Co-detection of respiratory pathogens in patients hospitalized with Coronavirus viral disease-2019 pneumonia

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Running title: Co-infection with respiratory pathogens in Covid-19.

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ABSTRACT

There is scarce information on the frequency of co-detection of respiratory pathogens (RP) in patients with Covid-19. Documentation of coinfections in Covid-19 pneumonia patients may be relevant for appropriate clinical and therapeutic management of patients. Between March 4th and March 28th, 2020, a total of 183 adult patients testing positive by SARS CoV-2 RT-PCR on respiratory specimens were hospitalized with interstitial pneumonia at our center, of whom 103 were tested for other RP by a multiplexed PCR assay. Three patients had a positive result for either one (n=2; Coronavirus HKU1 or Mycoplasma pneumoniae) or two targets (n=1; Influenza virus A (H3) and Respiratory syncytial virus B). Twenty-three patients testing negative by SARS CoV-2 RT-PCR and presentig with clinical, laboratory findings and imaging compatibe with Covid-19 pneumonia underwent RP screening. Of these, 6 (26%) had a positive result for a single RP. Our data indicate that despite the apparent rarity of coinfections in patients with Covid-19 pneumonia, routine testing for RP should be advised, since agents for which specific therapy can be prescribed may be detected.

Clinical, laboratory and imaging characteristics of Coronavirus disease 2019 (Covid-19) and risk factors associated with poor outcomes have been reported in various studies (1-5). Information on whether other respiratory pathogens (RP) were co-detected in these patients was not provided, despite the fact that coinfection with RP has been reported to occur in this clinical setting (6-9). Documentation of coinfections in Covid-19 pneumonia patients may be relevant not only for appropriate clinical and therapeutic management of patients, but also to precisely characterize disease features and delineate risk factors potentially impacting on clinical outcomes. Here, we report on our experience on this topic, gathered between March 4th and March 28th, 2020, at the Clinic University Hospital of Valencia, a tertiary teaching hospital with 586 beds which serves Clínico-Malvarrosa Health Department (attending 368.000 inhabitants in the northeast of the city). The study was approved by the Ethical Committee of University Clinic Hospital, INCLIVA, Valencia. Informed consent was not requested as laboratory analyses reported herein were conducted routinely in our patients following local guidelines.

A total of 183 adult patients testing positive by SARS CoV-2 RT-PCR on respiratory specimens were hospitalized with interstitial pneumonia, of whom 103 (64 males/39 females; median age, 64 years; range, 19-100 years) were tested for other RP by a multiplexed PCR assay (10) (Table 1). Three patients (2.9%) had positive results for either one (n=2; Coronavirus HKU1 or *Mycoplasma pneumoniae)* or two targets (n=1; Influenza virus A (H3) and Respiratory syncytial virus B). Twenty-three patients (14 males/9 females; median age, 54 years; range, 31-79 years) testing negative by SARS CoV-2 RT-PCR in a single respiratory specimen, presentig with clinical, laboratory

findings and imaging compatibe with Covid-19 pneumonia (1-5) underwent RP screening. Of these, 6 (26%) had a positive result for a single RP.

Coinfection with RP in adult patients with respiratory tract infections occurs commonly (11,12). In line with this, coinfection with RP was observed in 2 out of 12 patients (25%) presenting with upper or lower respiratory tract infections and no clinical suspicion of Covid-19 and testing positive for RP within the study period. The rate of co-detection of RP in the same time period of the preceding year was rather comparable (6 out of 18 patients testing positive for RP; 35%). Nevertheless, patients with Covid-19 pneumonia were found to be infrequently coinfected with other RP; this phenomenom could have been underestimated, since 26% of patients fulfilling criteria of this clinical entity (1-5) and testing negative by SARS CoV-2 RT-PCR, had RP detected in respiratory specimens. In this sense, false negative SARS CoV-2 PCR results in upper respiratory tract specimens occurs (13). The possibility of a viral interference phenomenon similar to that described for influenza virus A (sub)types should also be considered (14). Despite the apparent rarity of coinfections in patients with Covid-19 pneumonia, routine testing for RP should be advised, since agents for which specific therapy can be prescribed (i.e *Mycoplasma pneumoniae*, Influenza virus A, or Respiratory syncytial virus) may be detected. This approach may have a benefitial impact on patient survival.

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Conflicts of Interest

The authors declare no conflicts of interest

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REFERENCES

1. **Wang Z, Yang B, Li Q, Wen L, Zhang R**. 2020. Clinical Features of 69 Cases with Coronavirus Disease 2019 in Wuhan, China**.** Clin Infect Dis; Mar 16. pii: ciaa272. doi: 10.1093/cid/ciaa272. [Epub ahead of print].

