Respiratory dysfunction three months after severe COVID-19 is associated with gut microbiota alterations

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Running Title: Gut-lung axis in COVID-19

Abstract

Background: Although coronavirus disease 2019 (COVID-19) is primarily a respiratory infection, mounting evidence suggests that the gastrointestinal (GI) tract is involved in the disease, with gut barrier dysfunction and gut microbiota alterations being related to disease severity. Whether these alterations persist and are related to long-term respiratory dysfunction remains unknown.

Methods: Plasma was collected during hospital admission and after three months from the NOR-Solidarity trial (n = 181) and analysed for markers of gut barrier dysfunction and inflammation. At the three-month follow-up, pulmonary function was assessed by measuring the diffusing capacity of the lungs for carbon monoxide (DL_{CO}). Rectal swabs for gut microbiota analyses were collected (n = 97) and analysed by sequencing the 16S rRNA gene.

Results: Gut microbiota diversity was reduced in COVID-19 patients with respiratory dysfunction, defined as DL_{CO} below the lower limit of normal three months after hospitalisation. These patients also had an altered global gut microbiota composition, with reduced relative abundance of 20 bacterial taxa and increased abundance of five taxa, including *Veillonella,* potentially linked to fibrosis. During hospitalisation, increased plasma levels of lipopolysaccharide-binding protein (LBP) were strongly associated with respiratory failure, defined as pO_2/fiO_2-(P/F ratio) < 26.6 kPa. LBP levels remained elevated during and after hospitalisation and were associated with low-grade inflammation and respiratory dysfunction after three months.

Conclusion: Respiratory dysfunction after COVID-19 is associated with altered gut microbiota and persistently elevated LBP levels. Our results should be regarded as hypothesis-generating, pointing to a potential gut-lung axis that should be further investigated in relation to long-term pulmonary dysfunction and long COVID.

Keywords: SARS-CoV-2, microbiome, pulmonary function

Key points: Respiratory dysfunction three months after COVID-19 is associated with reduced biodiversity and gut microbiota alterations, along with persistently elevated LBP levels. Our findings indicate a potential gut-lung axis in relation to respiratory failure during hospitalisation and long-term COVID-19 morbidity.

Introduction

Although coronavirus disease 2019 (COVID-19) is primarily a viral respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), aberrant immune responses to the causative virus, resulting in systemic inflammation and multi-organ involvement, are central features in severe and critical disease. Mounting evidence suggests that the gastrointestinal (GI) tract is involved in the pathogenesis of COVID-19(1, 2).

The GI tract is the largest immunological organ in the body, and its resident microbiota modulates regional as well as systemic host immune responses. It has been hypothesised that the gut microbiota could be a mediator of host inflammatory immune responses during COVID-19, thereby contributing to the pronounced systemic inflammation observed in patients requiring hospitalisation(3, 4).

Recent studies suggest that severe SARS-CoV-2 infection could compromise the integrity of the gut-blood barrier, leading to enhanced leakage of microbial products, such as lipopolysaccharides (LPS), possibly affecting the host's response to COVID-19 through activation of the innate immune system(5, 6). In a small observational study of hospitalised patients with COVID-19, we recently reported that elevated plasma levels of LPS-binding protein (LBP) were associated with elevated cardiac markers(7). However, knowledge on the relationship between markers of gut barrier dysfunction and the degree of respiratory failure, particularly persistently impaired pulmonary dysfunction following COVID-19 hospitalisation, is scarce.

Patients with COVID-19 exhibit an altered gut microbiota composition(5, 8), and gut microbiome alterations have recently been found to be related to disease severity(9). Although these alterations persisted when investigated at a median of six days after a negative SARS-CoV-2 polymerase chain reaction (PCR) test, it is currently not known whether gut microbiota alterations or gut barrier dysfunction persists long term after hospitalisation. Moreover, whether microbiota-related

mechanisms could be involved in the persistent inflammation and pulmonary dysfunction observed in some patients during follow-up after hospitalisation for COVID-19 is unknown.

The NOR-Solidarity trial is an independent add-on study to the WHO Solidarity trial(10), which has recently reported no effects of hydroxychloroquine (HCQ) or remdesivir compared to standard of care (SoC) on clinical outcome, viral clearance, or systemic inflammation in hospitalised patients with COVID-19(11). In the present sub-study, we investigated whether gut microbiota collected three months after hospital admission, and levels of the gut barrier dysfunction marker LBP measured during and after hospitalisation, were related to pulmonary dysfunction after COVID-19.

Materials and methods

Study design and participants

NOR-Solidarity is a multicentre, open-label, adaptive randomised clinical trial evaluating the effect of antiviral drugs on hospitalised patients with COVID-19 admitted to 23 Norwegian hospitals(11). In addition, NOR-Solidarity included collections from a blood biobank and outpatient visits at a three-month follow-up after hospital admission. The study was approved by the Committee for Medical Research Ethics, Region Southeast Norway (118684) and the Norwegian Medicines Agency (20/04950-23) and registered at ClinicalTrials.gov (NCT04321616). Participants were included from 28 March until 5 October 2020, and all participants > 18 years of age admitted to the hospital with PCR-confirmed SARS-2-CoV-2 infection were eligible for inclusion. Exclusion criteria as described in the original study protocol included severe comorbidity (life expectancy < 3 months), high levels of liver transaminases (AST/ALT > 5 times the upper limit of normal), corrected QT interval time as assessed by electrocardiography > 470 ms, pregnancy, breast feeding, acute comorbidity occurrence in a 7-day period before inclusion, known intolerance to study drugs, concomitant medications interfering with the study drugs, and participation in a confounding trial(11). All participants provided informed consent prior to inclusion in the study.

