COVID-19

SARS-CoV-2 and influenza virus coinfections in the Tuscan population during the 2021/2022 influenza season

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Keywords

Coinfection • Population • Respiratory viruses

Summary

Introduction. *The 2021/2022 influenza season was not characterised by a well-defined incidence peak. As reported by the Italian National Institute of Health, a high value of incidence of influenza cases was recorded in week 13, but it was still lower than in other influenza seasons. This abnormal circulation was probably due to relaxation of the COVID-19 pandemic restriction measures, such as social distancing, smart-working, home leaning and the use of masks, which greatly reduced the circulation of respiratory-transmitted viruses, including human respiratory syncytial virus (HRSV). The symptoms of SARS-CoV-2 and influenza are quite similar, sharing the human-to-human transmission route via respiratory droplets.*

Methods. *The aim of this study was to estimate the rate of coinfection with influenza viruses and/or HRSV in SARS-CoV-2-positive subjects (N = 940) in a population of central Italy during the 2021/2022 season.*

Results. *A total of 54 cases of coinfection were detected during the study period, 51 cases (5.4%) of SARS-CoV-2 and influenza virus and three cases (0.3%) of SARS-CoV-2 and HRSV coinfection.*

Conclusions. *These results highlight the importance of continuous monitoring of the circulation of influenza virus and other respiratory viruses in the context of the COVID-19 pandemic.*

Introduction

SARS-CoV-2 is a non-segmented, enveloped, positive-sense RNA virus that began its worldwide spread in December 2019 [1]. The World Health Organization (WHO) declared the COVID-19 pandemic on March 2020. The virus spread all year round, showing peaks in winter and when social containment measures were relaxed [2]. Influenza is caused by a segmented, negative-sense RNA virus that gives rise to epidemics, mostly in the winter months. Of the four types of influenza viruses, influenza A (IAV) and B (IBV) are mainly responsible for seasonal influenza. Currently, A/H1N1 and A/H3N2 are the most widespread IAV subtypes circulating in the human population [3]. Human respiratory syncytial virus (HRSV) is a seasonal negative-sense RNA virus and prominent cause of acute lower respiratory tract infections in young children [4].

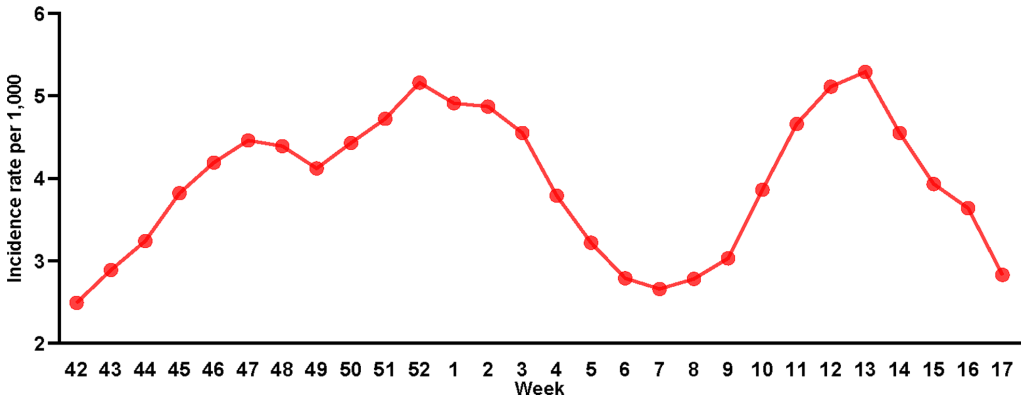
In response to the COVID-19 health emergency and given the absence of specific pharmacological therapies or highly effective vaccines to curb the spread of SARS-CoV-2, many countries adopted non-pharmaceutical mitigation strategies. These strategies included the use of personal protective equipment, implementation of social distancing measures, temporary closure of educational institutions and airports, and mandatory reporting of cases of infection, followed by isolation of affected individuals. This approach passively influenced the seasonal transmission patterns of airborne viruses, including influenza viruses and HRSV [5, 6].

