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INVESTIGATION OF THE SARS-CoV-2 INDUCED IMMUNE RESPONSES IN RHEUMATOID ARTHRITIS AND HEALTHCARE WORKERS

PhD thesis

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Table of Contents

1	Introduction	7
1.1	Rheumatoid arthritis (RA).....	8
1.1.1	Epidemiology	8
1.1.2	Systemic immune alterations and infection susceptibility in RA.....	8
1.1.2.1	Infections as triggers of autoimmunity and disease flares.....	10
1.1.2.2	Impact of antirheumatic therapies on immune responses to infections 10	
1.2	SARS-CoV-2 and COVID-19.....	11
1.2.1	Epidemiology	11
1.2.2	Pathophysiology of SARS-CoV-2 infection and clinical characteristics of COVID-19	11
1.2.3	SARS-CoV-2-specific immune responses	12
1.2.3.1	Innate and adaptive immune responses to SARS-CoV-2 infection	12
1.2.3.2	COVID-19 vaccine induced immune responses.....	13
1.2.3.3	SARS-CoV-2-specific immune responses in RA patients	14
1.3	Healthcare workers (HCWs) and RA patients as high-risk populations during the COVID-19 pandemic.....	15
2	Objectives.....	17
3	Methods.....	18
3.1	RA patients	18
3.2	HCWs	18
3.3	Assessment of SARS-CoV-2-specific antibodies (Elecsys® Anti-SARS-CoV-2 S assay, 95 Roche; Elecsys® Anti-SARS-CoV-2 N assay, 95 Roche; Euroimmun ELISA test)	19
3.4	Evaluation of IFN- γ T-cell responses (QuantiFERON (QIAGEN Group)) ...	20
3.5	Statistical analysis	21

3.5.1	Baseline study.....	21
3.5.2	Follow-up study.....	22
3.5.3	Study among HCWs	25
4	Results	26
4.1	Study population.....	26
4.1.1	Baseline study.....	26
4.1.2	Follow-up study.....	28
4.2	Anti-SARS-CoV-2 antibody response	30
4.2.1	Baseline study.....	30
4.2.2	Follow-up study.....	36
4.2.3	Study among HCWs	40
4.3	IFN- γ T-cell response	42
4.3.1	Baseline study.....	42
4.3.2	Follow-up study.....	45
5	Discussion	47
6	Conclusions	52
7	Summary	53
8	References	54
9	Bibliography of the candidate's publications.....	64
10	Acknowledgements	65

List of Abbreviations

ACE2 - angiotensin-converting enzyme
2

ACPA - anti-citrullinated protein
antibodies

anti-CPP - anti-cyclic citrullinated
peptide

ARDS - acute respiratory distress
syndrome

bDMARDs - biologic disease-
modifying antirheumatic drugs

CD... – cluster of differentiation

CI – confidence interval

COVID-19 - Coronavirus disease-19

CRP - C-reactive protein

csDMARDs - conventional synthetic
disease-modifying antirheumatic drugs

CVID - common variable
immunodeficiency

CW 4-8 - whole COVID virus-specific
CD4+ and CD8+ T-cell response

DAS28-CRP - disease activity score 28-
C-reactive protein

DMARDs - disease-modifying
antirheumatic drugs

E – envelope protein

ELISA - enzyme-linked immunosorbent
assay

GCS – glucocorticoids

HCW - healthcare worker

HRP - horseradish peroxidase

IFN - interferon

IFN- γ – interferon-gamma

Ig - immunoglobulin

IgA – immunoglobulin A

IgG – immunoglobulin G

IgM – immunoglobulin M

IL-1 β - interleukin-1beta

IL-6 - interleukin-6

IQR - interquartile range

JAK - Janus kinase

M – membrane protein

mRNA - messenger ribonucleic acid

MTX - methotrexate

NCP - nucleocapsid protein

PCR - polymerase chain reaction

RA - rheumatoid arthritis

RBD - receptor binding domain

RF - rheumatoid factor

RNA - ribonucleic acid

S – spike protein

SARS-CoV-2 - Severe Acute

Respiratory Syndrome Corona Virus 2

tsDMARDs - targeted synthetic disease-
modifying antirheumatic drugs

Th cell – T helper cell

TLRs - Toll-like receptors

TNF- α - tumor necrosis factor-alpha

T reg - regulatory T-cell

VV-ECMO - venovenous extracorporeal
membrane oxygenation

WHO - World Health Organization

1 **1 Introduction**

2 Rheumatoid arthritis (RA) is a chronic, systemic autoimmune disease characterized by
3 persistent synovial inflammation, progressive joint destruction and a wide array of extra-
4 articular manifestations. Affecting approximately 0.5–1% of the global population, RA
5 results from a complex interaction of genetic, environmental and immunologic factors.
6 Characteristics of RA pathogenesis include abnormal activation of both the innate and
7 adaptive immune systems, with crucial roles played by pro-inflammatory cytokines such
8 as tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6) and interleukin-1beta (IL-
9 1 β). It is known that RA patients exhibit increased susceptibility to infections due to both
10 the immune dysregulation associated with the disease and the immunosuppressive effects
11 of disease-modifying antirheumatic therapies (1).

12 In December 2019, a number of patients with pneumonia of unknown reason was
13 identified in Wuhan, China, shortly thereafter the World Health Organization (WHO) had
14 formally classified the current coronavirus outbreak as Coronavirus disease-19 (COVID-
15 19) (2), which was induced by the newly identified severe acute respiratory syndrome
16 coronavirus 2 (SARS-CoV-2). The COVID-19 pandemic had serious global health effects
17 and resulted in millions of deaths worldwide (3, 4). Beyond its respiratory manifestations,
18 SARS-CoV-2 is now recognized as a systemic virus with significant immunological
19 consequences, including cytokine release syndrome, lymphocyte dysregulation and
20 autoimmune phenomena (5). The virus's complex interactions with host immune
21 responses have raised questions regarding its impact on individuals with pre-existing
22 immune-mediated disorders, such as RA.

1.1 Rheumatoid arthritis (RA)

1.1.1 Epidemiology

The global prevalence of RA is estimated to be approximately 0.5% to 1% of the European and American adult population and it is more prevalent in women than in men, with a ratio of two to one. The disease commonly emerges between the ages of 30 and 60 (6). RA is linked to various long-term complications, including other chronic illnesses such as cardiovascular and respiratory conditions, osteoporosis and malignancies. It can also lead to physical and occupational impairment, mental health disorders and diminished quality of life (7-10).

1.1.2 Systemic immune alterations and infection susceptibility in RA

RA is a chronic systemic autoimmune disease characterized by persistent synovial inflammation, progressive cartilage destruction, bone erosion and extra-articular manifestations. Its immunopathogenesis is driven by sustained activation of antigen-presenting cells, dysregulated cytokine signaling, expansion of autoreactive CD4⁺ T helper cells (Th) - particularly Th1 and Th17 subsets - and impaired regulatory T-cell (Treg) function, resulting in breakdown of peripheral tolerance and chronic inflammation (11, 12). B-cell dysregulation further contributes through abnormal antigen presentation, cytokine production and generation of pathogenic autoantibodies, including rheumatoid factor (RF) and anti-citrullinated protein antibodies (ACPA), which are closely associated with disease severity and structural damage (13). In addition to driving autoimmunity, RA is associated with profound and persistent immune dysfunction that reduces host defense. Epidemiological studies consistently demonstrate an increased risk of infections in patients with RA compared with the general population, reflecting the influence of disease-related immune dysfunction on infection risk (1, 14).

Innate immune dysregulation in RA is characterized by chronic activation of monocytes, macrophages and neutrophils, with sustained overproduction of pro-inflammatory cytokines such as TNF- α , IL-6 and IL-1 β . Although essential for antimicrobial responses, prolonged cytokine exposure leads to immune imbalance, impaired leukocyte function and disrupted pathogen clearance, paradoxically increasing infection risk (15). Adaptive immune alterations further exacerbate immune insufficiency, including reduced naive T-cell output, decreased T-cell receptor repertoire diversity, accumulation of highly

differentiated memory T cells and qualitative defects in B-cell mediated antibody responses that impair effective neutralization of pathogens (12).

Collectively, RA represents a state of chronic immune activation coexisting with impaired immune competence, resulting in heightened susceptibility to infections. As illustrated in Figure 1, this vulnerability is amplified by the requirement for immunosuppressive therapies to control disease activity, while infections can, in turn, exacerbate inflammatory pathways and trigger disease flares, reinforcing a self-perpetuating cycle. These interconnected mechanisms provide a critical framework for understanding altered immune responses to infections such as SARS-CoV-2 in RA.

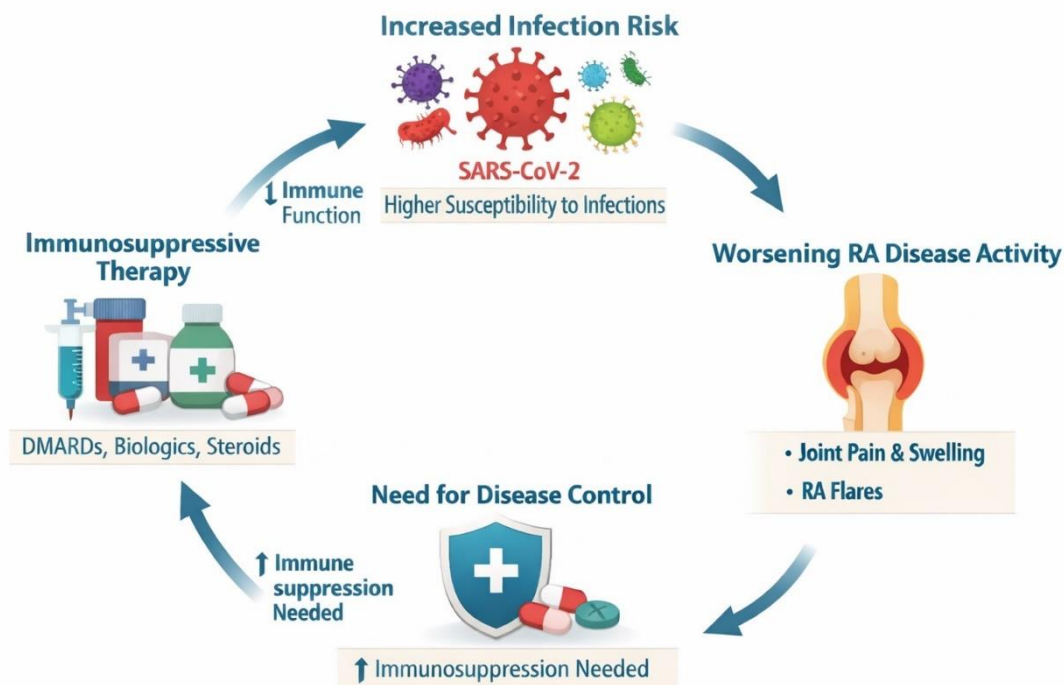


Figure 1. Conceptual model of the interaction between immunosuppression, infection risk and RA disease activity. Immunosuppressive therapies used in RA can reduce immune competence and increase susceptibility to infections, including SARS-CoV-2. Infections might induce systemic inflammation, exacerbate disease activity and trigger disease flares, potentially necessitating intensified immunosuppressive therapy to re-establish disease control, thereby sustaining a self-reinforcing cycle. DMARDs: disease-modifying antirheumatic drugs. *The figure was created using OpenAI GPT-5.3 based on the author's conceptual design.*

1.1.2.1 Infections as triggers of autoimmunity and disease flares

Experimental and clinical evidence supports the role of infections as important environmental factors capable of initiating autoimmunity and exacerbating established RA. In genetically susceptible individuals, infectious agents may contribute to RA development through multiple, non-exclusive mechanisms, including molecular mimicry, epitope spreading and infection-induced post-translational modification of self-antigens that enhance their immunogenicity (16). Viral infections are of particular interest because they can induce strong innate immune activation and sustained type I interferon (IFN) responses, thereby lowering the activation threshold of autoreactive T-, and B-cells (16). Several viruses, including Epstein–Barr virus and parvovirus B19, have been implicated in RA pathogenesis based on the detection of viral components in synovial tissue and evidence of cross-reactivity with citrullinated self-proteins. These findings suggest a potential role for viral infections in the transition from preclinical autoimmunity to clinically manifest RA (17, 18).

