Influenza co-infection associated with severity and mortality in COVID-19 patients

Bandar Alosaimi[1], Maaweya Hamed[2], Haitham Alkadi[3], Asif Naeem[3], Thamer Alanazi[4], Snaa Alrehily[5], Abdullah Almutairi[5], and Adnan Zafar[3]

[1]King Fahad Medical City Faculty of Medicine
[2]King Saud University College of Science
[3]King Fahad Medical City
[4]Zulfi General Hospital
[5]King Fahad General Hospital

September 11, 2020

Abstract

Background In COVID-19 patients, undetected co-infections may have severe clinical implications associated with increased hospitalization, varied treatment approaches and mortality. Therefore, we investigated the implications of viral and bacterial co-infection on COVID-19 clinical outcomes. Methods Nasopharyngeal samples were obtained from 48 COVID-19 patients (29% ICU and 71% non-ICU) and screened for the presence of 24 respiratory pathogens using six multiplex PCR panels. Results We found evidence of co-infection in 34 COVID-19 patients (71%). Influenza A H1N1 (n=17), Chlamydia pneumoniae (n=13) and human adenovirus (n=10) were the most commonly detected pathogens. Viral co-infection was associated with increased ICU admission (r=0.1) and higher mortality (OR 1.78, CI=0.38-8.28) compared to bacterial co-infections (OR 0.44, CI=0.08-2.45). Two thirds of COVID-19 critically ill patients who died, had a co-infection; and Influenza A H1N1 was the only pathogen for which a direct relationship with mortality was seen (r=0.2). Amongst comorbidities, co-infection in patients with diabetes was associated with a significantly higher mortality (p=0.02). We also found that Troponin T was strongly related (p=0.001) with ICU admission and could be used as a predictor of COVID-19 severity. Conclusions The similarity in clinical presentation for both COVID-19 and Influenza makes it difficult to assess their impact on ICU admission and mortality. Our study highlights the importance of screening for co-infecting viruses in COVID-19 patients, given the high prevalence of Influenza viruses. The detection of co-infections in COVID-19 cases shows the importance of flu vaccination and warrants its increased coverage to reduce the hospitalization and associated mortality.

Type of the Paper (original Article)

Influenza co-infection associated with severity and mortality in COVID-19 patients

Bandar Alosaimi[1,2,*], Maaweya E. Hamed [3], Haitham S. Alkadi [1], Asif Naeem [1],Thamer Alanazi [4], Snaa S. Alrehily [5], Abdullah Z. Almutairi [6], Adnan Zafar [7]

[1] Research Center, King Fahad Medical City, Riyadh, Saudi Arabia;[2] College of Medicine, King Fahad Medical City, Riyadh, Saudi Arabia.[3] College of Science, King Saud University, Department of Botany and Microbiology, Riyadh, Saudi Arabia.

[4] Laboratory and Blood Bank, Zulfi General Hospital, Zulfi, Saudi Arabia.

[5] Infection Diseases department, King Fahad Hospital, Medina, Saudi Arabia.

[6] Laboratory and Blood Bank, King Fahad Hospital, Medina, Saudi Arabia.

[7] Pediatric Pulmonology Department, King Fahad Medical City, Riyadh, Saudi Arabia.

* Correspondence: balosaimi@kfmc.med.sa

running title: Influenza A H1N1 and SARS-CoV-2 co-infection

Abstract:

Background

In COVID-19 patients, undetected co-infections may have severe clinical implications associated with increased hospitalization, varied treatment approaches and mortality. Therefore, we investigated the implications of viral and bacterial co-infection on COVID-19 clinical outcomes.

Methods

Nasopharyngeal samples were obtained from 48 COVID-19 patients (29% ICU and 71% non-ICU) and screened for the presence of 24 respiratory pathogens using six multiplex PCR panels.

Results

We found evidence of co-infection in 34 COVID-19 patients (71%). Influenza A H1N1 (n=17), Chlamydia pneumoniae (n=13) and human adenovirus (n=10) were the most commonly detected pathogens. Viral co-infection was associated with increased ICU admission (r=0.1) and higher mortality (OR 1.78, CI=0.38-8.28) compared to bacterial co-infections (OR 0.44, CI=0.08-2.45). Two thirds of COVID-19 critically ill patients who died, had a co-infection; and Influenza A H1N1 was the only pathogen for which a direct relationship with mortality was seen (r=0.2). Amongst comorbidities, co-infection in patients with diabetes was associated with a significantly higher mortality (p=0.02). We also found that Troponin T was strongly related (p=0.001) with ICU admission and could be used as a predictor of COVID-19 severity.

