Immunological imprinting by seasonal coronaviruses and previous herpesvirus infections in patients with long COVID

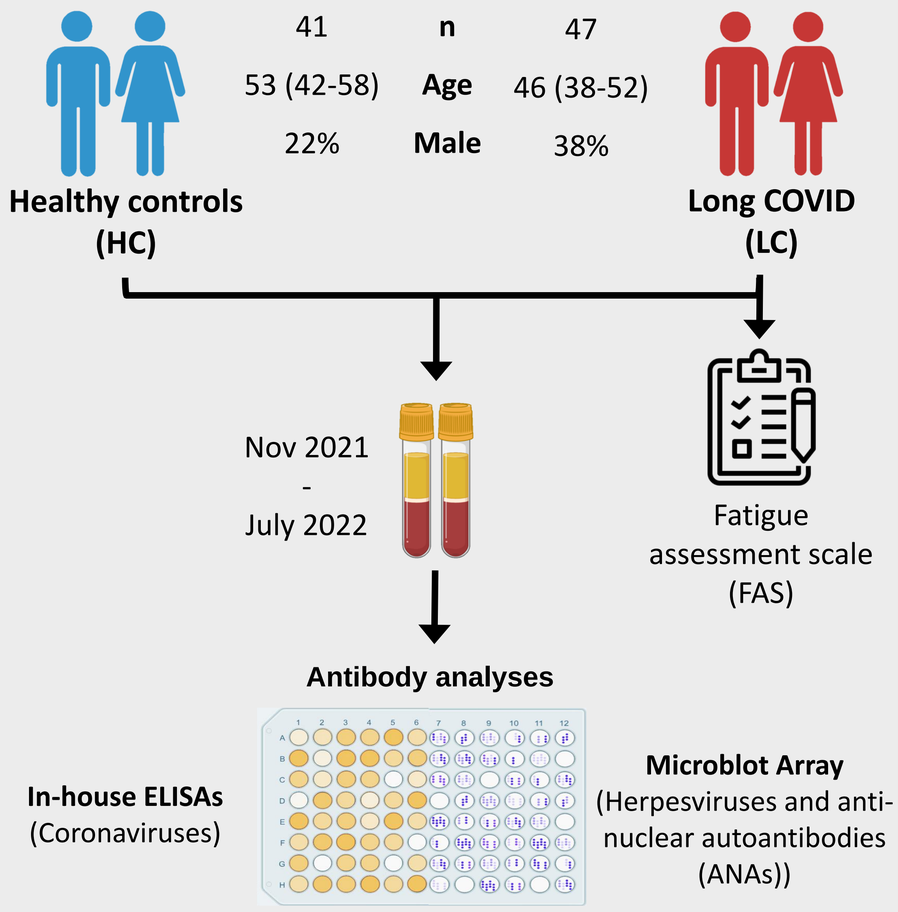
W. Ashwin Mak[1,2], Daphne Wapperom[1], Anne-Lotte Redel[3,4], Johannes G.M. Koeleman[1], Pieter W. Smit[1,5], Wai-Kwan Lam-Tse[6], Tom van der Poll[2], Hung-Jen Chen[2], Jeroen den Dunnen[2], Gert-Jan Braunstahl[3,4],David S.Y. Ong[1,7]

[1] Department of Medical Microbiology and Infection Control, Franciscus Hospital, Rotterdam; [2] Center for Experimental and Molecular Medicine, Amsterdam Institute for Infection and Immunity, Amsterdam University Medical Centers, Amsterdam; [3] Department of Pulmonology, Franciscus Hospital, Rotterdam; [4] Department of Respiratory Medicine, Erasmus MC, University Medical Center Rotterdam, Rotterdam; [5] Department of Medical Microbiology, Molecular Diagnostics Unit, Maasstad Hospital, Rotterdam; [6] Department of Rheumatology and Clinical Immunology, Franciscus Hospital, Rotterdam; [7] Department of Epidemiology, Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht; all the Netherlands

