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REVIEW

VIRAL RESPIRATORY CO-INFECTIONS. IMPORTANCE OF NEWLY DISCOVERED VIRUSES IN PAEDIATRIC RESPIRATORY PATHOLOGIES

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Abstract. Respiratory infections represent a major cause of morbidity and mortality in children. The roles of viruses such as respiratory syncytial virus, paraflu and adenovirus are well known in the etiology of respiratory infections. Lately, many studies displayed the implication of Rhinoviruses and Coronaviruses in inferior tract infections, where, sometimes, they can lead to a severe form of illness. Within the last decade, new respiratory viruses were discovered: human metapneumovirus, NL63 and HKU1 coronaviruses and human bocavirus. Viral co-infections are often seen in children hospitalized for acute respiratory infections, this causing more serious forms of disease that require hospital days. Multiplex PCR tests allow a simultaneous detection from the same sample of a large spectrum of viral and bacterial agents, which lay at the basis of respiratory infections.
Keywords: New respiratory viruses, viral co-infections, PCR-RT technologies

Introduction

Viral respiratory infections represent a major cause of morbidity and mortality in children who suffer every year between 3 and 8 viral respiratory infections. Their impact falls on the social and economic life of a country, through increased costs of medical care, namely: consultations, treatment, number of hospital bed days, school absenteeism.

Due to medical, social, demographic and economic implications, viral respiratory infections can be considered important public health problems which require surveillance and control measures.

Currently, around 200 viruses, antigenic distinguishable are known to belong to: Adenoviridae, Parvoviridae, Orthomyxoviridae, Paramyxoviridae, Picornaviridae, Coronaviridae families, which can determine acute respiratory illnesses [1].

Most of these viruses affect the superior respiratory tract, sometimes the lower respiratory tract, especially in children and elder persons. They can also cause self limiting infections localised in the superior respiratory ways and in the lower respiratory tract. Some infections acquired in childhood may lead to asthma or chronic respiratory dysfunctions.

Etiology: the role of respiratory syncytial, paraflu, flu viruses and adenoviruses in the etiology of respiratory infections are accepted at a large scale. Rhinoviruses and coronaviruses, previously known as etiological agents for rhinopharyngitis, caught scientists' attention, after their impact in infections of the inferior respiratory tract was proved. Sometimes, they cause severe disease forms.

During the last decade new respiratory viruses were discovered: human metapneumovirus, NL36 and KHKU1 coronaviruses as well as human bocaviruses which hold a large responsibility in pediatric respiratory infections.

Human metapneumovirus

As with the respiratory syncytial virus, human metapneumovirus (hMPV) belongs to Pneumovirinae sub-family of the Paramyxovirdae family and together with the avian metapneumovirus are classified in Metapneumovirus genre. Its first description appeared in Holland, in 2001 where it was isolated from stored nasopharyngeal secretions, by PCR [**2**].

Since then, the virus was identified in countries from all continents except for Antarctica. Hmpv

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is an enveloped RNA virus, to whom two major groups were attributed: A and B and 4 sub-groups: A_1, A_2, B_1, B_2.

Physiopathology: hMPV infections lead to: low level of cytokines response and a significantly increased LTh_2. The decreased level of cytokines is associated with a lower level of pulmonary inflammation determined by hMPV, compared to the one caused by RSV. The peak of viral load is detectable 4-5 after the virus has been caught. In most cases, viral clearance takes place the following 10-14 days after the infection. Unlike animals, where viable viral particles have been ceased even 2 month after the infection, in humans there are no reliable data on the viral persistence and its meaning.

Two recent studies observed the correlation between viral load (by PCR-RT) and clinical parameters:

One of the studies emphasised that increased VL levels are associated with the impact of the lower respiratory tract and can produce forms of disease that require hospitalisation [3]. The other study focused on the link between high viral load and high fever, increased use of bronchodilators and prolonged hospitalization, regardless of underlying chronic affections [4]

Similar assessments for the identification of RSV could not establish the same relation with the illness severity, implying different pathogen manifestations that the two related viruses.