Conclusions

The similarity in clinical presentation for both COVID-19 and Influenza makes it difficult to assess their impact on ICU admission and mortality. Our study highlights the importance of screening for co-infecting viruses in COVID-19 patients, given the high prevalence of Influenza viruses. The detection of co-infections in COVID-19 cases shows the importance of flu vaccination and warrants its increased coverage to reduce the hospitalization and associated mortality.

Keywords: SARS-CoV-2, COVID-19, Influenza A H1N1, co-infection, ICU, comorbidities, mortality.

1. Introduction

The newly emergent Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) continues to circulate outside of Wuhan, China since December 2019, and now exported to different countries all over the world [1]. At the time of writing this report, there were nearly quarter of a million of Coronavirus Disease-19 (COVID-19) confirmed cases ranking Saudi Arabia as the 14th highest in the world [2]. Most hospitalized patients needed admission to intensive care unit (ICU) and mortality reaches up to 50% in some cases [3]. Until now, twenty-two studies have reported co-infection in COVID 19 and of these 16 have evidence of viral co-infection [4]. The prevalence of critical cases with viral co-infection has been reported up to 35% [5]. Early literature reported that 50% of the patients who died had coexisting bacterial infection [6]. This is higher than what was previously seen during influenza pandemic in 2009 when 25% of patients with influenza infection had secondary bacterial co-infection [7].

SARS-CoV-2 is a single stranded RNA Betacoronavirus and belongs to the corona virus family [8]. Phylogenetic analysis has revealed that SARS-CoV-2 is closely related to SARS-CoV-1 and genetically distinct from MERS-CoV [9]. SARS-CoV-2 utilizes ACE-2 receptors in the lower airways which are also cellular receptors for other viruses in this group i.e. SARS-CoV and MERS-CoV [10]. Despite similar expression of ACE-2 receptors in different organs of the body, the most affected site is the lung tissue [11]. Influenza strains also cause lung damage by ACE-2 receptor mediated effects [12]. On the other hand, since the ACE-2 receptor

used by SARS-CoV-2 is an interferon-stimulated gene, it was hypothesized that type I and III interferons produced after bacterial infection may facilitate SARS-CoV-2 attachment [13].

During pandemics, the detection of the novel virus may lead to underreporting of other pathogens that could be the etiological agent contributing to the disease severity. Indeed, during the influenza A (H1N1) pdm09 pandemic, 44.3% of patients had unreported respiratory viruses [14]. Earlier studies indicated that common viral co-infections reported in COVID-19 patients include Influenza viruses, RSV and adenovirus [5,15]. Bacterial co-infection is more frequent than viral co-infection and it is homogeneously distributed in mild, moderate or severe illness [16]. The commonly known COVID-19 co-infecting bacteria are Mycoplasma pneumoniae, Pseudomonas aeruginosa, Hemophilus influenzae and Chlamydia pneumoniae [17]. These findings clearly emphasize on the importance of screening for other clinically important co-circulating respiratory pathogens contributing to the etiology of the disease.

The novelty of SARS-CoV-2 and the complicity of profound etiology of co-infection urged for consideration of comorbidities. COVID-19 patients with an underlying condition such as hypertension, diabetes, chronic kidney disease, and heart failure have been associated with COVID-19 disease severity [18]. Cardiovascular disease has a strong association with COVID-19 pneumonia (14.4%) [7,18] and other common comorbidities found in patients with SARS-CoV-2 include hypertension (18.6%) and diabetes (11.9%) [19]. Comorbidities were also linked with increased hospitalization, prolonged stay in ICU, and mortality. Hypertension was more prevalent in severe cases (47%) compared to diabetes (24%) and Respiratory diseases (10%) among other underlying conditions [18].

In conclusion, an extensive evidence revealed that viral infections predispose patients to subsequent bacterial co-infections [7]. This knowledge gap is puzzling as limited number of reports have described prevalence of bacterial and viral co-infections simultaneously. We hypothesized that undetected co-infections might have severe clinical implications associated with increased hospitalization, prolonged stay in ICU, and mortality. Therefore, our aim was, to investigate the presence of viral and bacterial co-infections in ICU and non-ICU COVID-19 patients.

2. Materials and Methods

2.1. Patients

Forty-eight extracted RNA samples were collected from COVID-19 positive patients, of which 14 were critical cases needing admission to the ICU, and 34 were mild cases. Nine patients died, (all were admitted to the ICU), and the rest survived. Thirteen patients were Saudi citizens and the rest were non Saudi (Table 1). Samples were collected from King Fahad Hospital, Medina, Saudi Arabia. This study was approved by the Institutional Review Board at King Fahad Medical City (IRB Log No. 20-160). Informed consent to participate was waived or not required since only remaining left-over specimens were used for this study.