Intervention and outcomes

In the NOR-Solidarity study (n=181), participants were randomised and allocated to one of three treatment arms: 1) local SoC; 2) SoC plus 800 mg of oral HCQ twice daily on day one, followed by 400 mg twice daily for up to nine days; or 3) SoC plus 200 mg of intravenous remdesivir on day one, followed by 100 mg daily for up to nine days. All study treatments were stopped at hospital discharge. Since the interventions had no effect on clinical outcome, viral clearance, or systemic inflammation(11), data from the different intervention arms were pooled for analyses in this sub-study to examine whether gut microbiota composition after three months, and marker of gut barrier dysfunction during hospitalisation and after three months, had any effect on (i) acute respiratory failure defined as pO_2/fiO_2-(P/F ratio) < 26.6 kPa (<200 mmHg) during hospitalisation, and (ii) respiratory dysfunction after three months defined as diffusing capacity of the lungs for carbon monoxide (DL_{CO}) below the lower limit of normal (LLN). To ensure the lack of influence of the different treatments, data were also adjusted for the treatment group.

Soluble LPS-binding protein measurements

Plasma levels of soluble LBP were measured using an enzyme immunoassay (EIA), as described in the Supplementary Methods.

Three-month follow-up

Three months after hospital admission, 149 participants attended a follow-up visit that included blood sampling for routine clinical biochemistry and biobanking(11). Rectal swabs were collected from a sub-group of participants (n=97). Lung function tests (n=108), consisting of spirometry and diffusion capacity of the lungs for carbon monoxide (DL_{CO}), were performed at each participating centre according to the guidelines of the European Respiratory Society and the American Thoracic Society (12, 13), as previously described(14). Studies have shown that DL_{CO} is the pulmonary measure most frequently affected after hospitalisation for COVID-19(14, 15). For the purpose of this study, we chose DL_{CO} as a measure of pulmonary function, as previously reported from the NOR-Solidarity

cohort(16). The $DL_{CO,}$ in per cent of predicted and the LLN, was calculated according to the Global Lung Function Initiative Network (GLI) (17), as previously described(14). Of the 108 participants who underwent lung function tests, 83 also underwent rectal swab collection.

Gut microbiota analyses

Rectal swabs were stored in a stabilising transportation medium (soluble Amies, Thermo Scientific™) and frozen at -80 °C until analysis. Faecal DNA was extracted using the QIAamp PowerFecal®Pro DNA Kit (Qiagen, Germany), with slight modifications. Briefly, 700 µL of faecal material was pelleted and homogenised in 800 µL of kit solution CD1 using a bead-beater (2x60 s at 5.5 ms, 20 °C) and further processed according to the manufacturer's protocol. Libraries for 16S rRNA amplicon sequencing were generated as previously described(18). Briefly, the hypervariable regions V3 and V4 of the 16S rRNA gene were amplified using dual-indexed universal primers 319F (forward) and 806R (reverse), and Phusion High-Fidelity PCR Master Mix with HF buffer (Thermo Fisher Scientific, USA). The PCR products were cleaned and normalised using a SequalPrep Normalization Plate Kit (Thermo Fisher Scientific, USA). Quality control and quantification of the pooled libraries were performed using an Agilent Bioanalyzer (Agilent Technologies, USA) and Kapa Library Quantification Kit (Kapa Biosystems, London, UK). Sequencing was performed at the Norwegian Sequencing Centre (Oslo, Norway) using the Illumina MiSeq platform and v3 kit (Illumina, San Diego, CA, USA), allowing for 300 bp paired-end reads.

Sequence processing and bioinformatics

Paired-end reads were filtered for Illumina Universal Adapters and PhiX, demultiplexed, quality trimmed, and merged using bbduk 38.90, je 1.2, cutadapt 3.3, and bbmerge 38.90, respectively. Denoising to amplicon sequence variants (ASVs) and taxonomic classification were performed using QIIME2 version 2021.2. Contaminants were filtered using the R package microDecon . Diversity analyses were performed on a rarefied (subsampled) dataset with an ASV count of 7123 per sample. Further details are provided in the Supplementary Methods.

Statistical analyses

Baseline characteristics are described as median (25th and 75th percentile [interquartile range]) for continuous variables and percentages for categorical variables. Group-wise comparisons were performed using a two-tailed *t*-test or Mann–Whitney *U* test for continuous data and chi-square or Fisher's exact test (two-sided) for categorical data. Predefined outcomes (acute respiratory failure defined as P/F ratio < 26.6 kPa during hospitalisation and respiratory dysfunction defined as DL_{CO} < LLN after three months of follow-up) were used as dichotomous variables. LBP levels were non-normally distributed and transformed using $\log_{10}$ for comparisons between groups with linear mixed model analysis, with subjects as random effects and time and respiratory failure as fixed effects (also as interaction). Treatment was included as a covariate. Multivariate analysis of LBP (defined as Z-score [standardised] $\log_{10}$ LBP) in relation to respiratory failure was performed using binary logistic regression, adjusting for the covariates age, sex, treatment, and known comorbidities in the base model. Correlation analyses were performed using Spearman's rho (ρ) owing to skewed distribution of the data. P-values were two-sided and considered significant at $p<0.05$. Group comparisons of LBP levels in relation to respiratory dysfunction were performed using a parametric t-test of $\log_{10}$-transformed data. SPSS release 26.0.0.1 and 27.0.0.0 (IBM® SPSS® Statistics, IBM Corporation, Armonk, NY, USA) were used for the statistical analysis.

