# Exploring seropositive rheumatoid arthritis: from immunological depths to clinical course

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# **The onset of rheumatoid arthritis after COVID-19 –** coincidence or connected?

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# Letter

COVID-19, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), can lead to severe inflammation and has been suggested to induce autoimmune phenomena. Multiple studies have reported autoantibodies in patients with COVID-19, particularly anti-cardiolipin, anti-β2 glycoprotein I and anti-nuclear antibodies (1, 2). Anti-citrullinated protein antibodies (ACPA) and flaring of rheumatoid arthritis (RA) after SARS-Cov-2 infection have also been described (1, 3). However, it is unclear how often ACPA occur after COVID-19 and whether they differ from ACPA normally found in RA patients.



We have therefore performed a detailed investigation into ACPA-positivity after COVID-19. To determine the seroprevalence of ACPA after COVID-19, ACPA was measured using routine tests or in-house enzyme-linked immunosorbent assay (ELISA) in 61 patients visiting the post-COVID outpatient clinic of the LUMC 5 weeks after hospitalization. None of the patients tested positive for ACPA, except two patients previously diagnosed with ACPA-positive RA. Thus, we could not observe an increase in ACPA-positivity after COVID-19.

Furthermore, we identified five patients across various Dutch rheumatology clinics presenting with polyarthritis compatible with RA after SARS-CoV-2 infection. To study the impact of COVID-19 on disease presentation, we closely examined their clinical phenotype and autoantibody characteristics (Supplementary table S1). All had suffered from moderate to severe COVID-19. On average, joint complaints started 6.6 weeks after infection, although two patients reported symptoms before infection. 4/5 patients fulfilled the ACR 2010 criteria for RA. Three patients were phenotypically very similar to regular new-onset RA patients. Patient 3 had a history of seronegative RA and had been in DMARD-free remission for 5 years. She flared 6 weeks after SARS-CoV-2 infection. Patient 2 had a remarkably different presentation. He was admitted with acute polyarthritis and high inflammatory markers 6 weeks after COVID-19. Pneumonia with reactive polyarthritis or septic polyarthritis were considered and treatment was started accordingly. ACPA-level was low positive. The patient died unexpectedly after two days and autopsy revealed dilating myocarditis of unclear underlying cause. No causative pathogen, nor compelling evidence of autoimmunity, could be identified.

Previous studies have shown that RA-patients are most often either seronegative or triple-positive for rheumatoid factor, ACPA and anti-carbamylated protein antibodies. ACPA IgM and IgA are most frequently found within patients positive for ACPA IgG (4). Autoantibody measurements on sera of the post-COVID polyarthritis patients using

in-house ELISA's (4), revealed patterns very similar to RA (figure 1A) with two patients being completely seronegative, and three patients positive for a range of autoantibodies at presentation. Sera prior to presentation to the rheumatologist are not available.

A unique feature of ACPA IgG in RA patients is that they harbour glycans not only in their Fc-part, but also in their variable domains (V-domains) (5). We analysed the ACPA IgG V-domain glycosylation profiles of the above-mentioned 3 ACPA-positive post-COVID patients and established RA patients (Supplementary table S1) using UHPLC (5). In all post-COVID samples, the percentage of ACPA V-domain glycosylation was increased compared to total IgG (figure 1B), similar to regular RA. Inflammatory conditions, among which COVID-19, can induces changes in the composition of antibody Fc-glycans (6). A detailed examination of the specific ACPA IgG V-domain glycan traits revealed a significant decrease in bisecting N-Acetylglucosamine containing moieties (G2FBS1, G2FBS2) after COVID-19 (figure 1C), comparable to patterns described for total IgG Fc-glycosylation post-COVID (6). The biological causes and consequences of these glycosylation changes currently remain unclear.

Limitations of this study include the small sample size and limited follow-up duration after COVID-19. Although autoantibody responses can develop rapidly after (SARS-Cov-2) infections, replication in a larger cohort with a longer follow-up would be valuable. Furthermore, part of the samples were measured on in-house instead of commercial tests. However, the characteristics of these assays appear very comparable based on previous experience.