The 2020/2021 influenza season in Italy was characterized by an initial phase of co-circulation of SARS-CoV-2 and influenza viruses, followed by a rapid decline in influenza transmission due to implementation of non-pharmaceutical measures. However, during the 2021/2022 influenza surveillance season (from week 42 of 2021 to week 17 of 2022), an increase in the incidence of influenza was recorded [7]. The epidemiological curve of influenza-like illness (ILI) cases showed a bimodal trend, peaking at week 52 of 2021 and weeks 12-13 of 2022, when positive samples again rose above the epidemic threshold of 10% positivity [8] (Fig. 1). The first wave was characterized as supported mainly by HRSV circulation, while influenza viruses dominated in the second wave [19].

An increasing number of studies show that patients affected with COVID-19 may also be coinfected with other respiratory pathogens [9-11]. Indeed, the coexistence of SARS-CoV-2 and influenza viruses led to cases of coinfection having more severe symptoms than infections with either virus alone [12].

The present study, performed during the 2021/2022 influenza season, investigated the prevalence of influenza or HRSV coinfections in SARS-CoV-2-positive subjects

Fig. 1. Incidence of influenza-like illness in Italy by week during the 2021/2022 influenza season, according to the Italian National Institute of Health [8].



in Tuscany (Italy), with the aim of underlining the importance of continuing epidemiological surveillance of other respiratory viruses in addition to SARS-CoV-2.

Materials and methods

Study design

Oropharyngeal swabs were collected by general practitioners, during the 2021/2022 influenza surveillance season (from week 46 of 2021 to week 17 of 2022) in Siena, Tuscany (Italy), and stored at -80°C. A total of 940 swabs were selected as having previously tested positive for SARS-CoV-2: 742 collected during the first influenza wave (week 46 of 2021 to week 4 of 2022) and 198 during the second (week 5 to week 17 of 2022). Information on the age and sex of the subject was available for 860 swabs. The median age of subjects was 30 years (range 1-65 years), 422 were male and 438 were female. Swabs were divided by age group as follows: 1-10 years (N = 52), 11-20 years (N = 146), 21-30 years (N = 260), 31-40 years (N = 147), 41-50 years (N = 125), and 51-65 years (N = 130). No information on COVID-19 or influenza vaccination status was available. Informed consent was submitted and signed by all patients who voluntarily underwent swabbing

Laboratory analysis

Total RNA was extracted from specimens by QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany). Real-time reverse transcriptase–polymerase chain reaction tests (RT-PCR) were performed for IAV, IBV and HRSV with Flu/HRSV kit (Siemens) on nasopharyngeal swabs of subjects who had already tested positive for SARS-CoV-2 by COVID-19 HT Screen (Clonit, Abbiategrasso, Italy). At the same time, one-step real-time RT-PCR was performed in a final volume of 25 µL (SuperScript III Platinum One-Step qRT-PCR Kit, Thermo Fisher Scientific, Waltham, MA, USA) to subtype for pandemic influenza virus A/H1N1 (Flu A/pH1N1) and seasonal influenza virus A/H3N2 (Flu A/H3N2) on samples positive for IAV. H3-For Primer: AAGCATTCCYAATGACAAACC, H3-Rev Primer: ATT GCR CCR AAT ATG CCT CTA GT, H3-Probe: Fam – 5' CAG GAT CAC A"T"A TGG GSC CTG TCC CAG – 3' SPACER – BHQ-1 and H1pdm-For Primer: GTG CTA TAA ACA CCA GYC TCC CAT T,H1pdm-Rev Primer: AGA YGG GAC ATT CCT CAA TCC TG, H1pdm-Probe: Fam – 5' TGG CCA GYC "T" CA ATT TTG TGC TTT TTA CAT A - 3' SPACER – BHQ-1, were used.