2.2. RNA Extraction and PCR

Nasopharyngeal swabs were collected from the patients and carried in a suitable fluid viral medium. The RNA extraction of SARS-CoV-2 was performed via a MagNA Pure 96 machine, using the MagNA Pure 96 DNA and Viral NA small volume kit, (Roche, Germany). The amplification RT-PCR was performed within a Roche LightCycler® 480 II instrument, using the RNA Process Control Kit Trial Pack (Roche, Germany) with an internal, positive, and negative controls.

2.3. Real time PCR panel for Co-infection

Eluted nucleic acid was stored at -80degC until use, and all reagents were stored at -20degC. The quantitative RT-PCR assay for respiratory pathogens was performed on 7500 Fast Real-Time PCR System (Thermo Fisher scientific, USA). Extracted nucleic acid was screened by RT/q-PCR with Fast Track Diagnostic (FTD) Respiratory pathogens 21 plus kit (Biomerieux, Luxemburg) following the manufacturer's protocol using six multiplex PCR for respiratory viruses and bacteria. The pathogens tested were influenza A (H1N1) virus (swine-lineage); influenza B virus; human rhinovirus; human coronaviruses NL63, 229E, OC43 and HKU1;

human parainfluenza viruses 1, 2, 3 and 4; human metapneumoviruses A/B; human bocavirus; human respiratory syncytial viruses A/B; human adenovirus; enterovirus; human parechovirus; Mycoplasma pneumoniae; Chlamydia pneumoniae; Staphylococcus aureus; Streptococcus pneumoniae; Haemophilus influenzae B. Six positive controls were performed with every run (five for each viral panel and one for bacterium) on multiplex PCR assay. Moreover, six negative controls (NC), provided in the kit, were incorporated with each run. Briefly, 10 µl of the extracted nucleic acid was used as a template in each reaction for the FTD Respiratory pathogens 21 plus multiplex PCR following the manufacturer's instructions. The thermal cycle amplification condition includes reverse transcription for 15 minutes at 42°C, denaturation for 3 minutes at 94°C followed by 40 cycles for 8 seconds at 94°C, and 34 seconds at 60°C. Specimens were determined to be pathogen positive or negative based on the manufacturer's interpretation criteria, and 12 samples were randomly chosen and repeated to confirm the results.

2.4. Data collection

Demographic and clinical data (Table 1) were collected, including the following clinical laboratory results: age, gender, history of chronic illness, Ct value, Dimar, CK, CK-MB, Trop, HB, Platalet, RBC, WBC, Nutrophile, Lymph, CRP, Pro calciponin, Glucose, ESR, LDL, AST, ALT, Ureae, Creatinine, LDH, Albumin, Total Protein and blood group.

Table 1: at the end of this file.

2.5. Statistical Analysis

Minitab version 19.0 software was used for statistical analysis. All data was expressed as continuous variables. Continuous data was expressed by median for normally distributed variable i.e. age; whereas absolute numbers were expressed as percentages. The paired t-test was used to compare continuous variables of normal distribution and non-normal distribution, respectively. We used Pearson Coefficient to show association between the variables. Relationship of one response and multiple predictors was examined using linear regression with best fitted model. Mortality was evaluated for bacterial and viral co-infections by using binary logistic expression and expressed as odds ratio. MANOVA (multivariate analysis of variance) was used to analyze mortality in the presence of co-infection and comorbidities and it was expressed as p-value. The patients were grouped by disease severity, comorbidities and co-infection or not. Factors were adjusted for age and gender. A 2-sided α of less than 0.05 was considered statistically significant.

3. Results

We investigated co-infection in 48 COVID-19 patients (including 37 males and 11 females), the male to female ratio was 3:1. Median age of our study population was 52 years (1-92). Fourteen patients (29%) needed admission to intensive care unit (ICU cases). The remaining 34 patients (71%) did not require any admission and were classified as non-ICU cases. We found co-infections in thirty-four (71%) patients. Although severity of disease was negatively correlated (r= -0.09) with presence of a co-infection (p=0.53), it had a positive correlation with co-infecting viruses (r=0.1, p=0.42) by Pearson Coefficient as shown in Figure 1. Furthermore, statistically significant inverse association was observed (r=-0.28, p=0.04) between bacterial co-infection and ICU admission. In other words, this association indicates less likelihood of ICU admission with bacterial co-infection. The most commonly found co-infecting virus was influenza A H1N1 in 17 patients (36%). Chlamydia pneumoniae was the most prevalent co-infecting bacteria found in 13 patients (28 %). Other organisms detected were adenovirus in 10 patients and S. aureus in 4 patients (Figure 1). It was noticed that 4/17 (23.5%) patients with H1N1 had coexisting Chlamydia pneumoniae.

