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Diagnosis of Respiratory Syncytial Virus (RSV) infection in children by Respiratory Panel utilized during the COVID-19 pandemic

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Abstract

Background. In the months of October and November 2021, there was throughout Italy and in our specific case in the area of Lucca and Versilia, a disturbing increase of SARS-CoV-2 infections and cases of Respiratory Syncytial Virus (RSV) in new-borns. The aim of this paper is to compare the cases of RSV infection diagnosed in recent years to the cases recorded during the SARS-CoV-2 pandemic to November 2022.

Materials and methods. The study consisted of evaluating the results of requests for RSV diagnosis from 2015 to November 2022, using molecular biology techniques.

Results. The data obtained show that the number of cases of RSV infection in children during the winter season had a constant trend from 2015 to 2019. From November 2020 to February 2021 there were no cases of RSV respiratory infections. Starting from September 2021, on the other hand, there was a resumption of cases of RSV infections in conjunction with an increase in the number of children affected by COVID-19. From January 2022, after a peak in cases of SARS-CoV-2 infection, there has been a decrease in RSV infections. From September 2022 to November 2022, there was no increase of cases of RSV infections in new-borns but on the contrary, there was a trend in respiratory infections comparable to the pre-pandemic period.

Conclusion. The data that emerged from the study conducted, show the onset of an outbreak of RSV in new-borns. This incidence is linked to the implementation of rigorous non-pharmacological public health interventions in 2020, aimed at combating COVID-19 infection. The use of the molecular panel made it possible to identifying the responsible agent and highlighting the most suitable clinical and therapeutic path.

Key words

- RSV
- respiratory infections
- SARS-CoV-2
- pandemic

INTRODUCTION

Clinically relevant respiratory infections, caused by a variety of viral and bacterial pathogens, commonly occur in both adults and children. Respiratory infections have the highest incidence at paediatric ages, particularly in the first years of life. In 80% of cases, the etiological agents of Recurrent Respiratory Infections (RRIs) are the Respiratory Syncytial Virus (RSV), the Influenza and Parainfluenza Viruses, the Adenovirus, the Rhinovirus and the Bocavirus [1]. The Respiratory Syncytial Virus is an RNA virus, classified as Pneumovirus, of which two subgroups have been identified, namely A and B. RSV is one of the respiratory viruses that circulates the most in the world and is the most common cause of bronchiolitis, an inflammation of the small airways of the lungs [2, 3]. Human Respiratory Syncytial Virus is present in the bronchi, bronchioles and pulmonary alveoli and can cause necrosis of epithelial cells and interstitial infiltrates of inflammatory mononuclear cells. The Respiratory Syncytial Virus affects all ages, but more severely infants in the first months of life and the elderly with multiple pathologies. There are people who are more at risk of contracting respiratory infections, such as children with heart disease, severe prematurity, or neurodevelopmental problems [4, 5]. In elderly people, with comorbidities, RSV is a dangerous virus like SARS-CoV-2, which can cause the premature death of the patient. Most children get infected at least

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once in their lifetime, usually in the first two years, but they do not always develop severe manifestations. Children can also be reinfected by the virus, because a first RSV infection does not make them completely immune; however, subsequent infections are milder than the first [6]. Infants or children in the first few months of life are at increased risk of developing the most severe form of the disease, with respiratory complications and pneumonia and consequent dangers to the baby's life. In infants, especially when premature, the first symptoms are apnoea or those typical of respiratory diseases such as colds, coughs, sore throats, and fever [7]. In the following days they may begin to manifest more serious symptoms including respiratory distress, a condition in which the use of oxygen is necessary [8, 9]. Respiratory Syncytial Virus usually has a seasonal peak, causing respiratory infections starting from mid-November and then lasting until the end of. The seasonality of the virus allows, therefore, in the case of premature babies, to program a pharmacological prophylaxis in order to prevent the onset of the most serious symptoms [10, 11]. Prophylaxes based on the use of monoclonal antibodies, which have a high cost and are reserved only for some special cases indicated by the guidelines, do not give immunity but confer protection from respiratory infections during the winter season. During the months of October and November 2021, throughout Italy and in our specific case in the areas of Lucca and Versilia, we observed a disturbing increase in cases of new-borns with SARS-CoV-2 infections and cases of Respiratory Syncytial Virus. This epidemic of RSV broke out earlier than in previous years. Numerous RSV outbreaks, with hospital admissions of paediatric patients, were recorded as early as the end of October 2021 when temperatures were not yet typically of winter. In the first weeks of December 2021, the ratio in terms of the number of hospitalizations of children for respiratory syncytial virus and those of COVID-19 was about 10 to 1. There were therefore 10 times more children who ended up in hospital for RSV than those who needed hospitalization for coronavirus SARS-CoV-2 infection [12, 13]. Since January 2022 a constant decrease in cases of RSV infection in young patients was observed until no cases were recorded during summer period. From October 2022 to November 2022 an increase of infections caused by RSV in children has been observed, but the number of cases reflecting the seasonality of respiratory infections [14, 15]. The introduction from September 2021 of the One-step RT Real-time PCR multiplex test for the screening of infectious agents, has made it possible to process a greater number of samples routinely and quickly for a correct differential diagnosis of RSV from other pathogens and guide the hospital pathways for the management of young patients. Using rapid diagnostic techniques also means reducing hospitalization times, hospital costs and often avoids the administration of inappropriate antibiotic therapy, thus preventing the phenomena of drug resistance [16, 17].

