



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Identification of biomarkers for post-COVID19 condition in paired pre- and post- infection samples from nationwide population cohorts

2026 Amsterdam ISLC-PAIS Conference
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Presentation overview



Project goals
Cohorts
Samples
Methods



Pilot study
results



Scaling up the
study: sample
selection



First observations
Challenges



Post COVID Biomarkers

- > The etiology of Post COVID remains unclear, which can cause underdiagnosis of cases. **Biomarkers are needed for the diagnosis and clinical management.**
- > **Main goal of our study: Identification of biomarkers for Post-COVID condition (PCC)**
 - Understanding the pathological mechanisms
 - Improving the diagnosis and treatments of PCC

Candidate biomarkers for Pulmonary/Respiratory LC

Immune cells
MDSCs, Memory-like NK, $\gamma\delta$ T CD27⁺
CD4⁺ GZMB⁺ perforin⁺
CD8⁺ GZMB⁺ perforin⁺
NK GZMB⁺ perforin⁺
 $\gamma\delta$ T GZMB⁺ perforin⁺
NK NKP30⁺
CD4⁺ perforin⁺
NK CD57⁺
CD8⁺/ $\gamma\delta$ T CD57⁺
NK/NKT/ $\gamma\delta$ T NKP30⁺
NK NKP46⁺
CD8⁺ CD27⁺ CD62L⁺
CD8⁺ CD27⁺ CD62L⁺
CD4⁺/CD8⁺/ $\gamma\delta$ KLRG-1⁺
CD4⁺/CD8⁺/NKT/ $\gamma\delta$ TIM-3⁺
NKT/ $\gamma\delta$ T NKB1⁺
CD4⁺ IL-17A⁺
CD4⁺/CD8⁺ IFN- γ ⁺
CD4⁺/CD8⁺ IL-2⁺
SARS-CoV-2 CD4⁺/CD8⁺ IFN- γ ⁺
SARS-CoV-2 CD4⁺/CD8⁺ TNF- α ⁺
SARS-CoV-2-S CD4⁺/CD8⁺ IFN- γ ⁺
SARS-CoV-2-S CD4⁺/CD8⁺ TNF- α ⁺
SARS-CoV-2-N CD4⁺/CD8⁺ TNF- α ⁺
SARS-CoV-2-M CD8⁺ TNF- α ⁺
SARS-CoV-2-N CD4⁺ TNF- α ⁺
SARS-CoV-2-N CD4⁺ IL-2⁺
SARS-CoV-2-S TNF- α ⁺ IFN- γ ⁺ IL-2⁺
SARS-CoV-2 CD8⁺ NKG7⁺ PRF1⁺ GNLY⁺
CST7⁺

Immunoglobulins
autoAb U1-snRNP
autoAb IFN- α 2
autoAb IFN- α 3
Cytokines: IL-6
APP: CRP
Blood cell counts:
WBC
Lymphocyte count
Hormones:
cortisol, cortisone
Metabolites
S-sulfocysteine
Pathogen molecules
EBV viremia
Others: Hemoglobin

Candidate biomarkers for Neurologic LC



Neural exosomes & proteins

GFAP, NDEV SNPH, NDEV NADH-ubiquinone oxidoreductase (CI-6), NDEV Cytochrome b-c1 oxidase (CIII-10), NDEV-MOTS-c, NDEV Humanin, NDEV VDAC1, NDEV NMDAR1, NDEV MCU, NDEV NCLX, NDEV LETM1

Metabolites

Kynurenine to Tryptophan ratio
S-sulfocysteine
Cytokines & Chemokines
IL-6, TNF- α , MCP-1
Immunoglobulins
SARS-CoV-2 N IgG
Pathogen molecules
EBV viremia
SARS-CoV-2 RNAemia (nasal swab)

Candidate biomarkers for GI LC

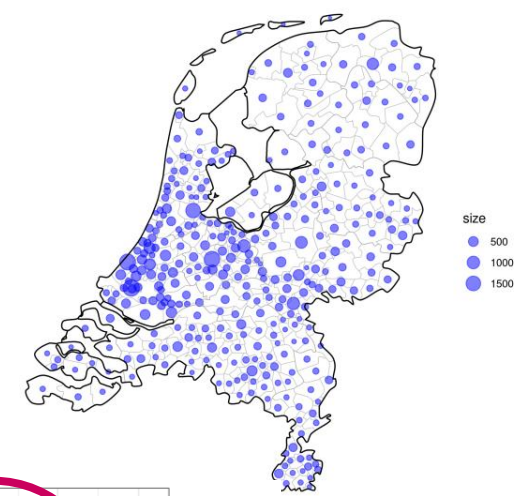
Immune cells

Cytotoxic TCR clonotypes CD8⁺
Cytotoxic TCR clonotypes CD4⁺
SARS-CoV-2 CD8⁺ NKG7⁺ PRF1⁺
GNLY⁺ CST7⁺
CMV CD8⁺ CTL
Immunoglobulins
autoAb La/SS-B
Pathogen molecule
Beta-glucan

