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Surveillance of the respiratory syncytial virus (RSV) outside infancy: impact of testing methods, a retrospective observational study

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Abstract

The European medicine agency has approved several vaccines to protect the elderly against respiratory syncytial virus (RSV) infections. However, differences of performances between antigen and PCR tests, especially in adults, can make monitoring RSV difficult. This study aims to assess the impact of the diagnostic methods chosen on the surveillance of RSV.

RSV and influenza test results obtained from July 2022 to June 2023 in a consolidated clinical laboratory in Brussels (Belgium) were collected. These results included antigen tests, quadruplex PCR tests and viral cultures on respiratory samples. Epidemiological trends related to the age of patients and the diagnostic methods were analysed.

Among 14,761 RSV tests, the overall number of positive tests for infants until 1 year of age peaked on 5 November 2022 (n=67/7 days) whereas it peaked on 22 December 2022 for adults (n=33/7 days). Positive antigen tests peaked on 7 November 2022 (n=56/7 days) whereas positive PCRs peaked on 19 December 2022 (n=36/7 days). Nevertheless, the positivity rate of RSV PCRs had peaked 1 month before. Infants were mainly diagnosed through antigen testing contrary to older patients. The influenza epidemic was likely the cause of the increased use of a quadruplex PCR leading to a delayed increase of the absolute number of PCRs positive for RSV.

This study shows that the use of different diagnostic methods could lead to an erroneous representation of RSV epidemiology in adults due to the lack of sensitivity of antigen detection. RSV surveillance for elderly should rely rather on molecular methods.

Keywords

Respiratory syncytial virus, surveillance, polymerase chain reaction, vaccines, antigen, adults

Introduction

The respiratory syncytial virus (RSV) is now recognized as an important cause of serious illness in the elderly [1] and at the time of writing, two vaccines recently approved by the European Union's health regulator are made available for the coming winter for older adults [2]. Therefore, monitoring the impact of the vaccination on this specific population requires efficient surveillance. The use of laboratory data to assess the occurrence of specific microorganisms in a population represents one of the most common established public health surveillance approach for infectious diseases [3]. Since 1983, Belgian authorities have implemented such strategy through the set-up of the Belgian Sentinel Network of Laboratories, which collect data on the epidemiology of 43 micro-organisms [4].

However, monitoring the number of RSV cases can be tricky because of (i) the difference of sensitivity of the affordable antigen testing between adults and infants [5], (ii) a probable lack of access to PCR tests as well as (iii) the disregard by clinicians on the impact of this pathogen in adults. Furthermore, (iv) the molecular detection of RSV is frequently paired with the detection of other respiratory viruses such as SARS-CoV-2 and/or influenza viruses [6], adding potentially another confusion bias in its surveillance. The aim of this study was to examine the testing data coming from a single large clinical microbiology laboratory for five hospitals in Brussels and to assess the impact of diagnostic methods on the surveillance of RSV during the winter 2022-2023.

Material and methods

Data were coming from a single consolidated clinical laboratory, the LHUB-ULB (Laboratoire Hospitalier Universitaire de Bruxelles—Universitair Laboratorium Brussel). It is a clinical laboratory serving five university hospitals (containing a capacity of around 3,000 beds) as well as a network of general practitioners in Brussels, Belgium, covering a service area of 700,000 inhabitants [7]. RSV positive test results as well as influenza positive PCR results were collected from July 2022 to June 2023. These results included RSV antigen detection tests (RSV *K*-set, Coris Bioconcept, Belgium), quadruplex PCR tests (Alinity *m* RESP-4-PLEX assay, Abbott molecular, USA) as well as viral cultures coming from respiratory samples which were routinely performed in addition of the antigen detection test. The quadruplex PCR test allowed the simultaneous detection of RSV, SARS-CoV-2, and influenza A and B viruses. In the routine surveillance perspective, all patients diagnosed with RSV or influenza infection by rapid antigen detection tests, molecular diagnostic tests or by viral culture are considered as notifiable cases of RSV or influenza infection in the frame of the Belgian sentinel network of laboratories. Multiple positive results for a same patient were deduplicated to keep only the first positive result per patient. Daily positivity rate for RSV and influenza PCR was calculated by using the number of non-deduplicated tests performed on the previous 7 days. The age of the patients at sampling date was also collected. Patients until 1 year old were considered as infants, patients from 2 to 14 years old were considered as children and patients from 15 years old and above were considered as adults. Epidemiological trends were analysed by cumulating daily positive tests per 7 days to minimize day-to-day and holiday-related fluctuations from 1 July 2022 to 30 June 2023.