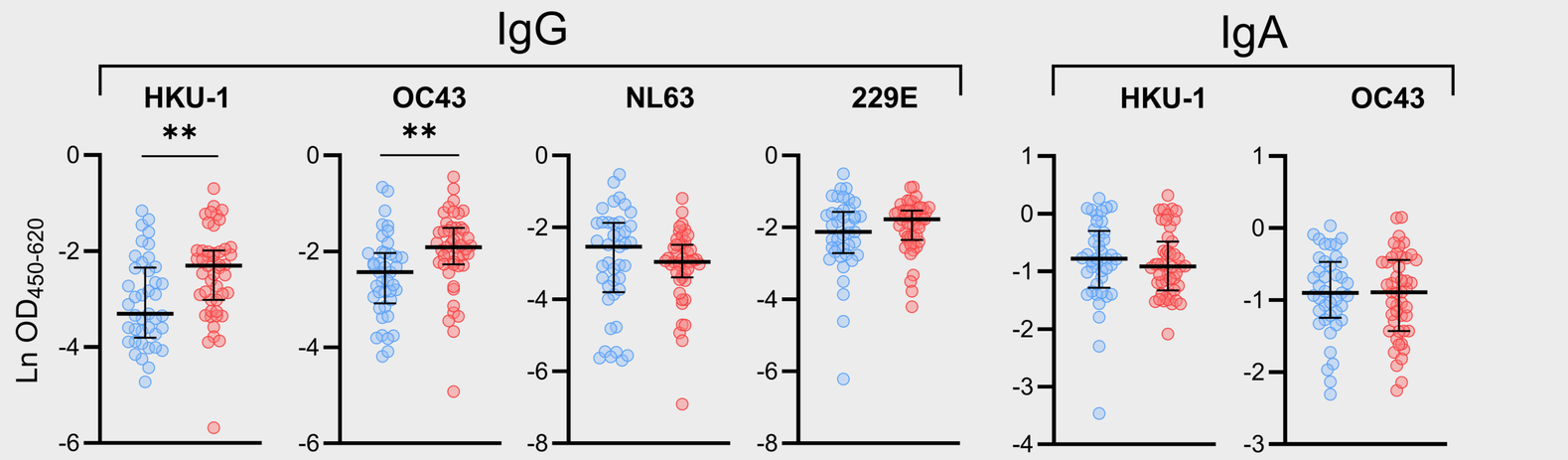
1. Introduction

- Up to 10% of individuals experience persistent symptoms after SARS-CoV-2 infection, termed **long COVID (LC)**, with a global economic impact estimated at 3 trillion USD.
- LC is a post-acute infection syndrome characterized by a wide spectrum of multi-organ symptoms, including fatigue, post-exertional malaise, neurocognitive impairments (brain fog), and many others.
- Proposed mechanisms underlying LC include SARS-CoV-2 persistence, herpesvirus reactivation, and autoimmunity.
- This study explores whether immunological imprinting from endemic coronaviruses, prior herpesvirus infections and reactivations, and autoimmunity contribute to LC.