SUBJECTS, MATERIALS AND METHODS

Subjects

The study done at the Clinical Chemical Analysis Laboratory of the San Luca Hospital in Lucca was carried out by considering the results of the requests for RSV diagnosis from 2015 until November 2022. A population of 500 patients, which included new-borns and children up to 11-12 years old with respiratory diseases was analysed. In past years, the search for Respiratory Syncytial Virus was performed using the RSV Card of Beta Diagnostici, subsequently, diagnostic platforms based on molecular biology techniques were used: Cepheid Xpert® Flu / RSV from Seegene Inc, FilmArray® multiplex PCR from BioFire Diagnostics Inc. and finally, the Seegene Allplex™ Respiratory Full Panel Assay kit.

Immunochromatographic method

The RSV Card is a qualitative lateral flow assay for the diagnosis of RSV antigen in nasopharyngeal specimens. The RSV antigens, if present in the sample, react with anti-RSV antibodies immobilized in the strip and combined with particles are capable of generating a colorimetric reaction. The time for the analysis and reporting is 15 minutes.

Molecular Biology Methods

A diagnostic platform used for RSV analysis is the FilmArray®, designed by BioFire Diagnostics, which uses a nested multiplex PCR (nmPCR) protocol for the identification of 16 viral and 4 bacterial targets (at gender and/or species), in a biological sample. The integrated FilmArray® platform is a closed system, which is based on the use of a disposable and compact pouch inside which the reactions of extraction, purification, and amplification of the nucleic acid of the eventual pathogen, present in a single sample, take place biologically. The execution time of the analysis is approximately one hour. The second platform in use is the Cepheid Xpert® Flu / RSV system, a diagnostic test in molecular biology, which allows one to identify the A, B and RSV Influenza Virus in just 20 minutes. Recently, an analytical platform for the diagnosis of symptomatic airway infections has been introduced, which permits examination for the presence of a wider variety of viruses and bacteria, from a single nasopharyngeal swab. The Seegene Allplex™ Respiratory Full Panel Assay kit is a multiplex One-step RT Real-time PCR assay, which in 22 samples simultaneously detects and identifies, 16 viruses, 7 bacteria and 3 subtypes of Flu A, agents of respiratory diseases. Analysis time is approximately four hours (*Table 1*).

RESULTS

From the analysis of the positive cases of RSV diagnosis over time, in particular before the pandemic period (end of February 2020), during the pandemic (from the lockdown until March 2022), and after the pandemic period (from April 2022 to November 2022), it clearly emerges that the Respiratory Syncytial Virus is a pathogen that causes seasonal winter epidemics (as shown in *Figure 1*). The data obtained from our study, starting from 2015, showed an increase in the cases recorded for RSV during the winter season, starting from November, with a peak in the month of January and then decreasing, with fewer cases of infections during the spring/

Table 1
The Seegene Allplex™ Respiratory Full Panel Assay that is composed of 4 different panels and is a multiplex One-step RT Real-time PCR assay to detect and identify, 16 viruses, 7 bacteria and 3 subtypes of Flu A