Candidate Biomarkers for Fatigue LC

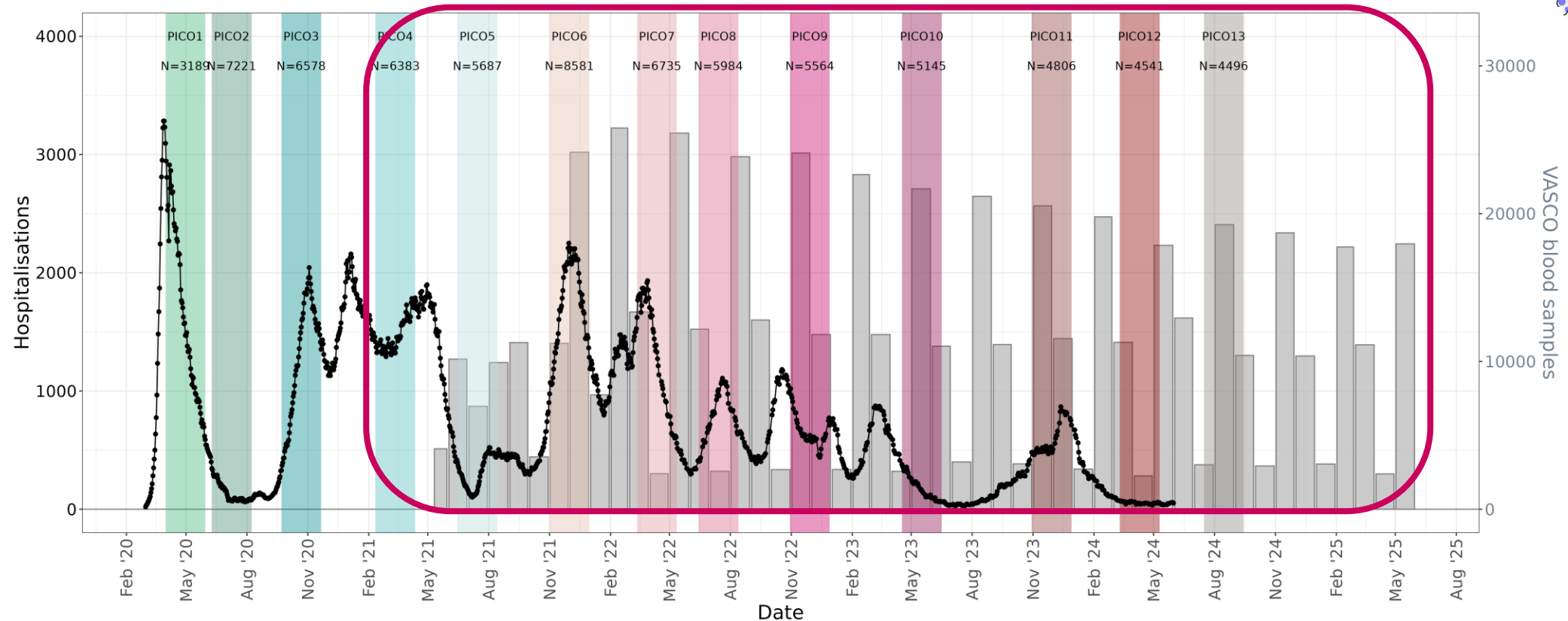
EBV viremia
endothelin-1 (ET-1), angiotensin-2 (Ang-2), Endothelial dysfunction (ED)