In established RA, infections are well-recognized triggers of disease flares. By inducing systemic inflammation and innate immune activation, they may impair immune regulatory mechanisms and promote synovial inflammatory responses (12, 15).

1.1.2.2 Impact of antirheumatic therapies on immune responses to infections

RA treatment largely depends on disease-modifying antirheumatic drugs (DMARDs), which effectively control inflammation and prevent joint damage, but substantially alter immune competence. Conventional synthetic DMARDs (csDMARDs), particularly methotrexate (MTX), broadly suppress lymphocyte proliferation, cytokine production and antigen-specific immune responses, contributing to increased infection susceptibility and impaired vaccine responses during long-term treatment. Glucocorticoids (GCS) promote dose-, and duration-dependent immunosuppressive effects on innate and adaptive immunity and are consistently associated with an elevated risk of serious infections, even at low chronic doses (12, 19, 20).

Biologic disease-modifying antirheumatic drugs (bDMARDs) and targeted synthetic disease-modifying antirheumatic drugs (tsDMARDs) provide more selective immune modulation, but confer distinct infectious risk profiles. TNF- α inhibitors impair macrophage activation and granuloma maintenance, increasing susceptibility to

intracellular pathogens and viral reactivation, whereas IL-6 receptor antagonist may attenuate acute-phase responses and mask early signs of infection (21). Janus kinase (JAK) inhibitors disrupt cytokine signaling pathways essential for antiviral defense and are associated with an increased incidence of viral infections, particularly herpes zoster (22).

Cumulative immunomodulatory therapy in RA can lead to secondary immunodeficiency arising from chronic inflammation, aging, comorbidities and prolonged or combination treatment. B-cell-targeted therapies, especially anti-CD20 monoclonal antibodies, can cause humoral immune defects including hypogammaglobulinemia, impaired vaccine responses and reduced immunological memory, with infection risk further amplified by simultaneous csDMARD or GCS use (22, 23). This therapy-induced immunodeficiency shares features with common variable immunodeficiency (CVID), highlighting the coexistence of immune suppression and immune activation in RA and supporting immunoglobulin (Ig) and vaccine-response monitoring in high-risk patients (24, 25).

1.2 SARS-CoV-2 and COVID-19

1.2.1 Epidemiology

SARS-CoV-2 is mainly spread through respiratory droplets during face-to-face interactions and to a lesser extent, through contact with contaminated surfaces (26). Since the start of the pandemic, SARS-CoV-2 virus has been marked by a significant number of genomic variations. These variations have resulted in significant changes across all structural proteins, driven by natural selection, leading to the emergence of variants with increased transmissibility and/or virulence with a possible devaluation of vaccine effectiveness. Since November 2021, the Omicron (B.1.1.529) coronavirus variant dominated the infection landscape, responsible for the majority of cases (27) and subsequently undergoing considerable genetic mutations (28). The rapid spread of variants is partly due to transmission by individuals with mild or asymptomatic infections (29).

1.2.2 Pathophysiology of SARS-CoV-2 infection and clinical characteristics of COVID-19

SARS-CoV-2 is a single-stranded ribonucleic acid (RNA) virus of the Betacoronavirus genus within the Coronaviridae family. The virion consists of four structural proteins:

spike (S), envelope (E), membrane (M) and nucleocapsid protein (NCP). The M protein coordinates virus particle formation, the E protein contributes to replication and infectivity and the NCP binds viral RNA and facilitates genome packaging (30). The S protein, composed of S1 and S2 subunits, mediates viral entry through interaction of the S1 receptor binding domain (RBD) with the angiotensin-converting enzyme 2 (ACE2) receptor expressed on host cells (31). SARS-CoV-2 primarily infects nasal and bronchial epithelial cells and alveolar pneumocytes, enabling efficient replication in the lower respiratory tract during early infection (32). Viral infection triggers the release of pro-inflammatory mediators from epithelial cells and alveolar macrophages, promoting immune cell recruitment, endothelial barrier disruption, impaired gas exchange and reduced oxygen diffusion - key features of COVID-19 (33). In severe disease, pulmonary edema and hyaline membrane formation reflect early acute respiratory distress syndrome (ARDS) (34), while endothelial inflammation and dysfunction can promote microvascular thrombosis and thromboembolic complications (35).

Clinically, COVID-19 exhibits marked heterogeneity, ranging from asymptomatic infection to severe pneumonia, ARDS, multiorgan failure and death. Disease severity is largely determined by host-related risk factors, including advanced age, male sex, obesity, malignancy, cardiovascular disease, diabetes, chronic lung disease and immunosuppression, all of which affect antiviral immunity and inflammatory regulation (36, 37). These observations emphasize that COVID-19 severity reflects the interaction between viral infection and pre-existing immune and metabolic dysfunction, underscoring the importance of risk stratification and targeted preventive strategies in vulnerable populations.

1.2.3 SARS-CoV-2-specific immune responses

As the number of COVID-19 patients increased, it became even more essential to identify, assess and understand the immune responses induced by SARS-CoV-2 infection or COVID-19 vaccines (38).

1.2.3.1 Innate and adaptive immune responses to SARS-CoV-2 infection

Following SARS-CoV-2 infection, the innate immune system is activated through pattern recognition receptors, including Toll-like receptors (TLRs), with TLR2 and TLR3 playing key roles in sensing viral components and inducing pro-inflammatory cytokines such as

TNF- α and type I IFNs (39). Adaptive immune responses elicited by natural infection or vaccination are critical for viral control (40-44). Humoral immunity is mediated by antibodies targeting viral antigens, primarily the S and NCP, which neutralize infection by blocking viral entry into ACE2-expressing cells (38). Although neutralizing antibodies typically develop rapidly and are maintained by long-lived plasma cells and memory B-cells in many viral infections, SARS-CoV-2 induced antibody responses tend to wane over time (45), with durability influenced by reinfection, booster vaccination and immunosuppressive therapies (46-49).

Cellular immunity plays a central role in antiviral defense against SARS-CoV-2. SARS-CoV-2-specific CD4⁺ T-cells are predominantly Th1-polarized and characterized by IFN- γ production, while CD8⁺ T-cells produce interferon-gamma (IFN- γ) and TNF- α and display cytotoxic activity (38, 50). During acute infection, SARS-CoV-2-specific T-cells exhibit an activated phenotype, transitioning to polyfunctional memory T-cells producing IFN- γ and TNF- α during convalescence (38, 50, 51). Both CD4⁺ and CD8⁺ T-cell responses develop in most patients within one to two weeks of symptom onset and decline toward baseline thereafter. Severe COVID-19 is associated with greater reductions in peripheral T-cell counts, whereas preserved T-cell responses correlate with reduced disease severity. Notably, T-cell immunity may partially compensate for insufficient neutralizing antibody responses and contribute to protection against severe disease (50, 52).

1.2.3.2 COVID-19 vaccine induced immune responses

Vaccination is a cornerstone of infection prevention in patients with autoimmune diseases, although vaccine-induced immunity is shaped by underlying immune dysregulation and immunomodulatory therapy. Autoimmune conditions are associated with impaired antigen presentation and dysregulated T-, and B-cell function, resulting in heterogeneous vaccine responses characterized by reduced antibody titers, impaired memory B-cell formation and variable cellular immunity compared with healthy individuals (53, 54). Immunosuppressive therapies further modulate vaccine responses: csDMARDs, such as MTX reduce immunogenicity by inhibiting lymphocyte proliferation, while B-cell-depleting therapies profoundly impair antibody production, although vaccine-induced T-cell responses are often partially preserved (55). In contrast, agents targeting cytokine signaling pathways, including JAK inhibitors, may also compromise cellular immune

responses (56). Evidence from COVID-19 vaccination has reinforced these observations, with reduced seroconversion rates reported in patients receiving B-cell-targeted therapies, GCS or combination immunosuppression (53), underscoring the importance of assessing both humoral and cellular immunity when optimizing vaccination strategies.

The rapid development of COVID-19 vaccines in response to the global pandemic introduced multiple effective platforms. Messenger ribonucleic acid (mRNA) vaccines, including BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna), use lipid nanoparticle-encapsulated mRNA encoding the SARS-CoV-2 spike protein and demonstrated high efficacy in preventing symptomatic COVID-19 in key clinical trials (50, 57). Viral vector vaccines, such as ChAdOx1 nCoV-19 (AstraZeneca), Gam-COVID-Vac (Sputnik V) and Ad26.COV2.S (Janssen), deliver spike protein genetic material via a replication-deficient viral vector, inducing both humoral and cellular immune responses (51, 58-60). Inactivated vaccines, including BBIBP-CorV (Sinopharm) and CoronaVac, contain chemically inactivated whole virus and elicit immune responses through preserved viral antigen structures (60).

1.2.3.3 SARS-CoV-2-specific immune responses in RA patients

RA is driven by immune dysregulation characterized by activation of antigen-presenting cells, expansion of autoreactive T-, and B-cells and production of autoantibodies such as RF and ACPAs (60, 61). Disease persistence is sustained by excessive cytokine production, which promotes chronic joint inflammation and systemic immune alterations (62, 63). SARS-CoV-2 similarly induces robust innate and adaptive immune responses, and in severe cases, dysregulated immunity results in a cytokine storm marked by elevated IL-6, IL-1 β and TNF- α - cytokines that also play central roles in RA pathogenesis, suggesting potential amplification of shared inflammatory pathways (15, 62).

Immunocompromised RA patients exhibit impaired adaptive immune responses and are at increased risk of SARS-CoV-2 infection and hospitalization, particularly in the presence of high disease activity, comorbidities or immunosuppressive therapy (12). csDMARDs, bDMARDs and tsDMARDs differentially modulate antiviral immunity and may influence both susceptibility to infection and vaccine responsiveness (64). Consequently, patients with RA are prioritized for primary and booster COVID-19

vaccination; however, vaccine-induced immunity is often attenuated under ongoing immunosuppression, necessitating earlier or more frequent booster strategies to achieve adequate protection (12, 64-67).

Beyond acute infection, SARS-CoV-2 has been implicated in triggering or unmasking autoimmune phenomena through mechanisms such as molecular mimicry, epitope spreading. Reports of RA-like inflammatory arthritis following COVID-19 raise the possibility that SARS-CoV-2 may act as an environmental trigger for RA in genetically predisposed individuals, although definitive causal relationships remain to be established (68).

