Chapter 12
Human Respiratory Syncytial Virus: Biology, Epidemiology, and Control

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1 Introduction

Acute respiratory infections (ARIs) are the most frequent infectious disease in humans, and the great majority of respiratory infections observed in medical practice around the world are of viral etiology [3, 34, 80]. During the period of 2000 to 2003, an estimate of 10,600,000 children under the age of 5 years died every year, and ARIs were responsible for nearly 19% of these deaths. Most of these fatalities were caused by bronchitis and pneumonia associated with viral infections [14, 57, 106, 119]. Viral respiratory infections are also associated with high morbidity in this age group worldwide. For example, 35% of hospitalized children in Brazil, 35% in Belgium, 22% in Italy, and 59% in the UK attending pediatric services were the result of viral respiratory infections [78, 84, 85, 87, 98].

Currently, the following viruses shall be considered causes of acute respiratory illness in children: human respiratory syncytial virus (HRSV); parainfluenza virus types 1, 2, 3, and 4 (PIV1, PIV2, PIV3, PIV4); influenza virus types A, B, C (IA, IB, IC); adenoviruses (ADV); coronaviruses HCoV-OC43, HCoV-229E, HCoV-HKU1, and HCoV-NL64; human rhinovirus (HRV); some subtypes of enterovirus (HEV-68); human metapneumovirus (hMPV); human bocavirus (HBoV); and WU and KI polyomavirus (Fig. 12.1). However, some viruses present high rates of co-detection,

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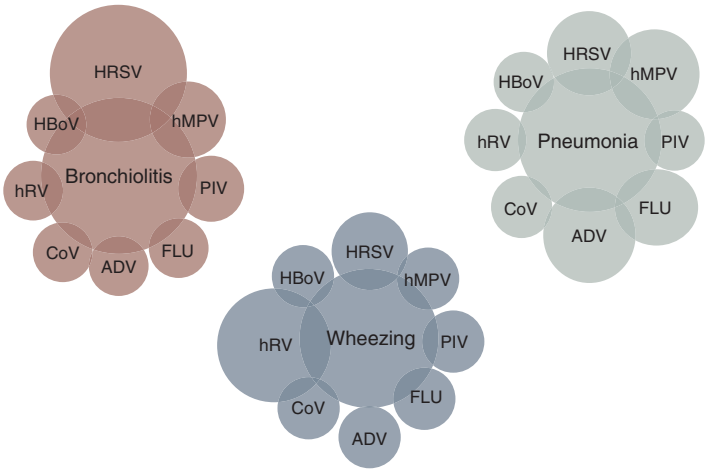

Fig. 12.1 Clinical association with major symptoms of disease and respiratory viruses. In South America, studies conducted in different regions of the country indicate the importance of viruses as etiological agents of low respiratory infection (LRI). These studies revealed the presence of different respiratory viruses in children and adults, such as human respiratory syncytial virus (HRSV), influenza A and B, HPIV, HAdV, HRV, hMPV, hBoV, and HCoV; the percentage of cases among children for some type of respiratory viruses ranged between 28.75% and 75%, whereas positivity in adults was 61.8% for at least one of the viruses studied [11, 15, 35, 39, 40, 85, 105, 111, 116]

as is the case of rhinovirus, enterovirus, human coronavirus and bocavirus, and polyomavirus, being questioned for its significance in the etiology of these infections [10, 45, 49, 64, 89, 91, 96, 114].

The seasonality of respiratory viruses is described in several studies, with some viral infections taking place throughout the year, such as influenza virus, with a predominance in the winter months [96], and others occurring chiefly in the late fall, winter, or early spring, such as HPIV, hMPV, HCoV, and HRSV [85, 99, 105, 114]. Adenoviruses are found worldwide and can circulate sporadically, endemically, or epidemically in the winter, spring, and early summer [103, 105].

Despite the great number of viral agents involved in respiratory infections and their importance, the HRSV is the leading cause of acute respiratory infections and one of the leading causes of hospitalization and death among children under 5 years of age worldwide. Each year, respiratory syncytial virus (RSV) infections lead to 2,100,000 outpatient attendances and 57,257 hospitalizations of children less than 5 years of age in the U.S. Additionally, RSV is responsible for 177,000 hospitalizations with 14,000 deaths among adults over 65 years of age [24].