PICO and VASCO nationwide prospective cohorts

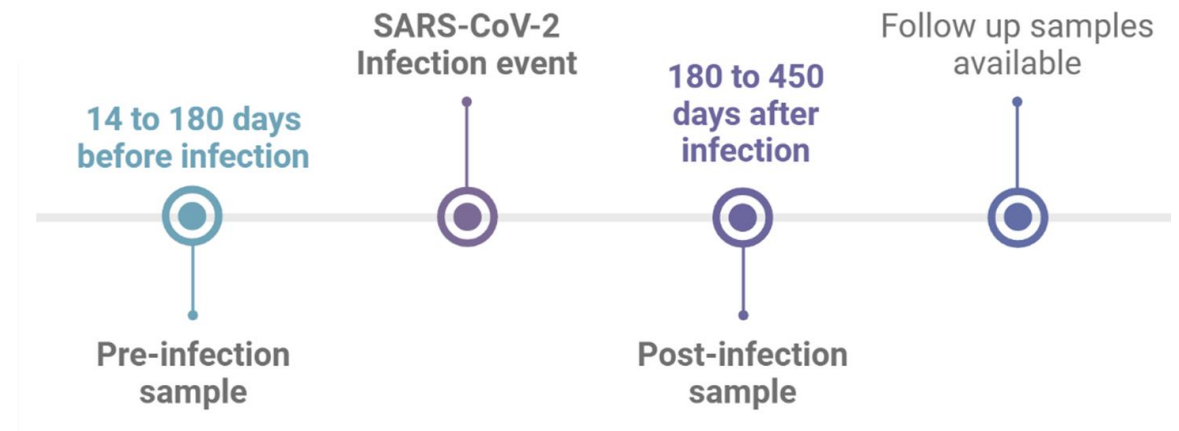
February 2021 – May 2025



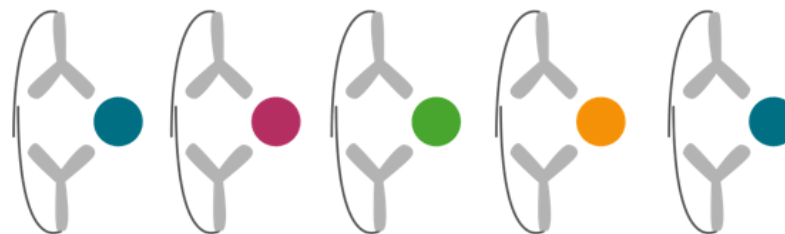


Samples and questionnaires

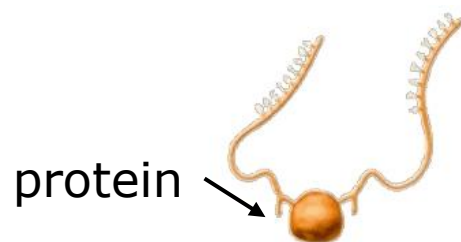
- > Sample set including paired samples collected **before** and **after** SARS-CoV-2 infection
- > Large number (~ 40 000) of cohort studies' participants
- > Fingerprick samples + questionnaires
 - **All participants:** Data on self-reported fatigue: Checklist Individual Strength (CIS)-Fatigue
 - **Subset of participants:** Self reporting of Post COVID, reporting other symptoms



Olink



Olink's technology: unique DNA oligo sequences report only matched DNA-pairs (e.g. 1A+1B). Cross-reactive events are not detected.



IMMUNO REACTION



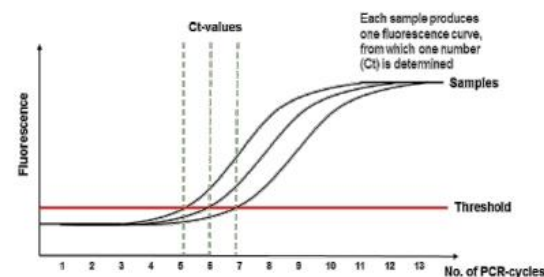
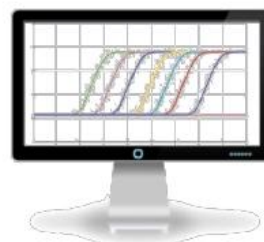
EXTENSION REACTION



PRE-AMPLIFICATION

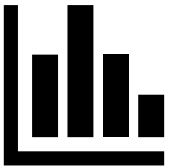


DETECTION



QC and GENERATION OF NPX

Concentration of proteins (biomarkers)



Pilot results

Pilot was performed to check:

- > Can we successfully measure proteins in a fingerprick?
- > Will we see differences between cases and controls?

Selection of:

- > **28** participants who developed severe fatigue after SARS-CoV-2 infection (**PCC**)
- > **12** sex and age matched convalescent controls (people who experienced infection but did not develop fatigue symptoms) (**CC**)
- > Pre-infection and post-infection timepoints of all selected participants

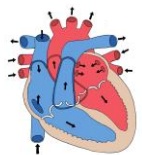
3 Olink panels, 276 assays in total:



