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A rare case of SARS-CoV-2 and influenza A virus super-infection

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ABSTRACT

We report the first Italian case of SARS-CoV-2 and influenza A virus super-infection. Laboratory diagnosis revealed the presence of both agents' RNA specific sequences by molecular methods and infectious influenza A virus by cell culture methods.

1. Case report

The emerging severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causing Coronavirus Disease 2019 (COVID-19) was revealed for the first time in December 2019 in Wuhan, China. To date, it is still spreading all over the world resulting in millions of cases of infection and deaths and still represents a relevant threat to public health [1,2]. Possible co-infections with known respiratory viruses could complicate recognition of the full disease extent and thus need an accurate laboratory diagnosis.

Despite a consistent prevalence of SARS-CoV-2 and a concomitant drop of the upper and lower respiratory tract infections caused by other known respiratory viruses during the present pandemic, co-infections including influenza A have been described and reported as potential worsening conditions to the clinical outcome of the disease [3–8]. A co-infection can be defined as concomitant infection, while super-infection as the sequential infection by 2 different pathogens [9,10].

We report a case of super-infection by influenza A H3 virus occurred in a SARS-CoV-2 positive young woman who had just given birth. The medical order reported the observation of a peak of hyperpyrexia and no antiviral/antibacterial prophylaxis/therapy administration with an outcome of residual interstitial pneumonia observed by CT scan. The first detection of SARS-CoV-2 RNA dated back to January 2022, while on 3 February 2022 influenza A H3 virus RNA, together with SARS-CoV-2 RNA, was revealed. To the best of our knowledge, no previous data of SARS-CoV-2 and influenza A H3 super-infection have been reported in Italy.

Laboratory diagnosis was performed on a nasal swab by multiplex real-time polymerase chain reaction and Retro-Transcriptase-real-time polymerase chain reaction assays and rapid and conventional cell culture methods.

The molecular techniques, allowing to simultaneously detect specific genomic sequences of up to 20 respiratory viruses (BioFire Respiratory Panel 2.1 plus, BioMérieux: adenovirus, influenza A virus H1, H3 and H1-2009, influenza B virus, parainfluenza virus 1–4, RSV, metapneumovirus, rhinovirus/enterovirus, coronavirus 229E, HKU1, NL63, OC43, MERS-CoV and SARS-CoV-2; Allplex Respiratory Panel 3 assay, Seegene: bocavirus 1–4), evidenced the presence of SARS-CoV-2 and Influenza A H3 RNA.

Concerning cell culture methods, the nasal swab was tested by: (1) immunofluorescence assay in exfoliated cells directly derived from the sample, to detect Respiratory Syncytial virus and Influenza antigens (monoclonal antibodies to Respiratory Syncytial Virus and Influenza A and B species; IMAGEN™ Respiratory Syncytial Virus kit and Influenza Virus A and B kit, OXOID), that was positive for Influenza virus; (2) the same immunofluorescence technique after a 24-hours incubation of inoculated cell monolayers grown on a chamber-slide to detect influenza (A and B), parainfluenza (1–3), RSV-specific

Abbreviations: COVID-19, coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; PCR, polymerase chain reaction; MDCK SIAT-1, Madin-Darby canine kidney cells stably transfected by the cDNA of human α-2,6-sialyltransferase receptor; p.i., post-infection; VERO, African green monkey kidney cells; IFN, interferon