Newborns, premature infants, and those with chronic lung disease are at greater risk of developing severe disease by infection with HRSV [48]. Despite their importance, there is no vaccine prophylaxis against HRSV infection or effective antiviral therapy available. Currently, in Latin America, only palivizumab (Pz) (Synagis; MedImmune, Gaithersburg, MD, USA) is being used in the prophylaxis and therapy of these infections [68].

2 History of the HRSV

In 1955, an outbreak of respiratory illness characterized by coughing, sneezing, and mucopurulent discharge was described in a colony of 20 chimpanzees at the Walter Reed Army Institute of Research (WRAIR) (Washington, DC, USA). During that episode, the RSV was isolated for the first time from a swab from the throat of a female chimpanzee and then called the chimpanzee coryza agent (CCA) [82]. Viral isolation was performed in liver cells, later being inoculated in various laboratory animals, mice, hamsters, rabbits, rats, and chimpanzees, the latter being the only ones to develop specific symptoms.

One of the attendants of the chimpanzees became sick and developed symptoms similar to those of the animals. Although the attempt at isolation of human respiratory syncytial virus was unsuccessful, an increase in antibody titer by complement fixation against CCA was detected. Parallel seroprevalence studies conducted in a human general population revealed the presence of antibodies to a new CCA agent in teenagers and adults.

The following year, Chanock and colleagues isolated a virus similar to the CCA of a child with pneumonia and another with croup, in Baltimore [25, 26]. The agent was named human respiratory syncytial virus, HRSV, to reflect its ability to form syncytia in cell culture and its tropism for the human respiratory tract.

Serological studies carried out at the time indicated that the majority of children in Baltimore had been infected with HRSV before 4 years of age. Similar investigations in diverse parts of the world indicated that the HRSV was associated with diseases of the lower respiratory tract [33]. Currently, HRSV is recognized as the viral agent more frequently related to cases of bronchiolitis and pneumonia during infancy and preschool age. About 95% of the children have the first HRSV infection in the first 2 years of life, and the peak incidence occurs in the first few months [3]. Approximately 40% of children develop symptoms of lower respiratory tract involvement during the first infection. Although reinfections are common during a lifetime, the clinical symptoms in older children and adults are less severe [55].

Some groups of patients are at risk of developing serious illness resulting from the lower respiratory tract infection by HRSV; these include children younger than 6 months of age, premature infants, immunodeficient children, and children with chronic lung disease or congenital heart disease [33]. There are also studies relating the HRSV to severe infections in the elderly [44, 117].

3 Classification

The human respiratory syncytial virus (HRSV) is a member of the order *Mononegavirales* (mono, from Greek, meaning “single, simple”; nega, from Latin, meaning “RNA negative polarity”; virales, from Latin, meaning virus), classified within the *Pneumoviridae* family and the genus *Orthopneumovirus*. Other members of the *Orthopneumovirus* genus are the bovine respiratory syncytial virus (BRSV) and the pneumonia virus of mice (murine pneumonia virus) [125].

4 Structure

The virion is pleomorphic with a diameter of 150–300 nm and is composed of an internal nucleocapsid of helical symmetry and an envelope derived from the host cytoplasmic membrane; viral glycoproteins that protrude from the envelope as 11- to 20-nm projections, separated by intervals of 6–10 nm, are involved in the processes of adherence and penetration of the virus. The viral genome is composed of a single-stranded RNA molecule, not segmented, and with negative polarity. Each infectious particle contains only one functional copy of the genome (Fig. 12.2) [33].

5 Genomic Organization

The virus contains a single-stranded negative-sense RNA genome with 15,222 nucleotides (nt), with molecular weight of 5×10^6 Da, which serves as a template for transcription of messenger RNAs (mRNAs), encoding for 11 proteins. The genome transcription takes place in the 5′ → 3′ direction. The 3′-region of the genomic RNA consists of a region of 44 nucleotides that presumably contains the viral promoter [81]. The first 30 nt in this region are highly susceptible to inactivation by the insertion or deletion of nucleotides. This region is followed by 10 genes that encode 11 proteins, in the following order: *NS1*, *NS2*, *N*, *P*, *M*, *SH*, *G*, *F*, *M2*, *L*. The last gene, *L*, is followed by a region that is more tolerant to the insertion or deletion of nucleotides [81].

