Journal of Clinical Virology 94 (2017) 72–78

Contents lists available at ScienceDirect

Journal of Clinical Virology

journal homepage: www.elsevier.com/locate/jcv

Prevalence and seasonality of six respiratory viruses during five consecutive epidemic seasons in Belgium☆,☆☆

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ARTICLE INFO

Keywords:
Respiratory tract infections
Respiratory syncytial virus
Orthomyxoviridae
Metapneumovirus
Adenoviridae
Seasons

ABSTRACT

Background: Acute Respiratory Infections (ARIs) are a major health problem, especially in young children and the elderly.
Objectives: Insights into the seasonality of respiratory viruses can help us understand when the burden on society is highest and which age groups are most vulnerable.
Study design: We monitored six respiratory viruses during five consecutive seasons (2011–2016) in Belgium. Patient specimens (n = 22876), tested for one or more of the following respiratory viruses, were included in this analysis: Influenza viruses (IAV & IBV), *Human respiratory syncytial virus* (hRSV), *Human metapneumovirus* (hMPV), Adenovirus (ADV) and *Human parainfluenza virus* (hPIV). Data were analysed for four age categories: < 6y, 6–17y, 18–64y and ≥65y.
Results: Children < 6y had the highest infection rates (39% positive vs. 20% positive adults) and the highest frequency of co-infections. hRSV (28%) and IAV (32%) caused the most common respiratory viral infections and followed, like hMPV, a seasonal pattern with winter peaks. hRSV followed an annual pattern with two peaks: first in young children and ± 7 weeks later in elderly. This phenomenon has not been described in literature so far. hPIV and ADV occurred throughout the year with higher rates in winter.
Conclusions: Children < 6y are most vulnerable for respiratory viral infections and have a higher risk for co-infections. hRSV and IAV are the most common respiratory infections with peaks during the winter season in Belgium.

1. Background

Acute respiratory infections (ARIs) are worldwide a major health problem, especially in young children and the elderly [1–3]. They have similar symptoms [4,5], are easily transmitted from one person to the next, and lead to high rates of morbidity, mortality and hospitalisation [6]. According to the WHO, ARIs were in 2015 responsible for 1,8 million deaths in children less than 5 years of age alone [7]. Respiratory viruses, including hRSV, are the leading cause of ARIs [8]. To gain a better understanding of the burden of respiratory viruses on society, it is necessary to learn more about their annual cycles and interactions with each other. Insights into the aetiology of respiratory viral infections can help us understand when the burden on society is highest and which subgroups of the population are most vulnerable for respiratory infections. Combined knowledge should allow a better prediction and management of major outbreaks. Hospitals could for instance anticipate the approaching threat in a pro-active way by vaccinating their staff and the most vulnerable patients in order to limit the spread within the hospital setting.

In this perspective, we analysed the occurrence of several respiratory viruses in Belgium over a 5-year period. Between 2011 and 2016, 22876 specimens from 13298 patients with symptoms of a

☆ Kaat Ramaekers, Els Keyaerts, Annabel Rector, Kurt Beuselinck, Marc Van Ranst and Annie Borremans declare that there is no association that might pose a conflict of interest. Katrien Lagrou reports grants, personal fees and non-financial support from MSD, personal fees and non-financial support from Pfizer, personal fees and non-financial support from Gilead, outside the submitted work.

☆☆ The data in this manuscript have been presented at the 10th international respiratory syncytial virus symposium "RSV16" that was organised in Patagonia, Argentina from Sept. 28th 2016 until Oct. 1st 2016. The abstract had number 272.

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http://dx.doi.org/10.1016/j.jcv.2017.07.011
Received 15 March 2017; Received in revised form 4 July 2017; Accepted 19 July 2017

respiratory infection were tested for one or more respiratory viruses as part of the routine clinical practice in the University Hospitals of Leuven. Test results for the following viruses were included in our analysis: Influenza viruses A and B (IAV, IBV), *Human respiratory syncytial virus* (hRSV), *Human metapneumovirus* (hMPV), Adenovirus (ADV) and *Human parainfluenza virus 1–4* (hPIV). The goals of this study are to estimate the prevalence of respiratory viral infections in the studied population, to identify the most common respiratory viruses and their relative frequency over time and to make a comparison between different age groups in the hospitalized population.

2. Study design

2.1. Patient population and data collection

Patients presenting with an ARI in the University Hospitals of Leuven are tested for different respiratory pathogens. For certain well-defined patient groups (e.g. transplant patients), there are clinical care paths in place to determine which tests will be performed at which moments during the treatment. For the majority of the patients presenting with an ARI however there is no pre-defined care path available, and in this case the choice of diagnostic tests depends upon the clinical judgement of the treating physician. In the context of its activities as an associated laboratory to the reference laboratory for respiratory pathogens in Belgium (Antwerp University Hospital), the lab for Clinical and Epidemiological Virology (KU Leuven) collects patient data regarding respiratory viruses [9]. The data used in this study were obtained from the University Hospitals of Leuven. Since the data analyses presented in this study were performed retrospectively and completely anonymous, ethical committee approval was not required.