1.3 Healthcare workers (HCWs) and RA patients as high-risk populations during the COVID-19 pandemic

HCWs have represented a population at increased risk of SARS-CoV-2 exposure since the beginning of the COVID-19 pandemic due to repeated contact with infected individuals and sustained viral circulation in healthcare settings. Early reports from affected regions documented substantial infection rates among HCWs, with occupational exposure identified as a major risk factor (69-71). Beyond individual morbidity, infections among HCWs threatened healthcare system capacity and continuity of care. The rapid development and application of mRNA vaccines marked a critical milestone in pandemic control. Clinical trials demonstrated the high efficacy of BNT162b2 in preventing symptomatic COVID-19 (57) and HCWs were prioritized for vaccination in many countries. Immunological studies have shown that mRNA vaccination induces robust humoral and cellular immune responses: anti-S and neutralizing antibody titers peak after the second vaccine dose (72), while SARS-CoV-2-specific CD4⁺ and CD8⁺ T-cell responses contribute to antiviral protection (73). However, antibody levels decline over time (74) and the emergence of viral variants with partial immune escape has raised concerns about post-vaccination infections (75).

While HCWs represent a population at increased occupational risk, their interaction with immunocompromised patient populations, including RA patients, introduces additional epidemiological and immunological complexity. RA is characterized by systemic immune dysregulation, chronic cytokine activation and altered adaptive immune responses (15). Furthermore, immunomodulatory therapies may attenuate antiviral immunity and reduce

vaccine immunogenicity, particularly affecting T-cell activation and humoral responses (20). Within healthcare systems, RA patients and HCWs therefore constitute a bidirectional transmission interface (Figure 2). Immunocompromised patients may exhibit prolonged viral shedding, increasing occupational exposure risk for HCWs, while infected HCWs - particularly during asymptomatic or presymptomatic phases - may transmit SARS-CoV-2 to vulnerable patients. This reciprocal dynamic represents a state of heightened epidemiological vulnerability. Consequently, investigating SARS-CoV-2 induced immune responses in both RA patients and HCWs, provides insight into host immunity under conditions of immune dysregulation and high exposure risk, thereby informing vaccination strategies and infection-prevention policies within healthcare settings.

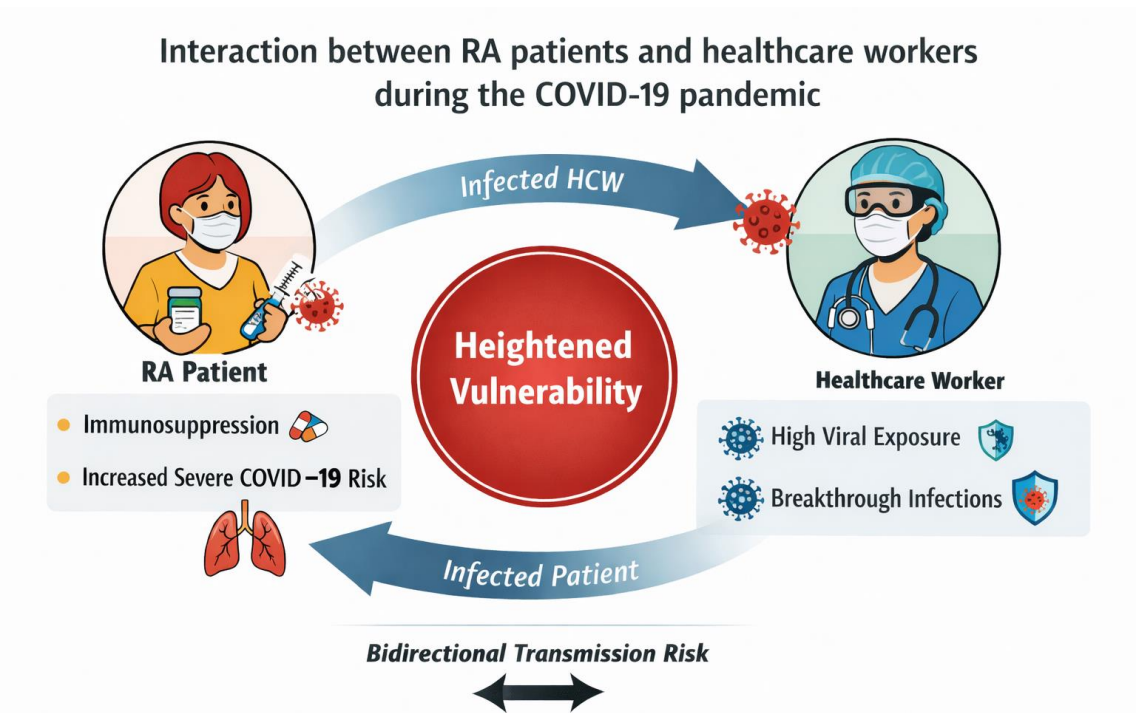


Figure 2. Interaction between RA patients and healthcare workers (HCWs) during the COVID-19 pandemic. Immune dysregulation and immunosuppressive therapy increase susceptibility in RA, while sustained occupational exposure elevates infection risk in HCWs. Arrows indicate two-way transmission, resulting in heightened epidemiological vulnerability. *The figure was created using OpenAI GPT-5.3 based on the author's conceptual design.*

2 Objectives

The primary objectives of this investigation were:

- to characterize the humoral and cellular immune responses to SARS-CoV-2 in RA patients and healthy individuals,
- to determine the factors that have significant impact on the SARS-CoV-2 induced immunity,
- to study the dynamic change and the longevity of SARS-CoV-2-specific immune responses in RA patients and healthy controls over prolonged time,
- to assess the effectiveness and the humoral immunogenicity of primary and booster BNT162b2 vaccination in HCWs.

3 Methods

3.1 RA patients

All patients diagnosed with RA were enrolled from the rheumatology outpatient department of Semmelweis University (Buda Hospital of the Hospitaller Order of Saint John of God) in Budapest, Hungary. The research was conducted with the approval number IV/2021-1/2021/EKU. Patients in the study were adults aged 18 years or older who received COVID-19 vaccination and/or had previously confirmed SARS-CoV-2 infection and were diagnosed with RA based on the classification criteria established by the American College of Rheumatology/European League Against Rheumatism in 2010 (76). 109 RA patients were included in the study during the initial period (08.2021-12.2021), while the data of 84 patients were analyzed during the follow-up period (10.2022-03.2023). A control group consisting of 43 healthy individuals was included who were vaccinated against COVID-19 and/or had previous SARS-CoV-2 infection. Peripheral blood samples were collected to assess both the humoral and the cellular immune responses elicited by SARS-CoV-2. All participants provided written, informed consent (77, 78).

3.2 HCWs

This prospective observational cohort study included HCWs of the Buda Hospital of the Hospitaller Order of St. John of God, Budapest, Hungary. Participants were employees aged ≥ 18 years who received two doses of the BNT162b2 COVID-19 vaccine between 01.2021 and 03.2021. Of 345 vaccinated individuals, 299 consented to follow-up; 294 were included in the final analysis after excluding those who did not complete primary immunization or had fewer than three antibody measurements. The cohort comprised physicians, nurses and other healthcare staff. Participants were stratified by prior SARS-CoV-2 infection status. Confirmed infection required compatible symptoms and positive polymerase chain reaction (PCR) or antigen testing. Presumed SARS-CoV-2 infection was defined by anti-NCP antibody positivity. Individuals without clinical history and persistently negative anti-NCP antibodies were classified as SARS-CoV-2 infection-naive.

A third (booster) dose was administered voluntarily, either homologous or heterologous, according to national recommendations. Participants were followed from 01.2021 to

06.2022 for SARS-CoV-2 infection, vaccine-related adverse events and humoral immune responses. For antibody kinetic analyses, individuals with intercurrent infection were excluded from the respective time-point analyses. The study was approved by the Hungarian Medical Research Council Scientific and Research Ethics Committee (ETT-TUKEB; IV-1287-1/2021/EKU) and conducted in accordance with the Declaration of Helsinki (79, 80).

3.3 Assessment of SARS-CoV-2-specific antibodies (Elecsys® Anti-SARS-CoV-2 S assay, 95 Roche; Elecsys® Anti-SARS-CoV-2 N assay, 95 Roche; Euroimmun ELISA test)

The Elecsys Anti-SARS-CoV-2 S immunoassay, developed by Roche, was utilized for analyzing SARS-CoV-2-specific antibodies. This assay, conducted on the Cobas e6000 instrument, is designed to identify antibodies directed against the RBD of the S protein of SARS-CoV-2 in human serum and plasma samples. Applying a recombinant protein representing the RBD of the S antigen, the assay follows a double-antigen sandwich format, effectively detecting high-affinity immunoglobulin A (IgA), G (IgG), M (IgM) against SARS-CoV-2 quantitatively. The assay's limit of quantification is 0.4 U/ml, indicating that results below this threshold are considered negative, while results equal to or above 0.8 U/ml are considered positive, as announced in the test documentation (81). The Elecsys Anti-SARS-CoV-2 N immunoassay was used to measure the level of anti-NCP in HCWs. In this group the positivity cut-off index (sample signal/cut-off signal) for the NCP antibody is ≥ 1.0 .

The Euroimmun enzyme-linked immunosorbent assay (ELISA) test was used to measure the level of specific antibodies (IgG) against the NCP from the SARS-CoV-2 virus in blood samples. This test offers a semi-quantitative evaluation, providing an approximate measurement rather than an exact value. Results are expressed as a ratio, comparing the absorbance of the sample to that of a standard calibrator. A ratio below 0.8 is interpreted as negative for the presence of antibodies, while ratios equal to or exceeding 1.1 are considered positive, indicating the presence of antibodies based on the test's guidelines (77, 78, 82).

3.4 Evaluation of IFN- γ T-cell responses (QuantiFERON (QIAGEN Group))

A small volume (1 ml) of heparinized whole blood was exposed to specific antigens (such as S1, S2 and RBD subdomains) known to trigger immune responses in particular types of T-cells (CD4⁺ and CD4⁺CD8⁺ T-cells). In one test tube, additional specific peptides were introduced to target various segments of the SARS-CoV-2 virus (spike, nucleocapsid and M protein domains), aiming to elicit a more comprehensive immune response from T-cells (referred to as Ag3). Following an incubation period at 37°C lasting 16-24 hours, the levels of IFN- γ were quantified using the ELISA QuantiFERON test, following the manufacturer's instructions (Figure 3) (83). While the manufacturer's data sheet suggested that an IFN- γ level ranging between 0.15 IU/ml and 0.2 IU/ml should be considered positive, for our study, we set the positive cut-off threshold at 0.15 IU/ml (77, 78).

