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FULL PAPER

Epidemiology and Clinical impact of single and multi-viral respiratory infections in post-pandemic era

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SUMMARY

Acute respiratory tract infections (ARI) are common diseases in children and adults and could cause severe infections in high-risk patients, like the immunocompromised and elderly, and are the leading cause of morbidity, hospitalization and mortality. This study aimed to explore the prevalence of respiratory viruses and the clinical impact of single- and multi-infection among hospitalized patients in various age groups. 3578 nasopharyngeal swabs (NPS) were analyzed for pathogen detection of acute respiratory tract infections. 930 out of 3578 NPS were diagnosed positive for at least one respiratory virus. The distribution of viral infections, prevalence and pathogen, differed significantly among age groups. Most RTI are observed in the age group over 65 years (50.6%) with a high SARS-CoV2 prevalence, following by group <5 years (25.6%), where the most frequently detected viruses were RSV, Rhinovirus, FluA-H3, MPV, and AdV. The co-infection rate also varies according to age and, in some cases, especially in older adults, could have severe clinical impact. This study emphasizes that it is important to know and analyze, in all age groups of hospitalized patients, the epidemiology of respiratory viruses, the prevalence of coinfections, and the clinical impact of various pathogens. Furthermore, in a clinical setting, the rapid diagnosis of respiratory infections by means of molecular tests is crucial not only to avoid hospital outbreaks, but also to allow early and optimal treatment to reduce morbidity and mortality.

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INTRODUCTION

Acute respiratory tract infections (ARI) are a major public health problem and a leading cause of morbidity, hospitalization, and death, especially in children and in elderly and immunocompromised patients (Gottlieb *et al*., 2019), representing an increased economic burden on the healthcare system. Moreover, in immunocompromised patients viral infectious may be shed for weeks. The emergence of SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) and the ongoing pandemic as a result of the viral spread brought attention to respiratory viruses and their role as viral pathogens as a cause of severe pneumonia (Cilloniz *et al*., 2022). Furthermore, respiratory viruses have been identified as agents capable of nosocomial transmission and can therefore be the cause of possible outbreaks. The severity of illness arising from viral infection varies from patient to patient, influenced by the virus itself and by genetic predisposition to severe illness (Zou *et al*., 2020). The main host factors related to severe outcome are age and previous comorbid conditions (e.g., immunosuppression, diabetes, hypertension, chronic cardiac, respiratory, and liver disease, chronic pulmonary disease) (Gao *et al*., 2021; Coleman *et al*., 2018).

Signs and symptoms of infection vary from self-limited upper respiratory tract infection (URTI) such as cold, sore throat or laryngo-tracheobronchitis to involvement of lower respiratory tract infection (LRTI) such as bronchiolitis, pneumoniae or acute respiratory distress syndrome (ARDS). Moreover, empirical antibiotics are improperly prescribed to patients with viral ARI while awaiting etiological diagnosis, and can have a negative impact on antibiotic resistance. Due to their mode of transmission, which is mainly by droplets, respiratory virus circulation within the community is high and dual infections within their sphere are widespread. In the pre-COVID-19 pandemic era, 10% of respiratory infections viruses were found to be co-infections (Sanz *et al*., 2021). Various studies observed an increased severity of disease in co-infected patients, especially elderly and high-risk

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patients (Mandelia *et al.*, 2021). Virus-virus interaction (either direct or immune-mediated) can have effects on disease severity, transmissibility, immunopathology, and vaccine effectiveness. The epidemiology of respiratory co-infection pairings is poorly understood. Knowledge on risk factors for co-infections and on possible changes in terms of clinical progression of the disease is important to assess the patient's prognosis. Data on virus prevalence and data on the most common co-infecting viruses in the post-pandemic era will help clinicians implement appropriate infection control measures and treat patients adequately, including by administering adequate antiviral therapy whenever available and appropriate.
The objective of this study is to evaluate the prevalence of individual respiratory viruses, co-infections, and clinical impact on patients in the various age groups.