Statistical analysis

The median ages of the total and influenza-positive populations were calculated. The number of SARS-CoV-2/influenza or HRSV coinfection cases by period of collection (first and second influenza waves) and age group (above or below median age, *i.e.* 30 years) was compared by the Yates corrected chi-square test. Statistical significance was set at $p < 0.05$ (two-tailed test). All statistical analysis was performed using GraphPad Prism 6 software.

Results

A total of 54 cases (5.7%) of coinfection were detected during the study period: 51 cases (5.4%) of SARS-CoV-2 and influenza viruses (43 for IAV and 8 for IBV) and three cases (0.3%) of SARS-CoV-2 and HRSV. Of the influenza cases, 34 cases of IAV were A/H3N2 subtype while the remaining 9 cases were not attributed to a subtype. The 8 cases of IBV were not subtyped.

As reported in Figure 2, most coinfections were detected during the first influenza wave (36/43 cases of IAV, 6/8 cases of IBV and 2/3 cases of HRSV), albeit showing no statistically significant difference with respect to the second wave.

For 860 samples, including all those in which an influenza virus or HRSV coinfection was detected, information on the sex and age of the subjects was available.

Fig. 2. Cases of coinfection with SARS-CoV-2 and human respiratory syncytial (HRSV), influenza A (IAV) or B (IBV) viruses from November 2021 to May 2022. Dashed vertical line separates first (left) and second (right) wave of influenza.

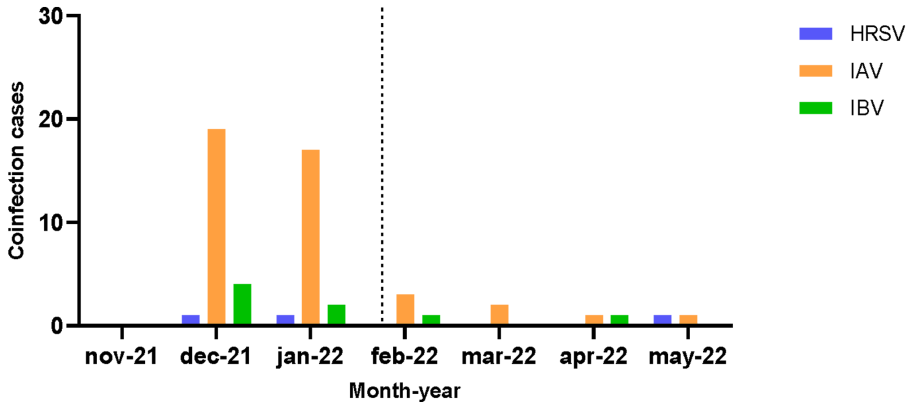
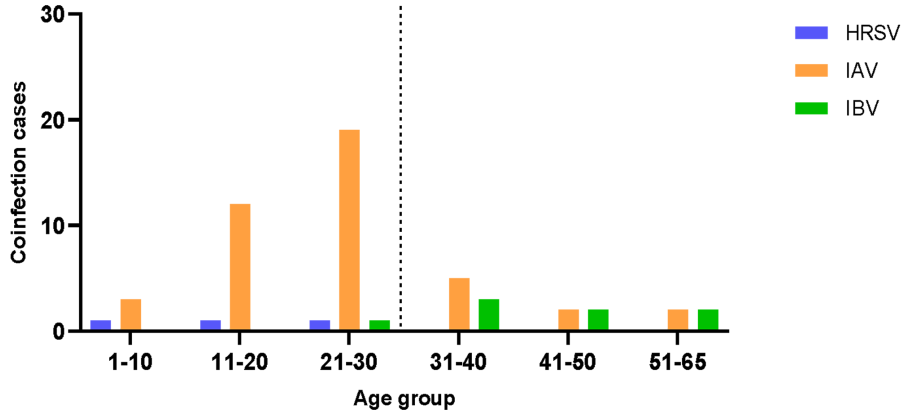


Fig. 3. Cases of coinfection with SARS-CoV-2 and human respiratory syncytial (HRSV), influenza A (IAV) or B (IBV) viruses by age group. Dashed vertical line indicates the median age of the study population (30 years).