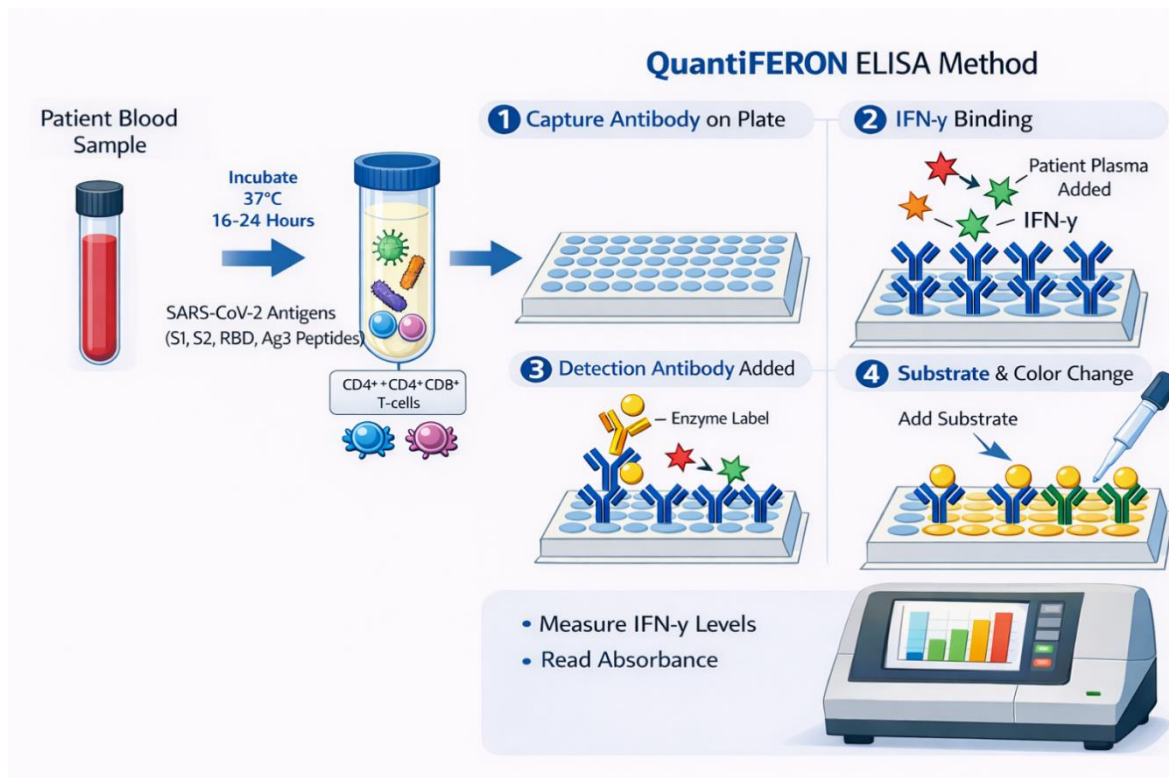


Figure 3. Assessment of SARS-CoV-2-specific interferon- γ (IFN- γ) T-cell responses using the QuantiFERON ELISA. Heparinized whole blood is incubated for 16-24 h at 37°C with SARS-CoV-2 antigen peptide pools (S1, S2, RBD, Ag3) to stimulate antigen-specific CD4⁺ and CD8⁺ T cells. Released IFN- γ in plasma is quantified using a QuantiFERON enzyme-linked immunosorbent assay (ELISA). Plasma is added to microplate wells pre-coated with anti-IFN- γ capture antibodies, allowing cytokine binding. A secondary enzyme-labeled detection antibody is then applied, followed by

substrate addition to generate a colorimetric reaction proportional to IFN- γ levels. Absorbance is measured using a microplate reader. *The figure was created using OpenAI GPT-5.3 based on the author's conceptual design.*

3.5 Statistical analysis

3.5.1 Baseline study

Demographic characteristics and immunogenicity outcomes were characterized using descriptive statistical methods. Categorical variables were descriptively characterized by counts and proportions, whereas continuous variables - including whole COVID virus-specific CD4+ and CD8+ T-cell response (CW 4-8) and anti-S antibody levels - were characterized as medians and interquartile ranges (IQR). Differences between healthy controls and RA patients were assessed using non-parametric statistical tests. The “Sampling Interval” was defined as the time elapsed between vaccination or documented SARS-CoV-2 infection and blood sample collection for immunological analyses. For reporting purposes, this interval was expressed in months (30 days per month); however, all statistical analyses were performed using the exact dates (in days). Age, sex, Sampling Interval and COVID-19 vaccine type were considered potential confounding variables. Primary outcome measures included anti-S antibody concentrations, representing humoral immune responses and CW 4-8, reflecting cellular immunity. The impact of the following explanatory variables on immunological outcomes was analyzed: cohort (healthy control vs. RA patient), MTX treatment, csDMARD therapy (MTX or leflunomide), systemic GCS treatment and targeted immunomodulatory therapy. To increase statistical power, targeted therapies were grouped according to shared mechanisms of action, including TNF- α inhibitors (etanercept, infliximab), CD20-targeting therapy (rituximab) and JAK inhibitors. A reference category was defined for healthy participants and RA patients not receiving targeted therapy. Vaccine type was categorized into five levels using commonly applied nomenclature in Hungary: BNT162b2, mRNA-1273, ChAdOx1-S, Gam-COVID-Vac and BBIBP-CorV. An additional category (“Infected”) included individuals with prior SARS-CoV-2 infection who had not received vaccination against COVID-19. Multivariable linear regression models with logarithmically transformed outcome variables were applied to account for confounding effects. The initial full model included cohort, age (years), sex (male/female), Sampling Interval (months), targeted therapy (four levels), vaccine type

(five levels), MTX use (yes/no) and GCS use (yes/no). Model selection was performed using best-subset regression implemented via the leaps package in R (84). Exponentiated regression coefficients were reported and interpreted as geometric mean ratios relative to the reference category or per unit increase for continuous predictors. Statistical significance was determined based on the regression coefficients of the explanatory variables. No adjustment for multiple hypothesis testing was applied. All analyses were conducted using R (85), tables and figures were generated using the gtsummary (86) and ggplot2 (87) packages (78).

3.5.2 Follow-up study

Time intervals were defined as follows: the “Sampling 1 interval” was the number of days between the most recent immunological event (COVID-19 vaccination or confirmed SARS-CoV-2 infection) and the first sampling; the “Sampling 2 interval” was the time in days between the first and second sampling; the “COVID interval” was the number of days between a confirmed SARS-CoV-2 infection (positive NCP IgG test) and the second sampling; and the “Vaccine interval” was the number of days between an additional COVID-19 vaccination during the interim period and the second sampling. The “Event interval” was defined as the number of days between the most recent interim event (vaccination against COVID-19 or SARS-CoV-2 infection) and the second sampling. In the absence of an interim event following Sampling 1, the “Event interval” was set equal to the “Sampling 2 interval”. Interval definitions and median (IQR) values are shown in Figure 4. Measurements were classified into “Sampling 1”, “Sampling 2”, “Sampling 2+ Vaccination” or “Sampling 2+Infection” subgroups based on events occurring between the two sampling points. Classification was determined by the most recent immunological event prior to the second sampling; participants who were vaccinated and subsequently infected were therefore assigned to the “Sampling 2+Infection” subgroup (Figure 5). Stratification was not applied to the first sampling period, as all participants had experienced at least one prior immunological event. Vaccine exposure differed between periods, with heterogeneous vaccine types used initially and only RNA-based vaccines administered during the interim period. Data presented in Figure 5 were analyzed using stepwise linear regression with non-significant variables (gender, study group, MTX use and low-dose GCS therapy) excluded from the final model.

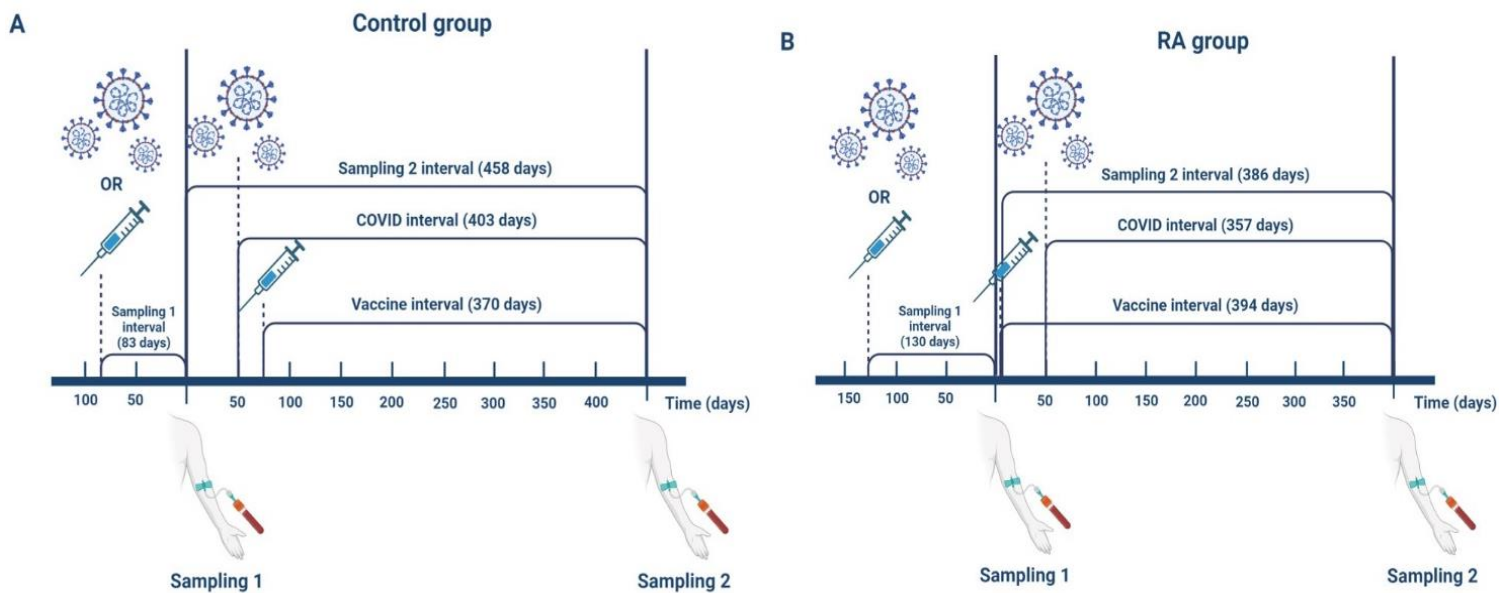


Figure 4. Interval definitions in the control (A) and RA (B) groups. Values are median (IQR). The “Sampling 1 interval” defines days from the most recent immunological event (SARS-CoV-2 infection or COVID-19 vaccination) to the first sampling (control: 83 [37–190]; RA: 130 [81–169]). The “Sampling 2 interval” defines days between the first and second sampling (control: 458 [410–547]; RA: 386 [311–448]). The “COVID interval” indicates days from confirmed SARS-CoV-2 infection (positive NCP IgG) to the second sampling (control: 403 [267–543]; RA: 357 [245–448]). The “Vaccine interval” denotes days from interim COVID-19 vaccination to the second sampling (control: 370 [330–405]; RA: 394 [342–453]). The “Event interval” is the time in days from the most recent interim event (COVID-19 vaccination or SARS-CoV-2 infection) to the second sampling, whichever occurred later (control: 194 [67–327]; RA: 321 [201–378]). If no interim event occurred after Sampling 1, the “Event interval” equaled the “Sampling 2 interval” (77).
Created with BioRender.com.