Figure 1: at the end of this file

Binary logistic regression was used to analyze the mortality association with viral and bacterial co-infections. The mortality rate was 19% (9/48), all of them were critically ill COVID-19 patients admitted to ICU, and two thirds of SARS-CoV-2 critically ill patients who died had co-infection (6/9). We found that viral co-infections (OR=1.78, CI=0.38-8.28) had higher mortality compared to bacterial co-infections (OR=0.44, CI=0.08-2.45) in COVID-19 patients. We observed that there was positive correlation between co-infecting

influenza H1N1 virus and mortality (r=0.2). On the other hand, co-infection with Chlamydia pneumoniae (r=-0.17) did not have any correlation with mortality in SARS-CoV-2 infected patients.

In terms of comorbidities, the prevalence of diabetes was 54% (26/48), cardiovascular disease 4% (2/48) and chronic kidney disease (CKD) 10.4% (5/48). Co-infection was present in 20/34 (58.8%) in diabetics, 2/34 (5.9%) in cardiovascular diseases and 4/34 (11.7%) in CKD. There was no significant association of co-infection with diabetes (p=0.25), cardiovascular disease (p=0.24) nor CKD (p=0.7). However, when we used MANOVA test to look at association of death and co-infection with diabetes, cardiovascular disease or CKD, it showed that statistically significant correlation was present between diabetes and death (p=0.02).

We also investigated the importance of different blood markers in COVID-19 patients (Table 1). Specifically, we examined association of d-dimer, lactate dehydrogenase (LDH) and Troponin T with the severity of disease. These markers have been interchangeably used to predict disease severity and the potential of ICU admission. Using linear regression, we found that Troponin T was strongly related (p=0.001) with disease severity compared to LDH (p=0.12) and d-dimer (p=0.25). This finding may imply that Troponin T could be used as a predictor for disease severity.

4. Discussion

In this study, we investigated the presence of co-infections in COVID-19 cases and analyzed their clinical and epidemiological characteristics. Viral and/or bacterial co-infections have been linked to disease severity, both directly, indirectly and through immunological response [20,21]. The occurrence of respiratory co-infections in this study was estimated to be as high as (71%) and two thirds of SARS-CoV-2 critically ill patients who died had co-infection. Influenza A H1N1 was the most common detected among the co-infecting viruses (64%). Several studies have partially reported the prevalence of COVID-19 pneumonia and influenza co-infection [22,23,24]. However, data on clinical significance of influenza A H1N1 co-infections with COVID-19 is limited. The similarity of clinical manifestations between the circulating respiratory viruses such as influenza A H1N1 and SARS-CoV-2 makes the differentiation very difficult [16,25]. Influenza A H1N1 dominance in our study population implies simultaneous outbreaks of two viruses and clearly emphasizes on the importance of screening for other clinically important co-circulating respiratory pathogens. Besides, numerous studies have shown viral co-infections being associated with disease severity, acute respiratory distress syndrome (ARDS) and even death. These studies show higher intensive care admission rates [5,23,26,27]. In this study, influenza A H1N1-COVID-19 co-infected patients were more severe and required ICU admission. Our results also showed a high case fatality rate among COVID-19 viral co-infections (r=0.2). The severity and higher case fatality among COVID-19 viral co-infected patients may be attributed to influenza A H1N1, which is known to induce a strong inflammatory cytokine/chemokine response (cytokine storm). Thus, the H1N1-COVID-19 co-infection could accelerate and play a major role in ARDS development. Our data showed that, during pandemics, focusing on the detection of the novel virus may lead to underreporting of other pathogens that could be the etiological agents contributing to disease severity.

