RESPIRATORY SYNCYTIAL VIRUS: FROM DISCOVERY TO TREATMENT

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ABSTRACT
To review the epidemiology, identification, clinical patterns and treatment of respiratory syncytial virus infection. Raised available studies in the MEDLINE database using the keywords respiratory syncytial virus, respiratory infection, bronchiolitis and indirect immunofluorescence in international studies since 1980. Respiratory Syncytial Virus (RSV) was first time isolated in 1956 and became the most frequently agent found, especially in the lower respiratory tract of children. It is responsible for significant morbidity and mortality in children younger than 18 months with risk factors such as prematurity and heart disease. It shows well-defined distribution along the year. Histopathology, immunohistochemistry and standardized molecular techniques to identify the virus have been described. The specific treatment of RSV infection is still limited. Antiviral agents as ribavirin showed limited effectiveness and restricted to use in a few patients with severe heart disease. Vaccines needs further studies before marketed. Humanized monoclonal antibody - palivizumab - is safe, well tolerated and the most cost-effective when used in children at risk for severe RSV infection. The high rate of children hospital admissions by RSV infection is a public health issue. Treatment is yet supportive and non specific according to severity. Epidemiologic surveillance, routine virus identification and accessibility to health centers are the key points to control infection by this virus.

Keywords: Respiratory syncytial virus, respiratory infection, bronchiolitis, prevention

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The Discovery
In 1956 the Respiratory Syncytial Virus (RSV) was identified for the first time from upper airway secretion of chimpanzee monkeys with bronchiolitis symptoms. It was known as the chimpanzee coryza agent (Chanock et al. 1957). At the same time some cases of airway infections in humans were associated to this agent, especially lower airway infection in children. In 1957, due to the virus characteristics, infecting the airway and generating gigantic syncytial cells in tissue culture, it was renamed as Respiratory Syncytial Virus, used still nowadays (Chanock et al. 1957).

Researches have confirmed its importance and frequency of the RSV involvement as a leading cause of airway infections in children worldwide.

RSV belongs to the family Paramyxoviridae - as the measles virus, mumps virus, parainfluenza group and more recently discovered Nipah and Hendra virus. The RSV’s subfamily is Pneumovirinae that includes the Human Metapneumovirus (hMPV) capable to create a very similar clinical presentation of the RSV, as bronchiolitis and pneumonia (Van Den Hoogen et al 2001, Lourenço et al 2005). Nonetheless, the hMPV infection is more common in children with congenital anomalies, as cardiopulmonary and it is associated to a greater ventilatory demand (Zhang et al. 2009).

The RSV particles have a median size ranging from 120 to 300nm with helical symmetry (Lourenço et al. 2005). The viral nucleocapsid, also known as core, is composed of structural proteins combined with the viral genome, which is a single RNA strand. It is capable to performing at least 10 different types of proteins, most of them with structural function and two non-structural proteins with still unknown function (Bricks 2001). The viral genome consists of 15222 nucleotides that in association with nucleocapsid proteins, form a ribonucleoprotein complex resistant to RNAse activity (Mohapatra & Lockey 2008).

The viral core is wrapped by an envelope that is formed by the plasma membrane of the host cell, composed by different types of glycoproteins. The Glicoprotein F and G are transmembrane proteins and
also play a role as primary surface antigens working as target of the host's immunological response. The F protein contributes for the virus penetration in the cell and cell membrane fusion, resulting in the multinucleated syncytial gigantic cells. The G protein is responsible for the virus and host cell binding. Meanwhile the F protein suffer few changes, the G protein presents great genetic and antigenic diversity in the RSV group (Timothy & James Jr 2008, Riccetto et al. 2009, Da Silva et al. 2008).

The RSV has great growth capacity at pH=7.5, and despite the temperature sensitivity, it can be found after as long periods as one hour in contaminated gloves with infected nasal secretions. Stability in the hospital environment contributes for being a nosocomial pathogen (Carvalho et al. 2007).

RSV is classified into two antigenic groups, A and B, initially on the basis of the reactivity of the virus with monoclonal antibodies directed against the attachment glycoprotein (G protein) and currently through genetic analyses (Riccetto et al. 2009). The groups have been further divided according genotypes. There are 11 RSV-A (ON1, GA1 to GA7, SAA1, NA1, and NA2) and 17 RSV-B (GB1 to GB4, SAB1 to SAB3, and BA1 to BA10) genotypes (Da Silva et al. 2008, Entemadi et al. 2013).

