Gut Pathogens

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Improved gut microbiota features after the resolution of SARS-CoV-2 infection

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Abstract

Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a tropism for the gastrointestinal tract and several studies have shown an alteration of the gut microbiota in hospitalized infected patients. However, long-term data on microbiota changes after recovery are lacking.

Methods: We enrolled 30 patients hospitalized for SARS-CoV-2-related pneumonia. Their gut microbiota was analyzed within 48 h from the admission and compared with (1) that of other patients admitted for suspected bacterial pneumonia (control group) (2) that obtained from the same subject 6 months after nasopharyngeal swab negativization.

Results: Gut microbiota alpha-diversity increased 6 months after the resolution of SARS-CoV-2 infection. Bacteroidetes relative abundance was higher ($\approx$ 36.8%) in patients with SARS-CoV-2, and declined to 18.7% when SARS-CoV-2 infection resolved (p = 0.004). Conversely, Firmicutes were prevalent ($\approx$ 75%) in controls and in samples collected after SARS-CoV-2 infection resolution (p = 0.001). Ruminococcaceae, Lachnospiraceae and *Blautia* increased after SARS-CoV-2 infection resolution, rebalancing the gut microbiota composition.

Conclusion: SARS-CoV-2 infection is associated with changes in the gut microbiome, which tend to be reversed in long-term period.

Keywords: Gut microbiota, SARS-CoV-2, COVID-19, Pneumonia

Background

Several studies to date have analyzed the gut microbiota of patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection both during and after disease resolution [1, 2]. However, information on long-term follow-up is lacking. Therefore, we evaluated changes to the gut microbiome six months after SARS-CoV-2 infection resolution in 30 Italian patients hospitalized for pneumonia in our center during the first wave of the pandemic.

Study design and results

Faecal samples of 31 SARS-CoV-2-positive patients were harvested within 48 h from admission and prior to transfer to the intensive care unit (ICU), in order to minimize any impact of the pharmaceutical treatment on the gut microbiome, and again six months after discharge. During this , patients were regularly interviewed and none of them reported any infection/antibiotic treatment or symptoms of new onset. Eighteen patients hospitalized in the same period for SARS-CoV-2-unerlated

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pneumonia served as control group. Following hospital admission, at the time of fecal specimen collection, both control group (94.7%) and patients showing SARS-CoV-2 related pneumonia (71%) were receiving the same antibiotic treatment schedule, as per Hospital protocol. Faecal samples analysis was conducted as described in the Additional file 4. Characteristics of the study population are shown in Table 1. Gut microbiota alpha-diversity was similar between patients affected by either SARS-CoV-2 or non-SARS-CoV-2 pneumonia; it showed a slight enhanced trend after SARS-CoV-2 negativization, increasing significantly   after the resolution of SARS-CoV-2 infection when compared with the control group ($p = 0.346$, $p = 0.043$ and $p = 0.048$, Kruskal–Wallis Test, for Shannon index, inverse Simpson index and Pielou's Evenness, respectively). In particular, equitability among bacterial species increased and appeared to be driven by microbial changes ensuing SARS-CoV-2 infection resolution (Fig. 1A). The PCoA of inter-individual variation based on weighted UniFrac distance showed a slender split among the study groups, although it was not statistically significant ($p = 0.114$, PERMANOVA; Additional file 1: Figure S1). As shown in Fig. 1B, Bacteroidetes relative abundance was higher ($\approx 36.8\%$) in SARS-CoV-2 positive patients than in those with SARS-CoV-2 negative pneumonia ($\approx 13.6\%$), and declined to 18.7% when SARS-CoV-2 infection resolved ($p = 0.004$,

Kruskal–Wallis Test). Conversely, Firmicutes were prevalent ($\approx 75\%$) in controls and in samples collected after SARS-CoV-2 infection resolution, while the relative abundance was lower ($\approx 50\%$) in SARS-CoV-2 positive pneumonia group ($p = 0.001$, Kruskal–Wallis Test).

