

Assessment of Gut Microbiota in Post-COVID-19 Patients

Ekaterina Sorokina, Ekaterina Chernevskaya

Federal Research and Clinical Center of Intensive Care Medicine and Rehabilitology, Moscow, Russia

INTRODUCTION & AIM

Gut dysbiosis, exacerbated by excessive antimicrobial use, is a significant concern in patients with post-COVID-19 syndrome, often persisting beyond the resolution of SARS-CoV-2 infection. Dysbiosis contributes to microbiota imbalance and antimicrobial resistance, necessitating alternative approaches for rehabilitating post-COVID-19 patients. Patients with COVID-19 had significant alterations in fecal microbiomes compared with controls, characterized by the enrichment of opportunistic pathogens and depletion of beneficial commensals at the time of hospitalization and at all time points during hospitalization. Deficiency of symbionts and gut dysbiosis persisted even after the elimination of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the resolution of respiratory symptoms [1].

The aim of the study was to reveal the clinical, laboratory, and gut disorders in patients with post-COVID-19 syndrome (n = 24) before and after taking part in the 14-day complex program of rehabilitation.

METHOD

A pilot study included 24 post-COVID-19 patients with gut dysbiosis (men: 45%). The ages of patients ranged from 46 to 80 years, with a median of 58 (51; 71) years. All patients had the following post-COVID-19 syndrome symptoms: shortness of breath, asthenic syndrome, rapid fatigue during little physical exertion, general weakness, and fatigue, pain in gastrointestinal tract. Post-COVID-19 rehabilitation is aimed at restoration of the respiratory system using a respiratory gymnastics complex with more than 10 different exercises according to the Strelnikova technique, which is based on active inhalation and passive exhalation and also, physical and motor activity (Terrenkur [2]) with clinical control of the patient's condition. Intestinal content samples were obtained on the first day of admission from the 24 patients, who had recovered from the disease and received outpatient or inpatient treatment for periods of two to six months by the time of the study. Antibacterial therapy was administered in most patients during the stage of acute illness (third-generation cephalosporins or amoxicillin in combination with beta-lactamase inhibitors). A complete blood count (Figure 1) including white blood cell count, red blood cell count, hemoglobin, hematocrit, and other parameters, was performed by a hematology analyzer (UniCel DxH800, Beckman Coulter, Brea, CA, USA). Coagulation test including D-dimer was performed on a coagulation analyzer (Sysmex CS-2000i, Kobe, Japan). Biomarkers, including interleukin-6 (IL-6) and neuron-specific enolase (NSE), were analyzed using electrochemiluminescence (Cobas e411, Roche, Basel, Switzerland).

The composition of the gut microbiota was analyzed using Colonoflor-16 (biocenosis) kits (AlphaLab, St. Petersburg, Russia) by real-time PCR detection (CFX 96, BioRad, Hercules, CA, USA). The Operating Instructions for Colonoflor-16 (biocenosis) kit, obtained from AlphaLab, Russia.

Biomaterial Collection Points (Intestinal contents + blood serum)

- ✓ Day 1 of rehabilitation admission
- ✓ Day 14 post-rehabilitation



Figure 1. Biomaterial Collection Points.

RESULTS & DISCUSSION

After 14 days of rehabilitation, there were no statistically significant changes in the results of the complete blood count, except for hemoglobin and hematocrit, although these parameters remained elevated in 21% of patients (n=5). Parameters of the coagulation test, including D-dimer, were out of reference ranges on admission in 4 of 24 patients, 13%. Two specific biomarkers (Table 1) were measured in serum samples using electrochemiluminescence. NSE levels were within reference values for most patients, although they were found to be statistically different at the two time points. IL-6 levels were higher than the reference value in 85% of patients at both time points.

Table 1. Specific Biomarkers (Electrochemiluminescent Analysis).

Biomarker	Reference Values	Patients on Admission	Patients after 14 Days	p-Value
Interleukin-6 (IL-6), pg/ml	<7	11.5 (8.2–15.6), 1.5–61.9 n (c > RV) = 19	12.3 (8.6–17.6), 2.6–58.2 n (c > RV) = 21	0.255
Neuron-specific enolase (NSE), ng/ml	<16.3	9.2 (4.9–11.8), 0.1–22.0 n (c > RV) = 1	9.5 (5.7–13.0), 0.1–48.0 n (c > RV) = 3	0.038

The levels of the *Bacteroides fragilis* group, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Candida* spp., *Staphylococcus aureus*, *Proteus vulgaris*/ *Proteus mirabilis*, *Enterococcus* spp., *Enterobacter* spp., and *Citrobacter* spp. exceeded the reference values by 1.5–2 times in the intestinal content samples of patients (Figure 2). Additionally, the presence of *Clostridium difficile*, *Clostridium perfringens*, *Fusobacterium nucleatum*, and *Parvimonas micra*, microorganisms that are absent in the gut microbiota of healthy subjects, was detected. *Bifidobacterium* spp. and *Escherichia coli* were subsequently decreased after rehabilitation ($p = 0.019$ and 0.023 , respectively).

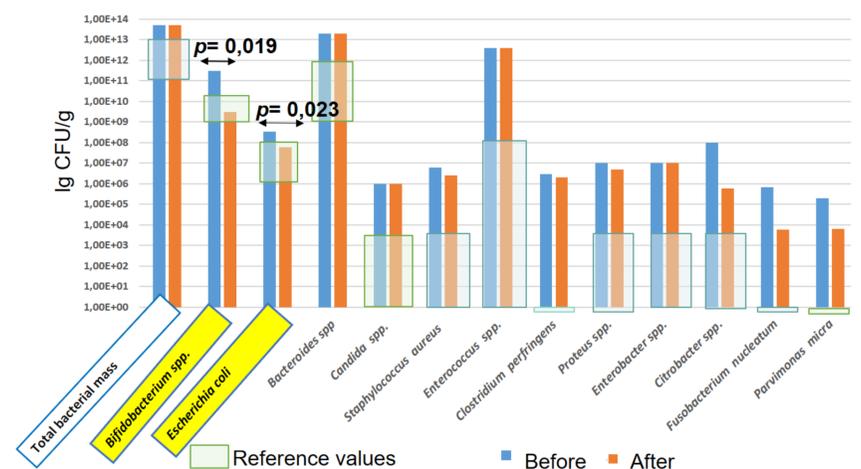


Figure 2. Dynamics of the taxonomic composition of intestinal contents in patients (n = 24).

CONCLUSION

A non-specific rehabilitation program for patients with post-COVID-19 syndrome over 14 days led to improvement health and respiratory function, but did not affect biochemical and dysbiotic disorders. Given the growing evidence linking dysbiosis to prolonged recovery with post-COVID-19 patients, a more judicious approach to antibiotic use is essential. Further research is needed to better understand the long-term consequences of antibiotic-induced dysbiosis with post-COVID-19 syndrome and to develop personalized treatment protocols that minimize harm while effectively managing infections.

FUTURE WORK / REFERENCES

1. Zhang, F.; Wan, Y.; Zuo, T.; Yeoh, Y.K.; Liu, Q.; Zhang, L.; Zhan, H.; Lu, W.; Xu, W.; Lui, G.C.Y.; et al. Prolonged Impairment of Short-Chain Fatty Acid and L-Isoleucine Biosynthesis in Gut Microbiome in Patients with COVID-19. *Gastroenterology* 2022, 162, 548–561.e4.
2. Jena, R. Effect of Strelnikova Exercise on Respiratory Parameters among Children with LRTI In Selected Hospital Bhubaneswar. *Eur. J. Mol. Clin. Med.* 2020, 7, 1058–1065