Allplex™ Respiratory Full Panel Assay			
Panel 1	**Panel 2**	**Panel 3**	**Panel 4**
Influenza A virus	Adenovirus	Bocavirus	Mycoplasma pneumoniae
Influenza B virus	Enterovirus	Rhinovirus	Chlamydophila pneumoniae
Respiratory syncytial virus A	Parainfluenza virus 1	Coronavirus NL63	Legionella pneumophila
Respiratory syncytial virus B	Parainfluenza virus 2	Coronavirus 229E	Haemophilus influenzae
Flu A-H1	Parainfluenza virus 3	Coronavirus OC43	Streptococcus pneumoniae
Flu A-H1pdm09	Parainfluenza virus 4	Internal Control	Bordetella pertussis
Flu A-H3	Metapneumovirus		Bordetella parapertussis
Internal Control	Internal Control		Internal Control

summer. The seasonal trend of RSV cases appears to be constant in the pre-pandemic years. On the other hand, no cases were recorded from November 2020 to February 2021, and this is related to the implementation of rigorous non-pharmacological public health interventions to combat COVID-19. From September 2021, on the other hand, there has been an increase in RSV cases and at the same time also of children, aged between a few months from birth up to 11 years, with SARS-CoV-2 infections. From the beginning of December 2021 to March 2022 there was another peak of SARS-CoV-2 infection involving a heterogeneous age group. At the same time, there was a steady decrease, starting from January 2022, in the number of respiratory infections caused by RSV in children. In September 2022 there was no anomalous increase in RSV cases as in 2021, but on the contrary, an increase of the cases RSV infection was detected up to November 2022 which correlates with a pathogen that causes seasonal winter epidemics (as shown in *Figure 2*).

DISCUSSION

The data that emerged from the study conducted in our laboratory describe a situation that corresponds to what has also happened in other countries in different continents of the Northern Hemisphere and the Southern Hemisphere [18-22]. In Italy, Finland, Belgium, the UK, and the USA the implementation of restrictions, starting from March 2020, coincided with an apparent sudden and earlier end of the RSV epidemic season, compared to previous years and almost no cases detected in the following months. In the Southern Hemisphere, SARS-CoV-2 restrictive measures were implemented just before winter and were maintained for different periods according to SARS-CoV-2 diffusion [23-25]. Starting from September 2021, in Italy and