2. **Mo P, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, Xiong Y, Cheng Z, Gao S, Liang K, Luo M, Chen T, Song S, Ma Z, Chen X, Zheng R, Cao Q, Wang F, Zhang Y.** 2020. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. Clin Infect Dis; Mar 16. pii: ciaa270. doi: 10.1093/cid/ciaa270. [Epub ahead of print].

3**. Wang D, Hu B, Hu C, Zhu F, Liu X, Zhang J, Wang B, Xiang H, Cheng Z, Xiong Y, Zhao Y, Li Y, Wang X, Peng Z**.. 2020. Clinical Characteristics of 138 Hospitalized Patients With 2019 Novel Coronavirus-Infected Pneumonia in Wuhan, China. JAMA; Feb 7. doi: 10.1001/jama.2020.1585. [Epub ahead of print].

4. **Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, Xiang J, Wang Y, Song B, Gu X, Guan L, Wei Y, Li H, Wu X, Xu J, Tu S, Zhang Y, Chen H, Cao B.** 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. Lancet; Mar 28;395(10229):1054-1062. doi: 10.1016/S0140-6736(20)30566-3. Epub 2020 Mar 11.

5. **Guan WJ, Ni ZY, Hu Y, Liang WH, Ou CQ, He JX, Liu L, Shan H, Lei CL, Hui DSC, Du B, Li LJ, Zeng G, Yuen KY, Chen RC, Tang CL, Wang T, Chen PY, Xiang J, Li SY, Wang JL, Liang ZJ, Peng YX, Wei L, Liu Y, Hu YH, Peng P, Wang JM, Liu JY, Chen Z, Li G, Zheng ZJ, Qiu SQ, Luo J, Ye CJ, Zhu SY, Zhong**

NS. 2020. Clinical Characteristics of Coronavirus Disease 2019 in China. N Engl J Med; Feb 28. doi: 10.1056/NEJMoa2002032. [Epub ahead of print].

6. **Wu X, Cai Y, Huang X, u X, Zhao L, Wang F, Li Q, Gu S, Xu T, Li Y, Lu B, Zhan Q.** 2020. Co-infection with SARS-CoV-2 and influenza A virus in patient with pneumonia. Co-infection with SARS-CoV-2 and Influenza A Virus in Patient with Pneumonia, China. Emerg Infect Dis;Mar 11;26(6). doi: 10.3201/eid2606.200299.

7. **Lin D, Liu L, Zhang M, Hu Y, Yang Q, Guo J, Guo Y, Dai Y, Xu Y, Cai Y, Chen X, Zhang Z, Huang K**. 2020. Co-infections of SARS-CoV-2 with multiple common respiratory pathogens in infected patients. Sci China Life Sci 2020; 63, http://doi.org/10.1007/s11427-020-1668-5.

8. **Touzard-Romo F, Tapé C, Lonks JR.** 2020. Co-infection with SARS-CoV-2 and Human Metapneumovirus I Med J (2013);Mar 19;103(2):75-76.

9. **Fan BE, Lim KGE, Chong VCL, Chan SSW, Ong KH, Kuperan P**. 2020. COVID-19 and Mycoplasma pneumoniae coinfection. Am J Hematol; Mar 15. doi: 10.1002/ajh.25785. [Epub ahead of print].

10. **Costa E, Rodríguez-Domínguez M, Clari MÁ, Giménez E, Galán JC, Navarro D.** 2015. Comparison of the performance of 2 commercial multiplex PCR platforms for detection of respiratory viruses in upper and lower tract respiratory specimens. Diagn Microbiol Infect Dis;82:40-43.

11. **Tanner H, Boxall E, Osman H.** 2012. Respiratory viral infections during the 2009-2010 winter season in Central England, UK: incidence and patterns of multiple virus co-infections Eur J Clin Microbiol Infect Dis;31:3001-3006.

12. **To KKW, Chan KH, Ho J.** 2019. Respiratory virus infection among hospitalized adult patients with or without clinically apparent respiratory infection: a prospective cohort study. Clin Microbiol Infect;25:1539-1545.

13. **Winichakoon P, Chaiwarith R, Liwsrisakun C, Salee P, Goonna A, Limsukon A, Kaewpoowat Q**. 2020. Negative Nasopharyngeal and Oropharyngeal Swab Does Not Rule Out COVID-19. J Clin Microbiol; Feb 26. pii: JCM.00297-20. doi: 10.1128/JCM.00297-20. [Epub ahead of print].

14. **Schultz-Cherry S**. 2015. Viral Interference: The Case of Influenza Viruses. J Infect Dis 212:1690-1691.