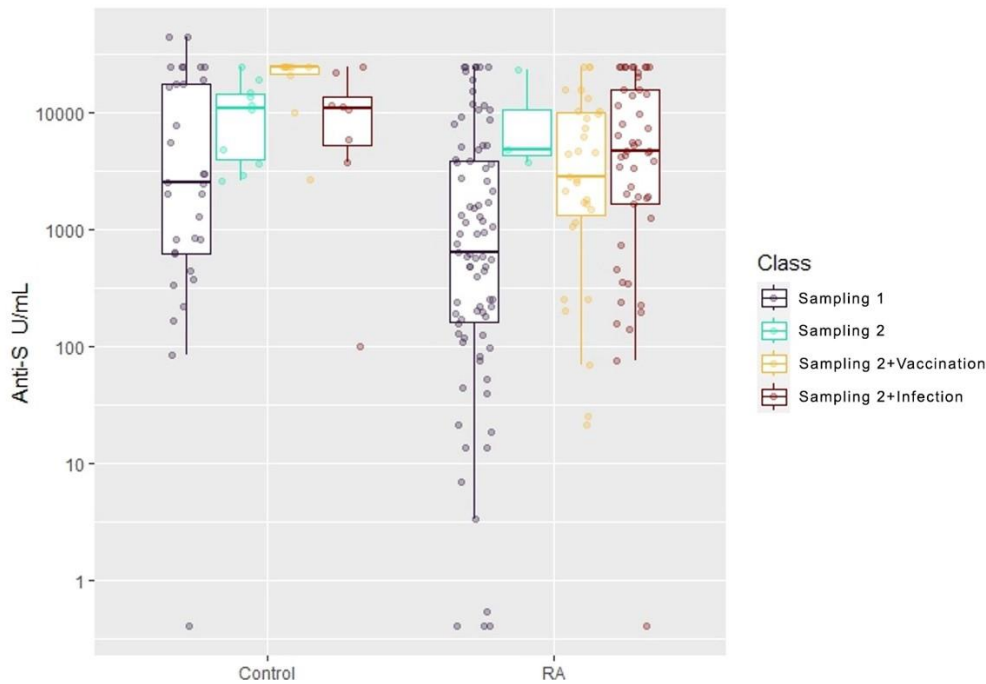


Figure 5. Boxplots of anti-spike (S) antibody levels by study group (healthy controls and RA patients) and subgroup. Participants were stratified by sampling period and interim events: “Sampling 1” includes first-period measurements; “Sampling 2” includes second-period measurements without interim COVID-19 vaccination or SARS-CoV-2 infection; “Sampling 2 + Vaccination” includes participants vaccinated between sampling points; and “Sampling 2 + Infection” includes participants with confirmed SARS-CoV-2 infection during the same interval. When both events occurred, classification was based on the most recent event (77).

Continuous variables were summarized as median (IQR) and analyzed using the Wilcoxon rank-sum or unpaired t test, as appropriate. Categorical variables were expressed as n (percent) and analyzed using the chi-square test. P-values are nominal and unadjusted for multiple hypothesis testing. Linear regression was used to assess associations between anti-S antibody concentrations and explanatory variables. The initial model included gender, age, study group (healthy control vs. RA), “Event interval”, type of most recent immunological event (COVID-19 vaccination or SARS-CoV-2 infection) and sampling period (Sampling 1 vs. Sampling 2). Anti-S concentrations were right-skewed and were therefore log-transformed prior to analysis. Anti-S half-life was estimated as $\log(0.5) / \text{“Event interval”}$, where “Event interval” refers to the estimated regression coefficient for the “Event interval” term. Because targeted therapy was

administered exclusively to RA patients, study group, age and targeted therapy were treated as interdependent variables and model interpretation focused on overall fit rather than individual coefficients. Two models were fitted: Model 1 including SARS-CoV-2 infection, COVID-19 vaccination, “Event interval” and “Sampling time point”; and Model 2, additionally including study group, age and targeted therapy. Models were compared using the F test, which demonstrated a significant improvement in fit ($F = 7.34$; $df = 208, 211$; $p = 0.0001059$), providing evidence of marked differences between the patient cohort and healthy volunteers. Associations between anti-S antibody levels and CW4–8 T-cell responses were evaluated using Spearman’s rank correlation. Correlation strength was classified as high ($\rho > 0.7$), moderate ($0.5 \leq \rho \leq 0.7$), or low ($\rho < 0.5$). All analyses were two-sided, with $p < 0.05$ considered statistically significant. Data analyses were conducted using R (85) and tables and figures were generated using the gtsummary (86) and ggplot2 (87) packages (77).

3.5.3 Study among HCWs

Continuous variables (S and NCP antibody levels) were expressed as mean $\pm$ standard deviation, while nonparametric data were summarized by their median values. The normality of the data distribution was assessed using the Shapiro–Wilk test and homogeneity of variances was evaluated with Levene’s test. For nonparametric distributions, group comparisons were performed using the Mann-Whitney test at a 95% confidence level. Observational data were analyzed using contingency tables, and independence between variables was examined with the χ^2 test. Binary logistic regression analysis was applied to identify variables influencing infection. All analyses were two-sided and the threshold for statistical significance was set at $p < 0.05$ ($\alpha = 0.05$). Statistical analyses were performed using Minitab (State College, PA, United States of America), IBM® SPSS® 28.0 (IBM Corporation, Armonk, NY, USA), and Statistica 13.5 (TIBCO Software Inc., Palo Alto, CA, USA) (79, 80).

4 Results

4.1 Study population

4.1.1 Baseline study

In the baseline study, we examined 109 RA patients and 43 healthy controls, the demographic information and the clinical characteristics of the participants are outlined in Table 1. We assessed the RA disease activity by clinical examination through the disease activity score 28-CRP (DAS28-CRP), with a median score of 2.6. DAS28-CRP scores above 5.1 indicate high disease activity, while scores between 3.2 and 5.1 indicate moderate activity, 2.6 to 3.2 suggest low activity and scores below 2.6 indicate remission (88). During the initial period we analyzed data related to the vaccination schedules of two COVID-19 vaccines for all participants. The majority of both RA patients and controls received BNT162b2 vaccine (75 RA patients and 34 controls), while 19 participants received mRNA-1273 vaccine. Additionally, participants who received vaccines using modified adenovirus technology, such as ChAdOx1s vaccine (6 participants) or Gam-COVID vaccine (5 participants), were included. Five participants received BBIBP-CorV vaccine. The median interval (IQR) between either the first day of confirmed SARS-CoV-2 infection or the day of the last vaccination against COVID-19 was 3.80 months for the control group and 3.67 months for the RA group. 75 RA patients (69%) and 23 controls (53%) were vaccinated against COVID-19 (no previous SARS-CoV-2 infection); 30 RA patients (28%) and 16 controls (37%) were vaccinated against COVID-19 and had previous SARS-CoV-2 infection 4 RA patients (3.7%) and 4 controls (9.3%) had previous SARS-CoV-2 infection but remained unvaccinated (Table 1). 33 RA patients had SARS-CoV-2 infection, all experiencing associated symptoms. Significant differences were noted in terms of age ($p<0.001$) and gender ($p<0.001$) between the two groups (Table 1). The majority of RA patients were treated with csDMARDs such as MTX, leflunomide, sulphasalazine, or hydroxychloroquine (N=80, 73%), with or without low-dose GCS (below 7.5 mg/day prednisone or equivalent), primarily with MTX (N=64, 59%). 25 patients (23%) received low-dose GCS therapy. In the RA group, 51 patients (47%) received TNF- α inhibitor treatment (adalimumab, etanercept, certolizumab, infliximab or golimumab), 15 (14%) were treated with IL-6 inhibitor (tocilizumab), 4 (3.7%) with rituximab therapy, and 5 (4.6%) with JAK inhibitors (baricitinib, tofacitinib), in all cases with or without DMARD/GCS (Table 1). To assess SARS-CoV-2-specific

immune responses in healthy controls, 36 individuals provided samples for serological analysis, and 39 provided samples for the analysis of T-cell specific responses (78).

Table 1. Patient characteristics during the baseline study. ¹n (%); Median (IQR). ²Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test. "Sampling Interval" represents the duration in months (1 month = 30 days) between a SARS-CoV-2 infection or the last COVID-19 vaccine dose and laboratory sample collection. csDMARDs: conventional synthetic disease-modifying antirheumatic drugs. GCS: glucocorticoids (78).

Variable	Healthy, N = 43 ¹	RA, N = 109 ¹	p-value ²
Gender			<0.001
Male	25 (58%)	14 (13%)	
Female	18 (42%)	95 (87%)	
Age	35 (29,48)	59 (46,66)	<0.001
Vaccination			<0.001
ChAdOx1s	1 (2.3%)	5 (4.6%)	
BNT162b2	34 (79%)	75 (69%)	
Gam-COVID	4 (9.3%)	1 (0.9%)	
mRNA-1273	0 (0%)	19 (17%)	
BBIBP-CorV	0 (0%)	5 (4.6%)	
Sampling Interval (months)	3.80 (1.73, 6.85)	3.67 (2.03, 5.50)	0.3
COVID status			0.13
Recovered from COVID-19 (unvaccinated)	4 (9.3%)	4 (3.7%)	
Recovered from COVID-19 and vaccinated	16 (37%)	30 (28%)	

Vaccinated (no COVID infection)	23 (53%)	75 (69%)	
Therapy			
Targeted therapies			
anti-CD20	-	4 (3.7%)	
IL-6 inhibitor	-	15 (14%)	
JAK inhibitor	-	5 (4.6%)	
TNF- α inhibitor	-	51 (47%)	
MTX	-	64 (59%)	
csDMARDs other than MTX	-	16 (15%)	
GCS	-	25 (23%)	

4.1.2 Follow-up study

In our follow-up study, 84 RA patients and 43 healthy controls were enrolled (Table 2). RA disease activity was measured by DAS28-CRP, with a median score of 2.6. Among RA patients, 63 individuals (75%) were treated with MTX, either as monotherapy or in combination with other medications. Additionally, 20 patients (24%) received low-dose GCS therapy (below 7.5 mg/day prednisone or its equivalent). The majority of RA patients (75 out of 84; 89%) received targeted therapy, including TNF- α inhibitors, IL-6 inhibitor, rituximab or JAK inhibitors. In the initial phase of our study, SARS-CoV-2 infection was confirmed through positive PCR test. In the second phase, SARS-CoV-2 infection was affirmed by detecting IgG antibodies against NCP as a marker of response. Throughout the interim period, only RNA-based vaccines like BNT162b2 vaccine and mRNA-1273 vaccine were administered. Figure 4 illustrates that during the follow-up phase, the duration between the events prompting the SARS-CoV-2-specific immune response and the time of sampling was notably extended compared to the initial period (77).

Table 2. Patient characteristics during the follow-up study. ¹TNF- α inhibitors, IL-6-inhibitor, rituximab, JAK inhibitors; ²During the initial phase of the study, SARS-CoV-2 infection was verified by a positive PCR test. ³In the second test part, the infection was confirmed through a positive NCP IgG antibody test. ⁴BNT162b2, mRNA-1273, ChAdOx1s, Gam-COVID, BBIBP-CorV COVID-vaccines were administrated. Continuous variables are reported as median (IQR) and analyzed with the Wilcoxon rank-sum test, whereas categorical variables are reported as n (%) and analyzed using the chi-square test. RA: rheumatoid arthritis; NCP: nucleocapsid protein; MTX: methotrexate (77).