The first nine genes are separated by inter-gene regions ranging from 1 to 52 nt in size, which apparently do not have an important role in the modulation of gene expression and show little conservation among isolates [31, 71]. The beginning of each gene contains a conserved signal (gene start signal) composed of nine nucleotides, 3′-CCCCGUUUA,

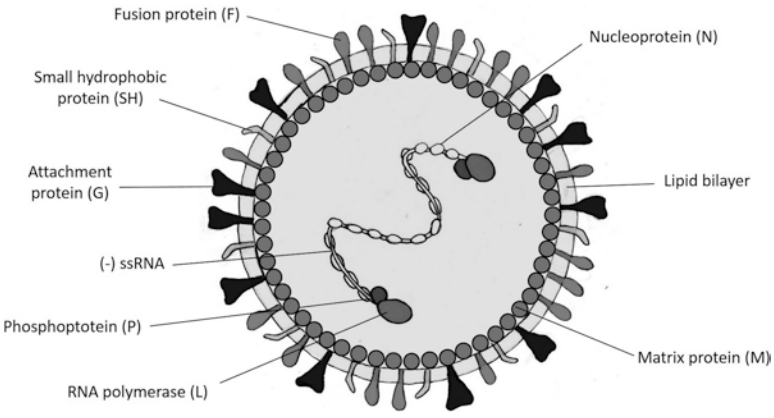

Fig. 12.2 Human respiratory syncytial virus (HRSV) virion structure

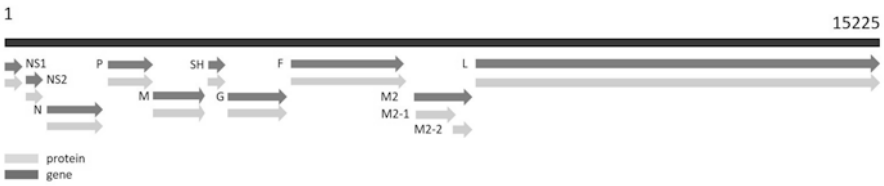

Fig. 12.3 Organization of the gene in the genome of HRSV. The genome is 15,225 nucleotides long, a single-stranded RNA with negative polarity. It has 10 genes encoding 11 proteins. The *M2* gene has two products: a nucleocapsid-associated transcription factor (M2-1) and another protein involved in genome replication (M2-2)

except for the *L* gene, which presents the following differences as underlined: 3′-*CCCUGUUUUA*. The genes end with a semi-conserved sign (gene end signal) composed of 12 or 13 nucleotides whose sequence is 3′-UCAAUNAAAUUU, which drives the end of transcription and polyadenylation. The last two genes, *M2* and *L*, have in common 68 nt. Consequently, the gene *L* has the initiation of the transcript inside the M2 gene [33]. The M2 has two ORFs (open reading frames), which give rise to proteins M2-1 and M2-2. The organization of the gene of HRSV is schematized in Fig. 12.3.

6 Proteins

In cells infected with HRSV, 11 proteins have been identified. Of these, 2 are nonstructural proteins, NS1 and NS2, present in abundance in the cells, but in small amounts in the virion. The others are structural proteins, M (matrix) and M2-1(transcriptiol elongation factor) proteins, N (nucleoprotein), P (phosphoprotein), L (large polymerase), and M22 nucleocapsid viral proteins, and 3 are surface glycoproteins G (attachment), F (fusion), and SH (small hydrophobic) [33]. The glycoproteins F and G are highly accessible to neutralizing antibodies, resulting in numerous changes in response to the host immune pressure [34] and therefore are the most studied.

The NS1 (molecular weight PM, 15.5 kDa) and NS2 (PM, 27 kDa) proteins have, respectively, 139 and 124 amino acids, and the genes that encode these have 532 and 503 nucleotides, respectively. Their functions are not well understood, but it is presumed that they are related to the structural regulation of RNA synthesis, the morphogenesis of the virion, or the interaction with the host cells [33].

The proteins P, L, and N are associated with genomic RNA and nucleocapsid, forming the ribonucleoprotein complex, considered as the minimum unit necessary for transcription and replication of the virus. The P protein is highly phosphorylated and acidic and has a key role in the regulation of the transcription and replication process. It has 241 amino acids and a molecular weight of 35 kDa, and the gene that encodes it has 914 nucleotides. The nucleoprotein N has 391 amino acids and a molecular weight of 43.4 kDa, and the gene that encodes it has 1203 nucleotides and is the main structural protein of the nucleocapsid, closely associated with the genomic RNA. The L protein, consisting of 2165 amino acids with a molecular weight of 250 kDa, is the largest viral protein. The gene that encodes the L protein has 6578 nucleotides [33].