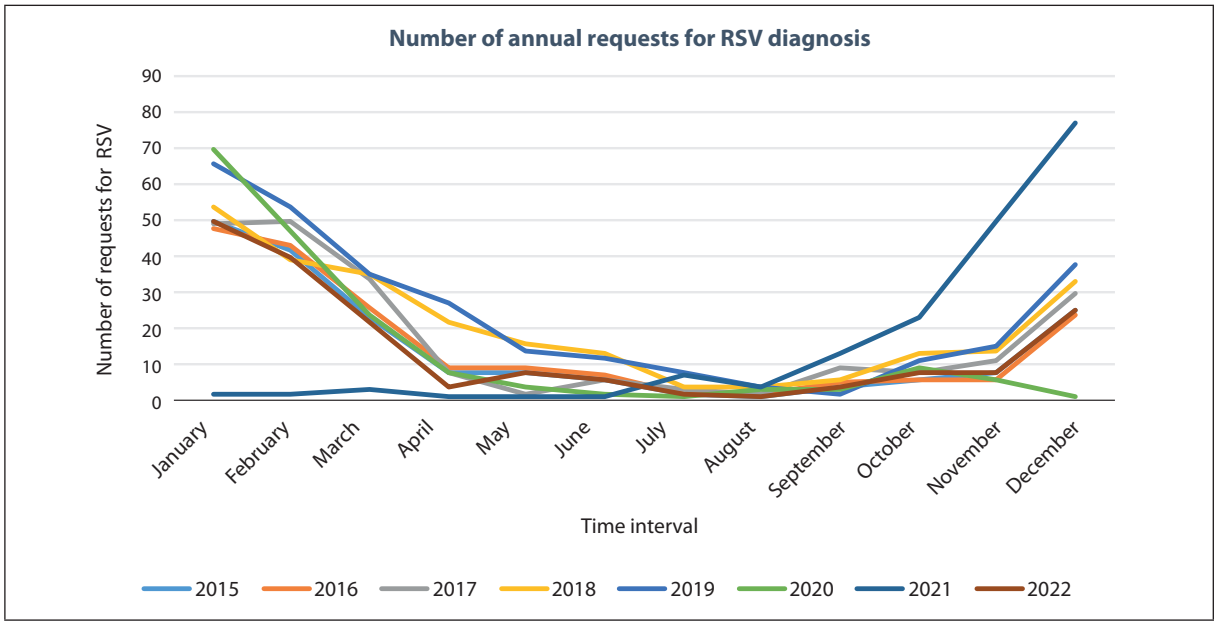

Figure 1
Number of annual requests for research of the Respiratory syncytial virus (RSV) genome using molecular biology techniques.

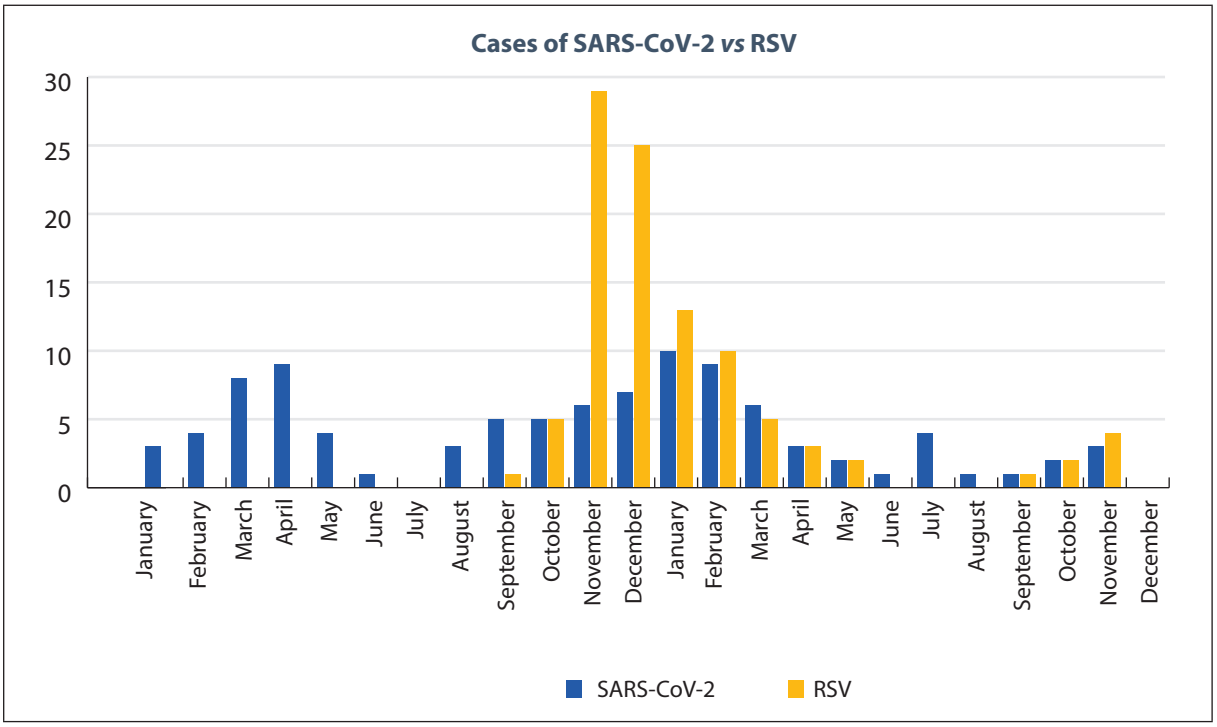

Figure 2
Comparison of the number of Respiratory syncytial virus (RSV) cases compared to SARS-CoV-2 infections in children, aged between a few months from birth up to 11 years old, during the pandemic and post-pandemic period up to November 2022.