Results

Baseline characteristics

The NOR-Solidarity trial design and main results have recently been published(11). A total of 181 randomised patients were included in the present study, and 149 completed the three months follow-up, of which 108 underwent pulmonary function testing and 97 underwent rectal swab collection for gut microbiota analyses (Fig. 1). Baseline characteristics for the main study group and the subgroups of patients with and without respiratory failure, as well as those with microbiota samples available, are given in Table 1. In the main study group, participants were 59 years old on average and mostly male (66%), and the median body mass index (BMI) was 27.4 kg/m^2 (27% obese,

BMI > 30 kg/m^2). The use of antibiotics during hospitalisation was reported in 48% of the participants, and 68% reported any known comorbidities, with hypertension (31%), obesity (27%), and diabetes mellitus (17%) as the most common, whereas only 6% reported the presence of chronic pulmonary disease.

Patients with respiratory failure were older and hospitalised for longer than patients without, with a higher need for intensive care unit (ICU) admission and invasive mechanical ventilation, more frequent use of antibiotics, and higher levels of inflammatory markers. The clinical characteristics at the three-month follow-up are presented in Supplementary Table 1. Patients with respiratory dysfunction after three months were older and had been hospitalised for longer than those without, with a higher frequency of pre-existing pulmonary disease and a greater need for ICU admission and invasive mechanical ventilation during hospitalisation. However, the levels of inflammatory markers after three months were similar in both the groups.

Respiratory dysfunction after three months is associated with altered gut microbiota composition and reduced bacterial diversity in previously hospitalised patients with COVID-19

At the three-month follow-up, 25 out of the 83 (30%) previously hospitalised patients with COVID-19, with both rectal swabs and DL_{CO} measurements available, had respiratory dysfunction defined as DL_{CO} < LLN. Notably, these patients had altered global gut microbiota composition as assessed by beta diversity (Bray–Curtis (Fig. 2A)). It is important to note that patients who received antibiotics during hospitalisation had overlapping global microbiota compositions with those not receiving antibiotics (Fig. 2B). Moreover, there was no statistical interaction between antibiotic use during hospitalisation and the association between global microbiota composition and respiratory dysfunction at the three-month follow-up, although such an interaction could not be ruled out due to the limited sample size.

Patients with respiratory dysfunction three months after hospital admission also had reduced bacterial alpha diversity (Faith's phylogenetic diversity, PD and observed ASVs) compared to patients

with respiratory function in the normal range (Fig. 2C-D). Moreover, alpha diversity measures were inversely associated with the need for invasive mechanical ventilation (Faith's PD and observed ASVs), length of hospitalisation (Shannon index), and diagnosis of diabetes mellitus, but were not associated with other comorbidities, age, sex, BMI, treatment groups, inflammatory markers, or viral load (Supplementary Table 2). Although not statistically significant, there was a tendency toward reduced bacterial alpha diversity in patients treated with antibiotics during hospitalisation.

At the taxonomic level, screening analysis using linear discriminant analysis effect size (LEfSe) suggested that respiratory dysfunction after three months was associated with increased relative abundance of 5 taxa and reduced relative abundance of 20 taxa (Fig. 3A-B), including *Erysipelotrichaceae UCG-003* and several members of the Lachnospiraceae family (*Coprococcus*, *Eubacterium_ventriosum_group*, *Lachnospiraceae_FCS020_group*, *Lachnospiraceae_ND3007_group*, *Fusicatenibacter*) and Ruminococcaceae family (*Subdoligranulum*, *Ruminococcus*), which are potential producers of butyrate, the main energy source for enterocytes. We subsequently performed the same analysis using the ALDEx2 algorithm (filtered to $p<0.05$), reducing the number of genera identified by both LEfSe and ALDEx2 as associated with respiratory dysfunction to three: reduced abundance of *Erysipelotrichaceae UCG-003* and increased abundance of *Veillonella* and *Flavonifractor* (Fig. 3C). The group-wise abundance distributions of the three genera are shown in Supplementary Fig. 3. The largest effect size and lowest p-value from ALDEx2 was found for *Veillonella*, an anaerobic opportunistic pathogen previously reported to be increased in patients with COVID-19(8) and associated with tissue fibrosis(19).

Circulating levels of LBP in relation to acute respiratory failure during hospitalisation

As microbiota traits associated with respiratory dysfunction were potentially related to a reduced capacity for butyrate production, which is vital for a functional gut barrier, we hypothesised that gut barrier dysfunction could also be related to respiratory failure in acute COVID-19. As microbiota samples from the acute phase of disease were not available, we explored whether levels of

circulating LBP were associated with acute respiratory failure defined as a P/F ratio < 26.6 kPa, which occurred in 60 patients (33%) during hospitalisation. LBP levels remained consistently elevated during hospitalisation in patients with acute respiratory failure compared to patients without, although with a declining trend over time (Fig. 4A). Moreover, LBP levels were consistently inversely associated with P/F ratio during the hospitalisation period ($p<0.001$, linear mixed model, intercept of all three time points; Fig. 4B). In multivariate logistic regression adjusted for age, sex, comorbidity, and treatment, LBP was associated with acute respiratory failure during hospitalisation (adjusted OR [aOR] 1.98 [1.15-3.40]) per standard deviation of $\log_{10}$ LBP. Further adjustment for smoking and antibiotic use had little impact on this association (aOR 1.77 [1.01-3.10]).

The association of LBP with respiratory dysfunction and inflammation after three months

Notably, LBP levels after three months remained significantly higher in patients than in age- and sex-matched healthy controls, where patients with respiratory dysfunction (DL_{CO} < LLN) had numerically higher levels than patients with normal respiratory function (Supplementary Fig. 1). Moreover, LBP levels correlated negatively with the percent predicted values of DL_{CO} (ρ=-0.27, p=0.031). Notably, we found no correlation between LBP and measures of microbiota diversity in the subgroup with both microbiota and lung function tests available. Plasma LBP levels at admission and after three months were significantly associated with several clinical markers of systemic inflammation, including CRP levels and white blood cell and neutrophil counts (Supplementary Fig. 2), although these inflammatory markers were not related to respiratory dysfunction after three months (Supplementary Table 1).