92

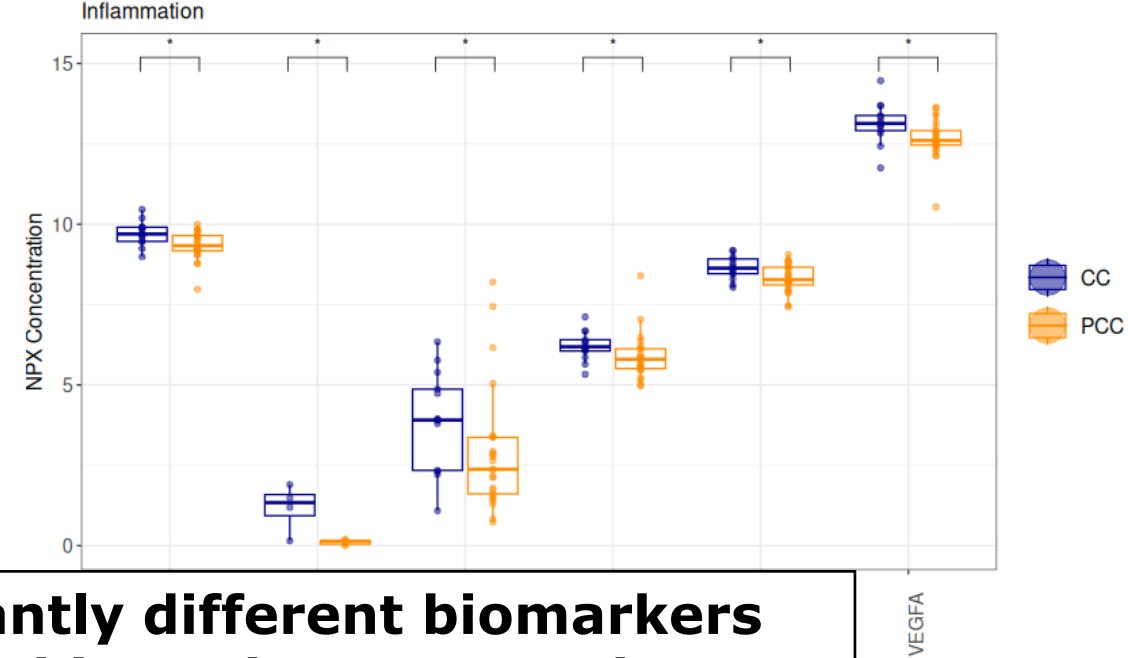
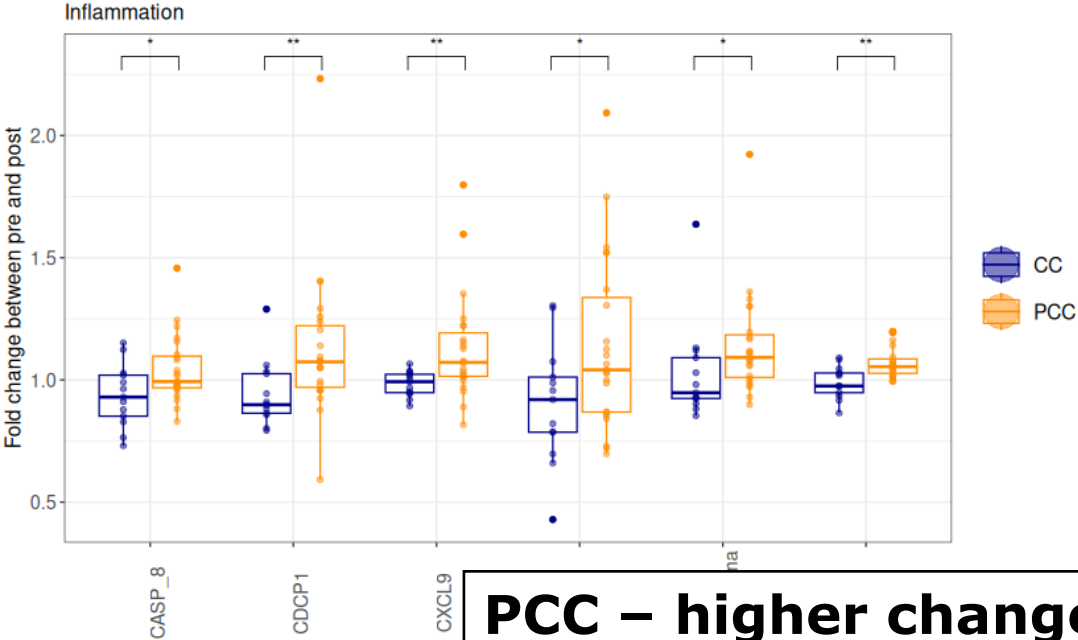