2.2. Clinical specimens

Mostly oronasopharyngeal swabs, but also bronchoalveolar lavages, sputum and bronchial or endotracheal aspirations were tested for the presence of respiratory viruses.

2.3. Virus detection

The molecular tests performed on the specimens are part of the standard of care in diagnostic procedures of the University Hospitals Leuven. Tests were performed in the Centre for Molecular Diagnostics (CEMOL) of the University Hospital Leuven upon patient- and sample specific request by the treating physician. Therefore, not all patients were tested for the same range of viruses. Influenza A/B and hRSV/hMPV detection were performed by in house developed duplex real-time PCR assays. On rare occasions, urgent samples were tested with the *Binax* quick antigen test for hRSV (Alere) [10]. hPIV was diagnosed with two separate duplex real-time PCRs: hPIV type 1/type 2 and hPIV type 3/type 4. ADV was fluorescently detected with a shell vial assay (cell culture) on Hela cells. In these analyses, test results were defined as positive or negative, no quantitative analyses were performed. Positive results were analysed per week and per age category. Weeks were calculated from Monday to Sunday, according to the week date system of the International Organisation for Standardisation (ISO).

2.4. Data analysis

The age of the tested patients ranged between a few days and 103 years old, which makes it a diverse study population. Patient sample data were divided into 4 age categories: infants and young children (< 6 years old); children and adolescents (6–17 years old); adults (18–64 years old) and elderly (≥65 years old). The investigated period includes 5 consecutive respiratory seasons, starting with the season 2011–2012 and ending with the season 2015–2016. In order to include winter peaks, an epidemic season is defined from week 30 up to week 29 of the following year, with exception of the last season included, 2015–2016, which is defined from week 30 to week 14. The analysis of the 2015–2016 season ended prematurely after week 14 due to a change in diagnostic test at the University Hospitals Leuven. Starting from week 15 of 2016, a new in-house developed respiratory panel assay was introduced in CEMOL. This respiratory panel consists of 12 different multiplex real-time PCRs and detects 28 different respiratory microorganisms, including 5 bacteria, 22 viruses and 1 fungus.

Adenovirus is known to reside in different parts of the human body and to cause different kinds of infection accordingly [11,12]. The non-respiratory samples were therefore excluded from the data set. We calculated the positivity rate per week, the onset, peak and end of each epidemic season, and the median for the 5 consecutive seasons for hRSV, IAV, IBV and hMPV. Additionally, we calculated the number of co-infections with 2 or more respiratory viruses. The onset and end of the epidemic seasons of hRSV, IAV and IBV were defined, respectively, as the first of 2 consecutive weeks with at least 10% positivity rate and the last of two consecutive weeks with less than 10% positivity rate [5]. The onset of the hMPV epidemic season was defined as the first of 2 consecutive weeks with at least 3% positivity rate and the end as the last of 3 consecutive weeks with less than 3% positivity rate [5]. For hPIV and ADV, the onset, end and peak could not be defined.

3. Results

3.1. Incidence and age distribution

Between week 30 of 2011 and week 14 of 2016, 22876 specimens were tested for one or more respiratory viruses to determine the cause of the patients' ARI. An average of 93 tests were performed per week, with peaks during the winter seasons (Fig. 1). Out of the 22876 specimens tested, 6104 (27%) were positive for at least one respiratory virus. The positivity rate of upper respiratory samples (URT) was higher (31%) than the positivity rate of lower respiratory samples (LRT) (14%). The most frequently detected viruses were IAV and hRSV, which were respectively found in 1981 (32%) and 1739 (28%) of the positive samples. Furthermore, 14% of the positive samples contained IBV, 13% hPIV, 9% ADV and 8% hMPV. With exception of the Influenza viruses,

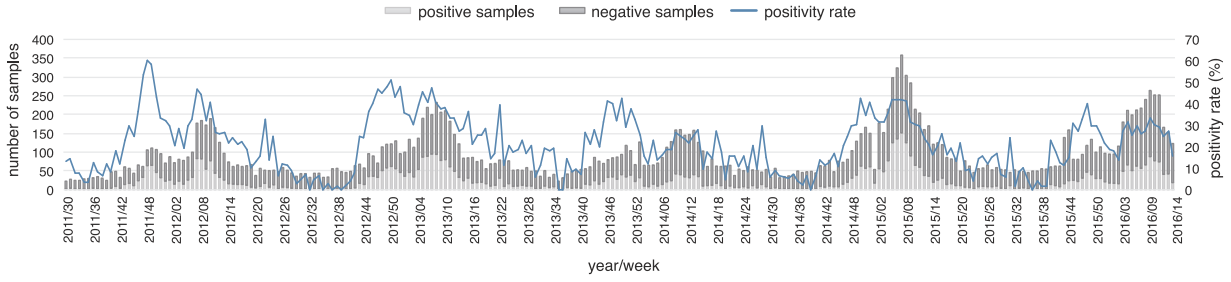

Fig. 1. Weekly number of processed specimens. Positive samples (light grey bars) versus negative samples (dark grey bars) and positivity rate (line graph) per week from week 30 of 2011 until week 14 of 2016. Positive samples can be positive for one or multiple viruses.