2. Methods

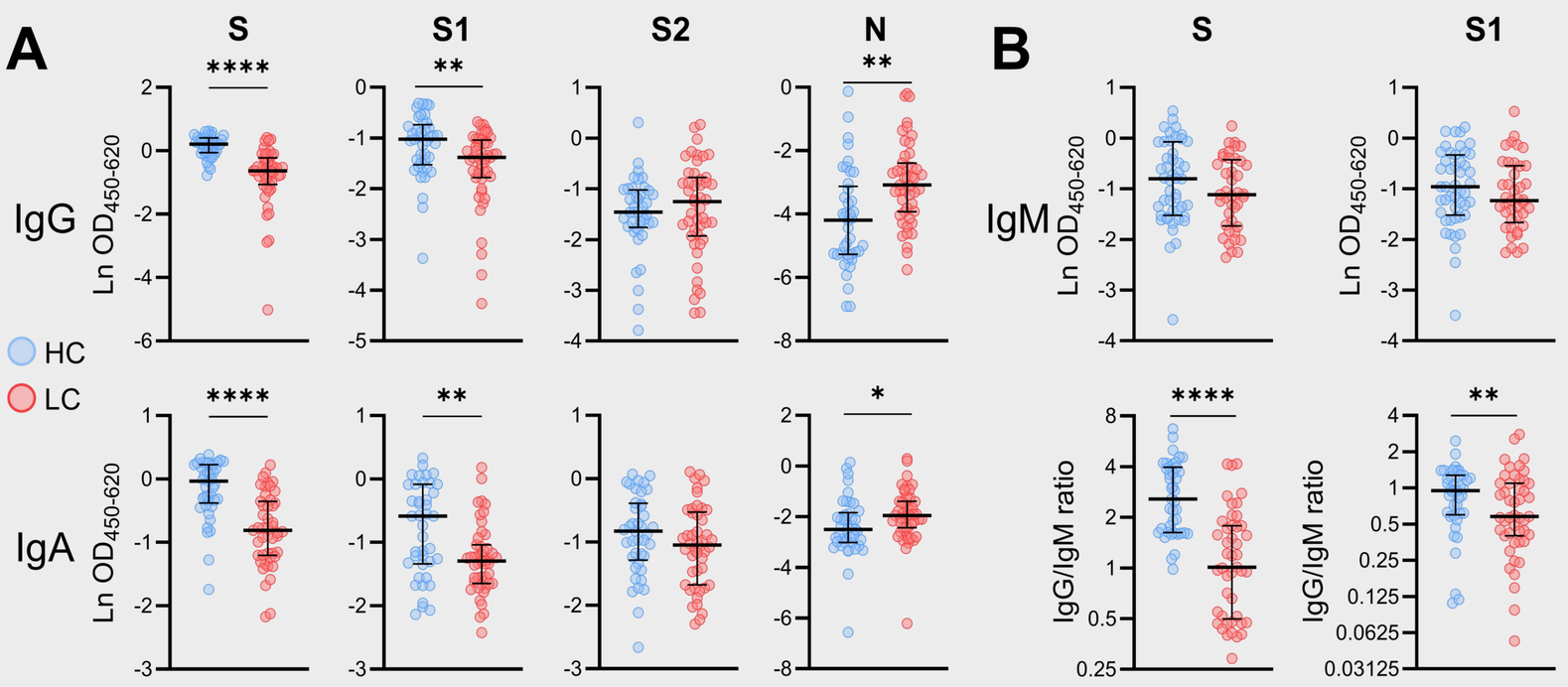


3. Increased endemic betacoronavirus anti-Spike IgG levels in LC patients



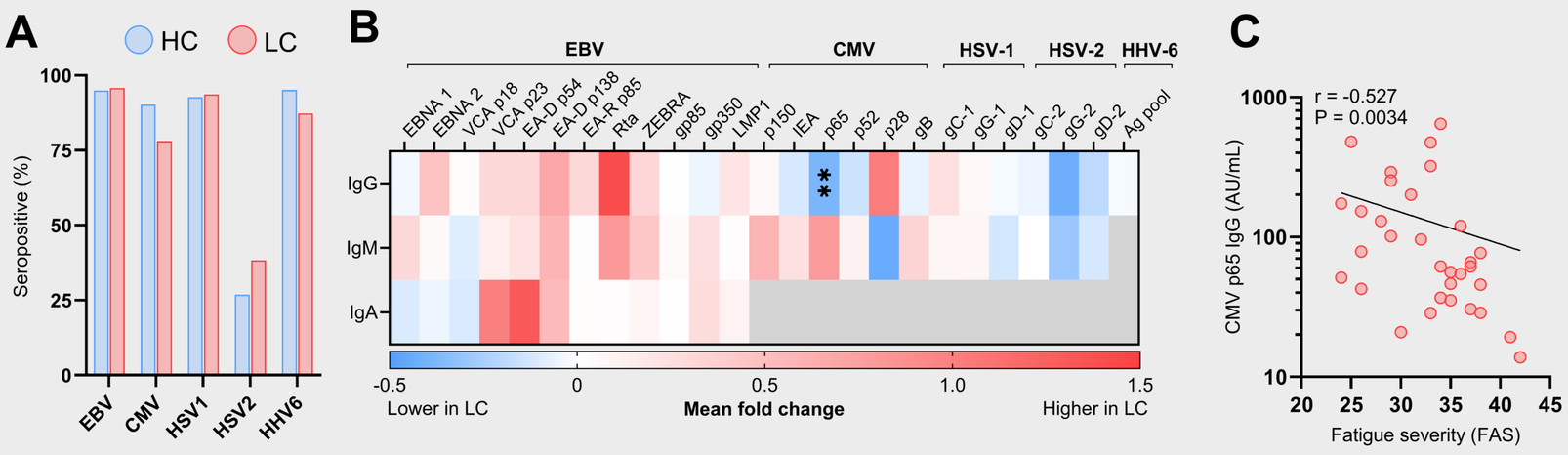
Serum IgG and IgA levels against the Spike protein of endemic coronaviruses in HC and LC cohorts. HKU-1 and OC43 are endemic betacoronaviruses, whereas NL63 and 229E are endemic alphacoronaviruses. Mann-Whitney U with adjusted p values for multiple comparisons (**p<0.01).

