Early Inflammatory Profiles Predict Maximal Disease Severity in COVID-19

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Background

- A proportion of individuals infected with SARS-CoV-2 will progress to severe disease requiring supplemental oxygen or intensive care
- Although risk factors for progression to severe disease are well defined, at an individual level predicting which patients will deteriorate remains challenging[1]
- Efficacy of available antiviral and immunomodulatory treatments for COVID-19 are dependent on appropriate timing within the disease course, and we have no biomarkers to guide optimal timing
- Improved understanding of the host inflammatory changes that precede the development of severe COVID-19 could help optimise delivery of available treatments and provide insights for the development of new therapies

Methods

- **Design:** We selected participants for the All Ireland Infectious Diseases Cohort study, a prospective, multicentre cohort study that recruits individuals with infectious diseases related issues attending participating hospitals in Ireland.
- **Population:** Individuals with PCR confirmed COVID-19 ≤10 days from symptom onset, including individuals in outpatient and inpatient settings
- **Laboratory analysis:** In cryopreserved plasma we measured 61 biomarkers as outlined below

Category	Markers analysed
Innate immune activation	hsCRP, IL-6, IL17A, IL-15, procalcitonin, sCD163, sCD14, MCP1, MIP1-alpha, IL-18, IP10(CXCL10), IL8, GM CSF, MDC, TNF R1, GDF15
T cell activation	CD40L
Th1 cytokines	IL1 beta, IL-2, IL-12p70, TNF alpha
Th2 cytokines	IL4, IL5, IL13, IL-33, TSLP
Interferons	IFN-α2, IFN-β, IFN-γ, IFN-λ1 (IL29), IFN-λ2 (IL28A), IFN-λ3 (IL-28B)
Endothelial Activation	ICAM-1, sVCAM-1, E-selectin, P selectin
Tissue repair	VEGF, EGF, PDGF-AA, PDGF-AB, TGF (alpha)
Coagulation	vWF, D-dimer, TPO, serpin E1/PAI1
Microbial translocation	Zonulin, beta D glucan, LBP
Immune regulation	IL1RA, PD-L1, IL-10, TNF R2
Obesity	Adiponectin, leptin, FABP4, resistin
Axonal injury	S100B
Alveolar epithelial injury	ST2, SPD, RAGE

- **Statistical analysis:**
- Data are presented as median (interquartile range) or number (%)
- We used principal component analysis (PCA) and unsupervised hierarchical clustering with k-means consolidation to derive biomarker derived clusters
- We used univariate and multivariate ordinal logistic regression to explore the relationship between cluster membership and maximal disease severity, adjusting for factors known to modify risk for COVID-19 including age, sex, ethnicity, BMI, hypertension, diabetes, immunosuppression, smoking and baseline anticoagulant use

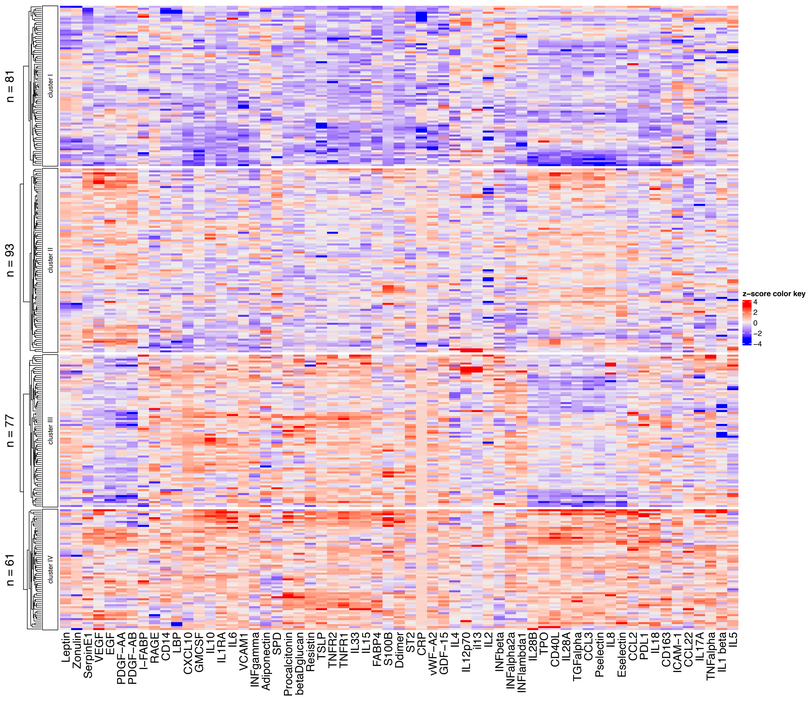
Results

- Between March 2020 and April 2021, we included 312 individuals for analysis
- PCA and cluster analysis demonstrated 4 distinct clusters. Demographics of the cohort and the 4 clusters are shown in Table 1