In conclusion, we found that the seroprevalence of ACPA is not increased after COVID-infection and that patients presenting with polyarthritis post-COVID resemble regular RA patients, both regarding clinical phenotype and autoantibody characteristics. Based on these data, it appears that RA post-COVID may be coincidence rather than connected.

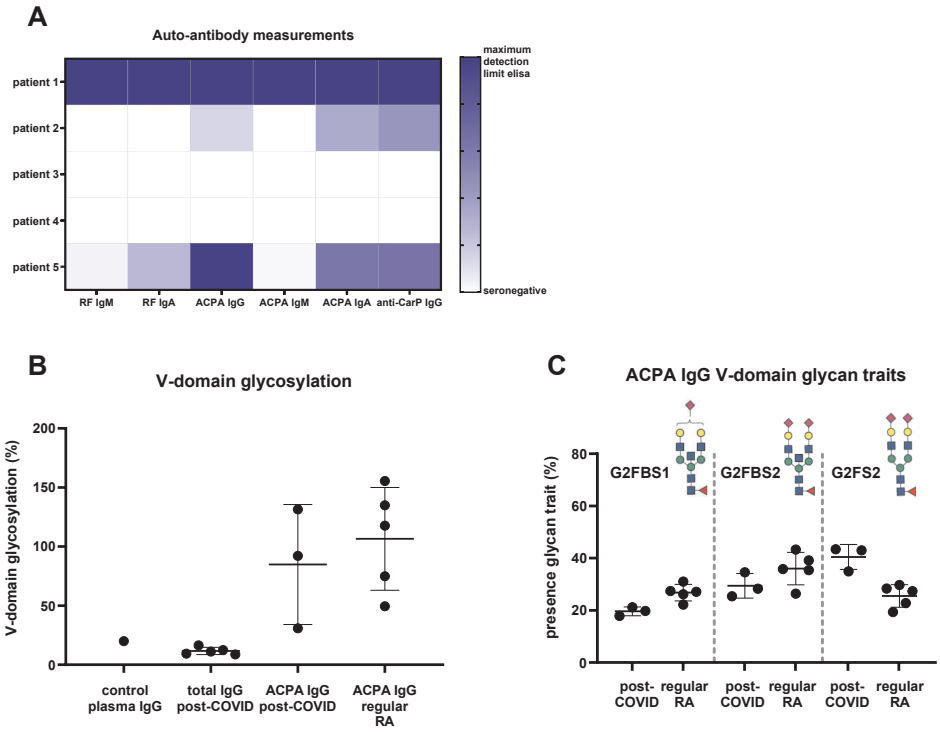


**Figure 1: Autoantibody positivity and (auto)antibody glycosylation.** A Auto-antibody measurements using in-house ELISA's: Rheumatoid factor (RF), anti-citrullinated protein antibody (ACPA) and anti-carbamylated protein antibody (anti-CarP) isotype levels per patient. White – seronegative, Gradient light to dark blue – low to highest levels, normalized against maximum detection limit ELISA per antibody isotype. B Percentage of variable domain glycosylation (mean, SD). Average value of duplicates plotted. V- domain glycosylation in ACPA IgG post-COVID is significantly increased compared to total IgG ($p<0.05$;Mann-Whitney U test), no significant difference between ACPA IgG V-domain glycosylation post-COVID and in regular RA (disease characteristics in supplementary table S1). C Percentage of specific glycan traits of all ACPA IgG V-domain glycans (mean, SD). Average value of duplicates plotted. Glycan trait G2FS2 without bisecting N-Acetylglucosamine is significantly increased, while G2FBS1, a glycan traits with bisecting N-Acetylglucosamine is significantly decreased post-COVID-19 ($p<0.05$; Mann-Whitney U test). Blue square – N-Acetylglucosamine (B when bisecting), green circle – mannose, red triangle – Fucose (F), yellow circle – galactose (G), purple diamond – sialic acid (S).