Epidemiology: hMPV is ubiquitous around the world. The first infection occurs in childhood, most children presenting seroconversion by the age of 5. At 10, 100% of the studied population is seropositive for hMPV; re-infections may occur, while infections can emerge in adults, too. hMPV is detected throughout the year, with the incidence peak towards the end of winter and beginning of spring, later than RSV peak and flu virus.

hMPV was detected in: 1-5% infections of superior respiratory ways, 12% of low respiratory ways infections, 1% of asymptomatic children. These values make hMPV the second cause of infection in the inferior respiratory ways, after RSV, also responsible for 10% of children hospitalisation for respiratory conditions.

Diagnosis: hMPV is difficult to cultivate on cell cultures (this explains its tardy identification despite its presence within human pathology, for over 50 years). Serological diagnosis is confirmed through ELISA test which also confirms seroconversion (serological tests are universally positive after first childhood). IF is another method of detection, but the most sensitive one is PCR from respiratory secretions, also known as the most spread diagnosis method everywhere. This procedure is also used to establish viral load.

Treatment: there is no specific antiviral treatment for hMPV. Treatment includes symptomatic drugs and respiratory assistance for severe forms.

Ribavirin has an extinguished antiviral action and, allegedly, it is efficient against in vitro hMPV. Tests performed so far on animals proved the decrease in viral replication and remission of inflammatory events, but it has not been tested on humans, yet.

Human bocavirus

In 2005 in Sweden, a new member of Parvoviridae family, named human bocavirus (HboV) was discovered, due to its similarity with canine and bovine parvovirues. This virus was discovered in respiratory secretions, through new molecular techniques- PCR **[5].** HboV was later discovered all around the world, with a rate of prevalence between 1.5% and 18% [6]. HboV is predominant in children and is associated with: inferior respiratory tract infections and gastrointestinal infections; it has small dimensions - 20 mm **[7]**, non-enveloped, with cleaved genomic DNA. Infections caused by HboV are present throughout the year, with incidence peak during winter.

Physiopathology: replication takes place at high titres, at the level of epithelial cells, in the respiratory tract. Viral DNA was detected in the peripheral blood of children suffering from respiratory infections, in both acute phases as well as in convalescence. Viral load decreases once symptoms efface. HboV is frequently identified in the presence of other viral infections. Often, viral load of the associated virus is lower than HboV, which speaks for the pathogenic importance of the latter. Allegedly, the presence of the associated virus favours viral replication of HboV.

Studys:

Study 1 deployed in Italy, during November 2004 – May 2007 [8]. The subjects included in the study were children below 14, admitted with several diagnostics: laryngotracheobronchitis, bronchiolitis, asthma, bronchopneumonia. The patients' lot contained 415 cases, which were tested for the presence of 14 respiratory viruses, by PCR. **Results:** for 214 cases (51.6%) pathogenic agents were identified: 28.9% RSV (120 children), 9.6% Rhinoviruses (40 children), 8.2% HboV (34 children); in 21 children HboV was identified in viral co-infection, especially with RSV. **Conclusions:** Age distribution exposed the highest rate of HboV infection in newborns: 4-12 months (88% of cases).

In the area of frequency, HboV ranks the third place, after RSV and Rhinovirus. HboV was the only pathogen agent for 4 cases of pneumonia and 6 cases of bronchiolitis.

Study 2 was deployed in Spain during Septem-

ber 2005 – August 2007 **[9].** The prospective study included 710 children below 14. The focus was on the frequency of HboV and other 15 respiratory viruses in nasal samples and on the clinical evolution of HboV determined infections, compared to those caused by common respiratory viruses. **Results:** In 435 cases (61.2%), etiological agents were identified in 99 samples (13.9%). As sole virus, HboV was detected in 5 cases. The age of patients with HboV infection was smaller or equal to 26 months (75%). Most common diagnosis was recurrent wheezing, followed by bronchiolitis. **Clinical comparisons:** In RSV infections, children have older ages and bronchiolitis are less frequent. Unlike adenovirus infections, fever occurs less within the HboV group, while compared to rhinovirus infections, hypoxia is sporadic for the same group. **Conclusions:** In the area of frequency, HboV ranks the 4th place, after RSV, Rhinovirus and Adenovirus. Clinically, HboV is associated with recurrent wheezing and bronchiolotis. In relation to common viruses infections, there are differences between clinical manifestations, especially from the diagnosis point of view, fever and age.