Unfortunately, the topic of co-infection is usually embedded within the characteristics of patients in COVID-19 studies. However, our study investigated the coexistence of a full panel of respiratory viruses and bacteria simultaneously in order to investigate whether viral infection predisposes patients to subsequent bacterial co-infection or not. Indeed, secondary bacterial co-infection is identified as the main cause of death in patients with viral pneumonia [7,28]. A study of common respiratory pathogens presenting as co-infections with COVID-19 from China revealed that the Mycoplasma pneumoniae and Legionella pneumophila were the most common bacteria detected among COVID-19 patients [29]. In this study, bacterial co-infection was present in 36% of patients and the most common bacterial co-infection among COVID-19 patients was Chlamydia pneumoniae with infection rate of (27%). Our findings appeared to be inconsistent with previous findings from China [29] which could be attributed to the diversity of geographical distribution of circulating respiratory bacteria. Nevertheless, the C. pneumoniae infection is a common cause of acute respiratory infections with seroprevalence of (34.1%) in patients with fatal COVID-19 [30]. However, inverse association was observed between bacterial co-infection and disease severity. This association indicates less likelihood of ICU admission with bacterial co-infection which may be attributed to empirical use of antibiotics

during the early onset of COVID-19 disease. It could be argued that COVID-19 patients co-infected with C. pneumoniae who are treated with antibiotics may have suppressed the opportunistic growth of potentially fatal secondary bacterial infections decreasing the likelihood of ICU admission.

Many risk factors including older age, diabetes mellitus, cardiovascular disease, elevated LDH levels, high levels of D-dimer and elevated inflammatory cytokines/ chemokines have been associated with adverse outcomes in COVID-19. In our study, the prevalence of diabetes was (54%) and significant correlation was present between death and co-infection with diabetes (p=0.02). This is expected as poor glycemic control predisposes to impaired innate and adaptive immunity which might lead to decreased viral clearance [31]. The Troponin complex is a predictor for coronary syndrome and myocardial infarction. The high levels of Troponin are significantly associated with acute myocardial infarction [32]. In this study, high levels of Troponin T were detected among COVID-19 patients. We found that Troponin T was strongly related (p=0.001) with disease severity compared to LDH (p=0.12) and d-dimer (p=0.25). This is explained by presence of ACE-2 receptors on myocardial cells and presence of myocardial injury in SARS-CoV-2 infection [33]. Several studies have revealed that the higher Troponin levels were increased in COVID-19 patients' ICU admission and in-hospital death [16,34,35]. Our results confirm the important role of Troponin in the COVID-19 severity. We think the Troponin levels can be used as a marker of COVID-19 severity and a predictor of cardiovascular events.

Our study has some limitations. First, only 48 COVID-19 patients were included. Second, our study did not include asymptomatic or pre-symptomatic cases or healthy non- COVID-19 controls. Third, important data about cardiovascular complications and echocardiography were not included. The impact of a secondary bacterial infections is less clear and cannot be established with the current study design. Future studies to overcome these limitations need to be considered.

In conclusion, the similarity in clinical presentation for both COVID-19 and Influenza makes it difficult to assess their impact on ICU admission and mortality. Our study highlights the importance of screening for co-infecting viruses in COVID-19 patients, given the high prevalence of Influenza viruses. The detection of co-infections in COVID-19 cases shows the importance of flu vaccination and warrants its increased coverage to reduce the hospitalization and associated mortality.

Author Contributions: B.A. contributed to study design, data analysis, results discussion, and manuscript writing and review; M.H. contributed to experimental design and work, and manuscript writing; H.A. conducted the experimental work; A.N. contributed to data analysis and manuscript writing; T.A. contributed to sample and data collection; S.A. performed results discussion and clinical interpretation; A.M. contributed to sample collection and data analysis; A.Z. contributed to Study design, clinical analysis of data, results discussion, manuscript writing and review. All authors read and approved the final manuscript.

Funding: This work was supported by the Research Center at King Fahad Medical city (Grant No 20-066). The funders of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Acknowledgments: The authors thank the Research Center at King Fahad Medical City for funding this study (Grant No 20-066). Special thanks to Dr. Omar Alhazmi, Mr. Mohammad A Alturkostani, Mr. Abdulaziz A Taleb, and Mr. Saeed Albalawi from the Regional Lab in Medina for their contribution in sample collection.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. WHO announces COVID-19 outbreak a pandemic. https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/news/news/2020/3/who-announces-covid-19-outbreak-a-pandemic.
2.

3. Saudi Arabia Coronavirus: 209,509 Cases and 1,916 Deaths - Worldometer. https://www.worldometers.info/coronavirus/country/saudi-arabia/.

4.

5. Liu, Y. et al. Clinical and biochemical indexes from 2019-nCoV infected patients linked to viral loads and lung injury. Sci China Life Sci 63, 364–374 (2020).

6. Kim D, Quinn J, Pinsky B, Shah NH, Brown I. Rates of Co-infection Between SARS-CoV-2 and Other Respiratory Pathogens. JAMA. 2020 May 26;323(20):2085–6.