Characteristic	Control, N =43	RA, N = 84	p-value
Sex			<0.001
Male	22 (51%)	13 (15%)	
Female	21 (49%)	71 (85%)	
Age	42 (33, 53)	63 (52, 69)	<0.001
Therapy status			
MTX	Not applicable	63 (75%)	
Glucocorticoid	Not applicable	20 (24%)	
Targeted therapy ¹	Not applicable	75 (89%)	
SARS-CoV-2 infection status			
SARS-Cov-2 positivity during Sampling 1 Positive PCR test ²	26 (60%)	38 (45%)	0.10
SARS-Cov-2 positivity during Sampling 2 Positive NCP IgG ³ ($\geq$ 1.1)	16 (57%)	29 (35%)	0.039
COVID-vaccination status⁴			
4 doses of COVID-vaccination	3 (11%)	12 (14%)	
3 doses of COVID-vaccination	21 (75%)	57 (68%)	

2 doses of COVID-vaccination	3 (11%)	13 (15%)	
1 dose of COVID-vaccination	0	1 (1%)	
Unvaccinated	1 (4%)	1 (1%)	

4.2 Anti-SARS-CoV-2 antibody response

4.2.1 Baseline study

In patients with RA, 94% (102/109) of them had positive anti-S antibody response, whereas in the control group, all 36 participants (100%) had positive response (Table 3). The median (IQR) concentration of anti-S antibodies in the RA group was 724 U/ml (99, 3909), as shown in Table 3 and Figure 6A. Figure 6B shows the levels of anti-S antibodies concerning different drug treatments, while Figure 6C illustrates the concentration of SARS-CoV-2-specific antibodies after various vaccine types in the whole study population. For those who received mRNA-1273 vaccine, their anti-S antibody levels were significantly higher (71.1 times, $p<0.001$) compared to unvaccinated individuals with prior SARS-CoV-2 infection (Table 4). Similarly, BNT162b2 vaccine increased anti-S antibody levels by 36 times ($p<0.001$) and ChAdOx1s vaccine raised them by 18.1 times ($p=0.037$) compared to those who were not vaccinated (Table 4). Additionally, Figure 7A, 7B indicates that individuals who were vaccinated after SARS-CoV-2 infection (red dots) had higher anti-S titers compared to those who were not previously infected (black dots), consistent with previous observations (89). Our optimized regression model, as detailed in Table 4, revealed significant effects of age and longer Sampling Interval on SARS-CoV-2-specific antibody levels in the whole study population. Specifically, anti-S antibody levels decreased by 25% ($p<0.001$) each month following SARS-CoV-2 infection or vaccination against COVID-19 (Figure 7A) and there was a 4% decrease associated with each year of age ($p=0.004$) (Figure 7B). Regarding different therapies, MTX therapy did not significantly impact the anti-S antibody response ($N=64$; $p=0.087$). However, anti-CD20 therapy notably decreased the SARS-CoV-2-specific antibody levels; rituximab-treated RA patients had 97% lower levels than expected compared to patients who were not treated with anti-CD20 ($N=4$; $p=0.002$) (Table 4). TNF- α inhibitors and IL-6 inhibitor also significantly decreased anti-

S antibody levels by 80% (N=51; p<0.001) and 74% (N=15; p=0.049), respectively (Table 4). By contrast, JAK inhibitors showed no significant effect on the SARS-CoV-2-specific antibody response (N=5; p=0.6) (Table 4). The covariate adjusted effects of the disease itself were 0.42 (i.e. 58% decrease; p=0.3) for anti-S antibody response. The estimated effect of the immunosuppressant drugs potentially can confound the disease as a contributing factor itself. However, the estimated negative effect on the anti-SARS CoV-2 responses remained essentially the same regardless whether the Group factor (control, RA) was included into the regression model or not. Furthermore, these results did not change when the analysis was narrowed down to the patient group (78).

Finally, the expected anti-S antibody curves following BNT162b2 vaccination for four hypothetical cases (S1-S4) are illustrated in Figure 8A. Case S4, a 75-year-old patient treated with TNF- α inhibitor therapy, showed the lowest expected anti-S antibody level due to older age and TNF- α inhibitor therapy (78).

Figure 9 represents the difference in anti-S immune response between anti-cyclic citrullinated peptide (anti-CCP) antibody negative and positive patients. It is important to note that these are observed data, while the statistical conclusion is drawn from covariate-adjusted data. For instance, it can be ruled out that the difference in anti-S antibody levels between anti-CCP positive and anti-CCP negative patients is due to an imbalance in treatment between the two groups (78).

Table 3. The humoral and the cellular immune responses elicited by SARS-CoV-2 during the baseline study. ¹n (%); Median (IQR). ²Pearson's Chi-squared test; Wilcoxon rank sum test; Fisher's exact test (78).

Variable	Healthy ¹	RA, N=109 ¹	p-value ²
Positive anti-S response (N) Total (N=36)	36 (100%)	102 (94%)	
anti-S response (U/ml)	1,708 (686, 6,579)	724 (99, 3,909)	0.017
Positive T-cell response (CD4+ or CD8+) (N) Total (N=39)	34 (87%)	57 (52%)	
COVID spike-specific CD4+ T-cell response (IU/ml)	0.41 (0.16, 1.07)	0.08 (0.02, 0.41)	<0.001

COVID spike-specific CD4+ and CD8+ T-cell response (IU/ml)	0.53 (0.30, 1.32)	0.10 (0.03, 0.46)	<0.001
Whole COVID virus-specific CD4+ and CD8+ T-cell response (IU/ml)	0.60 (0.30, 1.56)	0.16 (0.04, 0.94)	<0.001

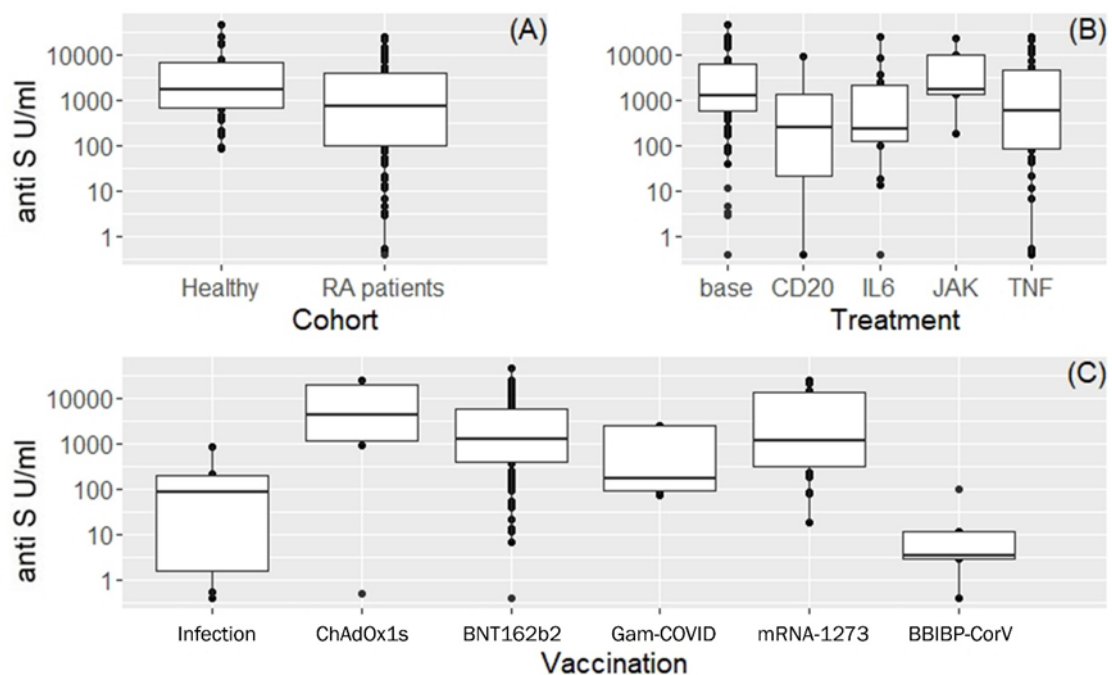


Figure 6. Box plots showing COVID-19 vaccine- or SARS-CoV-2 infection induced anti-spike (S) antibody titers by demographic and clinical characteristics. (A) Comparison of anti-S antibody titers between study cohorts. (B) Anti-S antibody titers by targeted treatment categories. The Base group includes healthy controls and RA patients receiving conventional synthetic disease-modifying antirheumatic drugs (csDMARDs) and/or glucocorticoids (GCS). (C) Anti-S antibody titers following different COVID-19 vaccines used in Hungary in RA patients and healthy controls (78).

Table 4. Results of the optimized multivariable regression analysis for anti-S antibody concentrations. TNF- α antagonist therapy was associated with an approximately 80% reduction in anti-S antibody levels relative to the reference group ($p < 0.001$), while IL-6 inhibitor treatment was associated with a 74% reduction ($p = 0.049$). CD20-targeted therapy showed the strongest effect, with antibody levels reduced to approximately 3%

of control values ($p=0.002$). Anti-S antibody concentrations declined by an estimated 25% per month after immunization ($p<0.001$) and by 4% per additional year of age ($p=0.004$) (78).

Characteristic	exp (Beta)	95% CI ¹	p-value
Sampling Interval	0.75	0.65, 0.86	<0.001
Vaccination			
ChAdOx1s	18.1	1.22, 269	0.037
BNT162b2	36.0	5.51, 235	<0.001
Gam-COVID	3.08	0.19, 49.9	0.4
mRNA-1273	71.1	8.74, 579	<0.001
BBIBP-CorV	0.25	0.02, 4.16	0.3
Age	0.96	0.93, 0.99	0.004
Targeted therapies			
anti-CD20	0.03	0.00, 0.26	0.002
IL-6 inhibitor	0.26	0.07, 0.98	0.049
JAK inhibitor	1.75	0.20, 15.2	0.6
TNF- α inhibitor	0.20	0.08, 0.49	<0.001
¹ CI = Confidence Interval			

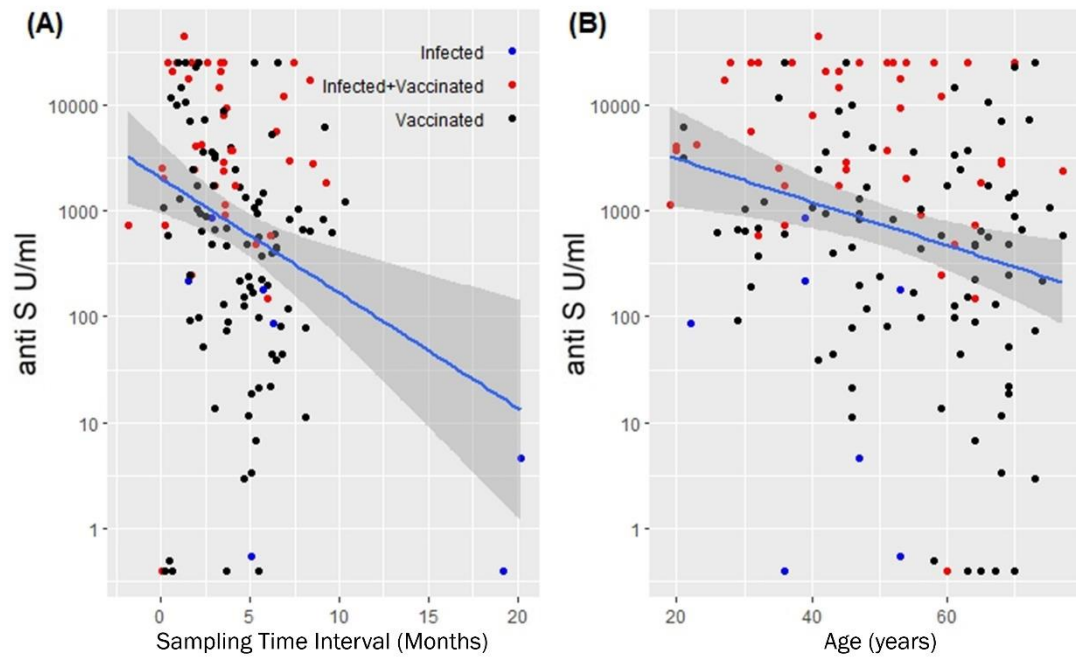


Figure 7. Regression analyses showing associations between anti-spike (S) antibody titers, time since COVID-19 vaccination (A) and age (B). Unadjusted regression lines with 95% confidence intervals are shown for the full study population. Adjusted models revealed a 25% monthly decline in antibody levels post-vaccination and a 4% decrease per year of age. Data points are color-coded according to subcohort (previously infected, infected and subsequently vaccinated and vaccinated without documented infection) (78).