# References

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# Supplementary information

**Supplementary table S1: Clinical characteristics of new-onset RA patients after SARS-CoV-2 infection.**

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Regular RA (n=5) |
|---|---|---|---|---|---|---|
| **Age, years** | 67 | 49 | 70 | 67 | 65 | 58 (36-67) mean (range) |
| **Gender** | Male | Male | Female | Female | Male | 2/5 male |
| **Smoking** | Never | Never | Former | Never | Current | 1 current (n=4) |
| **Hospitalisation due to COVID-19** | Yes | Yes (not in acute phase) | Yes | Yes | No | - |
| **ICU admission due to COVID-19** | Yes | No | No | No | No | - |
| **Joint symptoms before COVID-19** | Yes | No | Yes | No | No | - |
| **Time between positive COVID-19 -test and onset joint symptoms** | Already present | 6 weeks | 6 weeks | 14 weeks | 3 days | - |
| **ESR (mm/hour)** | 36# | 79# | 52# | 49# | 26# | 49 (35-79) mean (range) |
| **CRP (mg/l)** | 6 | 449# | 44# | 168# | 6 | - |
| **Fulfilment ACR 2010 criteria** | Yes | No | Yes | Yes | Yes | Yes (All) |
| **Swollen joint count 28** | 7 | 4 | 10 | 13 | 10 | 15 (2-22) mean (range) |
| **Tender joint count 28** | 7 | 4 | 1 | 3 | 8 | 6 (0-16) mean (range) |
| **Joints affected (small/large/both)** | Both | Both | Both | Small | Both | - |

**Supplementary table S1: Continued**

| | Patient 1 | Patient 2 | Patient 3 | Patient 4 | Patient 5 | Regular RA (n=5) |
|---|---|---|---|---|---|---|
| **Location affected joints** | Both extremities | Both extremities | Both extremities | Both extremities | Upper extremity | - |
| **Symmetric onset** | Yes | Yes | Yes | Yes | Unknown | - |
| **DAS28** | Unknown | Unknown | Unknown | 5.76 | 4.69 | 5.12 (2.98-6.49) mean (range) |
| **Additional information** | - | acute poly-arthritis 6 weeks after COVID-19, died unexpectedly 2 days later* | Previous diagnosis RA, 5 years DMARD-free remission | History of sarcoidosis | - | Disease duration 9.2 (3-26) years mean (range) |

Clinical characteristics of the five patients presenting with polyarthritis compatible with RA after SARS-CoV-2 infection, as compared to patients with established, regular RA whose serum was used for the glycosylation experiments. #elevated ESR/CRP according to local reference values

*Full case description: A 49-year-old male with morbid obesity was admitted with polyarthritis and very high inflammatory markers. He also had petechiae on the lower legs and proteinuria. 6 weeks before he experienced flu-like symptoms, after which he remained extremely fatigued and slightly short of breath. Based on serology these symptoms were most likely caused by COVID-19. The chest X-ray at the time of admission (6 weeks after COVID19-symptoms) showed a consolidation, which could be compatible with pneumonia on the right basal side. The working diagnosis was reactive polyarthritis with an (atypical) pneumonia or a septic polyarthritis with bacteraemia and the patient was treated empirically with ceftriaxone iv. He died unexpectedly after 2 days. Obduction showed a dilating myocarditis as cause of death, but the underlying cause of the myocarditis remains unclear. The nature of the myocardial infiltrate was not typical for viral myocarditis due to the diffuse localization, CD4+ T-cell dominance and many neutrophils. These histopathological findings, especially the presence of neutrophils, are also not compatible with an idiopathic inflammatory myopathy such as polymyositis. The myocardial infiltrate could be caused by focal ischemia under the influence of catecholamines secondary to sepsis. However, no causative pathogen or focus of infection could be identified. The blood and synovial fluid cultures, as well as the SarS-Cov-2 PCR on pulmonary and myocardial tissue were negative. Furthermore, the lung tissue showed oedema, but no signs of viral or bacterial infiltrate. No convincing evidence for an autoimmune disease could be found, but this can also not be completely excluded. Thus the underlying cause of death remains unclear.