Discussion

In this sub-study of the NOR-Solidarity trial, we investigated the potential gut-lung axis during and after hospitalisation for COVID-19. Our results can be summarised as follows: i) three months after hospitalisation for COVID-19, patients with respiratory dysfunction (DL_{CO} < LLN) showed a lower

microbial diversity and an altered global gut microbiota composition than patients with normal respiratory function; ii) these microbiota alterations included reduced abundance of *Erysipelotrichaceae UCG-003* and increased abundance of *Veillonella* and *Flavonifractor;* iii) during hospitalisation, increased plasma levels of LBP were significantly associated with acute respiratory failure; and iv) LBP levels remained elevated during and after hospitalisation, and were significantly associated with persistent low-grade inflammation and respiratory dysfunction at the three-month follow-up.

In a recent report, Yeoh *et al.* reported the downregulation of several gut commensals with known immunomodulatory potential in patients with COVID-19, including *Faecalibacterium prausnitzii* and *Eubacterium rectale*, which remained low in samples collected up to 30 days after disease resolution(9). Here, we extend these findings by showing that altered gut microbiota, including decreased alpha diversity three months after hospital admission, was associated with impaired pulmonary function at this time point. We also found that several members of the Lachnospiraceae and Ruminococcaceae families, such as *Coprococcus* and *Ruminococcus*, which are known producers of butyrate, were reduced in patients with COVID-19 with pulmonary impairment after three months. Butyrate has local immunomodulatory effects in the gut mucosa and, as the main energy substrate for enterocytes, is vital for gut barrier maintenance(20). Interestingly, LBP levels as a potential indirect marker of gut leakage were associated with impaired pulmonary function, not only during hospitalisation (P/F ratio < 26.6 kPa, respiratory failure), but also after three months (inverse correlation with DL_{CO}).

COVID-related dysbiosis reported by Yeoh *et al.* correlated with several inflammatory markers, in line with our finding that LBP is associated with acute inflammation during hospitalisation. Notably, we now show that LBP levels are associated with persistent low-grade inflammation even after three months. A potential role of gut barrier dysfunction as a driver of the multisystem inflammatory syndrome occurring in paediatric COVID-19 has been recently reported(21), and our findings suggest

that similar mechanisms could be relevant in adult patients. In some chronic inflammatory conditions related to immunodeficiencies, we had also seen a direct correlation between microbiota composition and markers of gut barrier function(22, 23), but this was not seen in patients with COVID-19 at three months.

Of note, LBP is an acute-phase protein produced in the liver(24), and as such, could be more related to acute inflammation than gut barrier alterations. Whether gut microbiota alterations were related to gut barrier dysfunction could not be determined in the present study. However, whereas pulmonary dysfunction was related to gut microbiota alterations and inversely correlated with LBP levels, no associations with inflammatory markers, including CRP levels, were detected at the three-month follow-up.

Other microbiota-related traits might also be relevant. The largest effect size observed in patients with pulmonary dysfunction was the increased relative abundance of *Veillonella*, which has previously been linked to several disease states of the lung and liver, where fibrosis is central to pathogenesis(19, 25, 26). Interestingly, an increased relative abundance of *Veillonella* has recently been reported in patients with COVID-19(27, 28). However, whether this bacterial genus is relevant for fibrosis development after COVID-19, either as a contributing factor or as a consequence of a fibrotic process, cannot be answered in an observational study. A future study would benefit from parallel evaluation of the respiratory tract microbiome, as *Veillonella* has been found to be enriched in airway samples of patients with idiopathic pulmonary fibrosis(29).

The present study has several limitations. Due to the logistic constraints of launching a randomised trial during the first wave of the pandemic, the gut microbiota samples and pulmonary function tests were only performed at the three-month follow-up. Therefore, we cannot relate long-term microbiota alterations to potential microbiota alterations or the potential gut-lung axis during hospitalisation. In addition, as the analysis of microbiota composition was performed in only one cohort, and without a validation panel, these data must be considered explorative.

Furthermore, whether the observed alterations in microbiota composition in this trial are related to COVID-19 *per se* or mirror other factors, such as pre-existing comorbidities, length of hospital stay, need for ICU, organ support, or other treatment, is not clear. However, our findings suggest a possible impact of invasive mechanical ventilation and length of hospitalisation on gut microbial diversity after three months, which could reflect disease severity, treatment during hospitalisation, or a combination. Notably, the use of antibiotics was significantly more frequent in patients with respiratory failure during hospitalisation. The use of antibiotics was also potentially related to lower alpha diversity measures in the gut microbiota samples, but not significantly, possibly due to the limited sample size. Although power calculations should preferably have been performed, this was not possible as the NOR-Solidarity trial was executed as an add-on trial of the WHO Solidarity trial, and the decision to stop further inclusion was made after advice from an independent data and safety monitoring board.

Finally, the compositional alterations found in the rectal specimens could have been supported by measuring butyrate levels in plasma or faecal samples. However, this was not performed because of the poor detection rate in previously analysed plasma samples of other patient cohorts(30), as well as the presence of preservation liquid in the rectal swab samples which precludes measurements of short-chain fatty acids.

The study also has obvious strengths, including standardised data capture in a randomised trial with longitudinal biobanking as well as comprehensive long-term follow-up with blood tests, microbiota sampling, and assessment of pulmonary function by DL_{CO}. To our knowledge, our study is the first to potentially link pulmonary function to gut microbiota alterations in COVID-19.

In conclusion, the decreased microbial diversity and compositional gut microbiota alterations in patients with respiratory dysfunction after three months, as well as the association of persistently raised LBP levels with these clinical features, point to a potential gut-lung axis in COVID-19. These observations could be related not only to acute respiratory failure during hospitalisation but also to

long-term COVID-19 morbidity. However, owing to power limitations, the cross-sectional nature of the microbiota and lung function assessments, and the interpretation of LBP as a gut leakage marker beyond an inflammatory marker, the present study should be considered exploratory. Nevertheless, our findings warrant further research on the potential role of gut microbiota composition and gut barrier dysfunction in relation to long-term pulmonary dysfunction and long COVID.

