LETTER TO THE EDITOR

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

Flavio De Maio1,2, Gianluca Ianiro3,4, Gaetano Coppola4, Francesco Santopalo4, Valeria Abbate3, Delia Mercedes Bianco1,2, Fabio Del Zompo4, Giuseppe De Matteis5, Massimo Leo4, Antonio Nesci5, Alberto Nicoletti4, Maurizio Pompili3,4, Giovanni Cammarota3,4, Brunella Posteraro6,2, Maurizio Sanguinetti1,2, Antonio Gasbarrini3,4 and Francesca Romana Ponziani3,4,7*

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 (∼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 (∼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-unrelated
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 anti-biotic treatment schedule, as per Hospital protocol. Fecal 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 (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 (≈36.8%) in SARS-CoV-2 positive patients than in those with SARS-CoV-2 negative pneumonia (≈13.6%), and declined to 18.7% when SARS-CoV-2 infection resolved (p = 0.004, Kruskal-Wallis Test). Conversely, Firmicutes were prevalent (≈75%) in controls and in samples collected after SARS-CoV-2 infection resolution, while the relative abundance was lower (≈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 Acidaminococcus were increased in those with SARS-CoV-2-unrelated pneumonia (p < 0.05, Kruskal-Wallis Test; Fig. 1D). Conversely, Lachnospiraceae (i.e., NK4A136 group, Fusicatibacter 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*        |                  |                  |         |
| 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 (PaO2/FiO2 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

* PaO2/FiO2 ratio 200–300, II PaO2/FiO2 ratio 100–200, I PaO2/FiO2 ratio < 100
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*)

![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](image-url)
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.

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Authors’ contributions
FDM analyzed samples, interpreted data, designed the study, wrote and approved the final version of the paper; FRP interpreted data, designed the study, wrote and approved the final version of the paper; DMB analyzed and approved the final version of the paper; All authors read and approved the final version of the paper. GC, FDZ, ML, AN collected samples, interpreted data; GI, FS, VA, GDM, AN, MP, GC, BP, MS, AG interpreted data, study, wrote and approved the final version of the paper; DMB analyzed and interpreted data, designed the study, wrote and approved the final version of the paper; FRP interpreted data, designed the study, wrote and approved the final version of the paper.

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Declarations

Consent for publication
Not applicable.

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

Author details
1 Dipartmento di Scienze di Laboratorio e Infettivologie, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy. 2 Dipartmento di Scienze Biotecnologiche di Base, Cliniche Intensivologiche e Perioperatorie, Università Cattolica del Sacro Cuore, Rome, Italy. 3 Dipartimento di Medicina e Chirurgia Traslazionale, Università Cattolica del Sacro Cuore, Rome, Italy. 4 CEMAD Digestive Disease Center, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy. 1* Internal Medicine, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Rome, Italy. 3 Dipartimento di Scienze Mediche e Chirurgiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy. 2 Division of Internal Medicine and Gastroenterology, Hepatology Unit, Fondazione Policlinico Universitario Agostino Gemelli IRCCS, Università Cattolica del Sacro Cuore, Rome, Italy.

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