSV bronchiolitis (64). It was however, found to reduce levels of neutrophilic inflammation in the airways of mice in a murine bronchiolitis model (145). A proof of concept study was therefore conducted in human infants investigating the effectiveness of azithromycin compared to placebo at reducing post-bronchiolitis wheeze and chronic airway inflammation in previously healthy infants aged 1-18 months (146). The results demonstrated that treatment with azithromycin for 14 days during the acute bronchiolitic illness prolonged the time to a third parent-reported wheezing episode and reduced the overall number of days with respiratory symptoms in the 50 weeks post treatment. However, the primary clinical outcome, of a reduction in the number of children experiencing 2 or more wheezing episodes was not met and the proportion of participants diagnosed with asthma during the follow up period was also no different. More promising were the findings of a 2007 double blind placebo control study from Turkey investigating the use of clarithromycin in infants (<7months) hospitalized with RSV bronchiolitis. Statistically significant reductions in length of stay, need for supplemental oxygen and bronchodilation were reported in addition to fewer re-admissions with wheeze in the subsequent 6 months. However, this study had a small sample size and further studies would be needed to validate the findings (147).

Several new therapeutic agents are currently in development pipeline. These fall broadly into four different groups: siRNA interference, immunoglobulins, fusion inhibitors and small molecules (96). Each aims to disrupt the production or action of proteins produced by RSV and thus limit its pathogenicity. Agents which have demonstrated efficacy include ALN-RSV01, which uses anti-sense mechanisms to disrupt viral protein synthesis and thus viral replication. Proof-of-concept studies in healthy adults experimentally inoculated with RSV demonstrated reduced infection rates with concomitant use of intra-nasal ALN-RSV01 (148) and use of the compound in lung transplant patients with RSV infection has been shown to reduce the rate of new or progressive

bronchiolitis obliterans syndrome at 180 days post infection (149).

Promising results have also been seen with the fusion inhibitor GS-5806 which reduced the viral load and severity of clinical disease in a double-blind placebo controlled viral challenge study in healthy adult volunteers (150). More recently, a placebo-controlled trial of a second antiviral (ALS-008176, that acts on the RSV polymerase L and inhibits viral replication in infected cells) enhanced viral clearance and reduced disease severity. Importantly, this compound appears to be effective even when administration was delayed until 12 hours after virus detection (151).

VACCINES & PREVENTION OF RSV

The lack of effective, disease-specific therapies for RSV alongside the significant global burden and cost of disease has stimulated huge interest and research in vaccine discovery. However, the development of RSV vaccines has been hampered by a number of factors. First, defects in both innate and adaptive neonatal immune responses make infantile vaccination problematic. In addition, the presence of maternal antibodies, despite offering valuable protection of the newborn child, may also suppress the development of intrinsic antibody responses (152).

Although certain immunisation strategies are effective in neonates (e.g. BCG for tuberculosis), many vaccines require repeated boosters and/or are delayed until the child's immune system is more mature (153). In addition to the challenges posed by the host response, RSV as a pathogen creates difficulties for vaccine development. In particular, natural infection does not result in durable immunity even in adults and re-infection with an identical strain of virus is commonplace (85). A successful vaccine therefore needs to induce immune responses more potently than natural infection whilst simultaneously preventing an excessive and pathological inflammatory response in the host.

Unfortunately, this balance was not achieved by early vaccine attempts. In the 1960s a formalin-inactivated vaccine was developed for use in infants and children. Although a similar approach was effective and safe with poliovirus immunisation, it resulted in insufficient neutralising antibody response and enhanced disease during subsequent natural infection. In one trial, nearly 80% of naturally infected

vaccinated children required hospitalisation and two died (154). As a result, progress in RSV vaccination development has been intensely cautious and slow.

Strategies to enable the investigation of the immune response to both natural infection with RSV as well as the response to a variety of potential vaccines have frequently relied on the use of animal models. Unfortunately, no single model has been sufficient to replicate the situation in human infection (155). The use of human RSV challenge has considerable advantages over animal models, but these too remain imperfect. Adults are not the primary target of RSV vaccines designed to prevent infantile bronchiolitis, although there may be a role for vaccination of pregnant women, adult care-givers and those adults at risk of severe RSV disease. However, challenge studies are currently limited to normal healthy adults and infants, pregnant women, immunocompromised or frail adults cannot be subjected to experimental infection. Ultimately, a single vaccination strategy for all target groups is unlikely to be preferred (155).