7.

8. Zhu X, Ge Y, Wu T, Zhao K, Chen Y, Wu B, Zhu F, Zhu B, Cui L. Co-infection with respiratory pathogens among COVID-2019 cases. Virus Res. 2020 Aug;285:198005. doi:10.1016/j.virusres.2020.198005. Epub 2020 May 11. PMID: 32408156; PMCID: PMC7213959.

9. Zhou, F. et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. The Lancet 395, 1054–1062 (2020).

10. Mcntyre, C. R. et al. The role of pneumonia and secondary bacterial infection in fatal and serious outcomes of pandemic influenza a(H1N1)pdm09. BMC Infect. Dis. 18, 637 (2018).

11. Gorbalenya, A. E. et al. The species Severe acute respiratory syndrome-related coronavirus : classifying 2019-nCoV and naming it SARS-CoV-2. Nature Microbiology 5, 536–544 (2020).

12. Lu, R. et al. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet 395, 565–574 (2020).

13. Devaux, C. A., Rolain, J.-M. & Raoult, D. ACE2 receptor polymorphism: Susceptibility to SARS-CoV-2, hypertension, multi-organ failure, and COVID-19 disease outcome. Journal of Microbiology, Immunology and Infection 53, 425–435 (2020).

14. Guo, G. et al. New Insights of Emerging SARS-CoV-2: Epidemiology, Etiology, Clinical Features, Clinical Treatment, and Prevention. Front. Cell Dev. Biol. 8, (2020).

15. Chen, L. & Hao, G. The role of angiotensin-converting enzyme 2 in coronaviruses/ influenza viruses and cardiovascular disease. Cardiovasc Res (2020) doi:10.1093/cvr/ cvaa093.

16. Bengoechea JA, Bamford CGG. SARS-CoV-2, bacterial co-infections, and AMR: the deadly trio in COVID-19? EMBO Molecular Medicine [Internet]. 2020 May 26 [cited 2020 Jun 13];n/a(n/a).

17. Ratnamohan VM, Taylor J, Zeng F, et al. Pandemic clinical case definitions are non-specific: multiple respiratory viruses circulating in the early phases of the 2009 influenza pandemic in New South Wales, Australia. Virol J. 2014;11:113.

18. Lansbury, L., Lim, B., Baskaran, V. & Lim, W. S. Co-infections in people with COVID-19: a systematic review and meta-analysis. Journal of Infection (2020) doi: 10.1016/j.jinf.2020.05.046.

19. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497–506.

20. Lai, C.-C., Wang, C.-Y. & Hsueh, P.-R. Co-infections among patients with COVID-19: The need for combination therapy with non-anti-SARS-CoV-2 agents? Journal of Microbiology, Immunology and Infection (2020) doi:10.1016/j.jmii.2020.05.013.

21. Gold MS, Daniel Sehayek, Gabrielli S, Zhang X, McCusker C & Ben-Shoshan M (2020) COVID-19 and comorbidities: a systematic review and meta-analysis, Postgraduate Medicine, DOI: 10.1080/00325481.2020.1786964

22. Mo P, Xing Y, Xiao Y, Deng L, Zhao Q, Wang H, et al. Clinical characteristics of refractory COVID-19 pneumonia in Wuhan, China. Clin Infect Dis [Internet]. 2020 Mar 16 [cited 2020 Jun 9]

23. DaPalma T, Doonan BP, Trager NM, Kasman LM. A systematic approach to virus-virus interactions. Virus Res. 2010;149(1):1-9. doi:10.1016/j.virusres.2010.01.002

24. Alosaimi B, Hamed ME, Naeem A, Alsharef AA, AlQahtani SY, AlDosari KM, Alamri AA, Al-Eisa K, Khojah T, Assiri AM, Enani MA. MERS-CoV infection is associated with downregulation of genes encoding Th1 and Th2 cytokines/chemokines and elevated inflammatory innate immune response in the lower respiratory tract. Cytokine. 2020 Feb;126:154895.

25. D'Abramo A, Lepore L, Palazzolo C, Barreca F, Liuzzi G, Lalle E, Nicastri E. Acute respiratory distress syndrome due to SARS-CoV-2 and Influenza A co-infection in an Italian patient: Mini-review of the literature. Int J Infect Dis. 2020 Jun 18;97:236-239. doi: 10.1016/j.ijid.2020.06.056. Epub ahead of print. PMID: 32565366; PMCID: PMC7301795.