The M proteins (PM, 27 kDa) and M2-1 (PM, 22 kDa) are internal and not glycosylated, possessing, respectively, 194 and 256 amino acids, and the genes that encode them have 958 and 961 nucleotides, respectively. The M protein mediates the association of nucleocapsid with the viral envelope [33], and the M2-1 acts on the elongation during transcription [32].

The SH protein is a small molecule (amino acids and 64 PM, 7.5 kDa), which is inserted in the cytoplasmic membrane of the host cell via a hydrophobic sequence, ranging from 14 to 41 amino acids. The function of this protein has not yet been clarified; however, because it is integrated in the membrane, it is assumed to be involved in adsorption, penetration, and denudation of the virus [33].

The glycoprotein F has 574 amino acids with a molecular weight of 70 kDa, and the gene that encodes it has 1903 nucleotides. Identified as a fusion protein, it is responsible for the attachment of the viral envelope with the plasma membrane of the host cell, releasing nucleocapsid directly within the cytoplasm. Also, it is responsible for the fusion of the cell infected with neighbor cells, favoring the formation of the syncytium [33].

The F protein is synthesized as an inactive precursor called F0, which consists of two domains, F2 (1–130 amino acids) and F1 (137–574 amino acids), and also has a cleaved peptide (131–136 amino acids). The F1 subunit is anchored to the membrane. The F1 subunit is relatively well preserved and is greatly affected in its function by deletions or substitutions of amino acids [33].

The glycoprotein G is a type II protein, which is anchored to the membrane next to its amino-terminal portion by a hydrophobic domain, non-cleaved, signal anchor type, that extends from residues 38 to 66 [73]. The G protein is 289 to 342 amino acids in length, depending on the viral strain. The G gene is composed of 918 to 1062 (group A) or 921 to 981 (group B) nucleotides [43, 102, 108].

The glycoprotein G is the viral attachment engaged in the adsorption of virus, and it has been shown that antibodies against the G glycoprotein inhibited the binding of virus to the cells [72]. The glycoprotein G is of special interest for showing the largest variability between the viral isolates [4, 52, 67] and can support large deletions or multiple amino acid substitutions without loss of function [43, 102]. This variability among strains of HRSV is a signature feature that can alter the pathogenicity and adaptation of the virus and contribute to the ability of the virus to cause repeated infections and outbreaks by escaping the immune system. The glycoproteins F and G are the most important proteins involved in a protective immune response [8, 66], and antibodies against them show strong neutralization activity in vitro [2, 123].

7 Replication

The cell receptor specific for the glycoprotein G was first identified by Krusat and Streckert [70], who showed that preincubation of the virus with heparin inhibited the infection in cell culture and that the G protein binds heparin. These results suggest that heparin or other glycosaminoglycans (GAGs) similar to heparin, present

on the cell surface, are involved in the binding of the virus to the cell. The binding site of the glycoprotein G to the heparin (or another GAG) was mapped between the 184 and 198 amino acids of the protein G for group A and among the 183 to 197 amino acids for group B. Martinez et al. [77] confirmed that the presence of these receptors is critical for the binding of the virus.

The virus enters into the cell through fusion with the cell membrane. After penetration, the viral envelope remains as part of the cell membrane. The nucleocapsid is released into the cytoplasm and begins the process of transcription of the viral genome by the viral polymerase. The genes are transcribed in sense 3′ → 5′ with a sign promoting to the 3′-side [33]. The peak of the synthesis of mRNAs occurs 16 h after infection, and the peak of proteins occurs at 18–20 h [6, 33]. In addition to the transcription and translation of proteins, another important step is the replication of the viral genome, which produces an intermediate positive (+ ssRNA), which will serve as a template to generate more copies of the viral genome (ssRNA). All the replication process takes place in the cytoplasm [33].

The maturation of the virus occurs in the first instance, with the combination of proteins N and P to the genomic RNA and subsequent addition of other auxiliary proteins to the nucleocapsid. The surface glycoproteins are inserted into the cytoplasmic membrane of the host cell. In the next step the matrix protein interacts by noncovalent forces to the cytoplasmic tails of the surface glycoprotein. The assembled internal structures of the virus interact with this surface and drive the budding, with the release of the virus, when the virus acquires the lipoprotein envelope [69].