Genetic study of protein sequence has supported this division and also has identified a great number of variations and lineages in each group. The G protein is the most variable viral protein with a minimally conserved ectodomain that contains 2 hypervariable regions (HVRs). The second HVR (HVR2) carries the C terminus of the protein and is commonly sequenced to exam the genetic variability of RSV strains within a given population (Da Silva et al. 2008). National (Da Silva et al. 2008) and international (Aamir et al. 2013, Entemadi et al. 2013) researches aimed to correlate epidemiological and clinical aspects of different groups (A or B) and several genotypes of RSV. They found variation by local research center and by current genotypes distribution.

Epidemiology and Seasonality

RSV is counted for significant mortality and morbidity in children younger than 18 months of life (Mohapatra & Lockey 2008, D’Elia et al. 2005, Straliotto et al. 2004, Lavia et al. 1992). Epidemiological studies have showed that RSV is responsible for 50% to 90% of hospital admissions in children with bronchiolitis and 5% to 40% from those related to pneumonia. It is considered the main agent responsible for children’s death during the winter season (Straliotto et al. 2004). Infants under 6 weeks and children with an underlying condition as prematurity, immunodeficiency, cardiac disease or chronic pulmonary conditions are more susceptible to hospitalization by RSV infection (Lavia et al. 1992).

Studies in several countries have showed that up to 90% of children around 2 years old presents antibodies against RSV, indicating that they have been infected at some time of their lives (Simões et al. 1999, Lavia et al. 1992, Pickering 2006, Robertson et al. 2004).

The combined action of CD4+ and CD8+ T cells have been involved in the development and healing of acute RSV infection, whereas antibodies can provide protection from re-infection. Despite eliciting an immune response that mediates clearance of the virus, immunity to the virus appears to wane over time and individuals remain susceptible to reinfection with RSV throughout their lifetime (Varga & Braciale 2013).

Several investigators in different regions of Brazil have studied and published epidemiological data about the frequency of the RSV infection in children (Table 1). The first studies date back to the 1980s and has been held periodically evaluating children under 5 years of age, hospitalized or in the emergency rooms of pediatric hospitals. The frequency of the RSV identification, isolated in secretions from nasopharyngeal wash (NPW), varied from 3% to 60% in these studies. A critical analysis is needed and the numerical data comparison should be careful since the identification methods were different and the figures are increasing in critical cases that resulted in hospitalization (D’Elia et al. 2005, Straliotto et al. 2004, Sutmöller et al.1983, Nascimento et al.1991, Sutmöller et al. 1995, Cintra et al. 2001, Vieira et al. 2001, Checon et al. 2002, Straliotto et al. 2002, Vieira et al. 2002, Moura et al. 2003, Bosso et al. 2004, Tsuchiya et al. 2005, Calegari et al. 2005, Nacul et al. 2005, Moura et al. 2006, Costa et al. 2006, Riccetto et al. 2006, Thomazelli et al. 2007, Vieira et al. 2007, Oliveira et al. 2008, Pecchini et al. 2008, Salomão et al. 2011).

In southern Brazil, the manifestation period of the virus is similar to other countries with temperate weather, with peaks in the winter and spring (D’Elia et al. 2005, Vieira et al. 2002, Calegari et al. 2005, Nacul et al. 2005). Up to 92% of the RSV infections occurs from April to June (Riccetto et al. 2006). Higher prevalence in this period was attributed to mother’s asymptomatic infections, crowded child environment, infections in children with chronic pulmonary diseases and immunodeficiency patients (Riccetto et al. 2006).

The RSV is among viruses with marked seasonality. Epidemics were related to low temperatures (Sutmöller et al. 1983), lower sun hours (Nascimento et al. 1991) and more precipitation (Straliotto et al. 2002, Vieira et al. 2001, Checon et al. 2001). As an example, in countries as Indonesia and Mozambique the cases of RSV occurred mainly during the rainy season, whereas in countries as Nigeria and South Africa the cases occurred mainly during the dry season (Robertson et al. 2004). Despite this season variation in the prevalence of the disease, many studies in different countries have demonstrated that both strains of RSV tends to circulate simultaneously however the proportion between them varied year after year. Other
studies performed in countries as United State, Finland and France confirmed the co-circulation of those two groups annually with different time patterns and variable prevalence rates among them (Robertson et al. 2004).

In Brazil, there were simultaneous circulation of different groups of RSV in the North, Southeast and South regions, and this epidemiological pattern was not observed in other regions. Despite the climatic diversity, factor which influences the characteristics of the airway infection, there are still missing data about the RSV viral outbreak in each region of the country. Knowledge of antigenic variations is important for diagnosis and definition of vaccine composition (Polack & Karron 2004).