No significant difference in the distribution of cases by sex was found, while Figure 3 shows the distribution of coinfection cases by age.

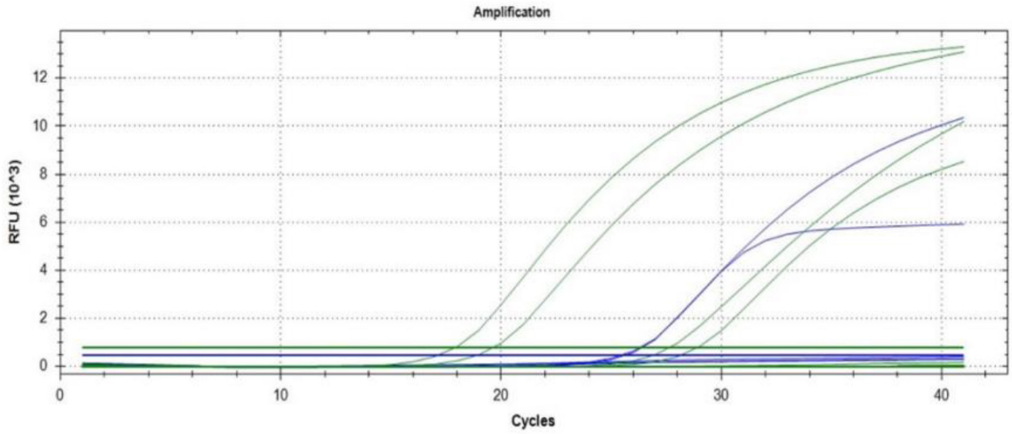
SARS-CoV-2 coinfections with HRSV were only detected in subjects under 30 years of age (median age of positive subjects 14 years), although the difference was not significant. Coinfections with IAV were detected in all age groups, but the prevalence was higher in subjects of 30 years and under (median age of positive subjects 23 years, $p = 0.0009$), while IBV was detected mainly in subjects of 30 years and over (median age of positive subjects 43 years, $p = 0.0494$). Some PCR results regarding Flu/HRSV kit (Siemens) are shown in Figure 4.

Discussion

In December 2019, identification of a new coronavirus in Wuhan, China, demanded a prompt response and global cooperation by health authorities [6]. The pandemic influenced the seasonal transmission patterns of airborne viruses. SARS-CoV-2 shares transmission through direct contact and airborne contagion as well as symptoms, including fever, cough, sore throat, fatigue, nasal congestion and respiratory distress, with the influenza virus, complicating differentiation of the two infections [5, 6]. Cases of coinfection with SARS-CoV-2 and influenza proved to have symptoms that were more severe [12]. After 2019/2020, the incidence of ILI in Italy declined due to non-pharmaceutical actions imposed for SARS-CoV-2. In the 2021/2022 season, an increase in the incidence of infections caused by influenza viruses was observed, with an overall positivity rate of 14.4% [19] although significantly lower than that observed before the COVID-19 pandemic. Indeed, the season was characterized by low circulation of influenza, the first cases being reported in week 52 of 2021. Circulation increased from week 8 of 2022, reaching a maximum in week 12 of the same year, when COVID-19 restrictions were relaxed [13].

In this study, we found a total of three cases of coinfection of SARS-CoV-2 and HRSV and 51 cases of coinfection of SARS-CoV-2 and influenza viruses: 43 cases of IAV and 8 cases of IBV. Ignoring influenza virus type, influenza coinfection cases were detected in 5.4% of the SARS-CoV-2-positive subjects tested in this study, a coinfection rate in line with those reported in other countries [14-16].

Fig. 4. Representative results of PCR. Green fluorescence for Influenza A positive samples, blue fluorescence for HRSV positive sample.