in our specific case in the areas of Lucca and Versilia, another epidemiological phenomenon has also been observed, a disturbing increase in cases of new-borns with respiratory infection of Respiratory Syncytial Virus. This epidemic broke out earlier than in previous years. In other countries of the northern hemisphere like in France, RSV arrived late in February 2021 and expanded during the spring with an outbreak of a duration comparable to the previous season, although of a lesser magnitude [26, 27]. In the USA, the RSV epidemic started at the end of March 2021 covering the spring–summer months, extending into the autumn in some states [28]. This epidemiological shift was previously observed in the southern hemisphere. In Australia and New Zealand, after a 2020 winter season with RSV virtually absent, an unusual reappearance of the virus was observed during the summer, with an even larger outbreak than previous epidemic seasons [29]. The data conducted in our laboratory has been highlighted that the association of SARS-CoV-2 infection and an increase in cases of children with RSV respiratory infections it was correlated to the fact that children were more vulnerable than usual to respiratory viruses and seasonal infections because they had been underexposed to germs during the measures decreed at the beginning of the pandemic (social distancing, the use of Personal Protective Equipment). Respiratory RSV infections did not spread during the COVID-19 lockdown and consequently pregnant women developed a reduced number of antibodies to be transmitted to their children, not having been exposed to the related infectious agents. With the easing of anti-Covid measures, the Respiratory Syncytial Virus has presented itself earlier and more aggressively, than would be expected in a normal winter season. In post-lockdown Italy there were several hospitalizations of children due to respiratory infections, a condition that supports the theory of immunity debt, a concept used to describe the poor protective immunity resulting from long periods of low exposure to a given pathogen, accumulated thanks to the measures adopted in times of pandemics. What we saw in that period was due to the boomerang effect of COVID-19. We assisted to a change in the immune system, particularly in younger children, as a result of major social and health changes related to the pandemic. Fortunately, the risk of death, unlike in developing countries, is exceptionally low in Italy, but the virus can leave permanent damage, starting with the development of asthma in adulthood which occurs in 50% of cases. Without seasonal exposure, therefore, immunity decreases and susceptibility to potentially more serious future infection increases (https://www.cdc.gov/flu/symptoms/flu-vs-covid19.htm). After an epidemic of RSV at the end of 2021 and a new peak of COVID-19, starting from the beginning of January 2022 there was a constant decrease over time in the number of cases of respiratory infections of RSV in children (https://www.cdc.gov/coronavirus/2019-ncov/long-term-effects/index.html.). This would seem to be associated with the easing of restriction measures to counter the spread of SARS-CoV-2 and the gradual return to pre-pandemic habits favouring, therefore, a lower susceptibility to seasonal respiratory viruses (https://www.cdc.gov/flu/season/faq-flu-season-2021-2022.htm.). The epidemio-

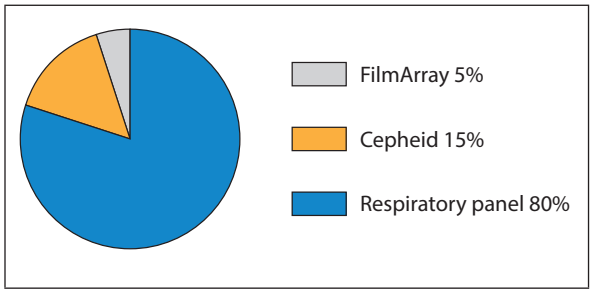

Figure 3
Percentage of diagnostic platforms use based on molecular biology techniques.

logical phenomenon that has been observed in these years, has highlighted the important role that the analytical laboratory in providing results quickly and thus help the clinician for the diagnosis. In order to optimise the number of samples to be processed, quickly and at moderate costs, it was decided to routinely introduce, in the Molecular Biology sector, a diagnostic platform based on the use of an analytical panel. Urgent requests for RSV diagnosis, on the other hand, are treated by analysing the nasopharyngeal swab with the Cepheid Xpert® Flu / RSV system or with the FilmArray® system from BioFire Diagnostics, faster but more expensive analysis techniques, permitting the analysis of a few samples simultaneously (as shown in *Figure 3*). With this type of panel, it was possible to perform the differential diagnosis of the two subgroups of the RSV, A and B. The pathology caused by subgroup A appears to be more serious and, in most cases, requires the hospitalization of the child.

CONCLUSION

We assisted a serious epidemic of Respiratory Syncytial Virus in new-borns, starting from September 2021. This incidence has been correlated to the implementation of rigorous non-pharmacological public health interventions in 2020, aimed at combating COVID-19 infection. The use of the molecular panel made it possible to draw attention to respiratory infections in children with similar clinical symptoms, thus identifying the responsible agent and highlighting the most suitable clinical and therapeutic path.

Conflict of interest statement

The Authors declare no conflicts of interest.

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