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Contributions

BV, TU, PA, JRH, and MT were responsible for study conception and execution of the present sub-study. ABD, TK, ICO, AMDR, KNH, PA, and MT were responsible for the management, coordination, research activity planning, and execution of the NOR-Solidarity trial. TL and OHS were responsible for the three-month follow-up protocol for pulmonary function. TBD, ABD, BH, TR, and PA coordinated collection and storage of the biobank material. AMDR, BS, KT, HH, AT, RM, MH, RE, ÅB, ABK, AJ, LT, HS, BRK, RBO, CMY, NVS, RH, SA, and BB were locally responsible for conducting the trial at the various included hospitals providing rectal swab material. BV performed DNA extraction and library preparation of microbiota samples. TU, TL, and AEM performed the gut leakage marker analyses. BV and TU performed the statistical analyses. KH performed the bioinformatics analyses of the microbiota data. BV and MT drafted the manuscript. TU, ABD, PA, and JRH critically revised the manuscript. All authors revised and approved the final version of the manuscript.

Conflicts of Interest Statement

The authors declare no competing interests.

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Figure legends

Figure 1. Flow-chart of patients included in the present add-on study based on the original NOR-Solidarity study protocol.

Figure 2. Gut microbiota diversity in patients with or without respiratory dysfunction at the three-month follow-up. Beta diversity by principal coordinate analysis showing Bray–Curtis distances separating patients with or without respiratory dysfunction (DLCO < LLN, n=83) (A), with overlapping microbiota composition in relation to antibiotic use (B). Alpha diversity measured with observed ASVs (C) and Faith`s PD (D) in patients with or without respiratory dysfunction. Abbreviations: ASVs, amplicon sequence variants; PD, phylogenetic diversity.

Figure 3. Gut microbial composition in patients with respiratory dysfunction at the three-month follow-up (DLCO < LLN, n=83). (A) LDA score of taxa abundance differences using LEfSe analysis. (B) Taxonomic cladogram highlighting differentially abundant taxa ($p<0.05$) by LEfSe. (C) Volcano plot from ALDEx2 analysis showing effect size representing the median of 'difference in clr (centred-log-ratio) values between groups divided by largest difference in clr values within group' on log_2-scale by p-value of differentially abundant genera. Abbreviations: LDA, linear discriminant analysis; LEfSe, linear discriminant analysis effect size.

Figure 4. Temporal profile of soluble lipopolysaccharide-binding protein (LBP) levels (n=144) in relation to acute respiratory failure (n=44) during hospitalisation (P/F ratio < 26.6 kPa) (A). The p-value reflects the overall effect of respiratory failure (RF) from the repeated measures regression analysis. Blue areas reflect levels in age- and sex-matched healthy controls (n=24). (B) Spearman correlations between LBP and P/F ratio at different time points (baseline orange; day 3–5, yellow green; day 7–10, brown) during hospitalisation. *$p<0.05$, **$p<0.005$. Observations per time point: BL, n=144; 3-5 days, n=134; 7-10 days, n=84.

Table legends

Table 1. Results are shown as median values (25^{th}-75^{th} percentile), unless otherwise specified. Respiratory failure during period of hospitalisation is defined as P/F ratio < 26.6 kPa. P-values refer to chi-square or Fisher's exact test (two-sided) for categorical data, or two-tailed *t*-test or Mann–Whitney *U* test for continuous data, comparing patients with and without respiratory failure during hospitalisation.

List of Supplementary materials

Supplementary figures

Supplementary Figure 1. Levels of LBP measured at the three-month follow-up in patients with or without persistent respiratory dysfunction.

LBP, lipopolysaccharide-binding protein

Supplementary Figure 2. Correlation heatmap of LBP levels with inflammation markers at corresponding time points.

LBP, lipopolysaccharide-binding protein

Supplementary Figure 3. Abundance distribution histograms of altered genera associated with respiratory dysfunction after three months.

Supplementary tables

Supplementary Table 1. Clinical characteristics at the three-month follow-up.

Supplementary Table 2. Associations of bacterial alpha diversity measures with clinical baseline characteristics.

Supplementary methods: Soluble LPS-binding protein (LBP) measurements, sequence processing, and bioinformatics (gut microbiota analyses)