8 Genetic Variability

The variability of the G protein is concentrated in the extracellular domain, where two variable regions have a high content of serine and threonine, between 69 to 164 and 207 to 298 amino acids, with approximately 56% divergence between groups A and B [66, 67]. Interspersing this region of high variability, there is a conserved region with a small segment of 13 amino acids (164–176) and four cysteine residues (C173, C176, C182, C186), which are well preserved in all samples of HRSV [97, 118], suggesting that this region is responsible for binding the virus to a cell receptor. However, data about the region for genotypes that emerged after 2010 are currently lacking.

The genotyping of HRSV-A and HRSV-B is based on the variability of the G-protein gene. For HRSV A, 11 genotypes were reported and designated as GA1, GA2, GA3, GA4, GA5, GA6, and GA7 [92, 93], SAA1 (South Africa, A1) [115], and more recently, NA1, NA2, NA3, and NA4 [102]. For HRSV-B, 17 genotypes have been described and designated as GB1, GB2, GB3, and GB4 [93], SAB1, SAB3 [115], BA1–BA6 (Argentina) [109], BA7–BA10 (Japan) [38], and B11 (Korea) [7]. Interestingly, strains belonging to genotype BA of HRSV-B exhibited duplication of 60 nucleotides (nt) in the second variable region protein gene *G*, but were not associated with more severe clinical manifestations [38, 108]. In Brazil, the only genotypes circulating currently from HRSV-A are NA2, NA3, and ON1 and BA genotyping from HRSV-B.

In 2012, Eshaghi et al. [43] detected in group A one repetition of 72 nucleotides (GTCAAGAGGAAACCCTCCACTCAACCACCTCCGAAG GCTATCTAAGCCCA TCACAAGTCTATACAACATCCG) in the C-terminal portion of the gene (G), being the largest duplication described in this group. This new genotype was called ON1 and was found in 10% of HRSV isolates. In 2013 this ON1 genotype was found in 75% of all isolates in Brazil [42, 80], and in 2015 the ON1 genotype had attained natural dominance and become the predominant genotype circulating in different areas of the world [107]. This area is specifically targeted for neutralizing antibodies, and these types of changes of structure can lead to changes in immunogenicity and pathogenicity of the virus. However, additional studies are still required to explore the pathogenicity, transmissibility, and replication of this new variant.

9 Epidemiology

In the 1990s several studies of molecular epidemiology were conducted based on partial sequences of genes *G* and *SH* and a restriction map of the *N* gene, enabling reaching some important conclusions about HRSV circulation:

1. The existence of several genotypes circulating concurrently in a single outbreak, with a predominance of one or two genotypes which tend to decrease in subsequent outbreaks until its disappearance [17, 19, 20, 27, 28, 30, 65, 75, 92, 93].
2. The genotypes of HRSV have worldwide distribution, and strains isolated in distinct communities and in different years may be more related to strains isolated in the same locality in two consecutive days, demonstrating a pattern of temporal and not necessarily geographic circulation [18, 50].
3. Within each strain (genotype) occurs a progressive buildup of amino acid changes [21].
4. Antigenic changes detected with a panel of anti-G monoclonal antibodies can be correlated with the position of the viruses in the phylogenetic trees [50].
5. The synonymous nucleotide substitutions have a uniform distribution over the *G* gene, and non-synonymous substitutions are accumulated in the two variable regions of the gene *G* [21, 50].

However, there are studies in which a minimal temporal variation in the gene encoding the G protein has been reported. A study performed in Cuba revealed the movement of extremely homogeneous samples during the 1994–1995 outbreaks, with a difference of just five nucleotides when compared to the sample long since isolated in 1956 [113].

The significance of the antigenic variation of HRSV groups in epidemiology is not yet clear. The antigenic dimorphism, although at modest rates, seems to contribute to the high incidence of reinfections during the first years of life. However, several reinfections in children involving viruses of the same group have been reported [60, 83]. In addition, there is no indication that reinfection with a heterologous group induces more serious clinical signs than reinfection with homologous samples [110].

The two groups (A and B) have been circulating concurrently in many epidemics for more than 20 years [12, 65], in diverse regions of the world, and with incidences that vary from year to year. Studies conducted in El Salvador, Santa Fe, and Buenos Aires in Argentina revealed the presence of both groups during outbreaks with prevalence of group A [23, 63, 121].

In some localities, such as Rochester and Boston in the U.S., Sapporo in Japan, and Rio de Janeiro, Porto Alegre, and Ribeirão Preto in Brazil, in addition to the co-circulation of the groups, the prevalence of groups A and B may switch over the years or show a balance of the frequencies of both groups [29, 56, 58, 104, 112].

