JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Coronavirus Pandemic

Bacterial and fungal secondary infections occurring in COVID-19 patients followed in intensive care: a retrospective study

Erhan Ozyurt[1], Aylin Erman Daloglu[2], Kerem Inanoglu[1], Nilgun Kavrut Ozturk[1], Sadik Ozmen[1]

[1] *Department of Anesthesiology and Reanimation, Antalya Training and Research Hospital, University of Health Sciences, Antalya, Turkey*
[2] *Department of Medical Microbiology, Antalya Training and Research Hospital, University of Health Sciences, Antalya, Turkey*

Abstract
Introduction: We aimed to investigate the effects of secondary bacterial and fungal infections on patient outcomes in patients followed up in the intensive care unit (ICU) due to coronavirus disease 2019 (COVID-19).
Methodology: We retrospectively analyzed reverse transcriptase polymerase chain reaction (RT-PCR) positive COVID-19 patients followed in the ICU of our hospital between March 2020 and June 2021, using the hospital information system. Demographic data, pathogens causing a secondary infection, onset time of secondary infection, and patient outcomes were recorded.
Results: A total of 251 RT-PCR positive patients who met the inclusion criteria were evaluated. The mean length of stay (LOS) in the ICU was 13.3 ± 9.6 days. During this period, 165 (65.7%) patients died. When blood, urine, respiratory tract, and catheter cultures were examined, the number of patients with growth in at least one culture was 129 (51.4%). There was growth in a total of 227 cultures. The highest culture positivity rate was observed in respiratory tract samples (n = 94, 41.4%). Gram-negative bacterial pathogens (n = 130, 58.4%) predominated. *Candida* spp. was more frequent in urine cultures. The median day of the occurrence of secondary infection was 10 (range: 6-15). Patients who developed secondary infection had a longer LOS and higher mortality rate than patients who did not ($p < 0.001$).
Conclusions: Gram-negative secondary infections, predominantly in respiratory tract cultures, occurred in COVID-19 patients followed in the ICU. As a result, the LOS was prolonged and mortality rates increased.

Key words: COVID-19; secondary infection; intensive care.

J Infect Dev Ctries 2024; 18(3):350-354. doi:10.3855/jidc.18480

(Received 04 May 2023 – Accepted 30 November 2023)

Introduction
The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues its impact all over the world due to the emergence of new variants. The vast majority of COVID-19 patients experience mild to moderate illness. Around 14-26% of patients need intensive care unit (ICU) stay due to severe respiratory failure occurring during the course of the disease [1].

As with previous influenza epidemics, all the attention is directed primarily to the treatment of the viral infection and its complications. However, secondary bacterial and fungal infections in these patients cause an increase in morbidity and mortality [1-3]. The length of stay (LOS) in the hospital is prolonged as a result of nosocomial infections. Thus, the resulting high costs and mortality rates strain healthcare systems [4,5].

In addition to the damage caused by COVID-19, invasive procedures such as mechanical ventilation and catheterization applied in immunosuppressed patients in the ICU may predispose them to infection [1,6]. The frequency of bacterial and fungal infections occurring during the ICU follow-up of COVID-19 patients varies between 8.1% and 60% [7].

There are regional differences in the pathogens that cause secondary infections during the pandemic. Since these pathogens cannot be fully identified, broad-spectrum antimicrobial drugs are used [5], which can cause antimicrobial resistance [1]. Therefore, identifying the pathogens that cause secondary infections can guide empirical antibacterial and antifungal drug guidelines to be used in COVID-19 patients [8].

Although there are studies examining bacterial and fungal infections that occur during ICU follow-up of COVID-19 patients, the number of studies examining all respiratory tract, blood, urine, and catheter cultures is very limited. Therefore, the primary outcome measure of the study was the frequency of bacterial and fungal pathogens seen in the respiratory tract, blood, urine, and catheter cultures. Additionally, the effect of secondary infections on patient outcomes was examined.