METHODS

3578 nasopharyngeal swabs (NPS) of hospitalized patients were analyzed for respiratory viruses. The patients were divided into 4 groups on the basis of age: <5 years (group 1), 5-18 years (group 2), 19-65 years (group 3), >65 years (group 4).
All NPS were analyzed by single PCR for SARS-CoV2 (Allplex, Seegene, Seoul, Republic of Korea) and by multiplex PCR Respiratory Panel 1, 2 and 3 (Allplex, Seegene, Seoul, Republic of Korea). Respiratory Panel 1 detected Influenza A virus (FluA), Influenza B virus (FluB), Human respiratory syncytial virus A (RSVA), Human respiratory syncytial virus B (RSV B) and subtyping of FluA virus (subtype H1, H3, and H1pdm09). Respiratory Panel 2 detected Human adenovirus (ADV), Human Metapneumovirus (MPV), Human enterovirus, Human parainfluenza virus 1(PIV1), Human parainfluenza virus 2 (PIV2), Human parainfluenza virus 3 (PIV3), and Human parainfluenza virus 4 (PIV4). Respiratory Panel 3 detected Human bocavirus 1/2/3/4, Human rhinovirus A/B/C (hRV), Human coronavirus 229E, Human coronavirus NL63, and Human coronavirus OC43.
930 out of 3578 NPS (pediatrics and adults) were diagnosed positive for at least respiratory viruses. The infections were classified as:

- *Severe Infections:* unabating high fever, respiratory failure, Acute Respiratory Distress Syndrome (ARDS), SO2 <92% and pO2 <70% bronchiolitis.
- *Non-Severe Infections:* fever, cough, cold, rhinitis, SO2 >92% and pO2 >70%.

The study was approved by the Institutional Ethics Committee of Cosenza Hospital by an internal procedure (24 June 2023) without assigning a number; all participants gave written informed consent.

Statistical Analysis

The Student's t test was used to compared single severe infections vs. multiple severe infections for every age group. The ANOVA test was used to compared prevalence of single or multiple infections among the groups.

RESULTS

From 1 September 2022 to 31 March 2023, a total of 930 patients were diagnosed positive for at least one respiratory virus. The distribution of viral infections, prevalence, and pathogen differed significantly among age groups (*Figure 1*): 238 out of 930 (25.6%) belonged to the <5 years group, 39 out of 930 (4.2%) to the 5-18 years group, 182 out of 930 (19.5%) to the 19-65 years group, and 471 out of 930 (50.6%) were> 65 years old.
In the <5 years group, the most frequently detected virus was RSV; rate of positivity was 40.4%, following by Rhinovirus (19.4%), FluA-H3 (8.3%), MPV and AdV (7.6%), and SARS-CoV2 (5.8%). In chil-