**Clinical Aspects**

RSV infection in children compromised lower respiratory tract and bronchiolitis was the most common condition (Wright & Piedimonte 2011, Bekhof et al. 2013, Thomazelli et al. 2007, Diniz et al. 2005). Pneumonia has been considered as virus infection too. Respiratory virus was found isolated in children's necropsies died by severe respiratory diseases (Chong DC et al. 2009).

* Cough, tachypnea, and wheezing were the most common clinical signs described in children with lower respiratory tract infection (LRTI) (Ferone 2014, Wright & Piedimonte 2011, Thomazelli 2007, Diniz 2005). Other studies showed shortness of breath (Salamão Júnior et al. 2011) and cyanosis (Riccetto et al. 2006) were more frequently in RSV positive patients than RSV negative. Fever was found in 70.2% of hospitalized children for LRTI (Ferone 2014, Wright & Piedimonte 2011, Thomazelli et al. 2007, Diniz et al. 2005). Pneumonia has been considered as virus infection too. Respiratory virus was found isolated in children’s necropsies died by severe respiratory diseases (Chong DC et al. 2009).

LRTI severity was measured by oxygen needs, time of oxygen needs, Intensive Care Unit (ICU) admission and mechanical ventilation. There were no differences in hospitalization time and mechanical ventilation needs among RSV+ patients and Adenovirus+ in the nasopharyngeal secretion (Ferone, 2014).

It was observed that the first infection by the RSV is commonly more severe, if there are history of prematurity, pulmonary dysplasia, chronic heart conditions, congenital or acquired immunodeficiency and cystic fibrosis, therefore this patients have higher risk of death (Mohapatra & Lockey 2008). Risk factors such as gestational age under 35 weeks, birth weight lower than 2500 grams and lower maternal education than 5 years, were found in patients with RSV infection (Riccetto et al. 2006).

Although previously healthy patients also developed severe RSV disease, and several factors have been described as predictors of severity in this disease: male gender, under 6 months of life, birth during the first half of the season of RSV, early breastfeeding cessation, previous episodes of wheezing, low maternal age, smokers in the family and home crowded, but not all studies confirmed this relationship (Riccetto, 2006).

Aimed to identify differences between infections severity among RSV groups, Cintra et al. 2001 evaluated 829 samples and there was no association of RSV group (A or B) and severity of disease.

When comparing clinical characteristics and RSV genotypes, Riccetto 2009 et al. found seasonality (April-May) as well oxygen saturation lower than 90% as signs indicating RSV infection. The most common genotypes were GA2, GA5 and SAA1 (RSV-A) and SAB1, SAB3 and BA (RSV-B), but there was no correlation between genotypes and clinical characteristics.

The RSV was identified in association with adenovirus, metapneumovirus, parainfluenza and influenza virus in children hospitalized for bronchiolitis and/or pneumonia (De Paulis et al. 2011, Bekhof et al. 2013, Bezerra et al. 2011, Da Silva et al.2013). There was no association between RSV co-infection or co-detection and clinical characteristics and severity of disease (De Paulis et al. 2011, Da Silva et al.2013).

The RSV is also one the causative agent of the post infection bronchiolitis obliterans (Teper et al. 2002). This disease is frequent in our environment and is one of the most important cause of chronic pulmonary obstructive disease in children, besides other etiologies as inhalation of toxic substances, aspiration syndromes and immunity diseases, and other infectious agents as adenovirus, influenza virus and parainfluenza. It was characterized by persistent wheezing, tachypnea, shortness of breath and cough for weeks or months after the initial infection (Teper et al. 2002)

**Identification**

The pathology analysis is a tool used for the identification of the etiologic agent of airway infections. Some authors have shown the advantages of making epidemiological researches with necropsies. When compared to population studies they reflect more reliable data with lower chance of bias seen in studies involving patients. The data obtained can be compared and reviewed more easily, neutral for the seasonality and other characteristics of some diseases (Mc Farlane et al. 1987).

Some virus presents typical cell inclusions that when seen in the microscope can define the etiologic diagnosis. Herpes simplex virus type 1 is known by the Cowdry’s type A intranuclear inclusions, which the nuclear center is transformed in a big acidophilic inclusion that is separated from the nuclear border by an artificial cliff. The Cytomegalovirus can cause cytomegalic inclusions in almost all organs. In the lungs presents as an interstitial pneumonitis with intracellular inclusions in the alveolar coating, endothelial cells from the septal capillary and alveolar macrophages (Mc Farlane et al. 1987, Demur et al. 2010, Chong et al 2009).
Regarding the histopathological aspects of RSV presents as an interstitial pneumonitis pattern, pneumonia or with the two patterns concomitance. Typical virus inclusions are not the classical finding of RSV (Cotran 1991).