Methodology

This study was approved by the local ethics committee (date: 5 August 2021, No: 11/5). COVID-19 patients who were over the age of 18 years and were followed up in the ICUs of our hospital between March 2020 and June 2021 were retrospectively analyzed, using the hospital information system. Patients with negative reverse transcriptase polymerase chain reaction (RT-PCR) or who were followed in the ICU for less than 48 hours were excluded from the study. Demographic data, co-morbidities, pathogens with growth in patient cultures, time of infection, LOS in the ICU, and patient outcomes were recorded. In order to evaluate the impact of secondary bacterial and/or fungal infections on patient outcome, LOS and mortality were compared between secondary infection positive (the number of patients with growth in at least one culture) and negative groups.

SARS-CoV-2 RT-PCR

All the patients had a diagnosis of COVID-19 confirmed by SARS-CoV-2 specific real-time reverse transcription RT-PCR testing, performed on nasopharyngeal throat swab specimens. RT-PCR tests of the samples were performed in routine studies with the BioSpeedy SARS-CoV-2 reverse-transcription RT-PCR kit (Bioeksen, Ankara, Turkey).

The decisions regarding the patients' management and microbiological cultures in the ICU were made by the ICU physician. Sputum, endotracheal aspirate, bronchoalveolar lavage fluid, blood, urine, and catheter samples were collected.

Culture

Blood culture samples sent from the ICU to the medical microbiology laboratory were incubated in a fully automatic blood culture device (BacT/ALERT, bioMerieux, Lyon, France). After the growth signal was obtained, sub-cultures were made on 5% sheep blood agar, eosin methylene blue (EMB) agar, chocolate agar, and Sabouraud dextrose agar. Urine, sputum/tracheal aspirate, and catheter samples were also directly inoculated on 5% sheep blood agar, EMB agar, chocolate agar, and Sabouraud Dextrose agar. The culture plates were incubated at 35 °C for 24-48 hours. The growths were evaluated at the end of the incubation period. Bacterial identification was performed with an automatic microbial identification system (VITEK MS, bioMerieux, Lyon, France) using the matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) technology.

Definitions

After RT-PCR positive diagnosis of COVID-19, positive pathogen detection in at least one of the microbiological samples (sputum, endotracheal aspirate, bronchoalveolar lavage fluid, blood, urine, or catheter) was defined as a secondary infection.

Study outcomes

The primary outcome measure of the study was the frequency of bacterial and fungal pathogens seen in the respiratory tract, blood, urine, and catheter cultures. In addition, we examined the effect of secondary infection on patient outcomes.

Statistical analysis

SPSS statistical package program (version 15.0; SPSS, Chicago, IL) was utilized for statistical analysis. The data obtained were evaluated using descriptive statistics (arithmetic mean, standard deviation, and percentage distribution). The Chi-square test was used to compare categorical variables. The Kolmogorov-Smirnov test was performed to determine normality of continuous variables. Mann-Whitney U test for the continuous variables with an abnormal distribution and independent t test for the continuous variables with a normal distribution were used to test the difference in means between two groups. In all analyses, the degree of statistical significance (p value) was determined as 0.05.

Results

During the study period, 265 RT-PCR positive patients were followed in the COVID-19 ICUs. However, 14 patients were excluded from the study because the LOS in the ICU was less than 48 hours. As a result, 251 patients who met the inclusion criteria were evaluated. The mean age of the patients was 68.2 ± 13.8 years. The majority of the patients were male (62.1%). The mean LOS was 13.3 ± 9.6 days. The median time between admission to the ICU and culture growth was 10 (range: 6-15) days. During the ICU follow-up, 165 (65.7%) patients died. Based on the culture results, patients with secondary infection had

Table 1. Demographic and clinical data of patients.

	All patients n = 251	**Bacterial/fungal infection (+) n = 129 (51.4)**	**Bacterial/fungal infection (-) n = 122 (48.6)**	***p* value**
Age (years)	68.2 (13.8)	69.6 (12.8)	66.8 (14.7)	0.100
Male /Female	156 (62.2) / 95 (37.8)	79 (61.2) / 50 (38.8)	77 (63.1) / 45 (36.9)	0.760
Comorbidity	189 (75.3)	98 (76)	31 (24)	0.800
LOS (days)	13.3 (9.6)	16.9 (10.5)	9.4 (6.8)	**< 0.001**
Mortality	165 (65.7)	106 (82.2)	59 (35.8)	**< 0.001**

Data expressed as mean ± standard deviation or n (%); LOS: length of stay.

longer LOS ($p < 0.001$) and higher mortality rates ($p < 0.001$) (Table 1).