Results

From 25 June 2022 to 30 June 2023, 14,761 RSV diagnostic tests (7,280 PCR and 7,581 antigen detection tests followed by viral culture) and 7,282 influenza PCR tests were performed (Table 1). Viral cultures yielded 123 RSV not detected by antigen detection test. One-hundred-sixty-two RSV PCR tests were performed on the same day of a negative antigen test, of which 19 yielded a positive result. Nine-hundred-forty-four patients had a positive RSV test during this period, including 608 (64.4%) infants. During the same period, 901 patients had a positive influenza PCR tests. The overall number of positive tests for RSV for infants peaked twice: on 5 November 2022 and on 26 November 2022 with 67 and 61 positive tests per 7 days, respectively (Figure 1). In the other hand, the overall number of RSV positive tests for adults peaked on 22 December 2022 with 33 positive tests per 7 days. For children, the overall number of positive tests for RSV was lower and reached its maximum at 12 positive tests per 7 days on 23 November 2022. When analysing the nature of the positive tests, the number of positive antigen tests peaked twice on 7 November 2022 and on 24 November 2022 with 56 and 55 positive tests per 7 day, respectively. By contrast, the number of positive RSV PCRs peaked on 19 December 2022 with 36 positive tests per 7 days. However, the positivity rate of RSV PCRs peaked 1 month before, on 17 November 2022, with 21.5% of PCR tests. This can be explained by the fact that the median age (interquartile range) for positive antigen tests was 0 (0 - 0) year whereas it was 50 (2 - 73) years for the positive PCR tests. The influenza epidemic, for which the number of positive PCRs peaked on 30 December 2022 with 181 positive tests per 7 days, likely indirectly drove the increasing numbers of positive PCRs for RSV after the epidemic peak observed for antigen testing and

infants. The positivity rate of influenza PCR tests peaked on 29 December 2022 at 43.8% and the positivity rate of RSV antigen detection tests peaked on 7 November 2022 at 46.0%.

Discussion

The multiplication of rapid detection methods for respiratory viruses, ranging from antigen tests [5] to rapid point-of-care multiplex PCRs [6], allowed for a broader detection of RSV. However, this can also complicate its surveillance. For the passive surveillance using RSV laboratory surveillance database, recommendations in Europe are to gather positive test results as well as the overall number of RSV tests and the type of test (antigen, PCR, culture…) [8]. This study highlights the importance of gathering these data as the number of cases on its own was not reflecting the actual epidemiological situation. It appeared there was a lag between the number of positive cases identified with antigen tests and those identified by PCR. These lag was artificially created by 2 factors: the lack of sensitivity of antigen tests for adults and the fact that the molecular diagnostic was using a quadruplex PCR which was also used for influenza and SARS-CoV-2 testing. The number of quadruplex PCR performed increased dramatically because of the spread of the influenza increasing the number of diagnosed RSV cases while its actual prevalence was decreasing.

In a previous study [9], we showed that for the PCR detection of SARS-CoV-2, the average cycle threshold values (Ct) of the positive PCRs varied in advance of the absolute number of positive results. Thus, when the number of positive PCRs peaked, the proportion of recently infected patients, hence contagious, was already decreasing for a few weeks. Indeed, due to the high sensitivity of the PCR, testing positive for a respiratory virus by PCR does not necessarily indicate illness. Antigen tests detect patients with a higher viral load [10] and are less impacted by this effect. In our setting, antigen testing was the favoured RSV diagnostic method for infants below 1 year old. This can be easily explained by the good performance of this test for this population [5], combined to its rapidity and easiness of use, making it available in on-site laboratory. Conversely, point-of-care PCRs are usually expensive while larger molecular diagnostic platforms, which allow a cheaper cost per test, are performed in our central laboratory during business hours, taking more time (a few hours), thus decreasing its interest for the rapid management of patients. Therefore, for the five partner hospitals of our clinical laboratory (LHUB-ULB), the diagnostic algorithm for the management of patients with influenza-like illness includes frequently the realization of a rapid antigenic diagnostic test as a first step. If the latter is negative and if the patient needs hospitalization, a molecular technique will be performed. Such strategy allows providing a rapid and sensitive diagnostic at the best cost, by avoiding the realization of an unnecessary molecular method. In addition, viral culture is performed on samples with a negative antigen test. These cultures are reimbursed by the Belgian national health insurance, as an alternative to molecular tests, which on the contrary are not reimbursed. They allow a confirmation diagnostic for mildly ill patients. However, beyond their epidemiological interest, the time-to-result of viral culture impede their clinical interest. In the context of epidemiological surveillance, it would also be interesting to focus on the positivity rate in each municipality located in the direct service area of the LHUB-ULB. This to see which populations are the most at risk of developing influenza but also to help hospital manager to target vulnerable populations or to rapidly identify clusters. As initially demonstrated by John Snow, such mapping proved their usefulness for showing differences in rates of disease between communities and for identifying clusters of disease [11].