Focusing on the twenty most represented bacterial genera, we observed specific patterns of *Bacteroides*, *Blautia* and *Enterococcus* variations among groups (Fig. 1C). Particularly, *Bacteroides* and *Enterococcus* genera appeared to correlate inversely, with the former genus being increased in SARS-CoV-2 positive patients compared to the other groups ($p = 0.003$), whereas the latter genus showed a decreasing trend ($p = 0.082$). Finally, *Blautia* increased after SARS-CoV-2 infection resolution, rebalancing the gut microbiota composition ($p = 0.029$). As expected, linear discriminant analysis (LDA) showed that bacterial elements belonging to Bacteroidetes (i. e., *Oscillibacter*, Ruminococcaceae DTU089, Bacteroidaceae, Bacteroidia, *Parabacteroides* and Tannerellaceae) were enriched in SARS-CoV-2 positive patients, whereas Lactobacillales, *Streptococcus, Staphylococcus and Acidoaminococcus* were increased in those with SARS-CoV-2-unrelated pneumonia ($p < 0.05$, Kruskal–Wallis Test; Fig. 1D). Conversely, Lachnospiraceae (i. e., NK4A136 group, *Fusicantibacter* and *Roseburia*) and Ruminococcaceae UCG-013 were increased after SARS-CoV-2 infection resolution. DESeq2 analysis reported substantially

Table 1 Clinical and demographic features of patients included in the study

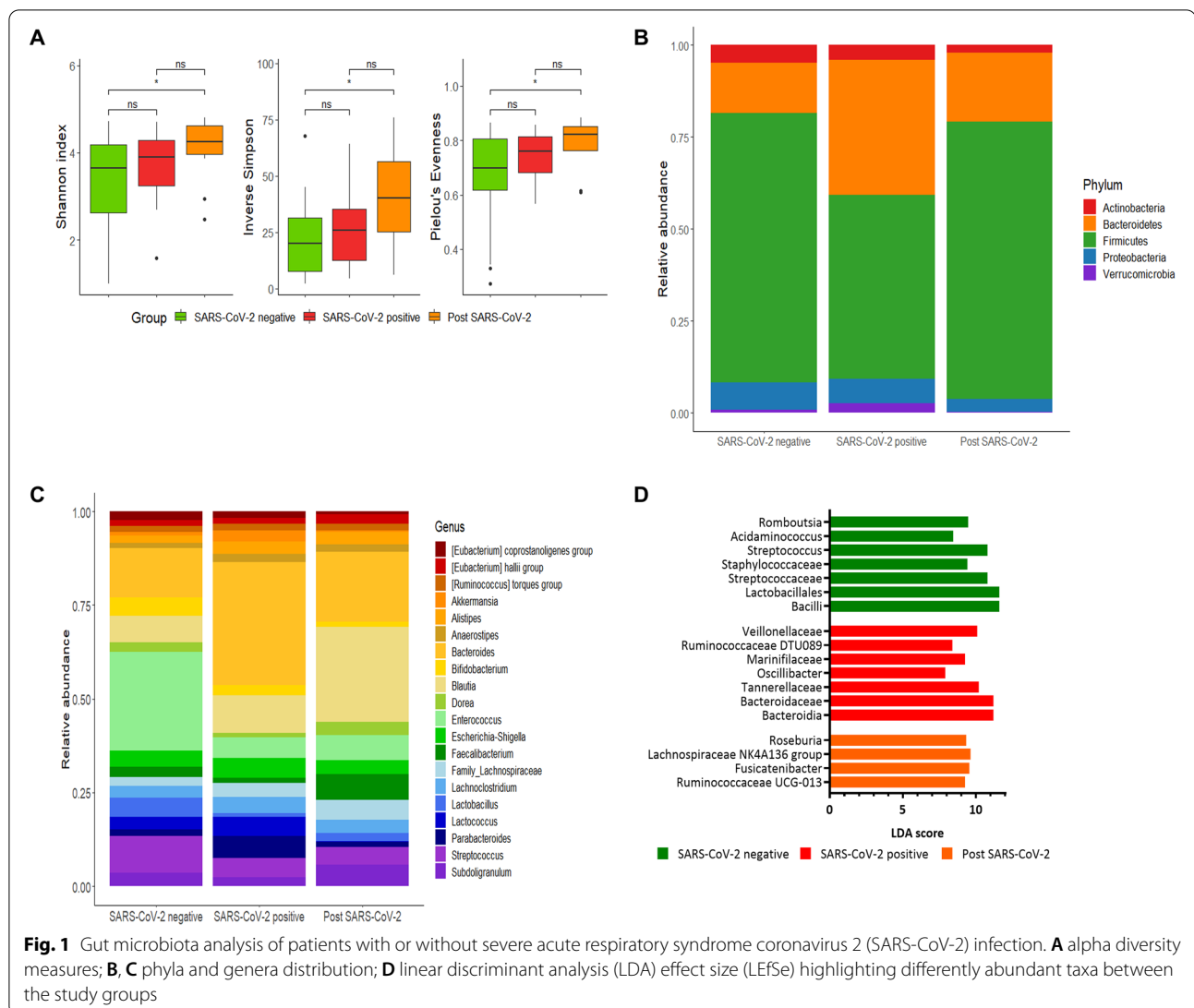|	SARS-CoV2 + (31)	SARS-CoV2 − (18)	p value
Age	66.7 ± 14.4	67.1 ± 17.5	0.372
Sex			
Males	23 (74.2%)	11 (61.1%)	0.357
Females	8 (25.8%)	7 (38.9%)	
Gastrointestinal symptoms	13 (41.9%)	5 (27.8%)	0.249
Respiratory failure*	22 (73.3%)	6 (33.3%)	0.012
ARDS categories[a]			
III	14 (63.6%)	5 (83.3%)	
II	7 (31.8%)	1 (16.7%)	
I	1 (4.5%)	0	
History of chronic lung disease	4 (12.9%)	3 (16.7%)	0.512
Treatment during hospitalization			
Antibiotics	22 (71%)	18 (94.7%)	0.05
Antivirals	27 (90%)	3 (16.7%)	0.00
Hydroxychloroquine	29 (93.5%)	2 (10.5%)	0.017
Anti-IL-6 receptor monoclonal antibodies	8 (25.8%)	0	