Blood, urine, respiratory tract, and catheter cultures of the patients were examined. The number of patients with growth in at least one culture was 129 (51.4%). The highest number of pathogens grew in respiratory tract cultures and the least number of pathogens were seen in catheter cultures.

Secondary infection positivity was observed in respiratory tract, urine, blood, and catheter samples. In blood, respiratory tract, and catheter cultures, the most frequently detected pathogens were Gram-negative bacteria, while in urine culture the most frequently detected pathogens were fungi. The most common Gram-negative bacteria were *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*. *Candida* spp. was cultured in all types of samples including urine (64.4%), catheter (38.5%), blood (31.3%), and respiratory tract (20.2%). In addition, two patients had *Aspergillus fumigatus* growth in respiratory tract cultures (Table 2).

There were patients in whom two or three pathogens could be found simultaneously in cultures, as well as patients in whom bacterial and fungal pathogens were detected together (Table 3).

Discussion

In this study, bacterial and fungal infections in COVID-19 patients followed in ICUs were examined. Blood, urine, respiratory tract, and catheter cultures were evaluated. The highest culture positivity rate was observed in respiratory tract samples. In this study, the rate of ICU patients infected with at least one pathogen was 51.4%. In blood, respiratory tract, and catheter cultures, the most common pathogens were Gram-negative bacteria, especially *Acinetobacter baumannii*. Furthermore, the most common pathogen detected in urine cultures was *Candida* spp. Patients with secondary infection had longer LOS and higher mortality rates.

The development of secondary infection in patients followed in the ICU is a common problem all over the world. As stated in the large EPIC III study, secondary infections may occur at a high rate of 65% in ICU patients [9]. Secondary infection increases the degree of systemic inflammation in the patient, leading to a more severe course of the disease, and thus may cause adverse effects in the ICU process. The number of pro-inflammatory cytokines associated with severe lung injury, such as IL-6, is significantly increased in COVID-19 patients [10]. Also, SARS-CoV-2 infection

Table 2. Distribution of pathogens in cultures.

	Blood 48 (21.1)	**Urine 59 (26)**	**Respiratory tract 94 (41.4)**	**Catheter 26 (11.5)**	**Total 227 (100)**
Gram-negative bacteria n (%)	28 (58.3)	17 (28.8)	72 (76.6)	13 (50)	130 (58.4)
Acinetobacter baumannii	14	4	39	8	65
Pseudomonas aeruginosa	2	1	13	1	17
Klebsiella pneumoniae	4	4	8	2	18
Stenotrophomonas maltophilia	2	0	7	2	11
Escherichia coli	3	7	3	0	13
Enterobacter spp.	1	0	1	0	2
Rhizobium radiobacter	1	0	0	0	1
Achromobacter xylosoxidans	1	0	0	0	1
Myroides spp.	0	1	0	0	1
Haemophilus influenzae	0	0	1	0	1
Gram-positive bacteria n (%)	5 (10.4)	4 (6.8)	3 (3.2)	3 (11.5)	15 (6.8)
Enterococcus spp.	3	4	1	3	11
Staphylococcus aureus	0	0	2	0	2
Mycobacterium flavescens	2	0	0	0	2
Fungi n (%)	15 (31.3)	38 (64.4)	19 (20.2)	10 (38.5)	82 (34.8)
Candida spp	15	38	17	10	80
Aspergillus fumigatus	0	0	2	0	2

Data expressed as n or n (%).

Table 3. Distribution of cultures with multiple pathogens.

