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MULTIPLE VIRAL CO-INFECTIONS IN PATIENTS WITH ACUTE RESPIRATORY INFECTION

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Abstract. Objective: To investigate the prevalence of respiratory viruses and the rate of viral co-infection in patients with acute respiratory disease hospitalized in the National Institute for Infectious Diseases "Prof Dr Matei Bals" in the winter season 2012/2013. Methods: Nasopharyngeal swabs were collected from patients with acute respiratory illness admitted from December 2012 to March 2013. The samples were tested with xTAG Respiratory Virus Panel Fast (Luminex). Results: A total of 227 samples were prelevated, most of them (77.3%) from paediatric (0-14 years old) patients. More than half (56.3%) of the samples were positive for at least one virus; the predominant viruses were influenza A and influenza B. Adenovirus, RSV and Rhinovirus/Enterovirus (RV/EV) were detected most frequently in children less than 5 years old. Eleven samples (8.5%) were positive for two viruses and one sample was positive for three viruses. Co-infection was significantly associated (p=0.01) with age under 5 years. Adenovirus and RV/EV were more frequently detected with other viruses, and all the viruses of the panel (influenza, parainfluenza, RSV, adenovirus, RV/EV, Coronavirus, Metapneumovirus, Bocavirus) were identified in co-infections. Conclusion: Co-detection of two or more respiratory viruses is not uncommon in paediatric patients with acute respiratory illness.
Keywords: respiratory viruses, influenza

Introduction

Acute viral respiratory infections are the most common human infections, several viruses being responsible: influenza viruses, rhinoviruses, coronaviruses, respiratory syncytial viruses (RSVs), parainfluenza viruses, adenoviruses, enteroviruses, human metapneumovirus (hMPV), human bocavirus (hBoV). The use of multiplex nucleic acid amplification techniques in the routine clinical practice revealed that co-infections are frequent in patients with acute respiratory infections. The co-infection rate reported by various studies is variable, due to differences in patient population, time of sample collection and screening methodologies [1,2].

The aim of our study was to investigate the prevalence of respiratory viruses and the rate of viral co-infections in patients with acute respiratory infection hospitalized in the National Institute for Infectious Diseases" Prof Dr Matei Bals" during the winter 2012/2013.

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Methods

We analyzed samples form patients with acute respiratory illness admitted in the National Institute for Infectious Diseases "Prof Dr Matei Bals", Bucharest, Romania, from December 2012 to March 2013. Informed consent was obtained from each patient or parent.

Nasopharyngeal swabs were collected at patient presentation. Aliquots of the respiratory specimens were tested freshly or after storage at -80°C.

Nucleic acids extraction was performed from 300 µL of respiratory sample, using the generic protocol of EasyMag system (Biomerieux, France).

Each sample was subsequently screened for the presence of respiratory viruses using the xTAG Respiratory Virus Panel (RVP) Fast (Luminex Molecular Diagnostics, Toronto, Canada), according to the manufacturer`s protocol. The RVP Fast test allows the detection of influenza A viruses (for matrix, seasonal H1 and H3), influenza B viruses, respiratory syncytial viruses (RSV) types 1 and 2,

human coronaviruses (hCoVs) NL63, 229E, OC43 and HKU1, parainfluenza viruses (PIV) types 1 to 4, human metapneumovirus (hMPV), enteroviruses and rhinoviruses (EV /RVs), adenovirus (ADV) types and human bocavirus (hBoV). The pandemic 2009 strain of influenza A virus was detected with the Influenza GeneXpert (Cepheid) real-time PCR assay.

Statistical analysis was performed using the $\chi 2$ test, and Fisher's exact test. A two-sided value of $p < 0.05$ was considered statistically significant.

Results

A total of 227 respiratory samples were received from December 2012 to March 2013, with most of them (80.3%) was February and March. At least one respiratory virus was found in more than half (56.3%) of these samples (figure 1). Overall, the more frequently detected viruses were influenza A (32.1%) and influenza B (32.8%), but other viruses were also identified: enteroviruses/rhinoviruses (15.6%), RSV (10.1%), adenoviruses (7%), human coronaviruses, parainfluenza virus types 1 and 3, human metapneumovirus (hMPV) and human bocavirus (hBoV) (less than 5% each).