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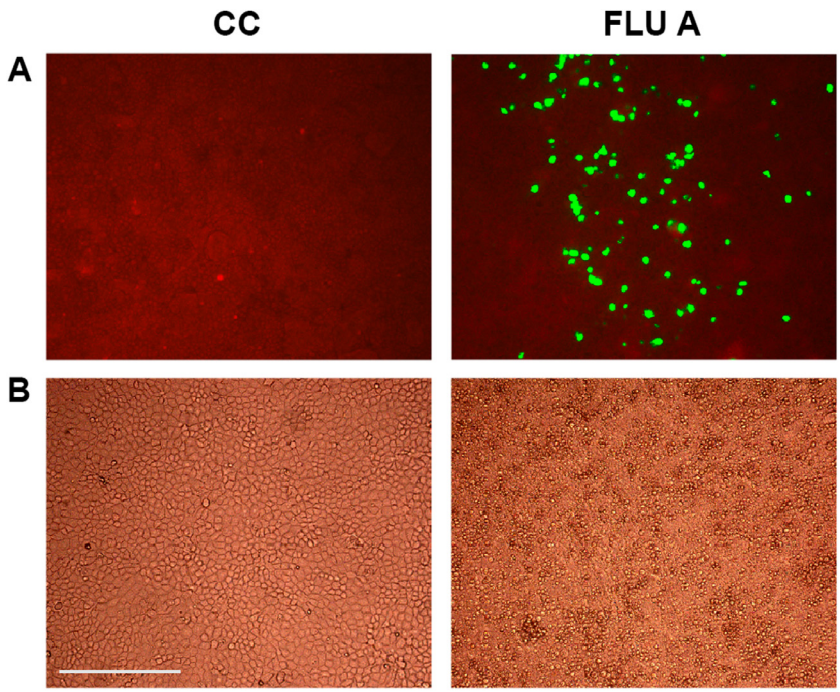

Fig. 1. (Panels A): Immunofluorescence analysis of Influenza A (FLU A) antigen expression in MDCK SIAT-1 cell monolayers at 24h p.i. (right panel) by rapid cell culture method. (Panels B): virus-induced cytopathic effect in MDCK SIAT-1 cell monolayers at day 4 p.i. (right panel) by conventional cell culture method. CC = control (uninfected) cells. Bar = 50 μm.

antigens (rapid cell culture method) performed as previously described [3,11,12]; it resulted positive for Influenza A virus (Fig. 1A); (3) conventional cell culture method by sample inoculation in different cell type monolayers to detect cytopathogenic respiratory viral agents by daily microscopic observation, according to previously described methods [3,11,12].

A cytopathic effect was detected in Madin-Darby canine kidney cells stably transfected by the cDNA of human α-2,6-sialyltransferase receptor (MDCK SIAT-1) at day 4 post-infection (Fig. 1B); the cytopathogenic agent was then identified as influenza A virus by immunofluorescence assay.

No cytopathic effect related to SARS-CoV-2 infection was evidenced in African green monkey kidney cells (VERO), a highly permissive cell line for the pandemic coronavirus [13], up to 2 weeks of microscopic observation.

Co-infections by SARS-CoV-2 and influenza A virus, a well-known viral agent associated to considerable morbidity and mortality worldwide, have been described during the 2019 to 2020 seasonal Influenza period, mostly in United States, China and Middle East [4–7].

On the other hand, most likely due to mask wearing and social distancing, significantly fewer cases of influenza, as well as other respiratory viruses and co-infections, were observed during the 2020 to 2021 Influenza season.

The peculiar features of the first Italian case, here reported, consisted in the fact that the young woman had already been infected by SARS-CoV-2 at the beginning of 2022, and then super-infected by influenza A virus 1 month later, with the first viral agent still persisting and detectable only as genomic RNA. The patient was reported as long COVID 19 on the medical order according to Center for Diseases Control and Prevention [14].

Despite the detection of the RNA alone cannot demonstrate the viability of SARS- CoV-2, and the prolonged RNA shedding could be associated with non-infectious viral recovery, infectious virus from patients with extended RNA shedding has been previously reported in particular for immunocompromised ones [15,16]. Indeed, recent studies demonstrate that SARS-CoV-2 infection is associated with accelerated replication, high viral RNA titers and isolation of infectious virus during the acute phase, which is followed by a rapid decrease after the first 2 to 3 weeks [17–20]. Nevertheless, the continuous detection of SARS-CoV-2 RNA in clinical specimens for long time could be due to slow replication of a persistent virus [16,17] and this cannot be excluded in the case here reported. Obviously, in this case, it cannot also be excluded that the long COVID observation could be partially affected by the Influenza infection 1 month later. Interestingly, using an animal model for SARS-CoV-2 infection, a strong correlation between the infection transmission and isolation of viable virus in cell cultures has been found; on the other hand, no correlation was observed between transmissibility and prolonged detection of viral RNA from the nasopharyngeal swab, further corroborating that infectivity decreases precipitously and prolonged detection of viral RNA does not necessarily mean that the individual is infectious [21].