Among IAV cases, 34 were caused by A/H3N2 subtype, in line with Italian and European data reporting it to be the predominant influenza subtype during the 2021/2022 season [17, 18].

The majority of SARS-CoV-2 and influenza coinfections were found during the first wave of the influenza season. A similar distribution was observed in a study conducted in children and adolescents hospitalized in the US, where influenza-associated hospitalizations characterized by influenza and SARS-CoV-2 coinfections were higher in December and January months [20]. IAV and SARS-CoV-2 coinfections were higher in the first months of the season also in a US study conducted in individuals from University of Missouri Health Care [21], suggesting that coinfection with SARS-CoV-2 Omicron variant may have been reduced compared to coinfections with Delta variant, due to different viral antagonism to IAVs.

In a surveillance study conducted on hospitalized subjects in Tuscany during the same season, three cases of SARS-CoV-2 and influenza coinfection were found in March in the second half of the influenza season [17]. The same study reported two cases of HRSV infection, one of which involved coinfection with SARS-CoV-2. Both cases occurred in subjects aged 30 years or under, in line with the results of our study. In contrast to our results, a report by Cong et al in a population older than 18 years, the median age of co-infected persons was older than the fifth year [25]. As reported in few studies, in patients with COVID-19-Influenza co-infection, the need for intervention with mechanical ventilation and hospital stay increase compared with patients infected with only one of the two respiratory viruses, suggesting an aggravation of the disease picture [23, 24]. Although it appears that the copresence of the two viruses does not result in increased mortality [25]

Our study has some limitations. Information on clinical features, medication use and outcomes, as well as vaccination status for COVID-19 and/or influenza was not available and sequencing of SARS-CoV-2- and influenza-positive samples was not performed. In addition, since in Italy administration of live attenuated influenza vaccines (LAIV) are authorised for use in persons aged between 2 and 18 years [22], we cannot exclude detection of influenza antigen from vaccinal strains in younger age groups swabs. Moreover, other respiratory viruses potentially coinfecting with SARS-CoV-2, such as parainfluenza viruses, were not included.

Few studies on respiratory virus epidemiology and coinfections have been conducted in Italy in the 2021/2022 season. The 2021/2022 influenza season was peculiar as it saw the partial resurgence of respiratory viruses after the advent of COVID-19, therefore the characterization of as much as possible the epidemiology of respiratory viruses and coinfections with SARS-CoV-2 is of outmost importance to understand the post-pandemic epidemiology of respiratory viruses.

Given the overlapping symptoms and epidemiology of the influenza virus and SARS-CoV-2, it remains of primary importance to conduct differential diagnosis of these major airborne-transmitted viruses to avert complications related to infection. The use of multiplex RT-PCR tests, as in our study, ensures a timely diagnosis and consequently an appropriate clinical approach to each patient. Despite its limitations, our study underscores the significance of continuous monitoring of the circulation of influenza viruses.

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Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

The data presented in this study are available on request from the corresponding author.

Conflict of interest statement

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Authors' contributions

IM, GM: conceptualization. IM: methodology. IM: validation. SM: formal analysis. GM, IV, LF, C.B: investigation. CMT, IM, IV, EM: resources. SM: data curation. GM: writing-original draft preparation. SM: writing-review and editing. SM: visualization. IM: supervision. IM: project administration. All authors have read and agreed to the published version of the manuscript.

References

[1] Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao GF, Tan W; China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med 2020;382:727-33. https://doi.org/10.1056/NEJMoa2001017.

[2] Civil Protection Department. Available at: https://www.protezionecivile.gov.it/en/ (Accessed on: 26/05/2022).

[3] Krammer F, Smith GJD, Fouchier RAM, Peiris M, Kedzierska K, Doherty PC, Palese P, Shaw ML, Treanor J, Webster RG, García-Sastre A. Influenza. Nat Rev Dis Primers 2018;4:3. https://doi.org/10.1038/s41572-018-0002-y.