Hospitalized COVID-19 patients
NOR-Solidarity study

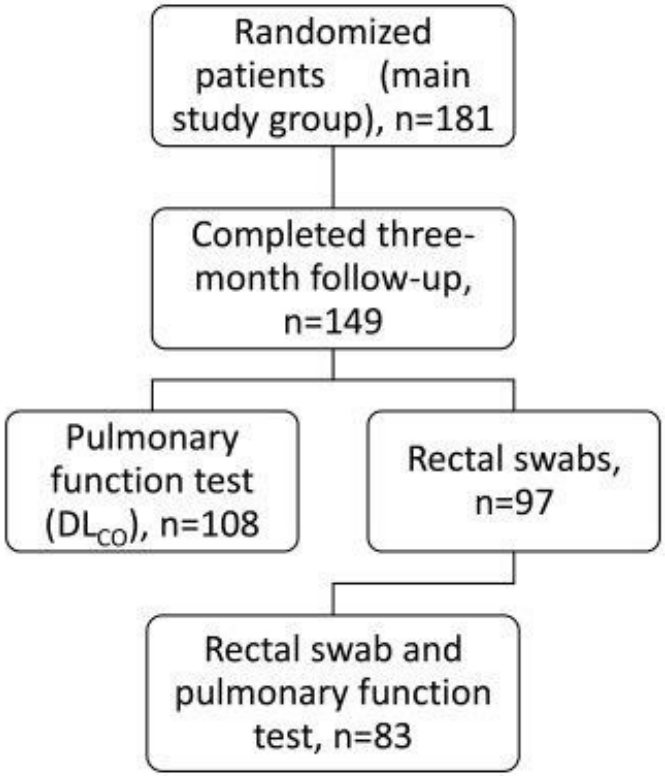
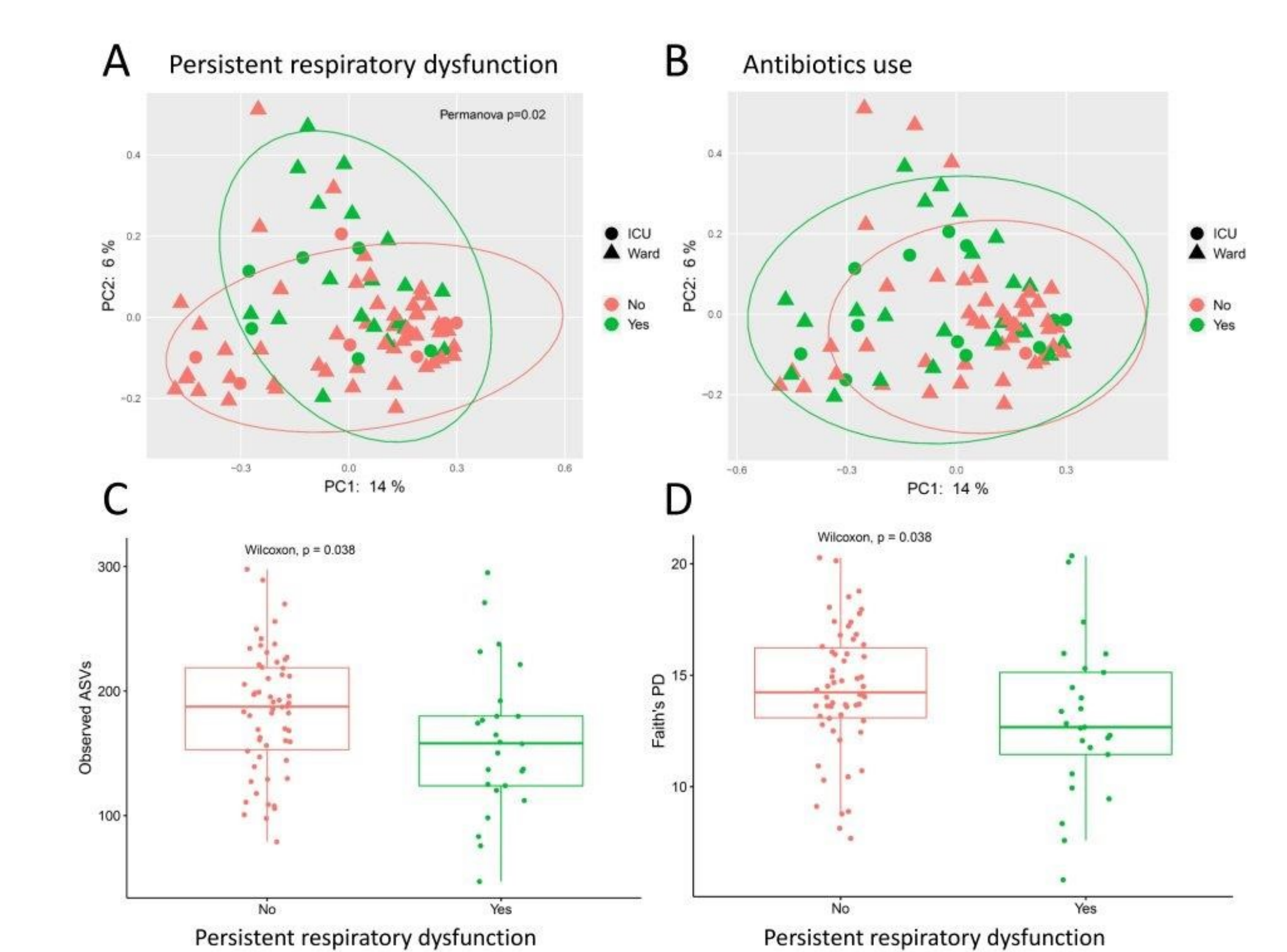