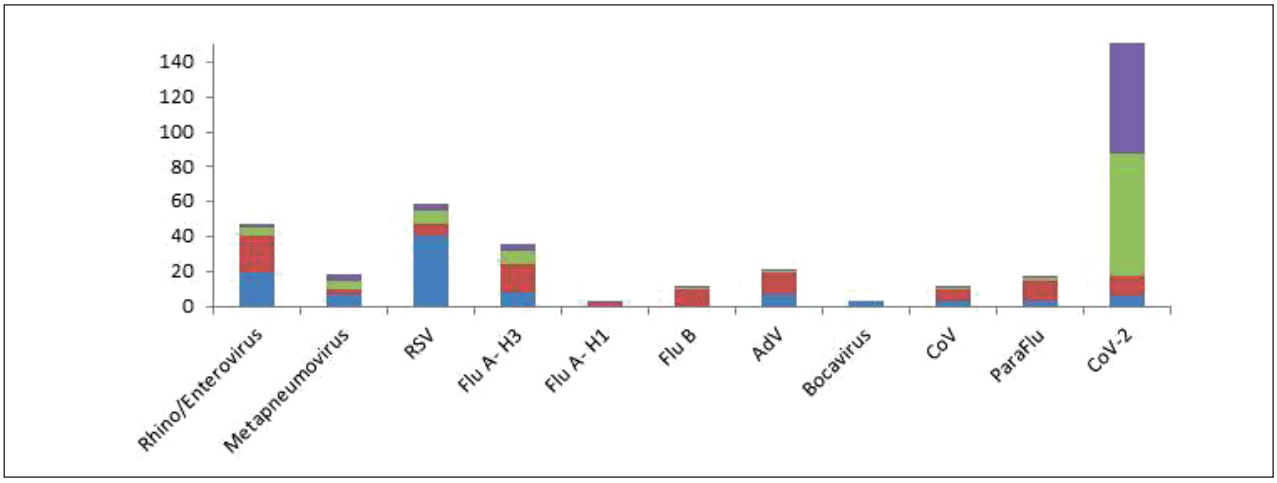

Figure 1 - *Pathogen positivity rate in different age group.*

dren under 5 years of age, severe infections were present in 71.45% with MPV (15/22), 56.2% of RSV (63/112) and 47.6% of ADV (10/21), while only 18.5% of hRV (10/54) patients had severe infections. In the 5-18 group, the rate of Rhinovirus (20.9%) was highest, followed by FluA-H3 (16.3%), SARS-CoV2 and AdV (11.6%), FluB (9.3%), and RSV (6.9%). In this age group, SI were found in one FluA-H3 positive patient, 1 hRV, 5 Paraflu, 4 AdV, and in all four Flu-B positive patients. In the **19-65** group, the most frequently detected virus was SARS-CoV2 (69.8%) followed by RSV and FluA-H3 (7.4%), and Rhinovirus (5.3%). In this group, SI were observed in FluA (H3) and MPV patients, followed by hRV and RSV positive. In the over 65 group, the rate of SARS-CoV2 reached 82%; in this group the rate of RSV was 4.6%, MPV 4.4%, FluA-H3 3.9% (*Figure 1*). In 100% of MPV positive patients, 90.9 % of RSV, and 89.5% of FluA (all single infections), symptoms were severe, while only 24.3% (96/299) of SARS-CoV2 positive (all single infection) patients had SI.

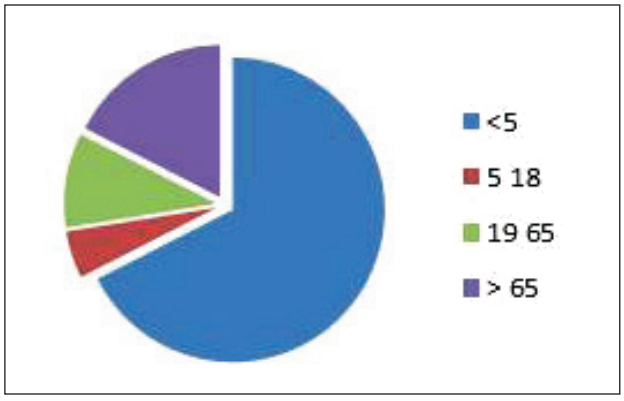

Figure 2 - *Coinfections rate in different age group.*

In total, more than one virus was identified in 80/930 of patients (8.6%), and the co-infections rate varied with age. The rate of coinfections was 67.5% (54/80) in the <5 group, 5% (4/80) in the 5-18 group, 10% (8/80) in the 19-65 group, and 17.5% (14/80) in the >65 group (*Figure 2*).

In the <5 group we observed that the viruses most frequently found in co-infections are RSV and Rhinovirus, following by ADV and MPV (figure 3). The most frequently encountered co-infections concerned hRV + RSV (35.2%), RSV + AdV (12.9%), RSV + MPV (9.2%), and hRV+ AdV (9.2%). In the coinfections, 4 out of 18 severe infections were for MPV, 13 out of 63for RSV positive, out of 10 for ADV 9, and 5 out of 10 for hRV (*Table 1*). Among all the co-infections found, the most serious symptoms were observed when there was simultaneous positivity for AdV and RSV.

In the **5-18** group, 3 out of 4 coinfections were caused by AdV + PIV, in 1 out of 4 by FluB+RSV. 4 out of 4 patients with co-infections showed severe infection.

In the **19-65** group, 5 out of 8 coinfections involved hRV (hRV+PIV3+MPV; hRV+ FluA (H3); hRV+RSV; hRV+OC43, SARS-CoV2+hRV+PIV), AdV+RSV were detected in 2 cases, and FluA (H1pdm09)+OC43 in 1 case. All co-infections detected showed clinical severity.

In the *over 65* group, the viruses most frequently found in co-infections were RSV (42.8%), followed by MPV and hRV (28.6%).

In all the elderly people recruited in our study, co-infections caused severe infection; 3 patients died.

Table 1 - *Distribution of respiratory virus in <5 years age group.*

	Severe Infections							
	<5 anni		*5-18*		*19-65*		*>65*	
	Single	*Multi*	*Single*	*Multi*	*Single*	*Multi*	*Single*	*Multi*
Rhinovirus	5/41	5/13	1/9	/	5/8	2/2	1/5	5/5
MPV	14/16	4/6	/	/	7/8	1/1	17/17	4/4
RSV	50/63	13/23	/	1/1	4/10	2/4	15/17	5/5
FluA-H3	5/19	1/5	1/7	/	10/13	1/1	17/18	/
FluA-H1	/	/	/	/	/	1/1	1/1	2/2
Flu-B	/	/	3/3	1/1	1/1	/	1/1	/
AdV	1/9	9/12	1/2	3/3	1/1	1/1	2/2	1
CoV	3/5	2/4	/	/	/	1/1	1/2	1/1
ParaFlu	1/7	1/2	2/2	3/3	1/2	1/1	3/3	2/2
Cov-2	1/8	6/9	/	/	25/132	/	96/395	/