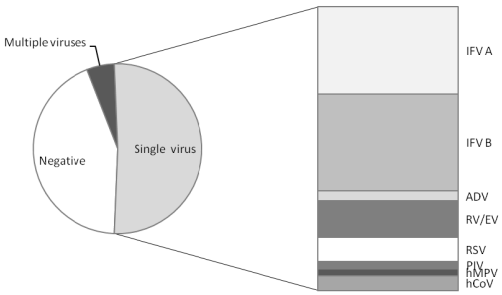

Figure 1. Proportion of samples with positive results for RVP Fast assay

Abreviations: IFV A - influenza A virus, IFV B - influenza B virus, ADV – adenovirus, RV /EV- rhinovirus/ enterovirus, RSV -respiratory syncytial virus, PIV - parainfluenza viruses, hCoV - human coronaviruses, hMPV - human metapneumovirus

Most of the samples (68.7%) were collected from paediatric (0-14 years old) patients, the 5-14 year-olds being the group. The positivity rate is highest in the under 5s children group (67.1%) and decreases gradually, the lowest level was observed in the group of adults aged 25 to 44 years (figure 2).

Influenza B virus and the pandemic 2009 strain of influenza A virus infected patients from all age groups; adenovirus and parainfluenza viruses were detected only in paediatric samples, with a higher rate in children younger than 5. Although the respiratory sincitial virus and enteroviruses

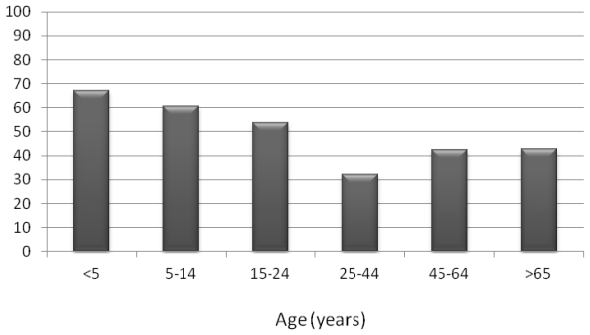

Figure 2. Proportion (%) of respiratory virus positive samples according to patient age

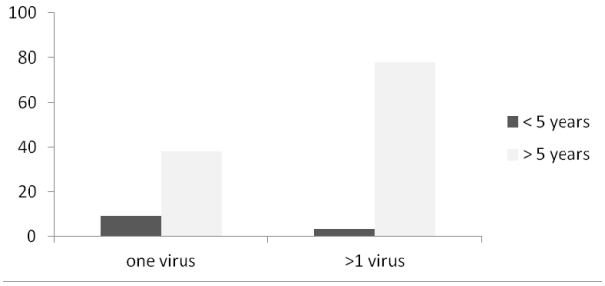

Figure 3. Number of samples with one virus versus multiple viruses detected, in children under 5 years old and older patients

/ rhinoviruses were detected mainly in children, there was no statisticalz significant difference when compared to adult infections.

Most of the positive samples were positive for one virus (90.6%), eleven samples (8.5%) for two viruses and one sample for three viruses (ADV + RSV + PIV3). Co-detection was significantly associated with age under 5 years (p=0.01, CI 95% 1.41-30.67) (figure 3). All the viruses identified by the Respiratory Viral Panel Fast were detected in co-infections, with ADVs and RV/EVs being more frequently involved (5 cases each) (Table I).