Differences in pathogenicity between the two groups are not clear. Hall et al. [56] and Imaz et al. [63] verified increased severity in children infected with group A, although Zelaya et al. [121] found greater severity in children infected with group B. Other authors did not observe significant differences in pathogenicity between the groups [29, 104].

In a study carried out in Bogota, Colombia, a total of 13,488 samples of children hospitalized with a diagnosis of respiratory infection were tested for RSV during 5 years and 4,559 (33.8%) were found positive. The average age of patients analyzed in the study was 9.2 ± 8.5 months, and 71.7% of cases of HRSV infection occurred in the period from March to May, whereas 50% of the bronchiolitis cases were diagnosed from April to June during the years of the study [47].

In Chile, HRSV are detected as a single pathogen at 74/124 (58.7%) samples of nasopharyngeal aspirate of patients, and 28/124 (22.6%) samples were co-detected with HRV. Hospitalization was necessary in 77% of positive cases of HRSV (57/74), and 44.6% of these cases were considered serious; 53.6% (15/28) of cases coinfected by both viruses were hospitalized, too, but this coinfection does not increase the severity of illness [74].

In Brazil, many studies have already been carried out to investigate the etiology of acute respiratory diseases [12]. During the period of 2003–2009, nasopharyngeal aspirates were examined in more than 2000 children less than 5 years old, and HRSV were found in at least 42% of positivity between respiratory viruses identified in children hospitalized with acute respiratory disease [85, 105].

In countries in southern Latin America such as Argentina and Uruguay, outbreaks of HRSV occur predominantly during the winter months [22, 61]. In tropical and subtropical climates, the outbreaks are not always well defined, although in Ceara, located in the northeast of Brazil, HRSV caused yearly seasonal epidemics, generally from February until July (Moura et al. 2013). In Brazil, in the cities of Rio de Janeiro and São Paulo, HRSV outbreaks start in autumn (ranging from March to April) and extend until winter (July–August), with peak incidence occurring usually in May (Table 12.1) [85, 105].

Fortunately, fatalities from infection by HRSV are uncommon, and estimates indicate that the number of deaths is around 200–500 a year, 80% of which are of children under 1 year of age. However, mortality may increase significantly in children who present some background that predisposes to more serious diseases, such as congenital heart diseases and lung diseases, and premature infants, in which mortality by HRSV infection is around 10%, 5.5%, and 4.6%, respectively [41, 100]. High mortality rates may also be observed in individuals with immunodeficiency,

Table 12.1 Occurrence of positivity for human respiratory syncytial virus (HRSV) in different cities of Latin America at different times of the year

Country	City	Seasonality	Average of HRSV positive (%)
Argentina	Buenos Aires	Autumn – winter	23–26
Brazil	Tropical	Summer – winter	21–4
	Subtropical	Autumn – winter	42
Chile	Santiago	Winter	22.6–52.7
Colombia	Cali	Throughout the year	33.8
	Medellín	Summer	41.7
Costa Rica	San Jose	Autumn	15–20
Mexico	Mexico City	Winter – spring	36–55
	San Luis Potosí	Autumn – winter	24.8–46.7
Venezuela	Caracas	Throughout the year	31.6–66
Uruguay	Montevideo	Winter	56
Guatemala	Santa Rosa and Quetzaltenango	Autumn	24

congenital or induced by chemotherapy against cancer [54] or from organ transplants, especially in the first 20 days after the transplant [94]. Among bone marrow transplant recipients, the mortality of those who become infected with HRSV can reach 45% [14, 57].

A study conducted in the U.S. revealed the occurrence of 14,000 to 62,000 annual hospitalizations of the elderly with pneumonia associated with HRSV, at a cost of approximately 150,000,000–680,000,000 dollars to the health system and causing about 1,500–6,700 deaths per year (5–20 deaths/100,000) [124].

10 Laboratory Diagnosis

The laboratory diagnosis of HRSV can be carried out by the direct detection of viruses, viral antigens, or the viral genome or, indirectly, based on the detection of specific antibodies. For the routine clinical laboratory diagnosis using respiratory secretions as biological samples, the procedures may include viral isolation in cell culture, antigen detection by immunofluorescence or enzyme-linked immunosorbent assay, and viral RNA detection by reverse transcriptase (RT)-polymerase chain reaction (PCR). The best samples are those obtained by aspiration or washing nasopharyngeal secretions [76, 79]. The viral particle present in the secretions is highly labile, and the samples should be kept refrigerated during transportation to the laboratory and processing before inoculation in cell cultures.