Table 1. Participant Demographics

	Whole cohort n = 312	Cluster 1 Less inflamed n = 81	Cluster 2 Tissue repair n = 93	Cluster 3 Tissue injury n = 77	Cluster 4 Systemic inflammation n = 61	P value
Age	62 (48-77)	37 (29-56)	59 (52-71)	73 (60-83)	72 (62-81)	<0.001
Male sex	170 (54%)	35 (43%)	55 (59%)	48 (62%)	32 (52%)	0.073
Non Caucasian ethnicity	63 (20%)	11 (14%)	19 (21%)	14 (18%)	14 (23%)	0.5
BMI	27.56 (24-32)	25 (23-28)	30 (25-32)	27 (24-30)	30 (26-33)	0.001
Ever smoked	99 (32%)	15 (19%)	30 (32%)	32 (42%)	22 (36%)	0.015
Hypertension	133 (42%)	11 (14%)	44 (48%)	44 (57%)	34 (56%)	<0.001
Diabetes	46 (15%)	4 (4.9%)	12 (13%)	13 (17%)	17 (28%)	0.002
Immunosuppressed	13 (4%)	1 (1.2%)	0 (0%)	8 (10%)	4 (6.6%)	0.0009
Baseline anticoagulation or anti-platelets	64 (20%)	6 (7.4%)	17 (18%)	22 (29%)	19 (31%)	0.001
Disease severity						
Mild	154 (49%)	64 (79%)	40 (43%)	23 (30%)	27 (44%)	<0.001
Moderate	66 (21%)	10 (12%)	28 (30%)	18 (23%)	10 (16%)	
Severe	92 (29%)	7 (8.6%)	25 (27%)	36 (47%)	24 (39%)	
Days from symptom onset	7 (4-9)	6 (4-8)	8 (5-9)	6 (4-8)	6 (3-8)	0.003
Death	36 (11%)	3 (4%)	3 (3%)	19 (25%)	11 (18%)	<0.001

➢ **Heatmap demonstrating the differences in biomarkers between clusters**

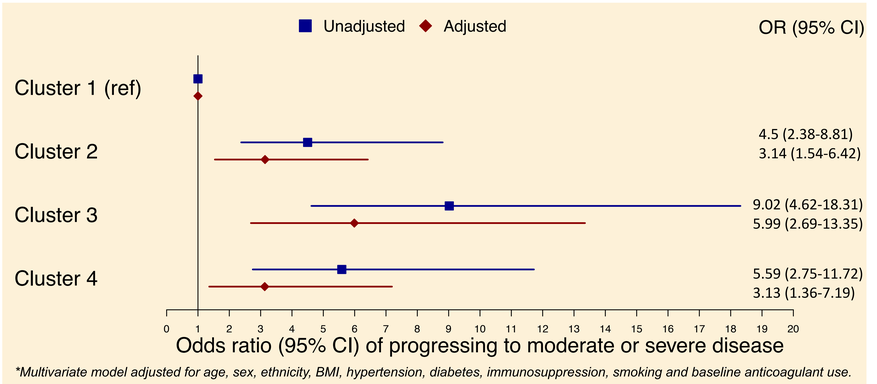


Results

- **Cluster 1 – Less inflamed.** This cluster was characterized by low levels of inflammation
- **Cluster 2 – Tissue repair.** Compared to cluster 1, this cluster had upregulation of growth factors (EGF, VEGF, PDGF, TGFα) and endothelial activation (e selectin, p selectin)
- **Cluster 3 – Tissue injury.** This cluster had the highest levels of markers of early alveolar epithelial injury (RAGE, ST2). Levels of innate immune activation (IL-6, procalcitonin, CRP, TNFα) and coagulation (d-dimer, TPO) were higher than clusters 1 and 2, but lower than cluster 4, and compared to cluster 4 this cluster had relative downregulation of growth factors, markers of endothelial activation and immune regulation (IL10, PDL1)
- **Cluster 4 – Systemic inflammation.** This cluster had the highest levels of markers of innate immune activation, coagulation and microbial translocation (I-FABP, Beta-D-glucan

➢ **Association between cluster membership and increasing maximal disease severity**

- In univariate analysis, compared to cluster 1, while all clusters had a higher odds of increased disease severity, cluster 3 (tissue injury) had the strongest association with more severe disease
- In multivariate analysis, adjusting for demographic variables associated with increased risk of severe COVID-19, membership in cluster 3 remained most strongly associated with more severe disease



**Multivariate model adjusted for age, sex, ethnicity, BMI, hypertension, diabetes, immunosuppression, smoking and baseline anticoagulant use.*

Conclusions

- **Distinct early inflammatory profiles predicted maximal disease severity independent of known risk factors for severe COVID-19**
- **A cluster with relative downregulation of growth factor and endothelial markers, and early evidence of alveolar epithelial injury, was associated with highest risk of disease progression**
- **Whether this inflammatory pattern reflects a dysregulated inflammatory response to SARS-CoV-2 that could improve targeted treatment requires further study**

References & Acknowledgments

1. Williamson EJ, Walker AJ, Bhaskaran K, et al. Factors associated with COVID-19-related death using OpenSAFELY. Nature. 2020 Aug;584(7821):430-436.

This study was funded by SFI grant number 20/COV/8549 and an unrestricted grant from Smurfit Kappa. GK was funded through a fellowship from the United States embassy in Ireland during the study. The authors gratefully acknowledge the investigators, study staff and all participants.