[4] Halabi KC, Wang H, Leber AL, Sánchez PJ, Ramilo O, Mejias A. Respiratory syncytial virus and SARS-CoV-2 coinfections in children. Pediatr Pulmonol 2022;57:3158-60. https://doi.org/10.1002/ppul.26127.

[5] Flerlage T, Boyd DF, Meliopoulos V, Thomas PG, Schultz-Cherry S. Influenza virus and SARS-CoV-2: pathogenesis and host responses in the respiratory tract. Nat Rev Microbiol 2021;19:425-41. https://doi.org/10.1038/s41579-021-00542-7.

[6] Ozaras R, Cirpin R, Duran A, Duman H, Arslan O, Bakcan Y, Kaya M, Mutlu H, Isayeva L, Kebanlı F, Deger BA, Bekeshev E, Kaya F, Bilir S. Influenza and COVID-19 coinfection: report of six cases and review of the literature. J Med Virol 2020;92:2657-65. https://doi.org/10.1002/jmv.26125.

[7] Istituto Superiore della Sanità, Epicentro. Available at: https://www.epicentro.iss.it/influenza/stagione-in-corso (Accessed on: 01/07/2022).

[8] Istituto Superiore della Sanità, Epicentro. Available at: https://www.epicentro.iss.it/influenza/flunews21-22#epi (Accessed on: 25/10/2023).

[9] Bai L, Zhao Y, Dong J, Liang S, Guo M, Liu X, Wang X, Huang Z, Sun X, Zhang Z, Dong L, Liu Q, Zheng Y, Niu D, Xiang M, Song K, Ye J, Zheng W, Tang Z, Tang M, Zhou Y, Shen C, Dai M, Zhou L, Chen Y, Yan H, Lan K, Xu K. Coinfection with influenza A virus enhances SARS-CoV-2 infectivity. Cell Res 2021;31:395-403. https://doi.org/10.1038/s41422-021-00473-1.

[10] Burrel S, Hausfater P, Dres M, Pourcher V, Luyt CE, Teyssou E, Soulié C, Calvez V, Marcelin AG, Boutolleau D. Co-infection of SARS-CoV-2 with other respiratory viruses and performance of lower respiratory tract samples for the diagnosis of COVID-19. Int J Infect Dis 2021;102:10-3. https://doi.org/10.1016/j.ijid.2020.10.040.

[11] Xiaojuan Z, Yiyue G, Tao W, Kangchen Z, Yin C, Bin W, Fengcai Z, Baoli Z, Lunbiao C. Co-infection with respiratory pathogens among COVID-2019 cases. Virus Res 2020;285:198005. https://doi.org/10.1016/j.virusres.2020.198005.

[12] Uyeki TM, Hui DS, Zambon M, Wentworth DE, Monto AS. Influenza. Lancet 2022;400:693-706. https://doi.org/10.1016/S0140-6736(22)00982-5.

[13] Rapporto Influnet. Available at: https://www.iss.it/site/rmi/influnet/pagine/rapportoinflunet.aspx (Accessed on: 09/02/2023).

[14] Steponavičienė A, Burokienė S, Ivaškevičienė I, Stacevičienė I, Vaičiūnienė D, Jankauskienė A. Influenza and respiratory syncytial virus infections in pediatric patients during the COVID-19 pandemic: a single-center experience. Children (Basel) 2023;10:126. https://doi.org/10.3390/children10010126.

[15] Trifonova I, Christova I, Madzharova I, Angelova S, Voleva S, Yordanova R, Tcherveniakova T, Krumova S, Korsun N. Clinical significance and role of coinfections with respiratory pathogens among individuals with confirmed severe acute respiratory syndrome coronavirus-2 infection. Front Public Health 2022;10:959319. https://doi.org/10.3389/fpubh.2022.959319. Saeedeh. E.; Mojtaba. K.; Barat. B.; Nayeb. F.D.; Zahra. N.; Tahereh. N.; Faezeh. S. The circulation of common respiratory viruses and their co-infection with severe respiratory syndrome coronavirus 2 before and after coronavirus disease of 2019 vaccination. Jundishapur J Microbiol 2022;15:e133326. Https://doi.org/10.5812/jjm-133326.