In this context, since in COVID-19 patients a diminished and delayed production of both interferon-λ and type I interferon was reported, it could be reasonably assumed that SARS-CoV-2 infection does not follow the conventional paradigm of antiviral immunity, [17,22,23] and this could account for SARS-CoV-2 persistence, also opening the route to co-infections, as in the case presented here, where only pandemic coronavirus RNA was detected, together with infectious influenza A virus. In conclusion, as demonstrated by the first case of SARS-CoV-2 detected in our area [13], to accomplish an accurate and complete laboratory diagnosis, molecular techniques should be used in parallel with viral culture, the latter representing the only reference laboratory method able to reveal the presence of cytopathogenic viral agents and demonstrate their infectivity.

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Declaration of Competing Interest

None.

CRediT authorship contribution statement

Maria-Cristina Arcangeletti: Investigation, Data curation, Writing – review & editing. **Flora De Conto:** Investigation, Data curation, Writing – review & editing. **Sara Montecchini:** Investigation, Data curation, Writing – review & editing. **Mirko Buttrini:** Investigation, Data curation, Writing – review & editing. **Clara Maccari:** Data curation, Visualization, Writing – review & editing. **Carlo Chezzi:** Writing – review & editing, Validation. **Adriana Calderaro:** Conceptualization, Writing – review & editing, Validation, Resources.

References

[1] Long B, Carius BM, Chavez S, Liang SY, Brady WJ, Koyfman A, et al. Clinical update on COVID-19 for the emergency clinician: presentation and evaluation. Am J Emerg Med 2022;54:46–57. doi: 10.1016/j.ajem.2022.01.028.

[2] Escobedo RA, Kaushal D, Singh DK. Insights into the changing landscape of Coronavirus disease 2019. Front Cell Infect Microbiol 2021;11:761521. doi: 10.3389/fcimb.2021.761521.

[3] Calderaro A, De Conto F, Buttrini M, Piccolo G, Montecchini S, Maccari C, et al. Human respiratory viruses, including SARS-CoV-2, circulating in the winter season 2019—2020 in Parma, Northern Italy. Int J Infect Dis 2021;102:79–84. doi: 10.1016/j.ijid.2020.09.1473.

[4] Wu X, Cai Y, Huang X, Yu X, Zhao L, Wang F, et al. Co-infection with SARS-CoV-2 and influenza a virus in patient with pneumonia, China. Emerg Infect Dis 2020;26:1324–6. doi: 10.3201/eid2606.200299.

[5] Lew S, Manes P, Smith B. Coinfection with SARS-CoV-2 and influenza a virus in a 32-year-old man. Am J Case Rep 2020;21:e926092. doi: 10.12659/AJCR.926092.

[6] Hashemi SA, Safamanesh S, Ghafouri M, Taghavi MR, Mohajer Zadeh Heydari MS, Namdar Ahmadabad H, et al. Co-infection with COVID-19 and influenza A virus in two died patients with acute respiratory syndrome, Bojnurd, Iran. J Med Virol 2020;92:2319–21. doi: 10.1002/jmv.26014.

[7] Hashemi SA, Safamanesh S, Ghasemzadeh-Moghaddam H, Ghafouri M, Azimian A. High prevalence of SARS-CoV-2 and influenza A virus (H1N1) coinfection in dead patients in Northeastern Iran. J Med Virol 2021;93:1008–12. doi: 10.1002/jmv.26364.

[8] Guan Z, Chen C, Li Y, Yan D, Zhang X, Jiang D, et al. Impact of coinfection with SARS-CoV-2 and influenza on disease severity: a systematic review and meta-analysis. Front Public Heal 2021;9:773130. doi: 10.3389/fpubh.2021.773130.

[9] Musuuza JS, Watson L, Parmasad V, Putman-Buehler N, Christensen L, Safdar N. Prevalence and outcomes of co-infection and superinfection with SARS-CoV-2 and other pathogens: a systematic review and meta-analysis. PLoS One 2021;16:e0251170. doi: 10.1371/journal.pone.0251170.