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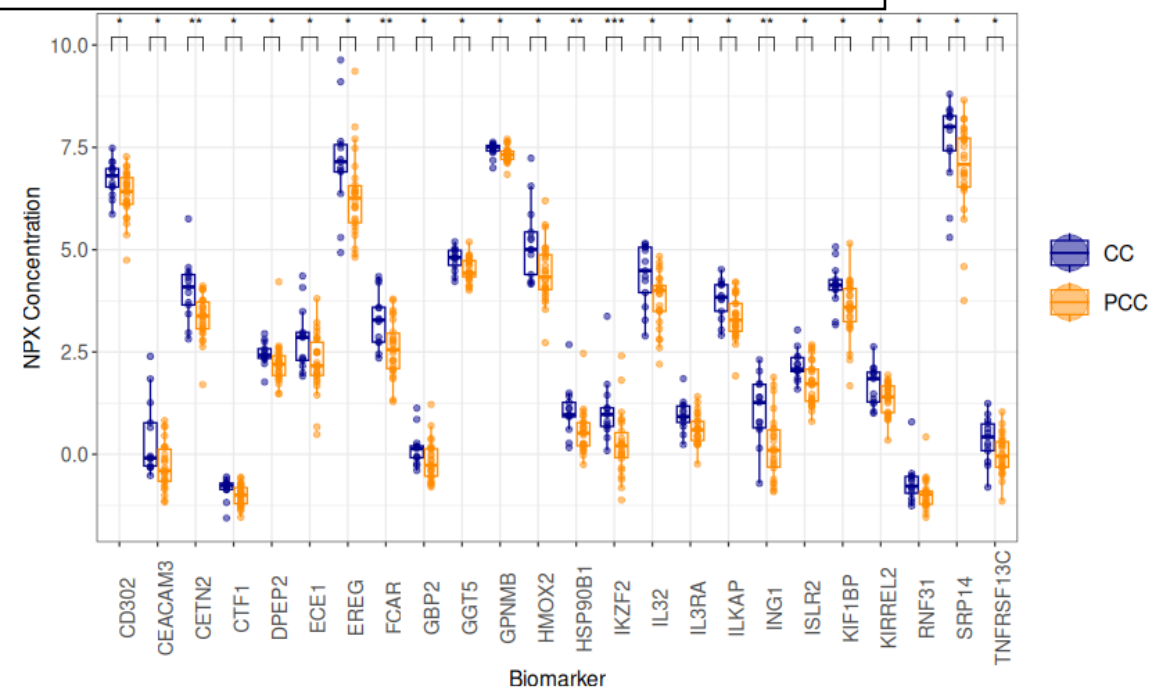
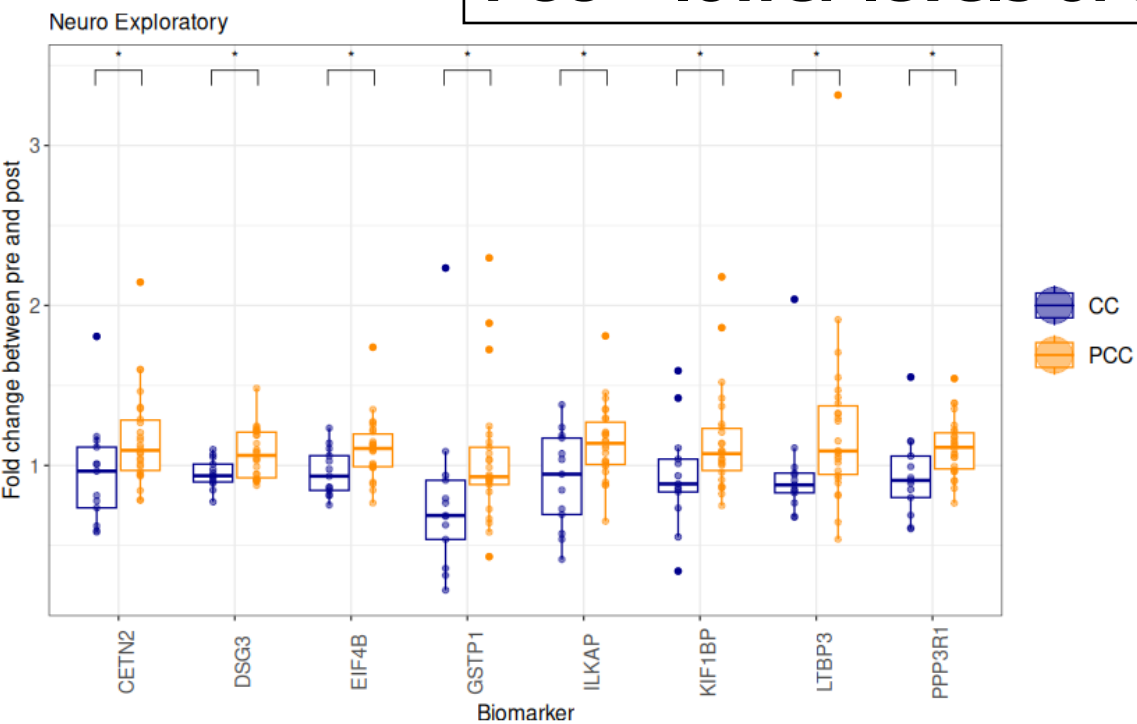


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N total significant differences between CC and PCC		
Adjusted p-value <= 0.05		
Panel	Absolute Difference Comparison	Pre Timepoint Comparison
Inflammation	5	6
Neuro Exploratory	10	24
Cardiometabolomic	1	23
TOTAL	16	53