Numeric variables are reported as mean ± standard deviation, categorical ones as frequencies and percentages

*Number of patients with respiratory failure during hospitalization (PaO_2/FiO_2 ratio < 300)

Significance was evaluated by Wilcoxon signed rank test and chi-square test (IBM SPSS Statistics)

SARS-CoV-2 severe acute respiratory syndrome coronavirus 2; *IL* interleukin

[a] III PaO_2/FiO_2 ratio 200–300, II PaO2/FiO2 ratio 100–200, I PaO2/FiO2 ratio < 100

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Fig. 1 Gut microbiota analysis of patients with or without severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. **A** alpha diversity measures; **B**, **C** phyla and genera distribution; **D** linear discriminant analysis (LDA) effect size (LEfSe) highlighting differently abundant taxa between the study groups

the same results (Additional file 2: Table S1, Additional file 3: Table S2).

Discussion

Our study conducted in a Western population confirms that gut microbiota alpha diversity is similar in patients with SARS-CoV-2 related or unrelated pneumonia. Nevertheless, we observed an increase in alpha-diversity after the resolution of SARS-CoV-2 acute infection. This may be related to the effects of SARS-CoV-2 on the gut microbiota, but the contribution of drugs (e.g., antibiotics) administered before hospitalization or during its initial phase may not be negligible. Nevertheless, alpha-diversity modification may be not sufficient to assess recovery of the healthy status.