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TABLE 1. Detection of respiratory pathogens by multiplexed PCR in the study patients

Respiratory pathogens[a]	Patients			
	SARS CoV-2 positive (n=103)[b]	SARS CoV-2 negative with clinical, laboratory and imaging compatible with Covid-19 (n=23)[c]	Respiratory infections with no suspicion of Covid-19 (n=48)[d]	Respiratory infections in season 2019 (n=50)[e]
One target	2	6	10	12
Adenovirus	0	0	1	0
Coronavirus HKU1	1	0	0	0
Coronavirus 229E	0	0	0	2

Coronavirus OC43	0	0	2	0
Coronavirus NL63	0	0	0	0
Human Bocavirus	0	0	0	0
Human Metapneumovirus	0	0	1	1
Influenza virus A	0	1	1	2
Mycoplasma pneumoniae	1	0	0	0
Parainfluenza virus 3	0	0	0	1
Respiratory Syncytial virus (A/B)	0	1	0	0
Rhinovirus-Enterovirus	0	4	5	6
Two targets	1	0	2	6
Adenovirus/Influenza virus A (H3)	0	-	0	2
Coronavirus 229E/Coronavirus NL63	0	-	0	1
Coronavirus 229E/Rhinovirus-Enterovirus	0	-	0	1
Human Metapneumovirus/Coronavirus 229E	0	-	2	0
Human Metapneumovirus/ Parainfluenza virus 3	0	-	0	2
Influenza virus A (H1)/Respiratory syncytial virus A	1	-	0	0
Naso and oropharyngeal swabs were collected with flocked swabs in universal transport				

medium (Beckton Dickinson, Sparks, MD,USA, or Copan Diagnostics, Murrieta, CA, USA). Tracheal aspirates, sputa and bronchoalveolar lavage fluids were transported undiluted. Specimens were received at the laboratory within 30 min of collection and were conserved at 4 ° C until processed (within 6 hours). Nucleic acid extraction was performed using the Qiagen EZ-1 Viral extraction kit or the DSP virus Pathogen Minikit on the EZ1 or QiaSymphony Robot instruments (Qiagen, Valencia, CA, USA), respectively.

[a]As determined by the NxTAG® Respiratory Pathogen Panel (Luminex Corp, Austin, Tx, USA), which includes the following targets: Adenovirus, Influenza A, Influenza A (H1), Influenza A (H3), Influenza B, Parainfluenza virus 1-4, Coronavirus HKU1, NL63,229E, OC43, Respiratory Syncytial Virus A, Respiratory Syncytial Virus B, Human Metapneumovirus, Human Bocavirus, Rhinovirus-Enterovirus, *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae*.

[b]Several commercially available assays were used for SARS CoV-19 detection, including the LightMix® Modular SARS and Wuhan CoV E-gene/LightMix® Modular Wuhan CoV RdRP-gene from TIB MOLBIOL GmHD, distributed by Roche Diagnostics (Pleasenton, CA, USA) on the Light Cycler 2.0 instrument, the SARS-COV-2 REALTIME PCR KIT from Vircell Diagnostics (Granada, Spain) or the REALQUALITY RQ-2019-nCoV from AB ANALITICA (Padua, Italy), both on the Applied Biosystems 7500 instrument. Detection of two SARS Cov-2 viral gene targets was considered a positive result. Respiratory specimens tested were the following: nasopharyngeal exudates (n=60), nasopharyngeal aspirates (n=21), oropharyngeal exudates (n=17), tracheal aspirates (n=3), and sputa (n=2).

[c]Respiratory specimens tested were the following: nasopharyngeal aspirates (n=14), nasopharyngeal exudates (n=4), tracheal aspirates (n=3), sputum (n=1), and bronchoalveloar lavage fluid (n=1).

[d]Patients in this group (31 males/17 females; median age, 61 years; range, 24-84 years) were screened for RP in respiratory specimens. In our center, as per protocol, onco-hematological patients with upper or lower respiratory tract infections and hospitalized patients with penumonia of probable viral origin are routinely tested for RP. Respiratory specimens tested from these patients were the following: nasopharyngeal aspirates (n=18), nasopharyngeal exudates (n=9), tracheal aspirates (n=8), sputa (n=4), and bronchoalveloar

lavage fluids (n=9).

[e]Patients in this group (28 males/22 females; median age, 58 years; range, 19-78 years) were screened for RP in respiratory specimens as per protocol (as detailed above). Respiratory specimens tested were the following: nasopharyngeal aspirates (n=24), nasopharyngeal exudates (n=5), tracheal aspirates (n=8), sputa (n=3), and bronchoalveloar lavage fluids (n=10).