	Blood	Urine	Respiratory tract	Catheter	Total
Two pathogens	23 (24)	23 (24)	33 (34.3)	17 (17.7)	96
Three pathogens	2 (28.6)	1 (14.2)	2 (28.6)	2 (28.6)	7
Bacterial and fungal pathogens	14 (22.2)	14 (22.2)	22 (34.9)	13 (20.7)	63

Data expressed as n or n (%).

can damage lymphocytes, particularly B cells, T cells, and natural killer cells, resulting in an impaired immune system during the disease [11]. Decreased lymphocytes and host immune function, and severe inflammation with the body's immune response may be the main cause of secondary infection [12]. In addition, immunosuppressant drugs such as tocilizumab and corticosteroids, which are used to prevent the cytokine storm that occurs in COVID-19, contribute to the development of secondary infections [13]. Moreover, long-term and immunosuppressed stay of patients in hospital and ICU and invasive procedures applied during this time also increase susceptibility to secondary infections [1,13,14].

In addition to infections developing in the respiratory tract, pathogens can also be detected in urine, catheter, and blood cultures of COVID-19 patients during ICU follow-up [15-17]. The pathogens responsible for secondary infections in COVID-19 patients seem to be the commonly encountered pathogens in ICUs [4]. Gram negative pathogens dominate the studies describing secondary infections in ICU [1,9]. Zhang *et al.* observed secondary infections in COVID-19 patients at a rate of 57%. The most common growth was in respiratory tract cultures. The most common pathogens were determined as 50% Gram-negative, 26% Gram-positive, 11% virus, and 7% fungi [18]. Furthermore, *Acinetobacter baumannii*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* are frequently isolated from COVID-19 patients, most commonly in respiratory tract cultures [5,7]. Unlike, *Escherichia coli* which is the most frequently detected bacteria in urine cultures [7], in this study, the most common pathogens in blood, respiratory tract, and catheter cultures were Gram-negative, especially *Acinetobacter baumanii*. At the same time, *Candida* spp. was the most common pathogen in urine cultures (Table 2). The high prevalence of opportunistic fungal pathogens in all cultures may be due to the suppression of the immune system of patients by viral sepsis due to COVID-19 [18].

The frequency of secondary infections seems to increase one week after ICU admission [14,19]. In their study, De Santis *et al.* observed that COVID-19 patients most frequently had secondary infection between 7-14 days after admission to the ICU [7]. In this study, growth in cultures occurred frequently from the 10th day after admission to the ICU. According to previous studies, infections occurring in COVID-19 patients followed in ICU increase both the LOS days and mortality rates [7,14]. In this study, similar to the literature, an increase was found in both LOS and mortality in patients with growth in any culture compared to patients without growth.

Studies have reported that age and male gender have an effect on mortality in patients with COVID-19 [20]. However, in their study, Li *et al*. found that age and gender did not affect mortality in the case of secondary infection [5]. Similarly, in this study, age and gender were found to have no effect on mortality in ICU patients with secondary infections (Table 1).

This study has some limitations. First of all, it is a single-center retrospective study. These results cannot be generalized because the flora of this hospital's ICU may differ from the flora of hospitals in other regions. Second, the effect of starting antibiotherapy when patients are first hospitalized for COVID-19 on the development of secondary infections is unknown. And lastly, the colonisation and the infection are not differentiated.

Conclusions

Thus, secondary infections occurring in COVID-19 patients followed up in ICUs seem to mostly originate from the respiratory tract. We detected mainly Gram-negative bacteria and especially *Acinetobacter baumannii* in cultures studied from body fluids. Therefore, we believe that if empirical antimicrobial therapy is to be started, it would be more appropriate to cover these pathogens. According to our study, secondary infections occurring in patients followed in the ICU due to COVID-19 seem to increase both LOS and mortality. Although more studies are needed to confirm these results, it would be a good decision to take strict measures to reduce the risk of infection.