Table 1. Baseline characteristics

	Main study group (n=181)	Microbiota sub-group (n=97)	Respiratory failure* (n=60)	No respiratory failure (n=121)	*P*
Age, years	59 (50-71)	57 (48-65)	62 (56-75)	55 (47-65)	**<0.001**
Male gender (%)	119 (66)	60 (62)	41 (68)	78 (65)	0.623
Body Mass Index, kg/m2	27.4 (24.7-30.9)	27.7 (25.0-31.6)	28.1 (25.2-31.9)	27.1 (24.5-29.8)	0.512
Antibiotics use (%)	87 (48)	41 (42)	42 (70)	44 (36)	**<0.001**
Treatment group					0.444
Standard of Care, SoC (%)	87 (48)	47 (49)	25 (42)	62 (51)	
SoC + Hydroxychloroquine (%)	52 (29)	33 (34)	20 (33)	32 (26)	
SoC + Remdesivir (%)	42 (23)	17 (18)	15 (25)	27 (22)	
Comorbidities					
Any known comorbidities (%)	122 (68)	66 (68)	42 (70)	80 (66)	0.611
Chronic pulmonary disease (%)	10 (6)	5 (5)	4 (7)	6 (5)	0.731
Hypertension (%)	55 (31)	28 (29)	21 (35)	34 (28)	0.308
Chronic cardiac disease (%)	28 (16)	12 (12)	13 (22)	15 (12)	0.124
Diabetes mellitus (%)	31 (17)	19 (20)	15 (25)	16 (13)	0.057
Obesity, BMI > 30 kg/m2 (%)	44 (27)	28 (30)	18 (30)	26 (22)	0.264
Symptom duration prior to admission, days	7 (5-10)	7 (5-10)	7 (5-10)	7 (5-10)	0.814
Lenght of hospitalization, days	6 (4-11)	6 (3-11)	13 (10-25)	5 (3-8)	**<0.001**
Admission to ICU (%)	35 (19)	18 (19)	34 (19)	1 (1)	**<0.001**
Invasive mechanical ventilation (%)	19 (10)	11 (10)	19 (32)	0	**<0.001**
P/F-ratio at admission, kPa	42.1 (32.0-48.1)	42.4 (33.4-49.6)	30.1 (23.4-38.6)	45.2 (40.1-51.9)	**<0.001**
C-reactive protein, mg/L	70.0 (36.3-70.0)	66.0 (33.5-122.5)	113 (65.5-163.5)	57 (28.3-110-8)	**<0.001**
White blood cell count, x 10^9/L	6.2 (4.7-8.6)	5.8 (4.3-7.6)	8.1 (5.5-9.8)	5.7 (4.5-7.2)	**<0.001**
Neutrophil count, x10^9/L	4.3 (3.0-6.6)	3.7 (2.4-6.2)	6.4 (4.0-8.0)	3.7 (2.7-5.6)	**<0.001**
Ferritin, µg/L	626 (325-1209)	608 (298-1017)	1015 (534-1481)	516 (257-1037)	**<0.001**
