ALTERED GUT MICROBIOTA AND RESPIRATORY DYSFUNCTION THREE MONTHS AFTER SEVERE COVID-19

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BACKGROUND

Although COVID-19 is primarily a respiratory infection, mounting evidence suggests that the 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 associate with long-term respiratory dysfunction is unknown.

METHODS

From the NOR-Solidarity trial[1], plasma was collected during hospital admission and after three months, and analyzed for markers of gut barrier dysfunction and inflammation. At the three-month follow-up, pulmonary function was assessed by measuring diffusing capacity of the lungs for carbon monoxide (DL_{CO}), and rectal swabs for gut microbiota analyses were collected and analyzed by sequencing of the 16S rRNA gene (V3-V4 region, Illumina MiSeq)).

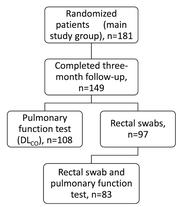


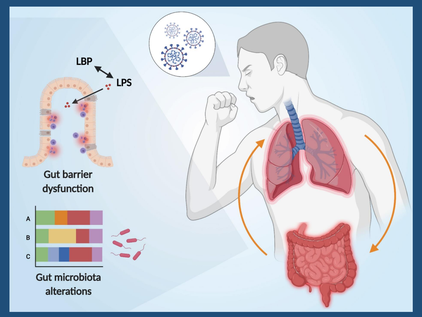
Figure 1. Flow-chart of patients included in the present add-on study based on the original NOR-Solidarity study protocol.

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**

Table 1. Baseline characteristics. Results are shown as median values (25th-75th percentile) unless otherwise specified. Respiratory failure during period of hospitalization, defined as P/F-ratio<26.6 kPa. P-values refer to chi-square or Fisher's exact test (2-sided) for categorical data, or 2-tailed t-test or Mann-Whitney U test for continuous data, comparing patients with and without respiratory failure during hospitalization.

Potential gut-lung axis in COVID-19



Respiratory dysfunction three months after COVID-19 is associated with reduced biodiversity and gut microbiota alterations, along with persistently elevated LBP levels.

SUMMARY

- Three months after hospitalization 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 (Figure 2).
- The microbiota alterations included reduced abundance of *Erysipelotrichaceae UCG-003* and increased abundance of *Veillonella* and *Flavonifractor* (Figure 3).
- During hospitalization, increased plasma levels of lipopolysaccharide-binding protein (LBP) were significantly associated with acute respiratory failure (Figure 4a).
- LBP levels remained elevated in patients compared with healthy controls after three months (Figure 4b), and were significantly associated with respiratory dysfunction and persistent low-grade inflammation at the three-month follow-up (Figure 4b and c).

RESULTS

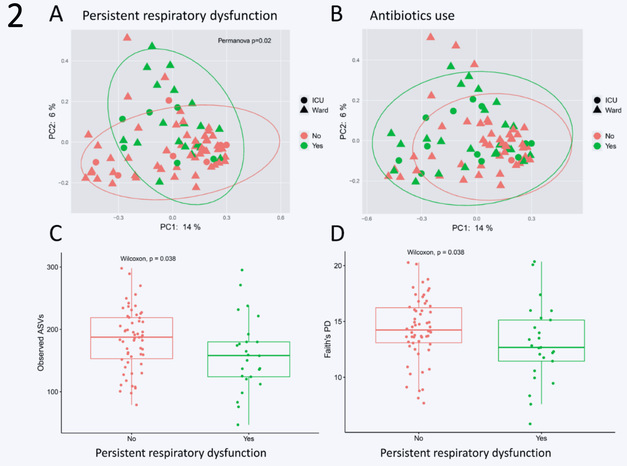


Figure 2. Gut microbiota diversity in patients with or without respiratory dysfunction at the three-month follow-up. Beta diversity by principal coordinate analysis showing Bray-Curtis distances separating patients with or without respiratory dysfunction (DLCO<LLN, n=83) and admittance to ICU or ward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

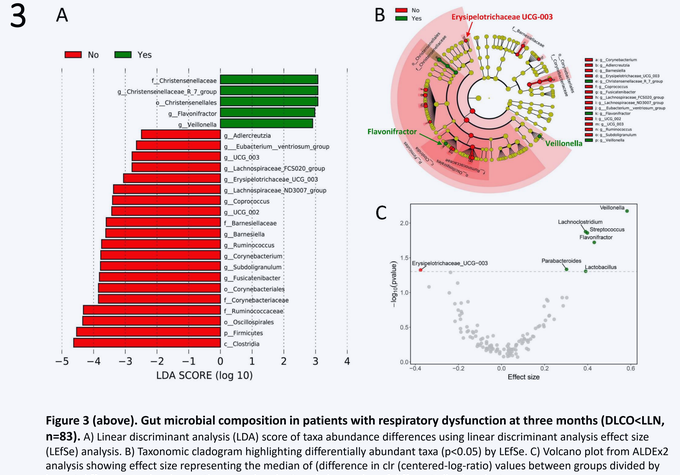


Figure 3 (above). Gut microbial composition in patients with respiratory dysfunction at three months (DLCO<LLN, n=83). A) Linear discriminant analysis (LDA) score of taxa abundance differences using linear discriminant analysis effect size (LEfSe) analysis. B) Taxonomic cladogram highlighting differentially abundant taxa (p<0.05) by LEfSe. C) Volcano plot from ALDEx2 analysis showing effect size representing the median of (difference in clr (centered-log-ratio) values between groups divided by largest difference in clr values within group) on log2-scale by p-value of differentially abundant genera.

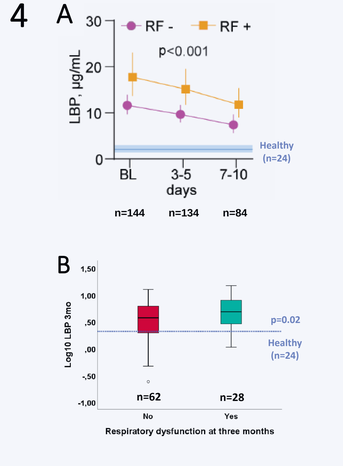


Figure 4 (above right). Soluble lipopolysaccharide-binding protein (LBP) levels in relation to acute respiratory failure and persistent respiratory dysfunction. A) Temporal profile of LBP in relation to acute respiratory failure (RF) (n=44) during hospitalization (P/F-ratio <26.6 kPa). P-value reflects the overall effect of RF from the repeated measures regression analysis. B) Numerically higher levels of LBP in patients with respiratory dysfunction (DLCO<LLN) compared to patients without (p=0.16). Linear regression analysis comparing patients to age- and sex-adjusted healthy controls (mean value). C) Correlation heatmap of LBP levels with inflammation markers at corresponding time points. Values are Spearman's ρ with *p<0.05, **p<0.01, ***p≤0.001.

CONCLUSIONS

- **Respiratory dysfunction after COVID-19 is associated with altered gut microbiota and persistently elevated LBP levels.**
- **Our results point to a potential gut-lung axis that should be further investigated in relation to long-term pulmonary dysfunction and long COVID.**

ADDITIONAL KEY INFORMATION

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