References

1. Sang L, Xi Y, Lin Z, Pan Y, Song B, Li CA, Zheng X, Zhong M, Jiang L, Pan C, Zhang W, Lv Z, Xia J, Chen N, Wu W, Xu Y, Chen S, Liu D, Liang W, Liu X, Liu X, Li S, Zhong N, Ye D, Xu Y, Zhang N, Zhang D, Li Y (2021) Secondary infection in severe and critical COVID-19 patients in China: a

multicenter retrospective study. Ann Palliat Med 10: 8557-8570. doi: 10.21037/apm-21-833.
2. Morris DE, Cleary DW, Clarke SC (2017) Secondary bacterial infections associated with influenza pandemics. Front Microbiol 8: 1041. doi: 10.3389/fmicb.2017.01041.
3. Morens DM, Taubenberger JK, Fauci AS (2008) Predominant role of bacterial pneumonia as a cause of death in pandemic influenza: implications for pandemic influenza preparedness. J Infect Dis 198: 962-970. doi: 10.1086/591708.
4. Liu HH, Yaron D, Piraino AS, Kapelusznik L (2021) Bacterial and fungal growth in sputum cultures from 165 COVID-19 pneumonia patients requiring intubation: evidence for antimicrobial resistance development and analysis of risk factors. Ann Clin Microbiol Antimicrob 20: 69. doi: 10.1186/s12941-021-00472-5.
5. Li J, Wang J, Yang Y, Cai P, Cao J, Cai X, Zhang Y (2020) Etiology and antimicrobial resistance of secondary bacterial infections in patients hospitalized with COVID-19 in Wuhan, China: a retrospective analysis. Antimicrob Resist Infect Control 9: 153. doi: 10.1186/s13756-020-00819-1.
6. Lansbury L, Lim B, Baskaran V, Lim WS (2020) Co-infections in people with COVID-19: a systematic review and meta-analysis. J Infect 81: 266-275. doi: 10.1016/j.jinf.2020.05.046.
7. De Santis V, Corona A, Vitale D, Nencini C, Potalivo A, Prete A, Zani G, Malfatto A, Tritapepe L, Taddei S, Locatelli A, Sambri V, Fusari M, Singer M (2022) Bacterial infections in critically ill patients with SARS-2-COVID-19 infection: results of a prospective observational multicenter study. Infection 50: 139-148. doi: 10.1007/s15010-021-01661-2.
8. Chen S, Zhu Q, Xiao Y, Wu C, Jiang Z, Liu L, Qu J (2021) Clinical and etiological analysis of co-infections and secondary infections in COVID-19 patients: an observational study. Clin Respir J 15: 815-825. doi: 10.1111/crj.13369.
9. Vincent JL, Sakr Y, Singer M, Martin-Loeches I, Machado FR, Marshall JC, Finfer S, Pelosi P, Brazzi L, Aditianingsih D, Timsit JF, Du B, Wittebole X, Maca J, Kannan S, Gorordo-Delsol LA, De Waele JJ, Mehta Y, Bonten MJM, Khanna AK, Kollef M, Human M, Angus DC, EPIC III Investigators (2020) Prevalence and outcomes of infection among patients in intensive care units in 2017. JAMA 323: 1478-1487. doi: 10.1001/jama.2020.2717.
10. Tan L, Wang Q, Zhang D, Ding J, Huang Q, Tang YQ, Wang Q, Miao H (2020) Lymphopenia predicts disease severity of COVID-19: a descriptive and predictive study. Signal Transduct Target Ther 5: 33. doi: 10.1038/s41392-020-0148-4.
11. Wang M, Luo L, Bu H, Xia H (2020) One case of coronavirus disease 2019 (COVID-19) in a patient co-infected by HIV with a low CD4(+) T-cell count. Int J Infect Dis 96: 148-150. doi: 10.1016/j.ijid.2020.04.060.
12. Luo Y, Xie Y, Zhang W, Lin Q, Tang G, Wu S, Huang M, Yin B, Huang J, Wei W, Yu J, Hou H, Mao L, Liu W, Wang F, Sun Z (2019) Combination of lymphocyte number and function in evaluating host immunity. Aging 11: 12685-12707. doi: 10.18632/aging.102595.