[16] Milano G, Capitani E, Camarri A, Bova G, Capecchi PL, Lazzeri G, Lipari D, Montomoli E, Manini I. Surveillance of influenza and other airborne transmission viruses during the 2021/2022 season in hospitalized subjects in Tuscany, Italy. Vaccines (Basel) 2023;11:776. https://doi.org/10.3390/vaccines11040776.

[17] Istituto Superiore della Sanità, Epicentro. Available at: https://www.epicentro.iss.it/influenza/flunews#vir (Accessed on: 26/05/2022).

[18] Istituto Superiore della Sanità, Epicentro. Available at: https://www.epicentro.iss.it/ben/2022/4/virus-influenzali-vaccinazioni-influnet-2021-2022 (Accessed on: 19/12/2023).

[19] Adams K, Tastad KJ, Huang S, Ujamaa D, Kniss K, Cummings C, Reingold A, Roland J, Austin E, Kawasaki B, Meek J, Yousey-Hindes K, Anderson EJ, Openo KP, Reeg L, Leegwater L, McMahon M, Bye E, Poblete M, Landis Z, Spina NL, Engesser K, Bennett NM, Gaitan MA, Shiltz E, Moran N, Sutton M, Abdullah N, Schaffner W, Talbot HK, Olsen K, Staten H, Taylor CA, Havers FP, Reed C, Budd A, Garg S, O'Halloran A, Brammer L. Prevalence of SARS-CoV-2 and influenza coinfection and clinical characteristics among children and adolescents aged < 18 years who were hospitalized or died with influenza – United States, 2021-22 influenza season. MMWR Morb Mortal Wkly Rep 2022;71:1589-96. https://doi.org/10.15585/mmwr.mm7150a4.

[20] Tang C, Boftsi M, Staudt L., Jane A. McElroy e, Tao Li f, Duong S, Adrienne Ohler g, Detlef Ritter h, Richard Hammer d,h, Jun Hang f, XiuFeng Wan. SARS-CoV-2 and influenza co-

infection: a cross-sectional study in central Missouri during the 2021-2022 influenza season. Virology 2022:576:105-10.https://doi.org/10.1016/j.virol.2022.09.009.

[21] Ministero della Salute, Available at: https://www.salute.gov.it/portale/p5_1_2.jsp?id=103&lingua=italiano#:~:text=%C3%88%20indicato%20dai%2018%20anni%20di%20et%C3%A0.&text=Il%20vaccino%20vivo%20attenuato%20%C3%A8,tra%202%20e%2018%20anni (Accessed on 19/12/2023).

[22] Garg I, Gangu K, Shuja H, Agahi A, Sharma H, Bobba A, Nasrullah A, Chourasia P, Pal S, Sheikh AB, Shekhar R. COVID-19 and influenza coinfection outcomes among hospitalized patients in the United States: a propensity matched analysis of national inpatient sample. Vaccines (Basel) 2022;10:2159. https://doi.org/10.3390/vaccines10122159.

[23] Swets MC, Russell CD, Harrison EM, Docherty AB, Lone N, Girvan M, Hardwick HE; ISARIC4C Investigators; Visser LG, Openshaw PJM, Groeneveld GH, Semple MG, Baillie JK. SARS-CoV-2 co-infection with influenza viruses, respiratory syncytial virus, or adenoviruses. Lancet 2022;399:1463-4. https://doi.org/10.1016/S0140-6736(22)00383-X.

[24] Cong B, Deng S, Wang X, Li Y. The role of respiratory co-infection with influenza or respiratory syncytial virus in the clinical severity of COVID-19 patients: a systematic review and meta-analysis. J Glob Health 2022;12:05040. https://doi.org/10.7189/jogh.12.05040.

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