26. Ding Q, Lu P, Fan Y, Xia Y, Liu M. The clinical characteristics of pneumonia patients coinfected with 2019 novel coronavirus and influenza virus in Wuhan, China. J Med Virol. 2020 Mar 20:10.1002/jmv.25781. doi: 10.1002/jmv.25781. Epub ahead of print. PMID: 32196707; PMCID: PMC7228290.

27. Konala VM, Adapa S, Gayam V, Naramala S, Daggubati SR, Kammari CB, Chenna A. Co-infection with Influenza A and COVID-19. Eur J Case Rep Intern Med. 2020 Apr 20;7(5):001656. doi: 10.12890/2020_001656. PMID: 32399452; PMCID: PMC7213830.

28. Wu, X., Cai, Y., Huang, X., Yu, X., Zhao, L., Wang, F. . . .Zhan, Q. (2020). Co-infection with SARS-CoV-2 and Influenza A Virus in Patient with Pneumonia, China. Emerging Infectious Diseases, 26(6), 1324-1326. https://dx.doi.org/10.3201/eid2606.200299.

29. Cuadrado-Payán E, Montagud-Marrahi E, Torres-Elorza M, Bodro M, Blasco M, Poch E, Soriano A, Piñeiro GJ. SARS-CoV-2 and influenza virus co-infection. Lancet. 2020 May 16;395(10236):e84. doi: 10.1016/S0140-6736(20)31052-7. Epub 2020 May 5. PMID: 32423586; PMCID: PMC7200126.

30. Hashemi SA, Safamanesh S, Ghafouri M, Taghavi MR, Mohajer Zadeh Heydari MS, Namdar Ahmadabad H, Ghasem Zadeh-Moghaddam H, Azimian A. Co-infection with COVID-19 and influenza A virus in two died patients with acute respiratory syndrome, Bojnurd, Iran. J Med Virol. 2020 May 15:10.1002/jmv.26014. doi: 10.1002/jmv.26014. Epub ahead of print. PMID: 32410338; PMCID: PMC7272908.

31. Guo L, Wei D, Zhang X, Wu Y, Li Q, Zhou M, Qu J. Clinical Features Predicting Mortality Risk in Patients With Viral Pneumonia: The MuLBSTA Score. Front Microbiol. 2019 Dec 3;10:2752. doi: 10.3389/fmicb.2019.02752. Erratum in: Front Microbiol. 2020 Jun 09;11:1304. PMID: 31849894; PMCID: PMC6901688.

32. Xing Q, Li GJ, Xing YH, Chen T, Li WJ, Ni W, et al. Precautions Are Needed for COVID-19 Patients with Co-infection of Common Respiratory Pathogens (3/2/2020). Available at SSRN: http://dx.doi.org/10.2139/ssrn.3550013.

33. Du Y, Tu L, Zhu P, et al. Clinical Features of 85 Fatal Cases of COVID-19 from Wuhan. A Retrospective Observational Study. Am J Respir Crit Care Med. 2020;201(11):1372-1379. doi:10.1164/rccm.202003-0543OC.

34. Gupta, R., Hussain, A. & Misra, A. Diabetes and COVID-19: evidence, current status and unanswered research questions. European Journal of Clinical Nutrition. 2020; 74, 864–870.

35. Omland T, de Lemos JA, Sabatine MS, Christophi CA, Rice MM, Jablonski KA, Tjora S, Domanski MJ, Gersh BJ, Rouleau JL, Pfeffer MA, Braunwald E; Prevention of Events with Angiotensin Converting Enzyme Inhibition (PEACE) Trial Investigators. A sensitive cardiac troponin T assay in stable

coronary artery disease. N Engl J Med. 2009 Dec 24;361(26):2538-47. doi: 10.1056/NEJMoa0805299. Epub 2009 Nov 25. PMID: 19940289; PMCID: PMC2997684.

36. Babapoor-Farrokhran S, Gill D, Walker J, Rasekhi RT, Bozorgnia B, Amanullah A. Myocardial injury and COVID-19: Possible mechanisms. Life Sci. 2020 Jul 15;253:117723. doi: 10.1016/j.lfs.2020.117723.

37. Guo T, Fan Y, Chen M, et al. Cardiovascular Implications of Fatal Outcomes of Patients With Coronavirus Disease 2019 (COVID-19) [published online ahead of print, 2020 Mar 27]. JAMA Cardiol. 2020;e201017. doi:10.1001/jamacardio.2020.1017.

38. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, Gong W, Liu X, Liang J, Zhao Q, Huang H, Yang B, Huang C. Association of Cardiac Injury With Mortality in Hospitalized Patients With COVID-19 in Wuhan, China. JAMA Cardiol. 2020 Mar 25:e200950. doi: 10.1001/jamacardio.2020.0950. Epub ahead of print. PMID: 32211816; PMCID: PMC7097841.

Table 1: Demographics and clinical characteristics of COVID-19 patients

Baseline variables	**All patients**	**Non-ICU**	**ICU**	***P*-value**
	(N=48)	N=34 (71%)	N=14 (29%)	
DEMOGRAPHICS				
Age				
Median	52±18	46±18	62±15	
Range	1-92	1-92	25-74	
Gender				
Men	37 (77%)	26 (70%)	11 (30%)	$p = .87$*
Women	11 (23%)	8 (73%)	3 (27%)	
CHARACTERISTICS				
Co-infection				
co-infection	34 (71%)	25 (74%)	9 (26%)	$p = .52$*
No co-infection	14 (29%)	9 (64%)	5 (36%)	
Multiple co-infection	11 (32%)	9 (82%)	2 (18%)	$p = .49$*
Single co-infection	23 (68%)	16 (71%)	7 (29%)	
Number of coexisting				
Viruses	30 (64%)	21 (70%)	9 (30%)	$p = .42$*
Bacteria	17 (36%)	15 (88%)	2 (12%)	$p = .04$**
Case Fatality Rate	9 (19%)	-	9 (100%)	
Comorbidities				
Cardiovascular disease	2 (4%)	0 (0%)	2 (100%)	$p = .22$*
Chronic Kidney disease	5 (10%)	4 (80%)	1 (20%)	$p = .67$*
Diabetes	26 (54%)	22 (85%)	4 (15%)	$p = .02$**
Serological markers (median)				
d-dimer (0-0.5)	1.6	1.7	1.4	$p = .25$*
LDH (98-192)	336	335	349	$p = .12$*
Troponin T (0-0.07)	0.01	0.01	0.05	$p < .05$**

*Not significant at $p < .05$ **Significant at $p > .05$

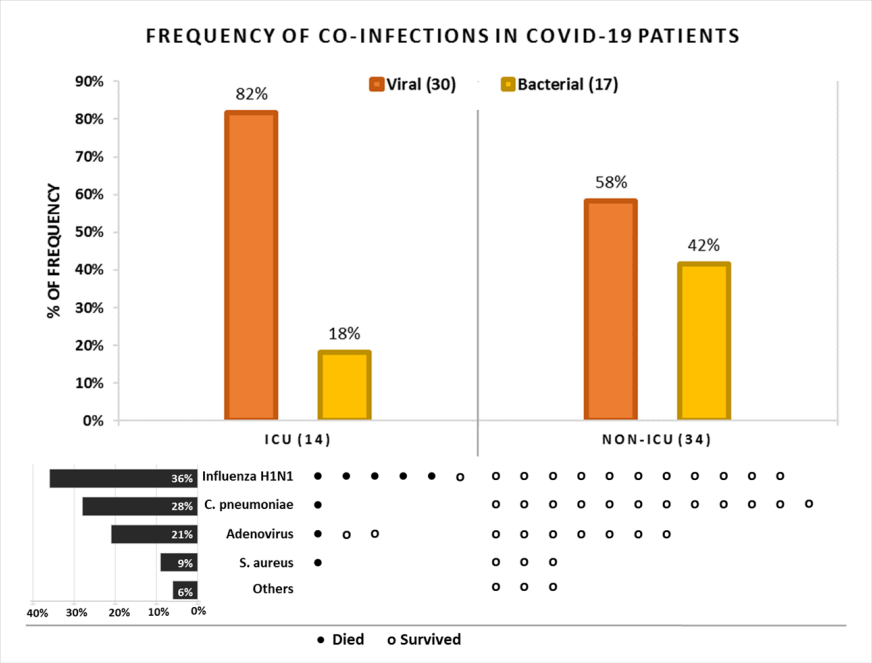

Figure 1: Frequency of Coexistence of Pathogens in COVID-19 Patients. The figure shows the frequency of viral vs bacterial co-infections in COVID-19 patients. The number of viruses detected in 14 ICU patients was 9 (82%) compared to 2 bacteria (18%). Remarkably, 8 coexisting pathogens (*) were detected in 6 dead patients. In 34 non-ICU patients, 36 coexisting pathogens were detected and none of them were involved in mortality (o). The percentages of the commonly co-infecting viruses and bacteria are also listed according to their prevalence. Remarkably, 5 out of 6 ICU patients died in the coexistence of influenza A H1N1.