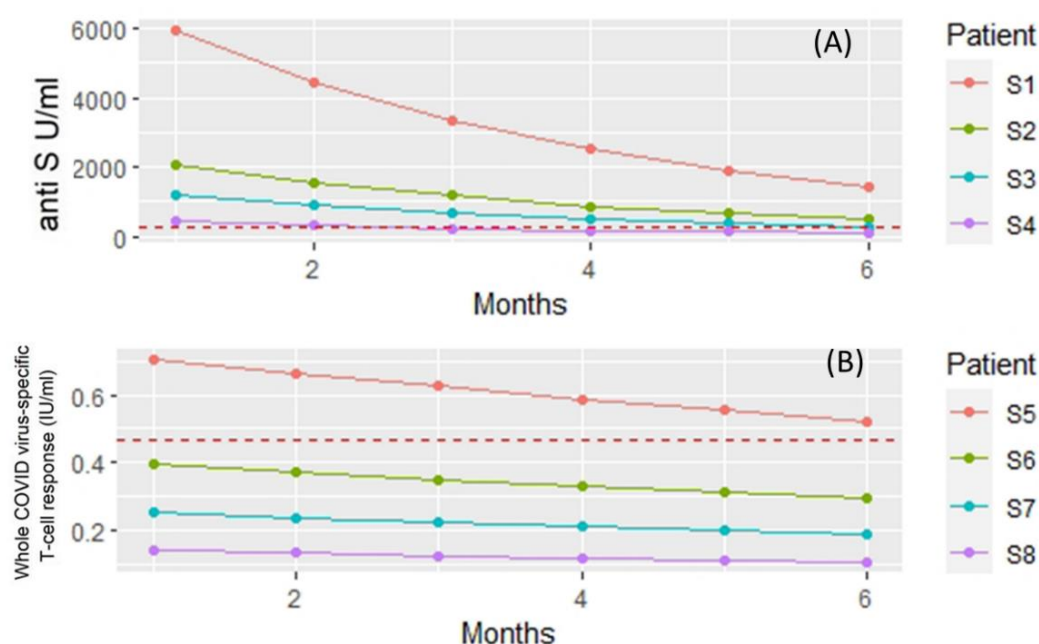


Figure 8. Model-predicted anti-spike (S) antibody and T-cell responses based on patient and treatment characteristics. Linear regression models were used to estimate immune responses for representative subjects. (A) Predicted anti-S concentrations following BNT162b2 vaccination by age and TNF- α inhibitor use: S1 patient: 50 years/no TNF- α inhibitor; S2 patient: 75 years/no TNF- α inhibitor; S3 patient: 50 years/TNF- α inhibitor; S4 patient: 75 years/TNF- α inhibitor. (B) Predicted SARS-CoV-2-specific CD4⁺ and CD8⁺ T-cell responses following BNT162b2 vaccination by age and rituximab use: S5 patient: 50 years/no rituximab; S6 patient: 75 years/no rituximab; S7 patient: 50 years/rituximab; S8 patient: 75 years/rituximab. Red dashed lines indicate mean responses in unvaccinated controls (78).

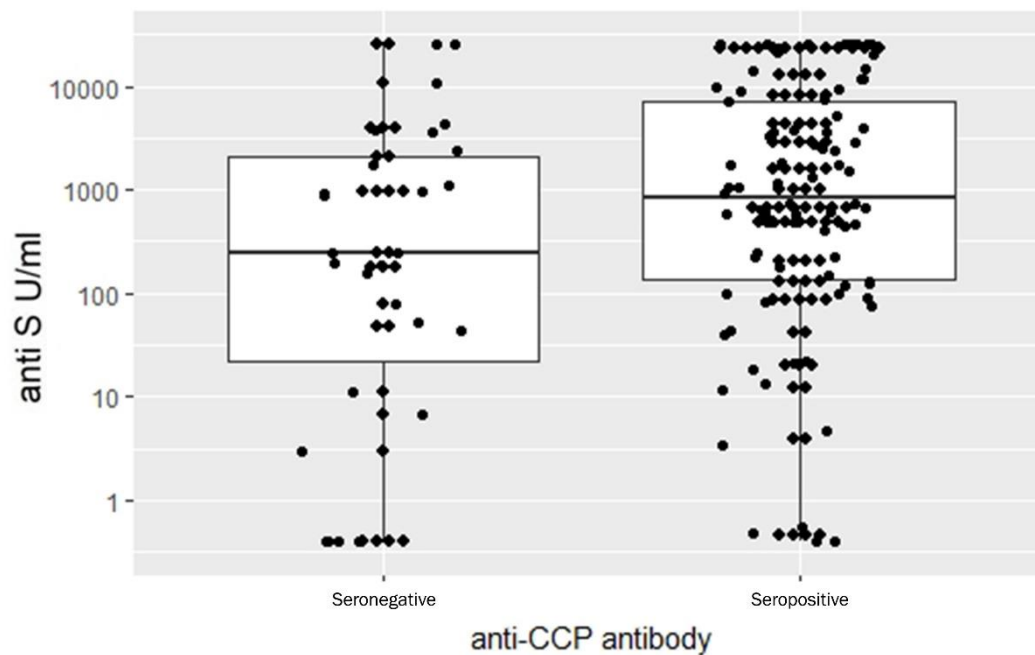


Figure 9. Comparison of anti-spike (S) levels between anti-cyclic citrullinated peptide (CCP) negative and seropositive RA patients. Observed data indicate a highly significant difference between the two groups ($p = 0.00165$) (78).

4.2.2 Follow-up study

Regardless of the sampling time point, RA patients had significantly lower anti-S antibody levels than healthy controls (Sampling 1: $p=0.008$; Sampling 2: $p<0.001$). At follow-up (Sampling 2), the median (IQR) anti-S antibody concentration was 4548 U/ml (1567–13949) in RA patients and 12813 U/ml (5632–25000) in healthy controls. Compared with Sampling 1, a significant increase in the SARS-CoV-2-specific humoral immune response was observed at Sampling 2 in both study groups (healthy controls: $p<0.05$; RA patients: $p<0.0001$, Wilcoxon signed-rank test) (Figure 10A). Specifically, 63% (53/84) of RA patients and 82% (14/17) of healthy individuals exhibited an increased SARS-CoV-2-specific immune response compared to their baseline results (shown in Figure 11). Table 5 shows that the effect of "Sampling time point," estimated at 9.34 (CI 95%: 4.58-19.00, $p<0.001$), suggests that anti-S concentrations during the second sampling period were several times higher than those during the first period after adjusting for covariates. Additionally, the effect of "Sampling time point" is magnified by a factor of 2.72 (CI 95%: 1.25-5.95, $p < 0.05$) if a study subject received a COVID-19

vaccination during the interim period (Figure 5). Moreover, individuals who had a confirmed SARS-CoV-2 infection during the interim period exhibited anti-S levels that were 2.1 times higher (CI 95%: 1.31-3.37) compared to those who did not have a SARS-CoV-2 infection (Figure 5). However, the effects of these two events gradually diminish over time, with an estimated half-life of 176.8 days (CI 95%: 51.9-301.8 days, $p < 0.01$). The multiplying factor for targeted therapy is 0.48 (CI 95% 0.25-0.94, $p < 0.05$), indicating that anti-S levels are 52% lower compared to matched patients. Additionally, anti-S concentrations decrease with age, with every 20-year difference corresponding to a decrease of approximately 45.6% (calculated as $100(1-0.9720)$) (77).

Figure 12A illustrates the number of weekly deaths caused by SARS-CoV-2 infection in Hungary between November 29, 2021, and November 28, 2022, versus the median of anti-S levels over time in each study population. Based on these data, there was an inverse relationship between the number of weekly deaths caused by SARS-CoV-2 infection in Hungary and the median of anti-S levels over time. The increased level of anti-S antibodies might offer an explanation for the decline in the number of weekly deaths caused by SARS-CoV-2 infection (77).

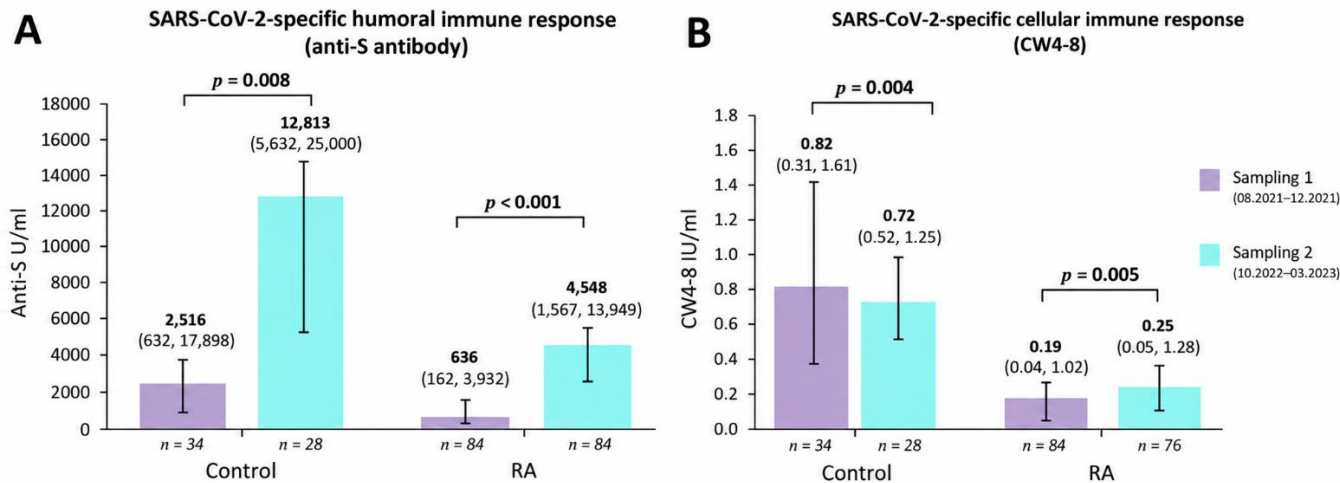


Figure 10. Comparative analysis of SARS-CoV-2-specific immune responses in healthy controls and rheumatoid arthritis (RA) patients. Continuous variables are presented as median (IQR) and compared using the Wilcoxon rank-sum test. (A) SARS-CoV-2-specific humoral immune response, expressed as anti-spike (S) antibody concentration (U/ml). Anti-S antibody levels were significantly lower in patients with RA than in healthy controls at both Sampling 1 (Control, $n = 34$; RA, $n = 84$; $p = 0.008$) and Sampling

2 (Control, $n = 28$; RA, $n = 84$; $p < 0.001$). (B) SARS-CoV-2-specific cellular immune response, assessed as the whole COVID virus-stimulated COVID-specific CD4+ and CD8+ T-cell response (CW4–8). The CW4–8 response was significantly lower in patients with RA than in healthy controls at Sampling 1 (Control, $n = 34$; RA, $n = 84$; $p = 0.004$) and Sampling 2 (Control, $n = 28$; RA, $n = 76$; $p = 0.005$). Bars represent median values and error bars indicate the interquartile range (IQR) (77). *The figure was created using OpenAI GPT-5.3 based on the author's conceptual design.*

