CYTOKINE PROFILE IN DIFFERENT POST-COVID-19 CONDITION PHENOTYPES

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BACKGROUND

- At least 10% of SARS-CoV-2 infected people suffer from persistent symptoms for >12 weeks, condition known as **post-COVID-19 condition (PCC)** or Long Covid.
- Reported symptomatology is diverse with >200 physical and neurological debilitating symptoms.
- The underlying pathophysiology of PCC is poorly understood.

OBJECTIVE

To analyze **pro-inflammatory cytokine levels as a potential mechanism underlying persistent symptomatology**.

METHODS

STUDY DESIGN AND PARTICIPANTS

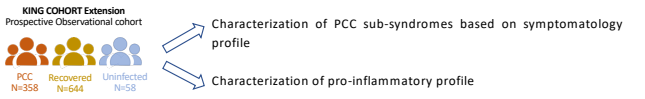


Table 1: PCC characteristics of the KING Cohort Extension

KING COHORT EXTENSION, PCC	N = 358
Sex (Female), n (%)	247 (69)
Age, median [IQR]	48 [40 - 56]
Hospitalization, n (%)	134 (37.4)
Severity – SO_2 < 94%, n (%)	86 (24)
RICU, n (%)	23 (6.4)
ICU, n (%)	16 (4.5)
N comorbidities, median [IQR]	2 [1 - 3]
N symptoms, median [IQR]	10 [6 -15]
Time from acute infection, months, median [IQR]	18.3 [12.2, 18.8]*

*Follow up from March 2020 – October 2021

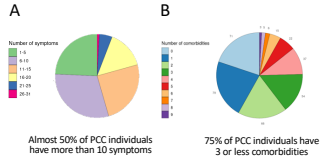


Figure 1. PCC characteristics of the KING Cohort Extension. (A) Number of symptoms and (B) Number of comorbidities.

CHARACTERIZATION OF PCC SUB-SYNDROMES

- Symptomatology-based hierarchical clustering of PCC individuals:
  -Gower distances were used to calculate similarity in symptomatology between PCC individuals.
  -Hierarchical clustering (Ward's minimum variance method) was used to identify different symptomatological profiles among PCC participants.

CHARACTERIZATION OF PRO-INFLAMMATORY PROFILE

- Cytokine profiling: Cytokine levels of randomly selected PCC, recovered and uninfected subjects (N=193, Table 2) were measured on EDTA plasma samples collected at least >6 months after acute infection, using the *30-Plex Panel for Luminex* (Life Technologies, CSF, EGF, GM-CSF, FGF-basic, IFN-α, MCP-1, HGF, IFN-γ, MIG, VEGF, IL-1β, MIP-1α, IL-1RA, MIP-1β, IL-2, RANTES, IL-2R, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12 (p40/p70), IL-13, IL-15, IL-17, TNF-α and Eotaxin) .

- Statistical analysis: Mann-Whitney t-test was used to compare PCC vs recovered groups and Kruskal-Wallis t-test for >2 groups comparisons (PCC vs Recovered vs Uninfected and within PCC clusters). FDR correction was applied for statistical significance (p-adj).

✓Some cytokines remained altered in all SARS-CoV-2 infected people *independently of persistent symptoms* after 6 months from acute infection.

✓ PCC cytokine profiles showed differences between clusters, suggesting a *distinct etiology of the different PCC subsyndromes*.

RESULTS

PCC SUB-SYNDROMES

Hierarchical clustering identified **5 different clusters** of individuals according to their symptomatology .

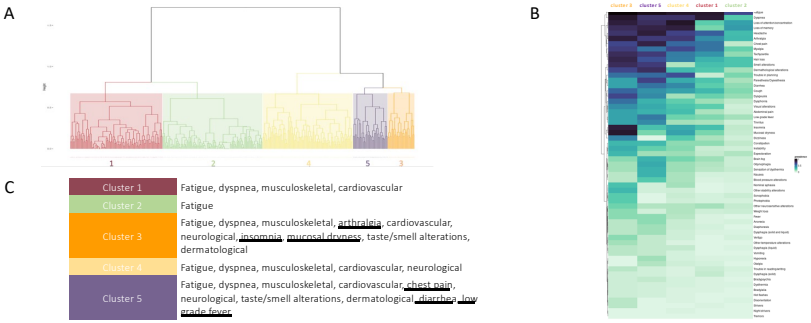


Figure 2. PCC participants from KING Cohort Extension (N=358) were sub-grouped according to their symptomatology identifying 5 different clusters. (A) Dendrogram (B) Heatmap showing symptoms prevalence within PCC clusters (C) Table showing the main symptoms of each PCC cluster.