13. Ripa M, Galli L, Poli A, Oltolini C, Spagnuolo V, Mastrangelo A, Muccini C, Monti G, De Luca G, Landoni G, Dagna L, Clementi M, Rovere Querini P, Ciceri F, Tresoldi M, Lazzarin A, Zangrillo A, Scarpellini P, Castagna A, COVID-BioB study group (2021) Secondary infections in patients hospitalized with COVID-19: incidence and predictive factors. Clin Microbiol Infect 27: 451-457. doi: 10.1016/j.cmi.2020.10.021.
14. Cohen R, Babushkin F, Finn T, Geller K, Alexander H, Datnow C, Uda M, Shapiro M, Paikin S, Lellouche J (2021) High rates of bacterial pulmonary co-infections and superinfections identified by multiplex PCR among critically ill COVID-19 patients. Microorganisms 9: 2483. doi: 10.3390/microorganisms9122483.
15. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, Satta G, Cooke G, Holmes A (2020) Bacterial and fungal coinfection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis 71: 2459-2468. doi: 10.1093/cid/ciaa530.
16. Jia L, Xie J, Zhao J, Cao D, Liang Y, Hou X, Wang L, Li Z (2017) Mechanisms of severe mortality-associated bacterial co-infections following influenza virus infection. Front Cell Infect Microbiol 7: 338. doi: 10.3389/fcimb.2017.00338.
17. Smith AM, McCullers JA (2014) Secondary bacterial infections in influenza virus infection pathogenesis. Curr Top Microbiol Immunol 385: 327-356. doi: 10.1007/82_2014_394.
18. Zhang H, Zhang Y, Wu J, Li Y, Zhou X, Li X, Chen H, Guo M, Chen S, Sun F, Mao R, Qiu C, Zhu Z, Ai J, Zhang W (2020) Risks and features of secondary infections in severe and critical ill COVID-19 patients. Emerg Microbes Infect 9: 1958-1964. doi: 10.1080/22221751.2020.1812437.
19. Gangneux JP, Dannaoui E, Fekkar A, Luyt CE, Botterel F, De Prost N, Tadie JM, Reizine F, Houze S, Timsit JF, Iriart X, Riu-Poulenc B, Sendid B, Nseir S, Persat F, Wallet F, Le Pape P, Canet E, Novara A, Manai M, Cateau E, Thille AW, Brun S, Cohen Y, Alanio A, Megarbane B, Cornet M, Terzi N, Lamhaut L, Sabourin E, Desoubeaux G, Ehrmann S, Hennequin C, Voiriot G, Nevez G, Aubron C, Letscher-Bru V, Meziani F, Blaize M, Mayaux J, Monsel A, Boquel F, Robert-Gangneux F, Le Tulzo Y, Seguin P, Guegan H, Autier B, Lesouhaitier M, Pelletier R, Belaz S, Bonnal C, Berry A, Leroy J, Francois N, Richard JC, Paulus S, Argaud L, Dupont D, Menotti J, Morio F, Soulie M, Schwebel C, Garnaud C, Guitard J, Le Gal S, Quinio D, Morcet J, Laviolle B, Zahar JR, Bougnoux ME (2022) Fungal infections in mechanically ventilated patients with COVID-19 during the first wave: the French multicentre MYCOVID study. Lancet Respir Med 10: 180-90. doi: 10.1016/S2213-2600(21)00442-2.
20. Li X, Xu S, Yu M, Wang K, Tao Y, Zhou Y, Shi J, Zhou M, Wu B, Yang Z, Zhang C, Yue J, Zhang Z, Renz H, Liu X, Xie J, Xie M, Zhao J (2020) Risk factors for severity and mortality in adult COVID-19 inpatients in Wuhan. J Allergy Clin Immunol 146: 110-118. doi: 10.1016/j.jaci.2020.04.006.

Corresponding author
Erhan Ozyurt, MD
Department of Anesthesiology and Reanimation, Antalya Training and Research Hospital,
University of Health Sciences, Varlık district Kazim Karabekir Street 07100 Antalya, Turkey
Tel: +90 505 317 17 09
Fax: +90 242 249 44 93
Email: eozyurt@hotmail.com

Conflict of interests: No conflict of interests is declared.