The isolation in cell culture, regarded as the gold standard, can be carried out in a wide variety of human and animal cell lines, but HEp-2 and HeLa cells are the most used [110; Perini et al. 2007]. The cytopathic effect usually appears within 3–7 days after inoculation and is characterized by the presence of large syncytia resulting from cell fusion (Fig. 12.4). Nevertheless, as viral isolation in cell culture is difficult, the

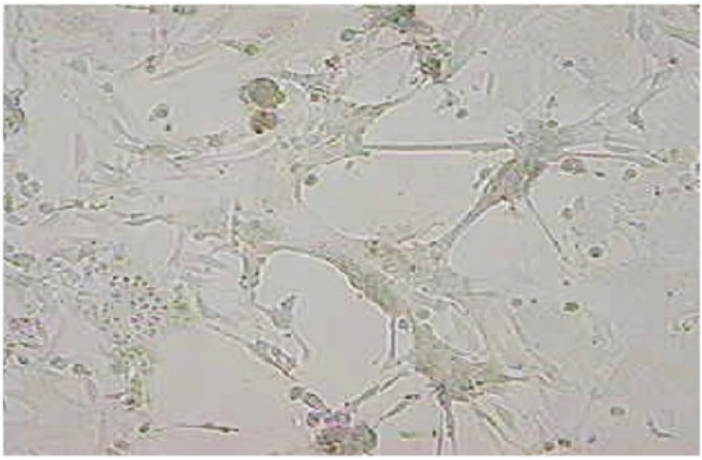

Fig. 12.4 Cytopathic effect of HRSV in HEp-2 cell line shows a large syncytium resulting from a fusion of the cells

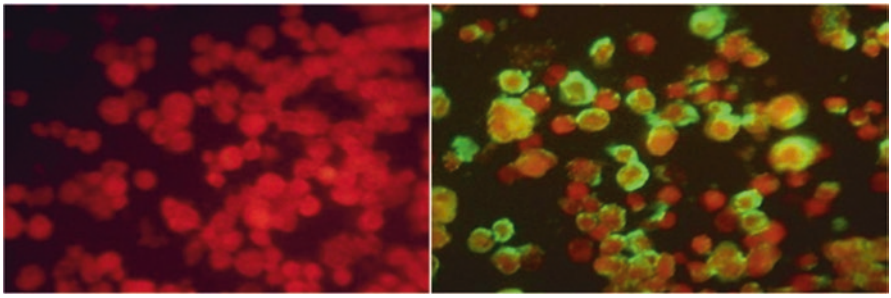

Fig. 12.5 Results of the indirect immunofluorescence (IFI) assay for detection of HRSV in nasopharyngeal aspirates. *Red*, negative results; *green*, positive detection of HRSV in the cytoplasm and membrane of the cell

diagnosis of infection is most often accomplished by detection of HRSV antigens in nasopharyngeal epithelial cells by immunofluorescence (Fig. 12.5) or enzyme-linked immunosorbent assay, faster methods that do not require the presence of infectious viral particles. These last two methods require the adequate preparation of the specimens by removing excess mucus. Finally, success of the immunofluorescence technique, aside from well-trained personnel and well-prepared samples, requires a minimum number of infected cells to enable a correct diagnosis [110].

Hughes et al. [62] compared the three diagnostic techniques for HRSV: isolation in cell culture, direct and indirect immunofluorescence (IFA), and enzyme-linked immunosorbent assay (ELISA). Both immunofluorescence-based methods detected more positive samples (showed higher sensitivity) than viral isolation. However, 15% of the samples found positive by viral isolation were negative by immunofluorescence, demonstrating the need for the use of at least two diagnostic methods.

The RT-PCR technique has been used both for the diagnosis [51, 59, 117] and for typing a sample to group A or B [53, 122]. It is considered a highly sensitive technique, especially useful in the diagnosis of infections, in which both the sample amount and the viral load in the sample are small, as is the case of samples taken from the elderly [117]. In the past decade, the molecular methods were considered as a gold standard, because of their specificity and ability of simultaneous detection of different viruses [90]. The advances in real time-RT-PCR (quantitative (q)RT-PCR) specificity and sensitivity for the detection of HRSV in clinical samples became more suitable for diagnosis in clinical laboratories [46].