Despite these hurdles, a large number of potential vaccines are under development and the likelihood of one or more successful vaccine reaching the market within the next decade is high (6,155). These vaccines fall into four broad categories: live attenuated, subunit, vector-based and particulate. Each targets different mechanisms of immunogenicity and thus would potentially suit different groups of at risk individuals. Strategies for optimising the effect of vaccines include the use of maternal vaccination to enable passive transfer of immunity *via* immunoglobulin to levels sufficient to provide effective protection, in an otherwise poorly immunogenic infant. Immunisation would need to be delivered to the mother in the second or third trimester, for the

transfer of passive immunisation to be optimal (152) which would be of less value for premature infants in whom trans-placental antibody transfer is inefficient. Immunisation of individuals in contact with vulnerable groups such as health care or nursery workers or older siblings may be another viable alternative to reduce virus transmission rates through herd-immunity.

Live attenuated vaccines show promise in infants, since they may safely induce local protective immunity. They may however be of limited immunogenicity and the possibility of pathological responses in (undiagnosed) immunodeficient states still remains a concern (153). Nevertheless, some success has been achieved and a variety of phase 1 and 2 clinical trials are currently underway. The leading contender in the field is Medi-559 (MedImmune/National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA). This has been well tolerated by healthy children and infants, with the majority of participants mounting a detectable serological response or shedding of vaccine virus as detected by PCR (7). Trials of other live-attenuated vaccines are ongoing (clinicaltrials.gov identifier numbers: NCT02040831, NCT01893554 and NCT01852266). Advantages of live attenuated vaccines include their ability to be delivered intranasally, to replicate even in the presence of passively delivered maternal antibody and their ability to stimulate multiple components of the immune system (152). Despite these attributes, the potential for reversion to wild-type and for secondary transmission from vaccinated child to contacts, as well as the difficulty in producing sufficient attenuation has slowed progression towards clinical use (155).

Panel 1: Outstanding Questions in the Field

- What innate and adaptive immune mechanisms determine the susceptibility of infants to bronchiolitis?
- Why do some people with RSV develop symptoms, whilst others remain asymptomatic?
- Why is it possible for adults (and children) to be re-infected with the same strain of RSV?
- What explains the variable findings with respect to the association between RSV and wheeze in later life?
- What are the best correlates of protection to use in vaccine studies?
- What are the ideal qualities of vaccines that target specific risk groups (i.e. pregnant women, adult care-givers, school age children and elderly persons)?
- How can animal models be improved still further?
- What public health measures are appropriate and feasible in resource-limited settings?

Some of these issues could be avoided with the use of subunit vaccines, which have no potential for infectivity in either vaccinees or their contacts. Unfortunately, they often fail to sufficiently stimulate a response in the immature infant immune system (96) and their optimal use may prove to be in boosting the adult response. Subunit vaccines, which contain purified proteins, with or without the addition of immune stimulating adjuvants, have been shown to be effective in inducing neutralising antibodies in the serum of adults (156) and may offer an effective option for the elderly and pregnant women (96). In seronegative individuals there is a possible risk of vaccine-enhanced disease and hence these vaccines would be unlikely to be useful in previously unexposed neonates. Particle based vaccines, which consist of multiple proteins arranged on virus/bacteria-like structures, mimic infective agents and may be able to induce durable immunity (155). Vector-based vaccines use other viruses or bacteria as a means of delivery of RSV genetic components. Viral protein production allows antigens to be made within the host, while the vector is rendered harmless (155). Much of the recent progress in vaccine development is due to improvements in our understanding of the structure and function of RSV, the immunogens that it makes and the protective and harmful immune responses that these raise in the host. A detailed appreciation of the host immune response to RSV, particularly the T and B-cell immune responses, are vital to future vaccine development.

Strategies for prevention of RSV mainly focus on the use of passive immunisation. Palivizumab, a humanised monoclonal antibody of RSV, is licensed for use in high risk babies during RSV season, in order to prevent disease in the lower respiratory tract. Large randomised controlled studies in the late 1990s showed a reduction of 55% in the rate of hospitalisation for RSV in preterm infants compared to placebo (157). Further studies have confirmed its efficacy and the American Academy of Paediatrics has recently produced advice as to which at risk infants benefit from treatment (158). Monthly administration of antibody is very costly, which largely limits its use and is of no value in established infection (155).