The findings in this report are subject to at least two limitations. First, sentinel surveillance based on LHUB-ULB data only might not provide a fully representative sample of the epidemiological situation in Belgium because influenza testing is mainly performed on inpatients and patients attending emergency department for respiratory symptoms. This was underlined by Jester et al. who highlighted that influenza surveillance relies on specimens collected from symptomatic patients during medical encounters, where the purpose of testing is primarily patient diagnosis rather than surveillance [12]. Furthermore, additional studies must be carried out to judge whether data unification from large consolidated laboratories located, for instance, in the 3 different regions of Belgium (Brussels, Wallonia, and Flanders), could be sufficient to describe the infectious events in Belgium, as we did previously for influenza [7]. At the European level, the interconnection of consolidated clinical microbiology laboratories – where each laboratory could be seen as a real-time sensor in its area – would move laboratory surveillance from public health structures to clinical laboratories [13]. Such a network, more directly linked to the field, demonstrated their abilities to adequately support public health responses during the COVID-19 pandemic. Second, because of their reimbursement by the National health insurance in Belgium, antigen testing and viral cultures are the main diagnostic methods used before molecular tests for RSV and influenza. At the time of writing, only the SARS-CoV-2 PCR is reimbursed in a limited number of indications (mainly symptomatic or fragile patients requiring admission), therefore clinical laboratories may perform a quadruplex PCR during influenza and

RSV season instead of a single SARS-CoV-2 PCR. The routine use of a quadruplex PCR increase the overall number of detected cases compared to targeted PCR.

In the frame of future RSV surveillance, especially with the distribution of vaccines and the development of therapeutic interventions, it seems important that decision makers favour tools which allow an efficient detection, hence, a better surveillance of RSV. Indeed, with the aging of the population, RSV might become a growing burden and surveillance or RSV will be of interest to promote vaccination [14]. If antigen testing can be used for infants due to its relative cost-effectiveness, this method is inaccurate for elder patients [5]. Patients requiring hospitalization should benefit from more expensive rapid multiplex PCRs to allow a better clinical management and relevant hygiene precautions [6]. As shown in this study, the intertwinement of influenza, RSV, and likely SARS-CoV-2 could clinically justify the systematic use of such multiplex PCRs during the epidemic season. Likewise, the surveillance of epidemic trends would benefit from data more accurate than the sole number of positive cases, such as positivity rate and maybe semi-quantitative approaches based on Ct values [15]. Indeed, As described for COVID-19, the use of Ct value of RT-PCR could help a better prediction of influenza and RSV trends [9]. Furthermore, the use of molecular diagnostic methods applied in the frame of syndromic approaches would also allow the detection of multiple respiratory pathogens and for some test subtype influenza viruses [16]. The overlap of the epidemics of RSV, influenza and COVID-19 during winter as well as the difficulties, especially for elderly, to clinically distinguish these infections [17], makes it more convenient for both laboratories and clinicians to use one multiplex assay. However, the use of such assay may lead to the increasing of RSV detection as a collateral effect of the spread of the other virus detected by it at a given time. This should be taken in consideration for the passive surveillance of respiratory viruses.

Table 1: Antigen detection (Ag) and nuclear acid amplification (PCR) tests performed for the detection of the respiratory syncytial viruses (RSV) and influenza viruses and patients age from 25 June 2023 to 30 June 2023.

Test	Ag RSV	PCR RSV	PCR Influenza
Overall			
Median age (years)	3	61	61
Interquartile range	0-52	37-75	37-75
Number of tests:	7581	7280	7282
- < 2 years old	3278	336	334
- 2-14 years old	1408	227	228
- >= 15 years old	2895	6717	6720
Positive			
Median age (years)	4	50	40
Interquartile range	0-55	2-73	26-63
Number of tests:	528	340	934
- < 2 years old	458	76	34
- 2-14 years old	44	35	66
- >= 15 years old	26	229	834
Negative			
Median age (years)	0	61	63
Interquartile range	0-0	38-75	41-76
Number of tests:	7053	6940	6348
- < 2 years old	2820	260	300
- 2-14 years old	1364	192	162
- >= 15 years old	2869	6488	5886

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Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Nicolas Yin. The first draft of the manuscript was written by Nicolas Yin and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study is an epidemiological retrospective observational study using aggregated anonymous data; therefore, no ethical approval was required.