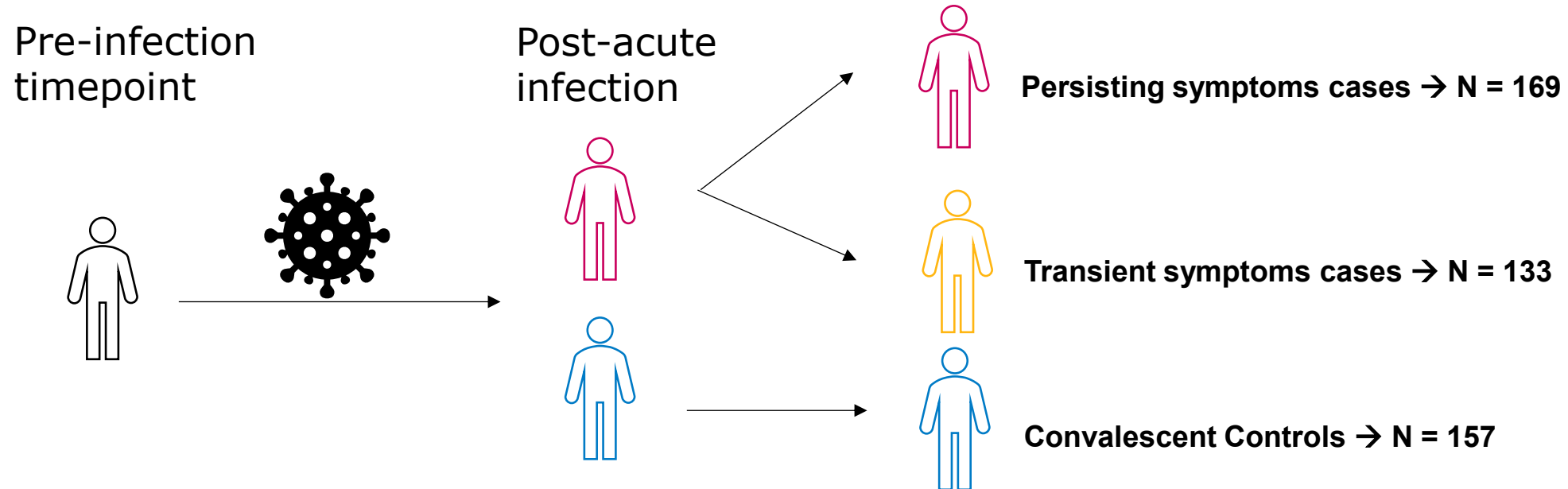
PCC – higher changes in significantly different biomarkers
PCC – lower levels of significant biomarkers at pre than CC





Scaling up the study: Olink Reveal and participant groups

Olink Reveal platform → ~1000 proteins!



