Associations between host microbiome and inflammation suggest role for host microbiome in driving COVID-19 Disease Severity

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Introduction

- Systemic inflammation and innate immune activation are associated with COVID-19 disease severity. Although alterations in gut microbiota are linked to systemic inflammation, the relationships between microbiome, inflammation and COVID-19 disease severity remain ill-defined

Objective

- To identify the interactions between host gut microbiome compositions and systemic immune responses and correlate these with COVID-19 disease severity

Methods

- Subjects with PCR confirmed COVID-19 were recruited to the All Ireland Infectious Diseases Cohort Study.
- Gut microbial diversity was explored by 16SrDNA analysis of stool, taxonomic repertoires derived and α-diversity (Shannon indices) and beta diversity (Envfit-based analysis of principal coordinates) measured.
- We measured 36 biomarkers using bead-based quantitative ELISA on the Luminex Magpix platform (Table 1)
- Principal component analysis (PCA) was performed followed by unsupervised hierarchical clustering (HC) to partition subjects into biomarker-derived clusters. Associations of microbial diversity and inflammatory biomarkers on clinical outcomes was explored using logistic regression and weighted gene correlation network analysis (WGCNA). Analysis was performed using R vers.4.2

Table 1: Custom Multiplex Biomarker panel*

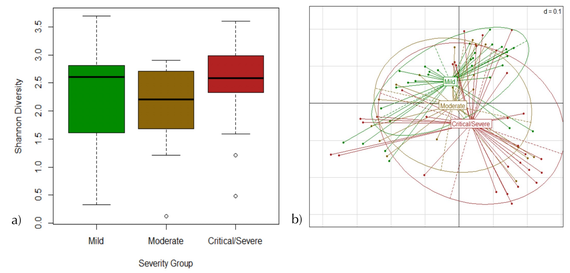
Systemic Inflammation	IL-2	Coagulation	D-dimer
	IL-6		Thrombopoietin
	IL-8		P-selectin
	TNF-alpha		CD40 Ligand
	IL- 1 beta	Innate Immune activation	MCP-1
	IL-17A		MIP-1 alpha
	CXCL10		IL-18
	S100B		IL-5
Immune regulation	IL-4		GM-CSF
	IL-10		CD163
	IL-13	Antiviral activity	IFN gamma
	PDL-1		IFN beta
	IL-1RA		IFN 2a
	IL-12p70		IFN lambda 1
Endothelial Function	E- selectin		IFN lambda 2
	ICAM-1		IFN lambda 3
	TGF- alpha	Angiogenesis	VEGF
			EGF
			PDGF-AA

*MCP, Monocyte Chemoattractant Protein ; MIP-1, Monocyte Inflammatory Protein-1; MPO, myeloperoxidase, PD-L1, Programmed Death Ligand-1; IL, Interleukin; IFN, Interferon; TNF, Tumor Necrosis Factor; VEGF, Vascular Endothelial Growth Factor; EGF, Epidermal Growth Factor; PDGF, Platelet-derived growth factor; ICAM, Intracellular Adhesion Molecule

Results

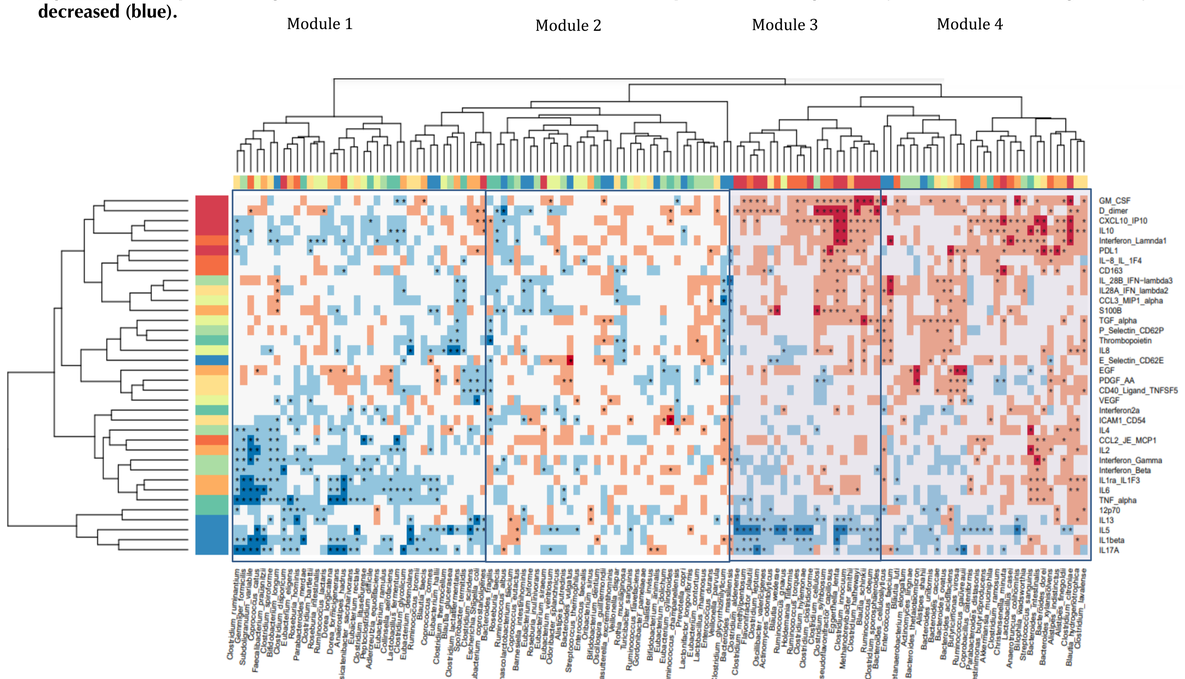
- Of 79 subjects recruited between April 2020 and July 2021, median (IQR) age was 64 (51,77) years, 58.8% were male; 88% Caucasian and 36% experienced mild, 22% moderate and 40% severe/critical disease respectively.
- On microbiome analysis, only beta diversity differed significantly between disease severity groups (figure 1) and 14 species significantly differentiated (p< 0.05) in severe/critical disease.
- Cluster analysis revealed three distinct biomarker-derived clusters that grouped participants according to clinical outcomes (table 2).
- These distinct inflammatory clusters were then correlated with microbiome profiles were identified using WGNA. These associations were grouped into 4 modules (Figure 2)