Immunohistochemical methods, the direct or indirect immunofluorescence have been studied and improved for the detection specific viral and bacterial antigens through the use of polyclonal and monoclonal specific antibodies. The use of immunofluorescence for virus investigation in oropharynx secretions is classical and is broadly described in clinical trials. Some variations were found in protocols that use this technique in paraffin material (Knott et al. 1994, Wang et al. 1995, Yun et al. 1995, Miyao et al. 1999).

The importance and applicability of immunofluorescence (IF) for viral antigen detection is a reality. Many authors have compared IF with other methods used for viral detection in tissue sample and cellular suspension. The advantages of IF relative to other methods were: the relative speed in obtaining the results, accessible cost for large scale, and feedback on the quality of collected material. The last one is an advantage that differentiates IF from the other compared methods. However this method requires trained personnel for handling samples and epifluorescence microscope reading. Readings are subjective and some virus with high antigenic variability make it difficult to identification with currently available antibodies. The indirect IF proved advantageous over the direct IF in relation to good signal quality and lowest occurrence of nonspecific background staining. IF has become useful method for detection of viral antigen, it is fast and cheaper when compared to other methods (Lanari et al. 2002, Forghani & Dennis 1989, Minnich & Ray 1982, Madeley & Peiris 2002).

New respiratory virus have been identified as Human Coronavirus, Human Bocavirus and porcine Influenza virus, and laboratories around the world developed new techniques to virus detection and follow behavior of these emerging virus (Mahony, 2008, Mahony et.al 2011).

Molecular tests based on nucleic acid amplification (NAAT) for respiratory viruses were the first to be developed and are widely used today. The most popular technique of NAAT is the Polymerase Chain Reaction (PCR) consisting of an enzymatic reaction using oligonucleotides flanking the target sequence to be amplified by the action of the enzyme DNA Polymerase (Mahony, 2008).

These tests are more sensitive than other laboratory diagnostic methods, including virus isolation in cell culture, the culture shell vial (SVC), antigen detection by direct fluorescent antibody (DFA), and fast enzyme immunoassay (EIA). NAAT provides fast, accurate and sensitive detection of respiratory viruses in clinical samples (Mahony, 2008, Mahony et.al 2011). Introduced few years ago in the routine of several laboratories, multiplex PCR is able to detect up to 19 different viruses in a single test. Many of these multiplex PCR tests are commercially available and came to be seen as the best choice for cost-effective for the detection of respiratory viruses (Mahony, 2008, Mahony et.al 2011, Mahony et al. 2009)

NAATs are highly sensitive and was in initial phase in virology laboratories, including PCR using nucleic acid sequence-based amplification (NASBA) and loop-mediated isothermal amplification (LAMP) already tested for RSV even produce results in up to 30 min (Mahony et al. 2013). These techniques and multiplex PCR still associated with fluidic microarrays using microbeads or DNA chips, will make up the roll of more modern techniques in detecting viral (Mahony, 2008, Mahony et al. 2011, Mahony et al. 2013).

Studies have shown the specificity and sensitivity of molecular assays and compared to cell cultures for RSV DFA and made of NAATs to be fast, accurate and sensitive method for the detection of choice currently RSV (Mahony, 2008, Mahony et al. 2009, Mahony et al 2011, Mahony et al. 2013).

Treatment

Surveys, some in phase III, are ongoing and promising new therapies for the treatment of infections caused by RSV. To date, the treatment of diseases caused by RSV is supportive and nonspecific (Olszewka & Openshaw 2008, Carbonell-Estrany et al 2010).

Ribavirin, inhibits the virus structure proteins synthesis, reducing its replication and the immunoglobulin E response (Carvalho et al. 2007, Ottolini & Hemming 1997) and represents the only licensed anti-viral drug for treating RSV infection. It has been associated with a mild decrease in infection with usual progression and it has a restricted indication for patients with T cell deficiency (Mohapatra & Lockey 2008). It is administered by aerosol form and was indicated for infants with severe bronchiolitis and immunocompromised patients, but has a limited use owing to variable efficacy, the risk of toxicity and high cost. Nowadays, ribavirin is restricted to use in a few patients with severe heart disease in a refere centers (Olszewka & Openshaw 2008, Krilov 2002, Murray et al. 2014).

There are no approved vaccines for RSV (Varga & Braciale 2013). The development of a vaccine has been worldwide limited after the 1960’s initial tests, when vaccination exacerbated the disease caused by naturally acquired virus.