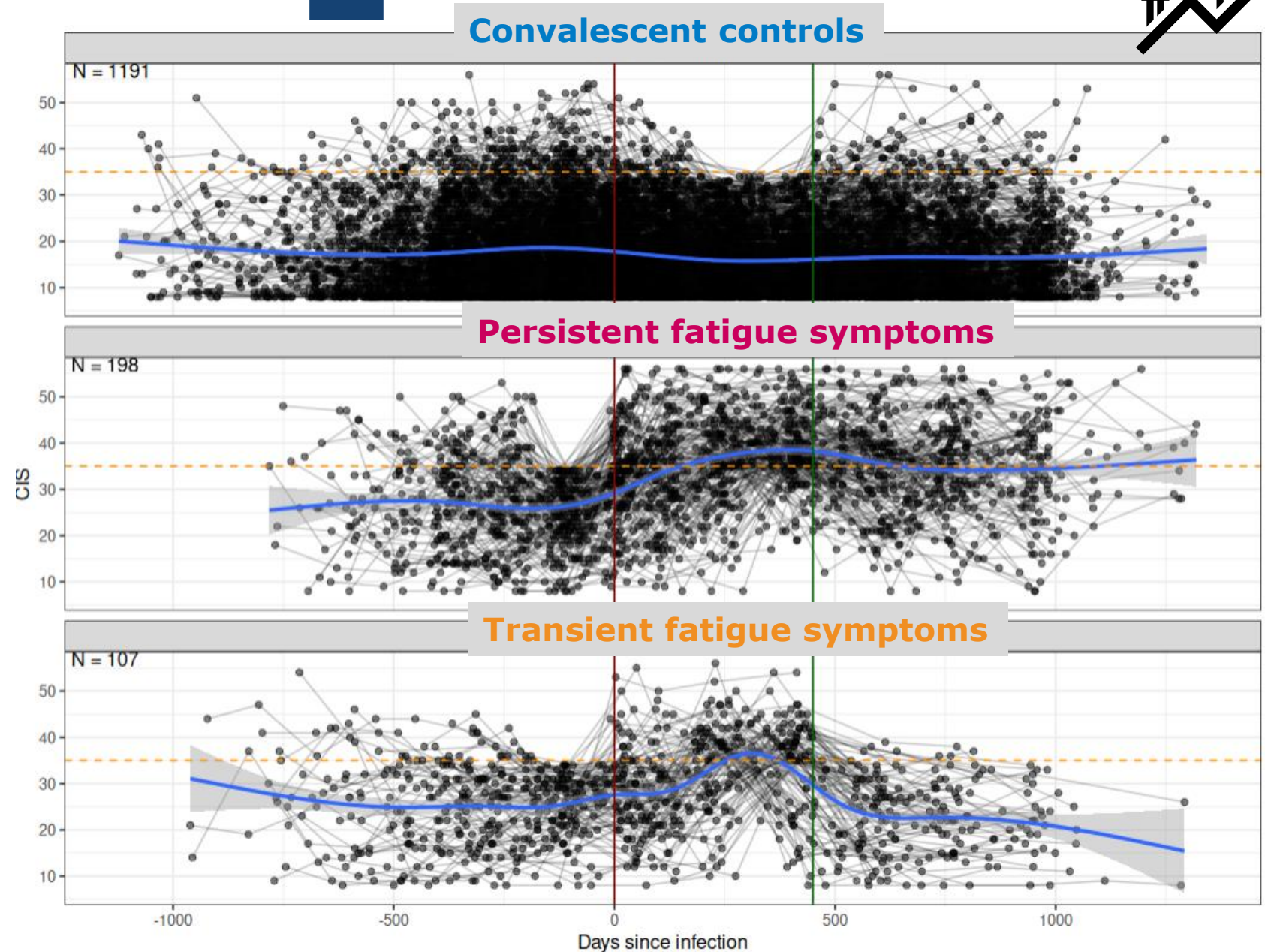


Selection of participants

Higher CIS-F → higher fatigue

CIS-F increase to severe fatigue after infection and persistence of it for more than 180 days → case

Various trajectories –
dividing the participants
into ones with persistent
and transient symptoms





Challenges of the scaled up study

- Many proteins → many tests → none of the assays remained significant after correction for multiple testing
- Potential problem: **high heterogeneity** of participants' individual protein signatures – also in the pre-infection (baseline) sample
- ➔ Still work in progress...



Summary

- › Our study has a unique setting of paired pre-infection, post-infection and further follow up samples.
- › The pilot study showed that it is possible to measure proteins in a little fingerprick sample using Olink.
- › The pilot study showed significant differences between participants with Post COVID and controls.
- › In our cohorts, we identified more participants with severe fatigue changes, which allowed us to scale up the study. We identified **various trajectories** of fatigue symptoms over time.
- › Scaling up the study both in terms of number of measured proteins and number of participants increased the complexity of analysis → **work in progress.**



Acknowledgements

National Institute for Public Health and the Environment (RIVM)

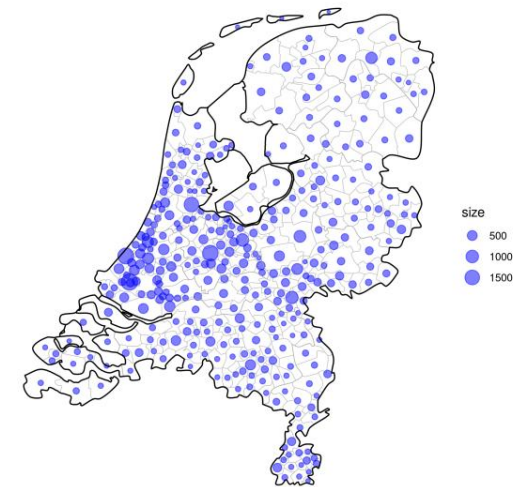
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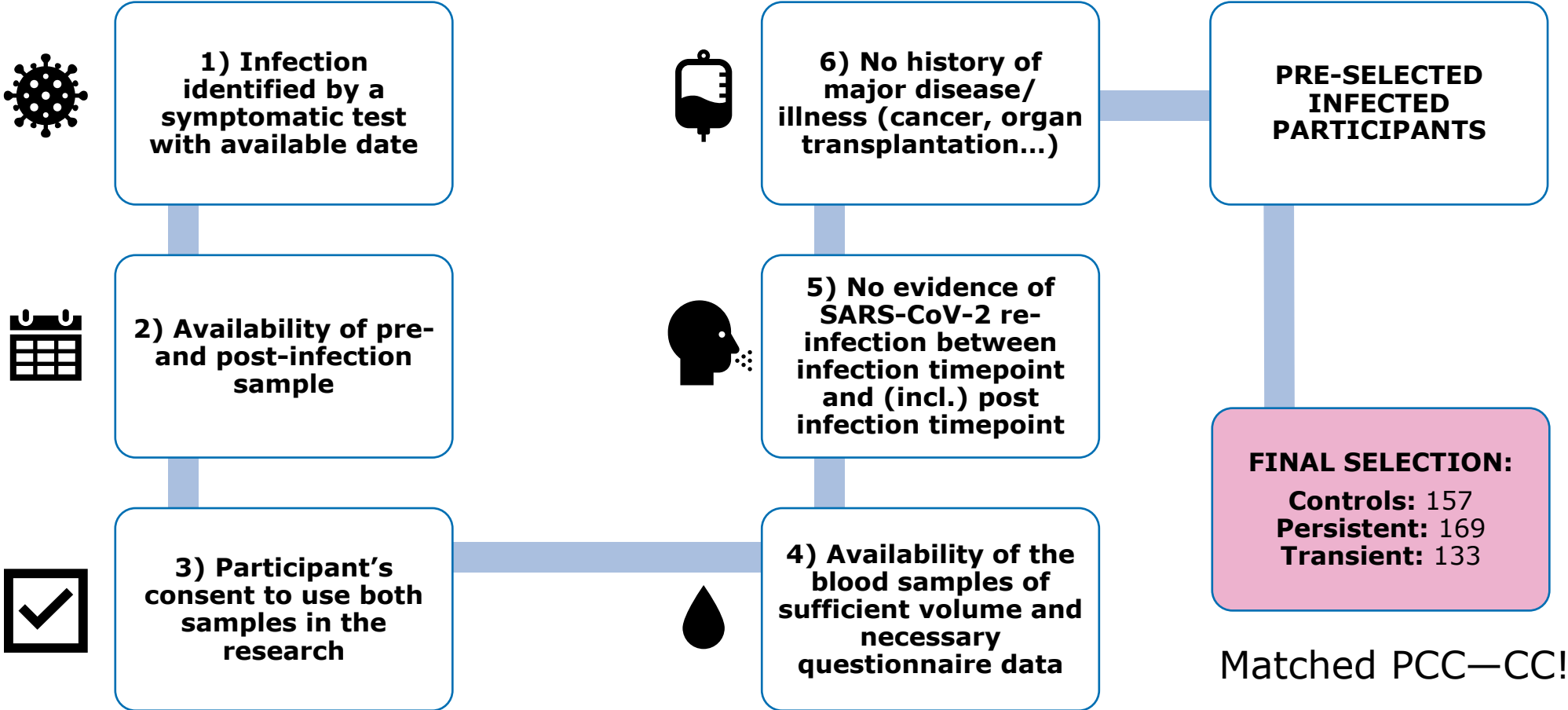
Post COVID Netwerk Nederland
PICO and VASCO participants