Study 3 took place in Spain, during September 2008- December 2006 **[10].** The prospective study included 908 children, less than 14 and surveyed the rate of incidence for HboV and another 15 viruses by screening nasopharyngeal samples, from children with respiratory problems and asymptomatic children. **Results**: At least one virus was caught at 587 patients (64.4%); HboV was identified at 153 cases (16.8%), occupying the second place by incidence rate, after RSV. HboV infection was detected throughout the entire year, with an incidence peak during winter months. 50% of HboV patients were over 14 months, with an usual diagnosis of recurrent clearing (51%), followed by bronchiolitis (30%). 55% of cases needed oxygen, while 70% experienced high fever. **Conclusions:** Besides respiratory ones, other clinical manifestations associated with HboV infection developed, namely rash petechiae and thrombocytopenia. In 99 cases (65%) of HboV infection, a viral co-infection was also present, but no significant differences were recorded within the clinical panel. HboV was identified at 5% of asymptomatic children.

Coronaviruses

CoV are enveloped RNA viruses, with a 75-160 mm diameter that are part of Coronaviridae family. Due to surface projections that cross the envelope, with a length of 12-24 mm and an end button, virions hold a unique morphology. These elements render the virus a crown form, visible through electronic microscopic images.

Currently, there are 5 types of known CoV associated with respiratory tract infections in humans. For many years, scientists had knowledge of only 2 types of CoV: HCoV 229E and HcoV-OC43, discovered in 1966-1967, as determinant agents for "common colds", respectively of auto-limited infections of superior respiratory ways [11].

After almost 40 years SARS occurred- Severe acute respiratory syndrome- adding another coronavirus to the list: SARS-CoV. During November 2002 – July 2003, 8096 cases of SARS were reported, of which 774 deaths increased the mortality rate up to 9.6%. Originally stemming from Guandong region in China, the virus spread around the world, with specific cases of infection in 37 countries.

Once CoV's high pathology proven, efforts were made to identify and describe new types, hence two new members of the CoV family:

- 2004: HCoV – NL 63 which subsequent studies identified in all parts of the world. It is associated with croup, namely with obstructed inferior respiratory ways targetting especially children younger than 2.
- 2005: the 5th coronoavirus was discovered HCoV – HKUD, in two female patients with pneumonia by a research team in Hong Kong.

Infections with coronovaruses can determine illnesses that require hospitalization in children, immunocompromised adults, and elder persons.

Studies carried out in Holland demonstrated that 13 newborns presented, after check-up, maternal anti bodies- NL63 and anti 229E, which disappeared by the time they turned 3 months. Seroconversion was detected in most children, by the age of 3,5 years **[12].**

Another study from Australia published in 2005 followed through PCR investigation the role of recently discovered viruses, respectively HCoV-HKU 1 and HboV in pediatric populations admitted in hospital for respiratory tract illness. The two viruses made a 21% contribution to the etiology of studied cases: HCoV-HKU 1 (31% of cases), HboV (5.6% of cases). These values are significantly higher than those reported by publications which exposed HCoV-HKU 1 in Hong Kong and HboV in Sweden.

Discovering these viruses opened the path to establish aetiology for an important number of respiratory ways infections, which, otherwise, would have remained undiagnosed, this being an important step for the study authors. They recommend including the new discovered viruses in the routine screening of respiratory infections **[13].**

Viral co-infections

A study deployed in **China**, published in 2009 in Virology Journal set as objectives to examine

epidemiology and clinical manifestations of mixed viral infections in children admitted for acute respiratory infections, during May 2008- April 2009 **[14]**. This retrospective study used IFI method to detect pathogen viral agents: RSV, Adenovirus, A and B Flu viruses, Paraflu virus 1,2 and 3. **Results:** Pathogen viral agents were discovered in 164 (51.9%) of 316 children included in the study. The exclusion criteria were the presence of any sign of bacterial infection. In 50 children (15.8%) one etiologic agent was detected, while in 114 children (36.1%), two or more agents were discovered. Most frequently observed etiological agent was flu virus A, for 97 cases, followed by flu virus B in 91 cases, adenovirus in 77 cases and hMPV in 53 cases. Of 114 cases of multiple infections, the most repeated association was with flu viruses A and B, followed by adenovirus+ flu virus A+flu virus B. **Conclusions**: although the study limits were set by the laboratory method, etiology for 51.9% of cases was established. The authors mention that a large part of research on double respiratory infections should use at least two identification processes. Brunstein showed that IFD combined with multiplex molecular tests is the best method to diagnose respiratory infections. Likewise, results show that there were no significant differences of associated clinical parameters connected to infections with one or multiple pathogen agents