Previous Chinese studies [3–5] compared the gut microbiota of patients with SARS-CoV-2-related and community-acquired pneumonia, confirming a surge of opportunistic pathogens and a reduction in commensals elements. Our data confirm Bacteroidetes enrichment in patients with COVID-19 [3]. Conversely, Firmicutes appeared to decline, whereas no significant variation was observed for Actinobacteria [3]. During SARS-CoV-2 infection, inflammation is a key determinant of disease severity [6–8]. Interestingly, cytokine storm is positively associated with *Bacteroides* relative abundance [3]. Conversely, *Blautia* with its anti-inflammatory properties may play an important role in the recovery from COVID-19 [9]. Indeed, interleukin-10 (IL-10) serum levels significantly decrease with the reduction of *Ruminococcus obeum* (otherwise *Blautia*)

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abundance [3], and this correlates with the failure in controlling host's inflammatory response. This study suffers of the limitations of the small sample size, but our results corroborate those achieved on populations with different ethnicity. Even though many factors can affect the gut microbiota, our findings support the hypothesis of a specific impact of SARS-CoV-2 infection on the gut microbiota.

Conclusions

This study supports the previous evidence that SARS-CoV-2 infection is associated with changes in the gut microbiome. However, many gut microbiome-related factors could influence the course of COVID-19, calling for more studies in the next future.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13099-021-00459-9.

Additional file 1. Principal coordinate analysis (PCoA) on weighted UniFrac distance of the gut microbiome of patient with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)-related pneumonia, who were tested before and after resolution of the infection, and patients with SARS-CoV-2 unrelated pneumonia.

Additional file 2. DESeq2 analysis.

Additional file 3. DESeq2 analysis.

Additional file 4. Supplementary methods.

Declarations

Ethics approval and consent to participate
The local ethics committee of the Fondazione Policlinico Universitario Agostino Gemelli IRCCS approved the study (ID 3042).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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References

1. Chhibber-Goel J, Gopinathan S, Sharma A. Interplay between severities of COVID-19 and the gut microbiome: implications of bacterial co-infections? Gut Pathog. 2021;13:14.
2. Yamamoto S, Saito M, Tamura A, Prawisuda D, Mizutani T, Yotsuyanagi H. The human microbiome and COVID-19: a systematic review. PLoS ONE. 2021. https://doi.org/10.1371/journal.pone.0253293.
3. Yeoh KY, Zuo T, Lui GCY, Zhang F, Liu Q, Li AY, et al. Gut microbiota composition reflects disease severity and dysfunctional immune responses in patients with COVID-19. Gut. 2021;70:698–706.
4. Zuo T, Zhang F, Lui GCY, Yeoh YK, Li AYL, Zhan H, et al. Alterations in gut microbiota of patients with COVID-19 during time of hospitalization. Gastroenterology. 2020;159(3):944-955.e8.
5. Gu S, Chen Y, Wu Z, Chen Y, Gao H, Lv L, et al. Alterations of the gut microbiota in patients with coronavirus disease 2019 or H1N1 influenza. Clin Infect Dis. 2020;71:2669–78.
6. Ponziani FR, Del Zompo F, Nesci A, Santopaolo F, Ianiro G, Pompili M, et al. Liver involvement is not associated with mortality: results from a large cohort of SARS-CoV-2-positive patients. Aliment Pharmacol Ther. 2020;52:1060–8.
7. Ponziani FR, Nesci A, Del Zompo F, Santopaolo F, Pompili M, Gasbarrini A, et al. Correlation between liver function tests abnormalities and interleukin-6 serum levels in patients with SARS-CoV-2 infection. Gastroenterology. 2021;160:1891–3.
8. Del Zompo F, De Siena M, Ianiro G, Gasbarrini A, Pompili M, Ponziani FR. Prevalence of liver injury and correlation with clinical outcomes in patients with COVID-19: systematic review with meta-analysis. Eur Rev Med Pharmacol Sci. 2020;24:13072–88.
9. Liu X, Mao B, Gu J, Wu J, Cui S, Wang G, et al. Blautia-a new functional genus with potential probiotic properties? Gut Microbes. 2021;13:1–21.

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