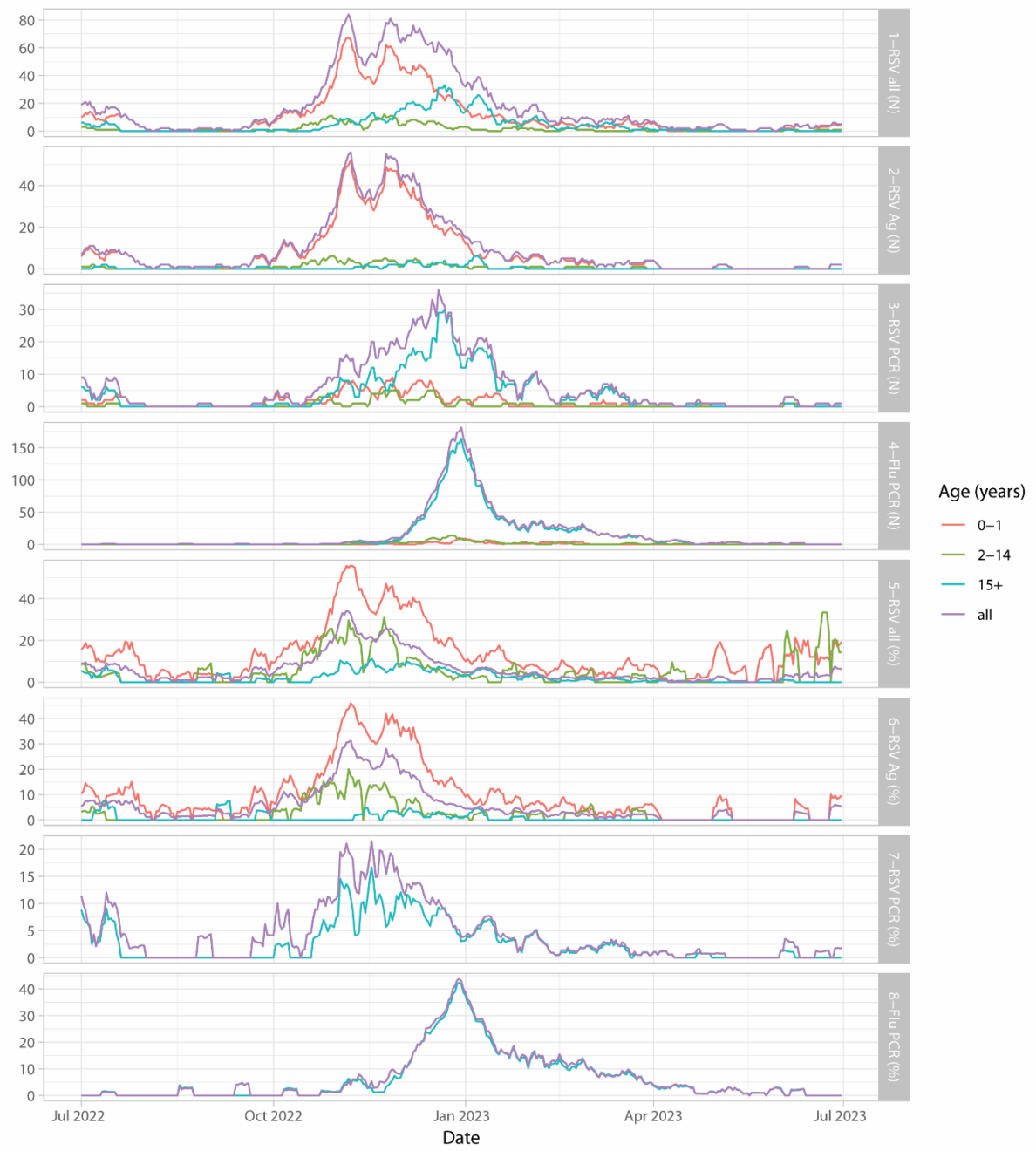
1–RSV all (N)
2–RSV Ag (N)
3–RSV PCR (N)
4–Flu PCR (N)
5–RSV all (%)
6–RSV Ag (%)
7–RSV PCR (%)
8–Flu PCR (%)
Jul 2022
Oct 2022
Jan 2023
Apr 2023
Jul 2023
Date
Age (years)
0–1
2–14
15+
all

Figure 1