Another **study** deployed in **Germany** in 2005-2006, published in 2008 by Pediatric Infections Diseases targeted the observation of respiratory viruses' incidence rate in 254 children, hospitalized in the Pediatric Ward, for acute respiratory infections, within 10 months **[15]**. In order to detect 12 viruses the method of diagnosis was PCR and PCR-RT. **Results**: 65% of subjects tested positive for at least one pathogen agent, 16% of the total cases were positive for at least two or more viruses.

The 254 cases presented:

- RSV - 112 cases (44%) (most detected virus)
- HboV – 49 cases (19.3%)
- Rhinovirus - 17 cases (6.7%)
- Viral Co-infections – 37 cases (10 bronchitis, 7 bronchiolitis, 17 pneumonias)

Most occurred viral co-infection was RSV + HboV in 10.6% of total patients.

Conclusions: the role of HboV is quite uncertain, hence the discussions whether it is a commensal or pathogen agent. Due to the fact that 6% of admitted patients were positive only for HboV, it was demonstrated that it alone can cause acute respiratory infections, as primary pathogen agent. Viral co-infections are predominant in children admitted for respiratory infections, determining severe forms of illness, which require days in hospital. PCR-RT technology allows a rapid detection, based on high sensitivity and accuracy, thus being considered as the ideal method to diagnose and evaluate viral co-infections. In this context, demonstrating the existence of viral co-infections raised many questions and generated controversies.

Some groups of researchers claim that mixed infections are associated to a severer form of disease, a theory confirmed by a study deployed in **Lyon, France** and published in 2008 [16]. The observation included 180 children with bronchioloitis during two seasons: 2003-2004 and 2004-2005. The clinical severity of bronchiolitis was defined by:

- Light forms – no hospitalization needed
- Mild forms – hospitalization in the Pediatric ward (92 cases)
- Severe forms – hospitalization in the Intensive Care Unit (88 cases), in need of oxygen and mechanical ventilation

Results: of 180 admitted cases, 44 presented mixed viral infections. Rates of co-infections differed in the two groups: from 92 children admitted in the Pediatric Ward, 14 presented double infections while from 88 children in ICU, 30 had these infections (34%). From the perspective of etiology, the highest rate of incidence occurred for RSV + Rhinovirus association (36 cases of 44 co-infections), most probable cause being the overlap between the viral epidemiological peaks of the two viruses within the respective seasons.

Other encountered associations between RSV and Rhinovirus with: HMPV, HCoV-NL63, Enteroviruses, Flu virus, Paraflu virus, Adenovirus.

Conclusions: double infections are less frequent in mild forms of disease. Viral co-infection represents a risk factor for the development of a severe form, the children who had it, presenting a risk- three times higher to be admitted in ICU, compared to those with a unique viral infection. Mixed respiratory infections determine severe forms of bronchiolitis, regardless of prematurity, age or other associated chronic diseases. The study emphasised the significant contribution of the rhinovirus to morbidity for children with bronchiolitis, in both monoinfections, RSV synergic pathologies and other respiratory viruses.

Further studies will probably provide answers to other queries concerning viremia, viral genotype or mixed infections as determining factors of severe bronchiolitis.

Conclusions

1. Respiratory infections are the main cause of illness and hospitalisation in children.

2. Alongside common viruses, during the former decade new viruses with an important role in respiratory infections pathogenesis were discovered by molecular processes. Thereby HMPV, NL63

and HKU1 Coronaviruses and Humm Bocavirus were included in the new diagnosis algorithms of respiratory infections.

3. Multiplex PCR tests allow a simultaneous detection from the same sample of a large spectrum of viral and bacterial agents, which lay at the basis of respiratory infections.

4. The identification of co-infections with 2 or 3 concomitant viruses explains the occurrence of severe illnesses, often treated in ICUs.

5. Multiplex PCR Technology contributes to a proper use of antibiotics and antiviral agents, as well as to a renewal of diagnosis and treatment guidelines for severe respiratory infections.

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