PRO-INFLAMMATORY PROFILE

Randomly unvaccinated individuals, 91 PCC (belonging to each cluster), 57 recovered and 45 uninfected, were selected for cytokine profiling (Table 2).

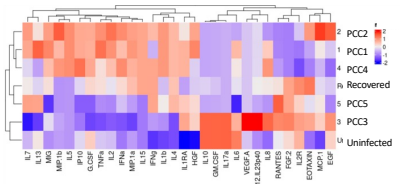


Table2: Characteristics of the participants of this study, randomly selected from de KING Cohort Extension

N=193	PCC	PCC1	PCC2	PCC3	PCC4	PCC5	Recovered	Uninfected
N (%)	91 (47)	16 (18)	23 (25)	12 (13)	21 (23)	19 (21)	57 (30)	45 (23)
Female, n (%)	69 (76)	10 (63)	15 (65)	10 (83)	16 (76)	18 (95)	32 (56)	30 (67)
Age, years, median [IQR]	50 [40-56]	50 [41-61]	51 [43-62]	50 [41-62]	52 [43-62]	49 [40-62]	49 [40-59]	50 [43-59]
Hospitalization, n(%)	21 (23)	3 (19)	4 (17)	1 (8)	8 (38)	5 (26)	31 (54)	-
Severe COVID-19, n (%)	14 (15)	3 (19)	4 (17)	0 (0)	6 (29)	1 (5)	16 (28)	-
Months after symptoms initiation, median [IQR]	8.1 [7-9]	7.8 [6-8]	7.4 [7-8]	11.1 [7-12]	8.2 [8-9]	8.8 [8-12]	7.5 [7-8]	-
Number of persistent symptoms, median [IQR]	7 [4-9]	3.5 [3-6]	3 [2-4]	12 [10-14]	8 [6-9]	8 [7-10]	-	-

Figure 3. Heatmap of cytokine concentration values (log 10 concentration, scaled using the mean and standard deviation of each analyte) with dendrogram showing hierarchical clustering within PCC clusters, recovered and uninfected groups.

CONCLUSIONS

- We have defined 5 subsyndromes based on patient symptomatic profile, going from a typical post-viral syndrome to severe illness with more than 20 simultaneous symptoms.
- Some cytokines remained altered in all SARS-CoV-2 infected participants independently of persistent symptoms after 6 months from acute infection.
- Differences between PCC and recovered individuals are limited after correction.
- Importantly, PCC cytokine profiles showed differences between clusters, which suggests different PCC subsyndromes with distinct etiology.

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Cytokine profiling PCC vs Recovered vs Uninfected: 14 cytokines were differentially expressed between the 3 studied groups.

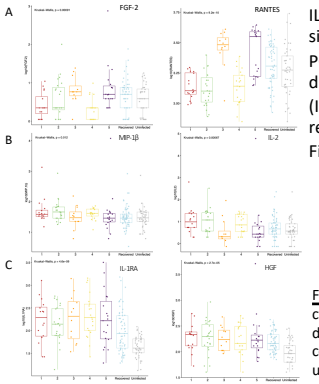
Infected vs Uninfected

Infected PCC vs Recovered

PCC vs Recov vs Uninfected

Cytokine	PCC-Recovered (p-adj)	PCC-Uninfected (p-adj)	Recovered-Uninfected (p-adj)
MCP-1	0.5203	0.0021	0.0211
IL-5	0.2324	0.0011	0.0499
HGF	0.218	1.54E-07	1.68E-04
MIG	0.9179	0.0132	0.0238
IL-1β	0.4349	0.0064	0.0642
IL-4	0.7100	0.0029	0.0140
RANTES	0.0446	0.4628	0.3324
MIP-1β	0.0331	0.0507	0.9127
IL-2R	0.0308	0.2617	0.5021
EOTAXIN	0.0566	0.1647	0.0053
IL-7	0.3941	0.0615	0.0210
IFN-α	0.2467	0.0105	0.1825
IL-8	0.6490	0.0199	0.0724
IL1RA	2.43E-03	9.24E-12	2.83E-04

Cytokine profiling within PCC clusters vs Recovered vs Uninfected: Within PCC clusters, FGF-basic and RANTES were elevated while IL-2 and MIP-1β were decreased in PCC3 and PCC5 compared to the other PCC clusters (Figure 4A and 4B, respectively).



IL-1 RA and HGF were similarly expressed within PCC clusters but significantly different with uninfected (IL-1RA and HGF) and recovered group (IL-1RA, Figure 4C).

Figure 4. Proinflammatory cytokines levels significantly different among PCC clusters compared with recovered and uninfected group.