As previously discussed, the potential benefits of RSV prevention with passive antibody could

extend beyond the effect on acute morbidity and mortality, with possible impacts on the occurrence of longer term sequelae such as recurrent wheeze (97). Of the newer monoclonal antibody candidates, motavizumab seemed the most promising (99), however its development has been halted due to non-superiority to palivizumab and adverse skin reactions. It is to be hoped that other monoclonals can be marketed for RSV prevention at lower cost.

CONCLUSION

A summary of some of the outstanding questions in the field of RSV research are outlined in panel 1 and the key take-home messages of the current research on RSV are outlined in panel 2. RSV infection is currently ubiquitous during early childhood, causing a vast burden of disease throughout the world. The link between severe childhood infections and the development of longer-term respiratory ill health is intriguing, but as yet poorly mapped and understood and the proposed causal link between bronchiolitis, the later development of recurrent infantile wheeze and the diagnosis of childhood asthma remains to be established with certainty. While infections with many common and important respiratory pathogens (e.g. influenza virus, *Haemophilus influenzae*, *Streptococcus pneumoniae*) are vaccine-preventable, RSV has so far eluded vaccinologists. There is now a race on between those developing a large number of vaccines that aim to induce long-lasting protection. Field trials of both vaccines and antiviral drugs in real-life settings are keenly awaited, but for now the treatment of RSV disease remains largely supportive. The advent of antivirals and vaccines that reduce viral load and disease, coupled with increasingly sensitive molecular techniques to diagnose infection at an early stage, may potentially greatly reduce the global impact of RSV infection over the coming few years. Ultimately, such measures may have an impact on RSV's ability to circulate in communities, changing the population dynamics of infection and altering the global grip of this virus.

Panel 2: Key take-home messages

- RSV is the leading cause of bronchiolitis in infants and excess winter deaths in the elderly.
- It is estimated to cause 30 million acute respiratory infections in children and in excess of 60,000 deaths worldwide each year.
- RSV causes upper and lower respiratory tract infections in both children and adults. Infants and young children, frail elderly and those with chronic cardio-respiratory disease or immunocompromise are at greatest risk of morbidity and mortality.
- RSV disease is predominantly a winter illness. Its incidence peaks in December to January in Northern European countries and February to March in Southern Europe.
- Susceptibility to severe RSV disease is increased by exposure to tobacco smoke, air pollution, crowding (in day care, nursing home or hospitals) and the presence of school-age older siblings.
- RSV infection in children has been linked to the development of asthma and atopy although a causal relationship remains unproven.
- RSV has been classified into two distinct subgroups (A and B) based on antigenic variability with the suggestion that subtype A causes more severe disease. Genetic sequence analysis is the most accurate way of identifying viral subtypes for research purposes.
- A key characteristic of RSV is its ability to evade immunity and thus to re-infect despite limited antigenic variation.
- Laboratory diagnosis of RSV is not essential but can facilitate appropriate antibiotic stewardship.
- Nasopharyngeal aspirate is the gold standard for collection of samples with flocked nasal swabs an acceptable alternative in community settings. Immunocompromised patients should have lower airway sampling and PCR diagnosis to increase the chances of a positive yield.
- Culture remains the gold standard for diagnosing RSV but is being overtaken by fast molecular techniques. Rapid antigen detection kits are relatively inexpensive, easy point of care testing but limited by lower sensitivity and poor reliability in adults. Nucleic acid detection (PCR) now offers high sensitivity and increasingly quick turnaround times.
- Hand decontamination with alcohol or soap is important in preventing nosocomial transmission.
- Appropriate supportive treatment with fluids, oxygenation and ventilatory support remains the cornerstone of RSV management.
- Salbutamol, adrenaline and systemic corticosteroids should not be routinely administered to infants with bronchiolitis.
- There are several very promising anti-viral drugs and vaccines in development, but their impact in clinical settings is currently difficult to anticipate. Promising vaccination strategies include the use of maternal vaccines, inactivated or live vaccines for neonates and sub-unit vaccines for the elderly.

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