No significant difference was observed comparing the number of severe single infections with that of severe mixed infections, the clinical impact of the co-infections is comparable to that of the infections caused by the single viruses. The prevalence of single infections in the various age groups showed a significant differences in >65 vs 18-65 group, 19-65 vs 5-18 group and <5 vs 18-65 group. The prevalence of mutiple infections in the various age groups showed a significant differences in <5 vs 18-65 and 19-65 group. No significant differences were found among other group.

CONCLUSIONS

This study showed that the distribution of viral infections in single and multi-detection, in hospitalized patients, differed among age groups. According to data in the literature, most ARI are observed in the over 65 age group (50.6%), followed by the <5group (25.6%), 19-65 group (19.5%), and 5-18 group (4.2%), confirming that a decline in immune functions in older adults and immature immune functions in children increase susceptibility to infections and risk of hospitalization. Differences were observed between pathogen distribution and clinical impact. In pediatric patients under 5 years of age, RSV, hRV, MPV, AdV, and FluA are the most common viruses (Zhu et al., 2021; Avolio et al., 2022), and in this group SARS-CoV2 is 6th among respiratory viruses. In pediatric patients, severe infections were observed, in particular for RSV and MPV. The percentage of positivity of SARS- CoV2 increased with age, and despite the presence of the vaccine continues to be the most frequent, reaching a very high rate in adult patients: 69.8% in the 19-65 group and over 82% in the >65 group. It is important to stress that the clinical impact of SARS-CoV2 is serious only in the presence of comorbidities and risk factors like organ transplant, diabetes, and in immunocompromised patients.

RSV, MPV and FluA were frequently encountered in older adult patients. These viruses showed lower hospitalized percentages than in pediatric patients but an increase in severe infections in the >65 group. Although there are few hospitalized cases of hRV in the 19-65 and > 65 group, the infections related to this virus were severe. The 5-18 group had the lowest number of hospitalized patients for respiratory infections.

Co-infections were significantly more widespread in pediatric samples. Rates of co-detections were especially elevated under the age of 5 (67.5%). In this study, high percentages of ADV, RSV, and hRV were found in coinfections. Conversely, FluA, SARS CoV-2, and MPV had the lowest co-infection rates. In pediatric patients, clinical significance is pathogen dependent in most cases, and severe infections did not show an increase in coinfection. The presence of AdV in multi-detection seemed to cause an increase in severe infections compared to single AdV infection. In the few cases found in the other groups, the clinical impact of coinfections appeared more severe, but no significant differences were found. Given the low prevalence of coinfections observed in other groups, it is difficult to establish with certainty the impact of co-infections on clinical outcome. Further studies are needed to establish the clinical significance of co-infections in both pediatric and adult patients.

This study highlighted that the implementation of early diagnosis of viral respiratory infections, by single- and multi-detection, is important for several reasons.

In the community setting, optimal knowledge of the epidemiology of viral infections, could help promote and implement the administration of available vaccines, in particular in high-risk patients.

In clinical practice, implementing rapid viral PCR tests is crucial to distinguish bacterial from viral infection, allow adequate therapy, and avoid the improper use of antibiotics in both children and adults, thereby promoting optimal treatment. Ribavirin and palivizumab could be used to reduce RSV disease burden in high-risk patients (Domachowske *et al*., 2021; Fragkou *et al*., 2021). Several studies report that the timing of treatment initiation is a critical aspect in treating the early stage of several influenza and SARS-CoV2 infections (Metlay *et al*., 2019; Lamontagne et al., 2020; Mastroianni *et al.,* 2021).

Furthermore, an accurate and rapid diagnosis of respiratory viruses, in a clinical setting, could help isolate the patient as soon as possible and prevent airborne transmission to avoid hospital outbreaks, representing an important infection control measure.

Authors' contributions

Mauro M.V.: designed the work, interpreted data, drafted the work.

Greco S.: acquired data and gave final approval of the version to be published.

Pellegrini M., Campagna T., Caprino F., Elia N.: acquired data and gave final approval of the version to be published

Mastroianni A., Greco F.: revised the work.

All authors read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest.

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