Figure 1: Measures of a) alpha-diversity and b) beta-diversity in COVID-19 disease



- Module 1 composed of several butyrate-producing and anti-inflammatory bacteria, together with lower levels of circulating cytokines, IL-4, IL-6, IL-2, GM- CSF and antiviral IFN-λ and IFN-β levels (figure 2) . This module correlated to biomarker cluster 1 (table 2), composed of a younger cohort, with predominantly mild/ moderate clinical outcomes.

Figure 2: Heatmap illustrating the serum immune mediators and microbiome species that are significantly increase (red) and significantly decreased (blue).



Results continued

Table 2: Clinical Characterisation by clusters

Cluster	Cluster 1, N = 36[1]	Cluster 2, N= 16[1]	Cluster 3 N = 20[1]	p-value[2]
Sex				0.15
Female	16 (44%)	9 (56%)	5 (25%)	
Male	20 (56%)	7(44%)	15 (75%)	
Age	56 (44, 69)	60 (51, 65)	77 (68, 80)	<0.001
BMI	30 (26, 32)	27 (24, 32)	27 (22, 30)	0.3
Ethnicity				0.6
Asian	2 (5.6%)	1 (6.2%)	0	
Caucasian	32 (89%)	13 (81%)	17 (85%)	
Other	2 (5.6%)	2 (12%)	3 (15%)	
History of comorbidities	29 (81%)	13 (81%)	16 (80%)	>0.9
Hypertension	11 (31%)	7 (44%)	7 (35%)	0.7
Diabetes	3 (8.3%)	2 (12%)	6 (30%)	0.11
Respiratory disease	11 (31%)	4 (25%)	2 (10%)	0.2
Heart disease	5 (14%)	5 (31%)	5 (25%)	0.3
Renal disease	1 (2.8%)	1 (6.2%)	0 (0%)	0.5
Liver disease	0 (0%)	1 (6.2%)	2 (10%)	0.12
Obesity	12 (33%)	5 (31%)	6 (30%)	>0.9
Malignancy	2 (5.6%)	3 (19%)	6 (30%)	0.04
Immunosuppression	1 (2.8%)	1 (6.2%)	1 (5%)	0.8
Neurological disease	4 (11%)	0 (0%)	1 (5%)	0.5
Other comorbidities	16 (44%)	9 (50%)	9 (45%)	0.7
Antibiotic use	17 (53%)	12 (60%)	29 (81%)	0.048
Antiviral use	3 (9.4%)	7 (35%)	16 (44%)	0.006
Immunosuppressives	3 (9.4%)	10 (50%)	25(69%)	<0.001
WHO Severity				>0.9
Mild	12 (33%)	4 (25%0	6 (30%)	
Moderate	10 (38%0	4 (25%)	4 (20%	
Severe/ Critical	14 (39%)	8 (50%)	10 (50%)	
Died	0 (0%)	1 (6.2%)	3 (15%)	0.007

[1] n(%); Mean (SD), [2] Pearson's Chi-squared test; Kruskal-Wallis rank sum test; Fischer's exact test. Immunosuppressives – Dexamethasone; Methylprednisolone; Prednisolone.

- Modules 3 and 4 represent an inflamed cohort, with upregulation of several pathogenic bacteria such as *Clostridium hathewayi*, *Enterococcus faecium, Coprobacillus, Eggerthella and Actinomyces spp.*
- These modules also showed an increased expression of IL-6, TNF-α, PDL-1, S100B, GM-CSF, D-dimer, E-selectin, MCP-1 and IL-8 as well as activated antiviral IFNλ 2 and IFNλ3.
- These modules correlate to our biomarker cluster 3, an older cohort with higher severe/critical disease outcomes. This suggest a dysregulated inflammatory response, correlating this "cytokine storm" with an inflamed microbiome environment.

Conclusions

- We have identified distinct gut and circulating biomarker inflammatory patterns in COVID-19 disease that are associated with clinical outcomes.
- This study provides further insights into links between host microbiome, inflammatory responses to SARS-CoV-2 infection and clinical COVID-19 disease severity, suggesting a role for the microbiome in shaping distinct host inflammatory responses to infection.