[10] Oliva J, Terrier O. Viral and bacterial co-infections in the lungs: dangerous liaisons. Viruses 2021;13. doi: 10.3390/v13091725.

[11] De Conto F, Conversano F, Medici MC, Ferraglia F, Pinardi F, Arcangeletti MC, et al. Epidemiology of human respiratory viruses in children with acute respiratory tract infection in a 3-year hospital-based survey in Northern Italy. Diagn Microbiol Infect Dis 2019;94:260–7. doi: 10.1016/j.diagmicrobio.2019.01.008.

[12] De Conto F, Conversano F, Razin SV, Belletti S, Arcangeletti MC, Chezzi C, et al. Host-cell dependent role of phosphorylated keratin 8 during influenza A/NWS/33 virus (H1N1) infection in mammalian cells. Virus Res 2021;295:198333. doi: 10.1016/j.virusres.2021.198333.

[13] Calderaro A, Arcangeletti MC, De Conto F, Buttrini M, Montagna P, Montecchini S, et al. SARS-CoV-2 infection diagnosed only by cell culture isolation before the local outbreak in an Italian seven-week-old suckling baby. Int J Infect Dis 2020;96:387–9. doi: 10.1016/j.ijid.2020.05.035.

[14] Centers for Disease Control and Prevention. Post-COVID conditions 2021. [17th February 2022] Available from: https://www.cdc.gov/coronavirus/2019-ncov/long-term-effects/index.html.

[15] Kim JY, Bae J-Y, Bae S, Cha HH, Kwon J-S, Suh MH, et al. Diagnostic usefulness of subgenomic RNA detection of viable SARS-CoV-2 in patients with COVID-19. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis 2022;28:101–6. doi: 10.1016/j.cmi.2021.08.009.

[16] Morris CP, Luo CH, Sachithanandham J, Li M, Schwartz M, Gaston DC, et al. Large scale SARS-CoV-2 molecular testing and genomic surveillance reveal prolonged infections, protracted RNA shedding, and viral reinfections. Front Cell Infect Microbiol 2022;12:809407. doi: 10.3389/fcimb.2022.809407.

[17] Desimmie BA, Raru YY, Awadh HM, He P, Teka S, Willenburg KS. Insights into SARS-CoV-2 persistence and its relevance. Viruses 2021;13. doi: 10.3390/v13061025.

[18] Wölfel R, Corman VM, Guggemos W, Seilmaier M, Zange S, Müller MA, et al. Virological assessment of hospitalized patients with COVID-2019. Nature 2020;581:465–9. doi: 10.1038/s41586-020-2196-x.

[19] Bullard J, Dust K, Funk D, Strong JE, Alexander D, Garnett L, et al. Predicting infectious severe acute respiratory syndrome Coronavirus 2 from diagnostic samples. Clin Infect Dis an Off Publ Infect Dis Soc Am 2020;71:2663–6. doi: 10.1093/cid/ciaa638.

[20] Liu W-D, Chang S-Y, Wang J-T, Tsai M-J, Hung C-C, Hsu C-L, et al. Prolonged virus shedding even after seroconversion in a patient with COVID-19. J Infect 2020;81:318–56. doi: 10.1016/j.jinf.2020.03.063.

[21] Sia SF, Yan L-M, Chin AWH, Fung K, Choy K-T, Wong AYL, et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. Nature 2020;583:834–8. doi: 10.1038/s41586-020-2342-5.

[22] Galani I-E, Rovina N, Lampropoulou V, Triantafyllia V, Manioudaki M, Pavlos E, et al. Untuned antiviral immunity in COVID-19 revealed by temporal type I/III interferon patterns and flu comparison. Nat Immunol 2021;22:32–40. doi: 10.1038/s41590-020-00840-x.

[23] Kim Y-M, Shin E-C. Type I and III interferon responses in SARS-CoV-2 infection. Exp Mol Med 2021;53:750–60. doi: 10.1038/s12276-021-00592-0.