The rapid antigen detection tests (RADTs) are dipstick-based immunoassays that allow for the rapid, qualitative detection of RSV antigen (viral fusion protein) directly from nasopharyngeal swab, nasopharyngeal aspirate, or nasal/nasopharyngeal wash specimens from symptomatic pediatric patients. The RADTs provide a result in 15 min, compared to approximately 90 min for a conventional IFA test and 2–3 h for ELISA [80]. Rapid tests may also be used as a point-of-care assay. These methods, although effective, may present several drawbacks, including price and skilled personnel. All these issues pose a challenge to hospitals and pediatric clinics to apply the best medical management for monitoring or treatment of children with suspected infection.

Serological diagnoses can be made through neutralization assays, complement fixation, or determination of class-specific immunoglobulins (IgG, IgM) by ELISA or immunofluorescence techniques. The diagnosis is based on the increase in antibody titer between acute and convalescent titers, performed in serum or saliva [110, 120]. The serology offers limited value in the diagnosis of primary infection in children less than 6 months of age because 40% of these cases present no increase in antibody titer. However, in infants and adults, the serology is regarded as a good indicator of reinfections [55]. The serology, therefore, is not the most appropriate method for diagnosis of infection by HRSV, having, however, great importance in clinical and epidemiological studies [36].

11 Treatment and Prevention

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a nucleoside analogue of the guanosine, licensed since 1986, is the treatment of choice for RSV. Its use is indicated in the form of aerosol for the treatment of serious diseases caused by HRSV. Although several studies have demonstrated the effectiveness of ribavirin in inhibiting replication of the virus and the improvement of clinical conditions, resulting in a decrease in the need for supplemental oxygen and mechanical ventilation in children with lower respiratory tract infection, chronic lung disease, and infection in immunocompromised individuals, lately there has been controversy about the benefits of its use. Since 1989, several studies have appeared indicating that the use of ribavirin has minimal effect on disease outcome caused by HRSV, not showing evidence of decreased duration of hospitalization or the need of supporting therapy, in addition to the high cost and extended treatment (12 h or more of inhalation) [13, 37].

Several drug candidates have been studied in the past decades, including several inhibitors, targeting different HRSV proteins. Despite these efforts, until the present time there has been no antiviral drug approved for treatment (Heylen et al. 2017).

Development of an RSV vaccine has been hampered by the incidence of enhanced respiratory disease (ERD) following vaccination with formalin-inactivated RSV in the 1960s. Since its failure, multiple live virus vaccines have been developed, as well as other vaccine platforms, including virus-like particles, peptide-based vaccines, protein subunit vaccines, and plasmid DNA-based vaccines. Many of these vaccines have been evaluated in animals, and a few have been studied in humans. None, however, has shown sufficient promise to move toward licensure. It is clear that a better understanding of virus and host factors that contribute to both disease and protective immunity is still necessary to develop safe and effective RSV vaccines.

Alternative approaches to identify vaccine-relevant epitopes include the identification of neutralizing RSV protein epitopes to which a protective immune response can be safely generated and the development of modern pre- and post-RSV fusion (F) protein subunits. One obstacle to developing an RSV vaccine has been the difficulty in inducing long-term protective immunity, as evidenced by the repeated infections throughout life and the incomplete protection afforded to recipients of immune prophylaxis. In addition, an immunogenic approach targeted to a single neutralizing epitope mapped to the site A region may generate a focused immune response against RSV F, but in general, the polyclonal response generated by site A-based vaccines has been characterized by poor binding to intact RSV F protein, modest in vitro neutralization, and no evidence of protection to RSV challenges in vivo.

Palivizumab (Pz) (Synagis; MedImmune) is a humanized IgG monoclonal antibody that neutralizes HRSV through interaction with the HRSV F glycoprotein. Pz is the only FDA-approved prophylaxis against HRSV infection [5, 101]. Five monthly Pz injections spanning the annual HRSV epidemic period have been shown to reduce hospitalizations among high-risk children in the U.S. However, the quasispecies nature of RNA viruses allows rapid emergence of escape mutants to the immune pressure. The increasing use of Pz in high-risk children and immunocompromised patients provides opportunities for Pz-resistant mutants to arise and persist among humans [1, 9, 86, 88, 123]. However, little is known of these mutations in patients who did not use Pz.

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