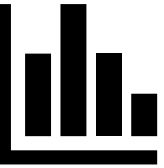


Thank you! Are there any questions?



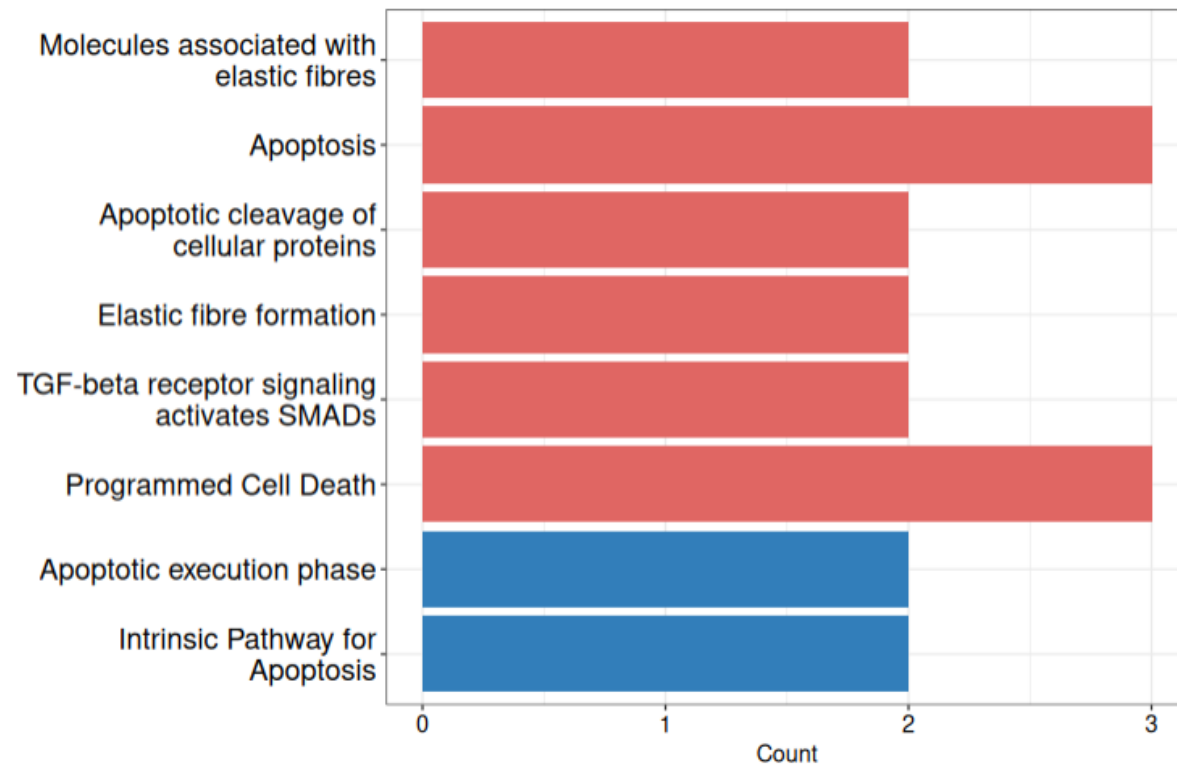
Selection criteria of case and convalescent control participants





Pathway analysis using Reactome

DELTA



PRE TIMEPOINT

- > Only signaling by interleukins pathway significant