D-dimer, mg/L	0.7 (0.5-1.2)	0.7 (0.4-1.1)	1.1 (0.5-1.7)	0.6 (0.4-0.9)	**<0.001**
Procalcitonin, µg/L	0.13 (0.10-0.21)	0.10 (0.10-0.20)	0.20 (0.12-0.51)	0.10 (0.10-0.17)	**<0.001**
Viral load ($log_{10/}$1000)	1.9 (0.7-3.1)	1.7 (0.0-2.9)	2.5 (1.0-3.2)	1.7 (0.6-3.1)	0.111
Lipopolysaccharide-binding protein, µg/mL	16.3 (8.5-26.1)	17.1 (7.3-26.3)	23.5 (12.1-32.3)	12.9 (7.2-21.6)	**<0.001**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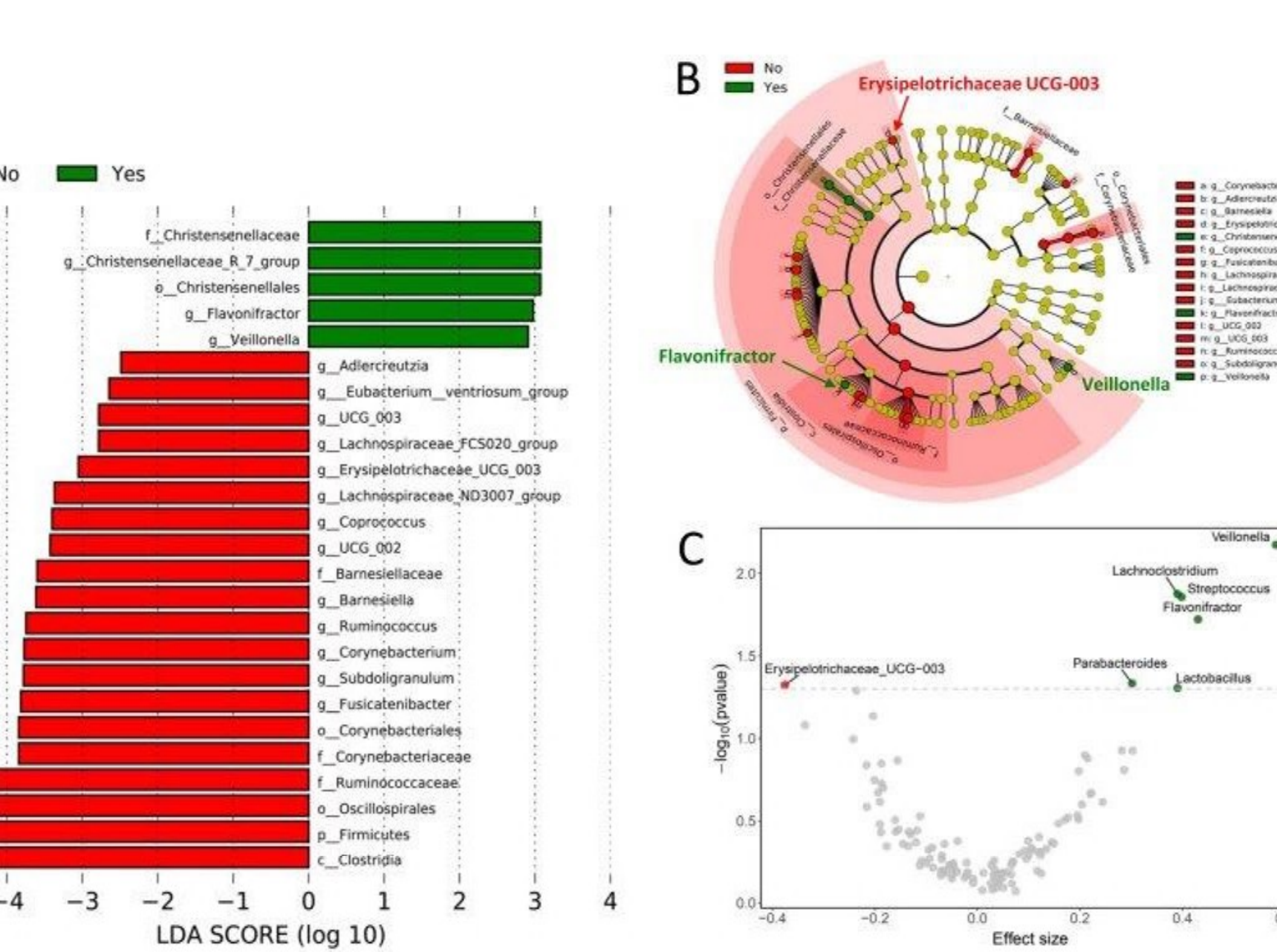
Persistent respiratory dysfunction
Permanova p=0.02
PC2: 6 %
PC1: 14 %
ICU
Ward
No
Yes
B
Antibiotics use
PC2: 6 %
PC1: 14 %
ICU
Ward
No
Yes
C
Wilcoxon, p = 0.038
Observed ASVs
No
Yes
Persistent respiratory dysfunction
D
Wilcoxon, p = 0.038
Faith's PD
No
Yes
Persistent respiratory dysfunction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Yes
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o__Christensenellales
g__Flavonifractor
g__Veillonella
g__Adlercreutzia
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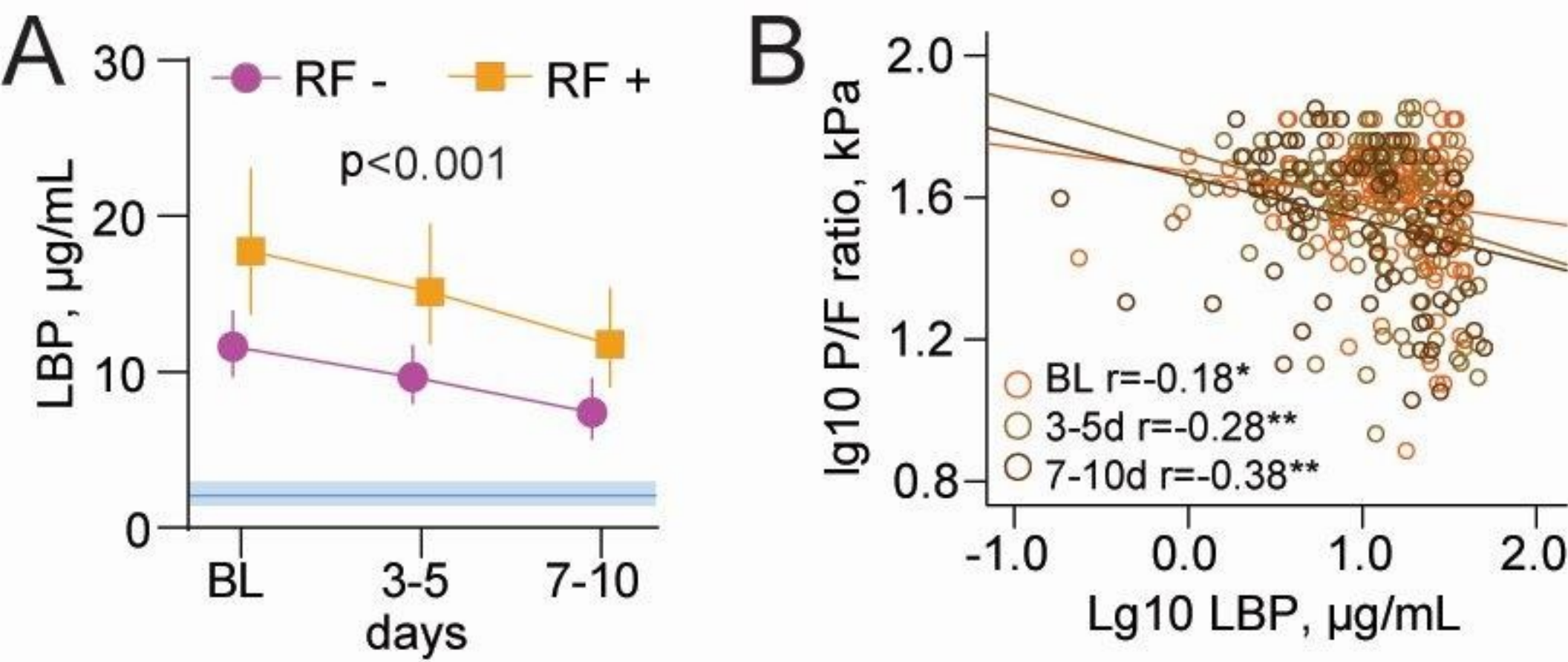
g__UCG_003
g__Lachnospiraceae_FCS020_group
g__Erysipelotrichaceae_UCG_003
g__Lachnospiraceae_ND3007_group
g__Coprococcus
g__UCG_002
f__Barnesiellaceae
g__Barnesiella
g__Ruminococcus
g__Corynebacterium
g__Subdoligranulum
g__Fusicatenibacter
o__Corynebacteriales
f__Corynebacteriaceae
f__Ruminococcaceae
o__Oscillospirales
p__Firmicutes
c__Clostridia
−4
−3
−2
−1
0
1
2
3
4
LDA SCORE (log 10)
B
No
Yes
Erysipelotrichaceae UCG-003
Flavonifractor
Veillonella
C
Veillonella
Lachnoclostridium
Streptococcus
Flavonifractor
Erysipelotrichaceae_UCG−003
Parabacteroides
Lactobacillus
2.0
1.5
1.0
0.5
0.0
−log10(pvalue)
−0.4
−0.2
0.0
0.2
0.4
Effect size


A
30
20
10
0
LBP, µg/mL
RF -
RF +
p<0.001
BL
3-5
7-10
days
B
2.0
1.6
1.2
0.8
lg10 P/F ratio, kPa
BL r=-0.18*
3-5d r=-0.28**
7-10d r=-0.38**
-1.0
0.0
1.0
2.0
Lg10 LBP, µg/mL