4. Lower SARS-CoV-2 anti-Spike IgG and IgA, but increased anti-Nucleocapsid IgG and anti-Spike IgM levels in LC patients



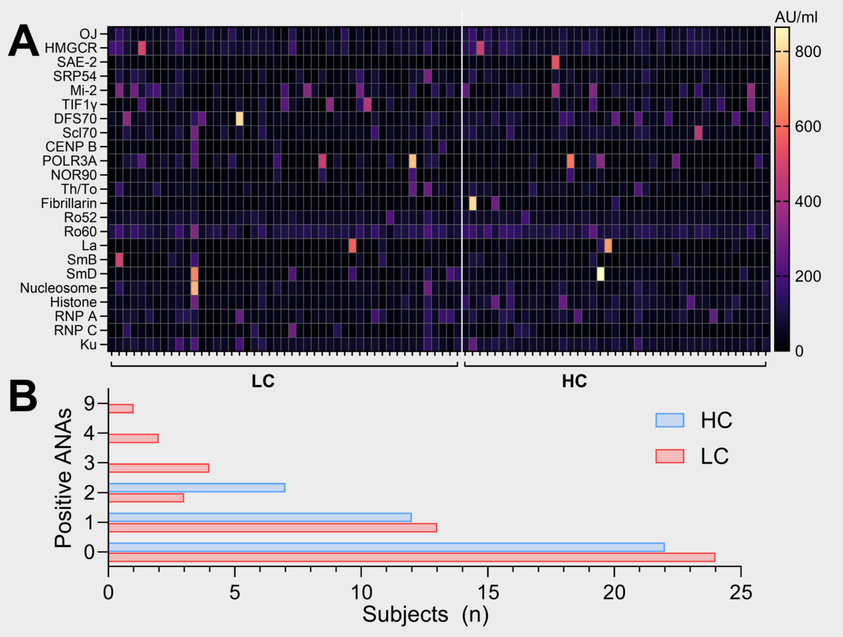
SARS-CoV-2 serum IgG, IgA, and IgM antibody levels in HC and LC cohorts. (A) IgG and IgA levels against SARS-CoV-2 full Spike (S) protein, S subunit 1 (S1), S subunit 2 (S2), and Nucleocapsid (N) protein. **(B)** Anti-S and anti-S1 IgM levels and IgG/IgM ratios. Mann-Whitney U with adjusted p values for multiple comparisons (*p<0.05; **p<0.01; ****p<0.0001).

5. LC patients have decreased CMV p65 IgG levels which associate with fatigue severity



Serum antibody levels against human herpesviruses in HC and LC cohorts. (A) Seropositivity rates for Epstein-Barr virus (EBV), cytomegalovirus (CMV), herpes simplex virus type 1 (HSV1) and type 2 (HSV2), and human herpesvirus 6 (HHV6). **(B)** Mean fold change of antibody levels specific to various herpesvirus antigens. Mann-Whitney U with adjusted p values for multiple comparisons (**p<0.01). **(C)** Correlation between CMV p65 IgG levels and fatigue severity in LC patients as assessed with the FAS questionnaire. Spearman's rank correlation.

6. LC patients do not express specific anti-nuclear autoantibodies



Serum ANA profile in HC and LC cohorts. (A) Heatmap showing quantitative levels of ANAs related to several systemic autoimmune diseases. **(B)** The number of positive ANAs in HC and LC patients.

7. Conclusion

- **LC patients exhibit altered coronavirus antibody profiles, potentially driven by impaired isotype switching of SARS-CoV-2-specific antibodies and immunological imprinting (original antigenic sin) by prior betacoronavirus infections.** These mechanisms may underlie the observed reduction in S1-specific IgG levels, contributing to viral or antigen persistence in LC.
- Lower CMV p65 IgG levels correlate with increased fatigue severity, highlighting a potential link between herpesvirus-specific immunity and LC symptoms.
- Further investigation into these mechanisms is essential to elucidate LC pathophysiology and develop therapeutic strategies.

franciscus
Amsterdam UMC
LinkedIn

a.mak@franciscus.nl