The ineffectiveness of the natural infection to induce long-term immunity, circulating a variety of genotypes and the fact of a person can develop the same disease genotype more than once, has made the development of RSV vaccines even more challenging (Varga & Braciale 2013).
Polack & Karron em 2004 demonstrated studies with intranasal vaccine compounded by genetic modified organism, a bovine parainfluenza virus inserted with protein F sequence from RSV. It doesn’t have infecting ability in humans and can stimulate antibodies production against the ligand protein of RSV. This type of vaccine still remain experimental.

A variety of vaccines for active immunization against RSV are now in clinical trials. Two promising new antivirals are currently in phase I/II trials to verify effectiveness in preventing severe RSV LRTI (Murray et al. 2014).

Vaccine play role in decreasing severe cases of lower airway infection by RSV (Mohopatra & Lockey 2008, Robertson et al. 2004, Varga e Braciale 2013, Murray et al 2014) and would attenuate development of subsequent recurrent wheeze in children (Stein & Martinez 2010).

Immunoprophylaxis against RSV is described as active and passive approaches. The intravenous infusion of specific immunoglobulin against RSV can attain concentration of antibodies 6 times higher than neutralizing antibodies. The Prevent Study that included 54 centers and 510 children have shown that the immunoglobulin is safe, well tolerated and can reduce in 41% the hospitalization in preterm and children with bronchopulmonary dysplasia, and could be administered for children with congenital heart disease (The PREVENT Study Group 1997).

The passive immunoprophylaxis by monoclonal humanized antibodies - palivizumab, has some success for the protection of individuals with high risk of RSV infection (Carvalho et al. 2007, Ottolini & Hemming 1997).

The palivizumab, works in binding with the A epitope of RSV’s F fusion protein, preventing the virus fusion with cell receptors, the important mechanism of the RSV pathogenesis (Simões & Groothius 2002). Many safety, effectiveness and cost-benefit studies have been developed and published (Tavares et al. 2005). Passive immunization has been effective and can reduce the hospitalization related to RSV rates up to 55%, especially in preterm children (Vieira et al. 2001, The PREVENT Study Group 1997). The American Academy of Pediatrics recommends that immunoprophylaxis with humanized monoclonal antibodies to be considered in some specific group of children that are prone to the risk of severe RSV infection (American Academy of Pediatrics 1998), such as infants and children with bronchopulmonary dysplasia, prematurity, and hemodynamically significant congenital heart disease (The Impact-RSV Study Group 1998, Pickering 2006). These groups are also considered of greater risk by the Brazilian Society of Immunization (SBIm) guidelines on the use of antibodies (Vieira et al. 2007, Sociedade Brasileira de Imunizações - SBIm 2013, America Academy of Pediatrics 1998).

Studies with a novel monoclonal antibody developed from the affinity maturation of palivizumab with the fusion protein (F) RSV - Motavizumab – are being conducted. Estrany-Carbonell et al. 2010 analyzed the effects of Motavizumab in a large number of preterm found that this was relatively higher in the lower of the low respiratory infections receiving care, with no significant difference in adverse effects when the drugs were compared. Although several promising studies, this therapy is expensive to be used on large scale and for all countries (Simões & Groothius 2002, Rudraraju et al. 2013, Murray et al. 2014).

RSV infects children in their first months of life, which theoretically should be protected by high levels of maternal antibodies (Vieira et al. 2007). One possible explanation for this phenomenon would be the existence of a great variety of circulating genotypes of RSV, whose levels also vary over the years. Some researches believe that a child can be infected by a subgroup which the mother has no antibodies. This hypothesis is supported by the fact that the prophylactic use of synthetic immunoglobulin can protect even young children against infections (Vieira et al. 2007).

Currently strong evidence appoints to the fact that re-infection could occur and probably for the same genotypes of RSV, which usually results in milder disease (Rudraraju et al. 2013). Risks of infection are inversely associated with serum neutralizing antibody titers-specific (Rudraraju et al. 2013).

There is also evidence that RSV may cause persistent infection. Persistence has been demonstrated in animals. Persistent year-round RSV detection in patients with chronic obstructive pulmonary disease (COPD) is associated with airway inflammation and accelerated decline in FEV1 (forced expiratory volume of the first second), an important parameter of pulmonary function. (Olszewka & Openshaw 2008, Valarcher et al.2001).

These and other factors discussed, do the anti-viral treatment and development of vaccines a complex issues.

In the absence of active immunization available, the criteria still remain restricted to the use of passive immunization and specific treatments for trial. The approach of RSV infection remains at the level of prevention, focusing on reducing pre-natal risk factors, as prematurity, passive smoking and improving hygiene practices.

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