Discussions

Several viruses can cause acute respiratory infections of variable severities and clinical manifestations, which are sometimes difficult to differentiate from those produced by bacteria. Therefore, a rapid viral diagnosis is needed in order to avoid unnecessary antibiotic treatments and to set up adequate infection control measures. The use of PCR based multiplex tests in routine clinical practice led to a more frequent identification of several viral infections, including those caused by rhinovirus, human metapneumovirus, human coronavirus, that were previously under-diagnosed. Simultaneous detection in respiratory specimens of more than one virus became a common situation, most of the studies

	IFV A/H1 pdm09	lFV A/ H3	IFV B	RSV	PIV 1	PIV 3	hMPV	hCov	ADV	RV/EV	hBoV
IFV A/H1 pdm09											
lFV A/H3											
IFV B	1										
RSV											
PIV 1											
PIV 3				*							
hMPV			1								
hCoV	1										
ADV	1	1		1+*							
RV/EV			1	1	1				1		
hBoV										1	
Total	3	1	3	3	1	1	1	1	5	5	1

Tabel I. Number of positive samples with multiple co-detected viruses

Abreviations: IFV A - influenza A virus, IFV B - influenza B virus, ADV – adenovirus, RV /EV- rhinovirus/ enterovirus, RSV -respiratory syncytial virus, PIV - parainfluenza viruses, hCoV - human coronaviruses, hMPV - human metapneumovirus, hBoV – human bocavirus

* **ADV + RSV + PIV3**

reporting dual viral infection in 5 to 47% of patients with acute respiratory symptoms and even multiple viral co-infections in some cases [1-5].

In our study 5.3% of the tested patients had a dual or multiple viral infections, this rather low proportion being probably correlated with the overall positivity rate (56.3%). We consider that this rate might be improved by several factors, like reduced delay between onset of symptoms and medical presentation or the consistent use of optimal swabs and media for sample collection and transport.

The clinical significance of viral co-infections is still controversial, the majority of studies reporting that patients infected with single or two respiratory viruses have a similar prognosis, as reflected by the hospitalization time, ICU admission and need of oxygen therapy [3, 5]. Other studies found differences in clinical severity between single and dual viral respiratory infections in children, but the impact of these dual infections appears to depend on which viruses co-infect [2, 6]. For example, when adenovirus or rhinovirus where detected during RSV infection, there was no increase in severity; however, co-infection with both hMPV and RSV increased the intensive care unit admission rate [6]. Also, the severity of the disease is lower in patients with influenza co-infected with rhinovirus and substantially higher in non-rhinovirus co-infections, without changes in influenza viral titer [2].

These conflicting results might be explained by several factors, such as the interactions between the pathogenic mechanisms triggered by different viruses. This might be sustained by the observation that the distribution of viruses in co-detections is not totally random, some studies suggesting a negative association between influenza A and other respiratory viruses, and positive associations between adenovirus and RSV, parainfluenza and rhinovirus, and rhinovirus with parainfluenza and RSV, although statistical significance was not reached in all cases [1].

In our study all the viruses identified by RVP Fast test were co-detected at least once, with ADV and rhinovirus/enterovirus being more frequently present. We found a similar proportion of influenza A infections but an association between influenza A and specific viruses was not observed but could not be excluded, due to the limited number of samples with more than one virus analyzed. Because of the low numbers of each specific co-infection combination, we could not assess an association between them. Overall, the proportion of multiple viral detections is significantly higher in children aged less than 5 years than in older age groups.

The clinical significance of viral co-detections is challenging also because the presence of viral nucleic acid is not per se an evidence of an active infection. Due to the high sensitivity of PCR, viral nucleic acid from recent infections might be detected after the offset of clinical symptoms, so at least in some cases multiple infections could be serial single infections. This limitation is difficult to overcome since most of these viruses are difficult to grow in cell culture. Some studies suggested that quantitative reverse transcription (RT)-PCR might be useful to differentiate true infections from detections and even to assess the disease severity, at least for some viruses [7, 8].

An important result of our study is the detection of H1N1 2009 pandemic strain in 90% of the samples positive for influenza A in the winter season 2012/2013, which is similar to other reports [2].

Conclusions

The use of PCR based, multiplex tests in clinical settings allows viral co-detection. In our study co-infections with two or more respiratory viruses are more often detected in paediatric patients with acute respiratory illness.

Conflicts of interests: none declared.

This work was partially presented at the 5th European Congress of Virology, Lyon, September 2013.

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