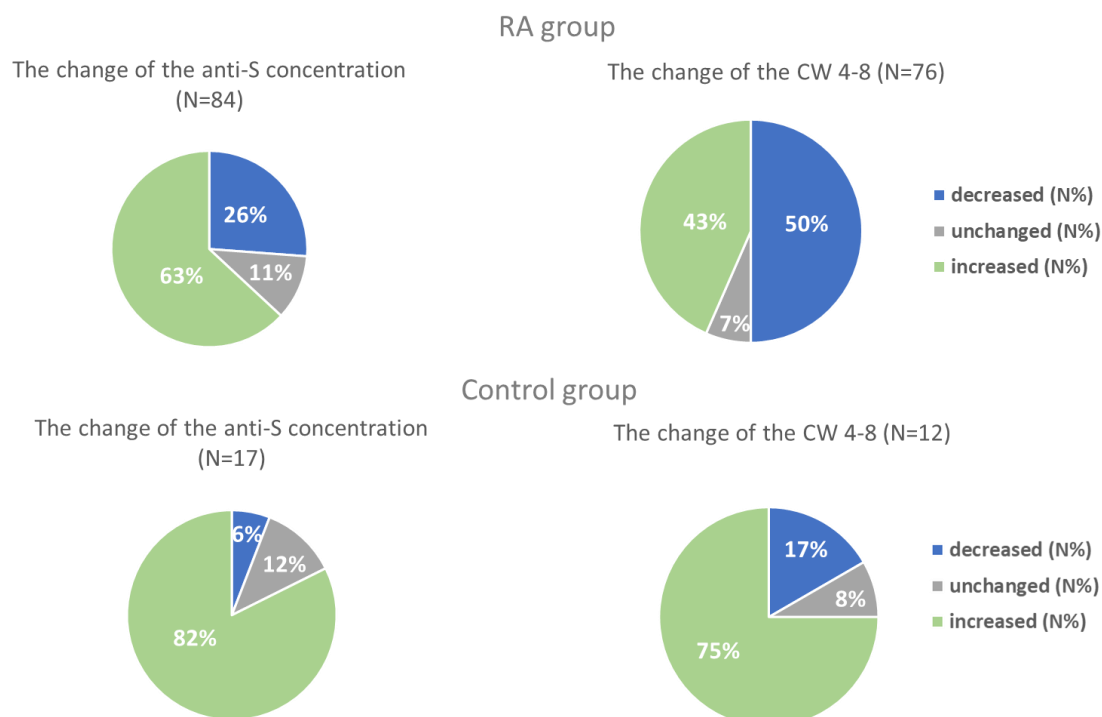


Figure 11. Changes in anti-spike (S) concentrations and whole COVID virus-specific CD4+ and CD8+ T-cell response (CW 4-8) in both study groups. Results from Sampling 1 and Sampling 2 were compared to assess temporal variation. Only participants with paired measurements available at both sampling time points were included in this analysis. A change of $\geq 10\%$ was considered an increase or decrease, while values within $\pm 10\%$ were classified as unchanged (77).

Table 5. Key determinants of anti-S antibody levels in each study cohort. Sampling time point refers to Sampling 1 or Sampling 2. For categorical variables, regression coefficients are expressed as multiplicative factors indicating the fold change in anti-S antibody concentration associated with the presence versus absence of the given factor.

For age, the coefficient represents the percent change per year. CI: Confidence Interval (77).

Characteristic	exp (Beta) (95% CI)	p-value
Vaccination against COVID-19	2.72 (1.25 to 5.95)	0.013
SARS-CoV-2 infection	2.10 (1.17 to 3.76)	0.014
Sampling time point	9.34 (4.58 to 19.0)	<0.001
Targeted therapy	0.48 (0.25 to 0.94)	0.033
Age	0.97 (0.94 to 0.99)	0.004

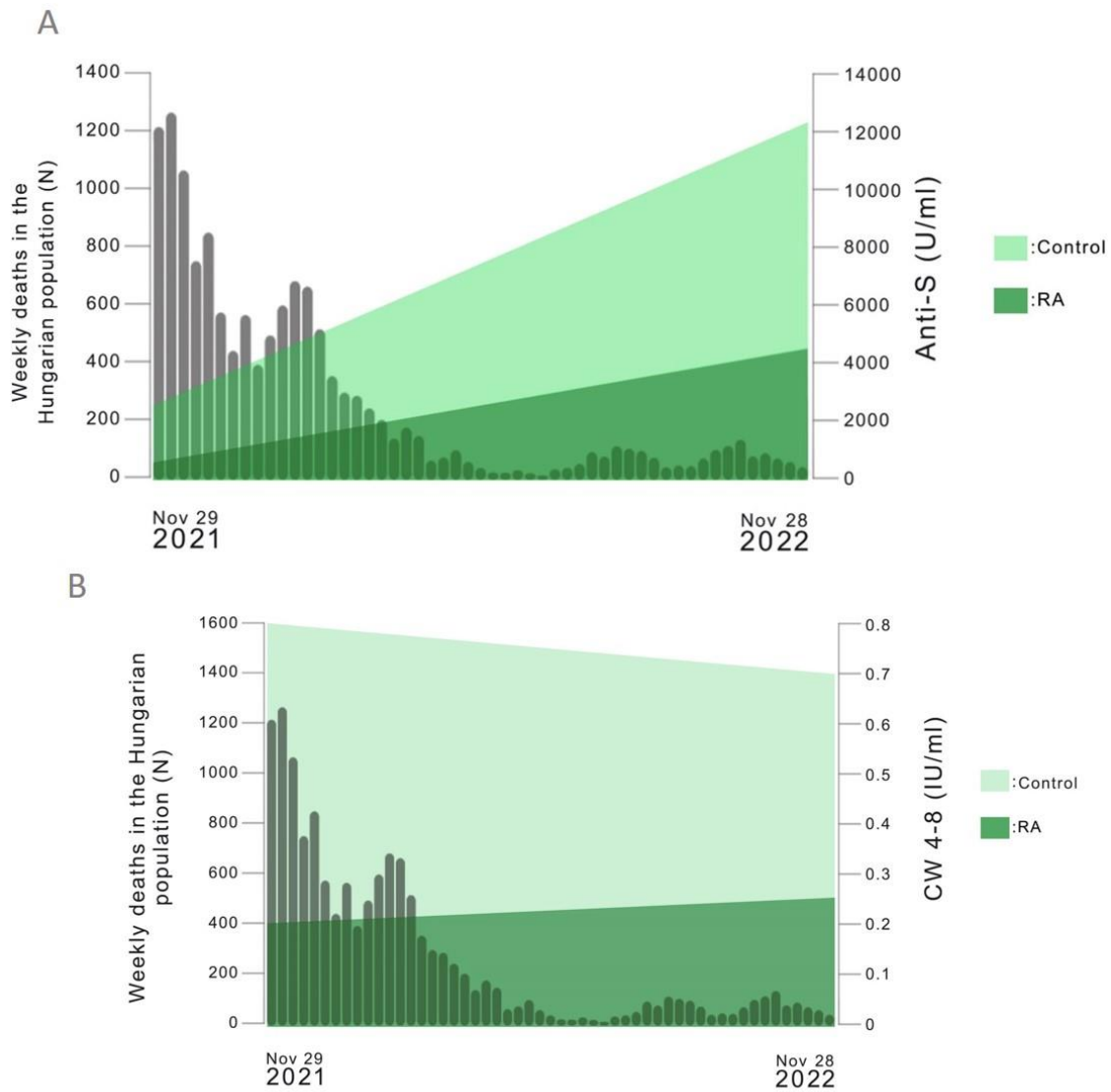


Figure 12. (A) Weekly COVID-19-related mortality in the Hungarian population plotted against the median anti-spike (S) antibody concentrations over time in each study group (<https://covid19.who.int/region/euro/country/hu>). Median values from Sampling 1 and Sampling 2 are connected by lines. (B) Weekly COVID-19-related mortality in Hungary plotted against the whole COVID virus-specific CD4+ and CD8+ T-cell response (CW 4–8) over time in each study group (<https://covid19.who.int/region/euro/country/hu>). Median values from Sampling 1 and Sampling 2 are connected by lines (77).

4.2.3 Study among HCWs

In the first study, we investigated the short-term immunogenicity and clinical effectiveness of the BNT162b2 mRNA vaccine in 295 HCWs during the primary

vaccination campaign. Participants were stratified according to prior SARS-CoV-2 infection status. During the three-month follow-up period after completion of the two-dose vaccination series, new SARS-CoV-2 infections were not detected among vaccinated individuals. Vaccine-related adverse events were mild and self-limiting. We observed that HCWs with a previous SARS-CoV-2 infection, exhibited significantly higher anti-NCP antibody levels and a markedly stronger anti-S antibody response already after the first vaccine dose compared with infection-naïve individuals. In contrast, participants without prior infection demonstrated only modest anti-S antibody production following the first dose, followed by an approximately 500-fold increase after the second dose. These findings indicate that previous SARS-CoV-2 infection acts as a strong immunological primer, whereas two vaccine doses are required to induce robust humoral immunity in infection-naïve individuals (80).

In the second study, we extended the observation period until June 2022 to assess long-term infection incidence, antibody kinetics and the impact of booster vaccination in 294 HCWs. During follow-up, SARS-CoV-2 infection occurred in 41% of participants, predominantly during epidemic waves dominated by the Omicron variant. The majority of infections were clinically mild and showed rapid resolution. Peak anti-S antibody levels were observed one month following the second dose of the primary vaccination series, followed by a progressive decrease over time. Booster immunization resulted in a robust amplification of the anti-S humoral response followed by a gradual decline. Exceptionally high antibody responses were consistently associated with prior SARS-CoV-2 infection. Although the third vaccination dose showed limited effectiveness in preventing Omicron-associated infections, it was associated with a milder clinical course of disease (79).

Overall, our findings demonstrate that mRNA-based COVID-19 vaccination is safe and highly immunogenic among HCWs. Prior SARS-CoV-2 infection substantially enhances vaccine-induced humoral immune responses. While booster vaccination does not fully prevent SARS-CoV-2 infection, it significantly augments antibody levels and contributes to reduced disease severity, supporting its role in protecting high-risk populations.

4.3 IFN- γ T-cell response

4.3.1 Baseline study

A significant correlation was noted between the level of anti-S antibodies and the COVID-specific T-cell response ($r=0.51$, $p<0.001$), as illustrated in Figure 13. By investigating the T-cell mediated immune response following stimulation of the three specific antigens (i: spike antigen stimulating COVID-specific CD4+; ii: spike antigen -stimulating COVID-specific CD4+ and CD8+ and iii: whole COVID virus stimulating COVID specific CD4+ and CD8+ T-cell responses) facilitated us to consider the overall COVID-induced cellular immune response. T-cell specific response markers- COVID spike-specific CD4+, COVID spike-specific CD4+ and CD8+, whole COVID virus-specific CD4+ and CD8+ T-cell response - strongly correlated with each other ($r = 0.87, 0.95, 0.9$ respectively, $p<0.001$) (Figure 13). Consequently, we further analyzed the whole COVID virus-specific CD4+ and CD8+ T-cell response, as the other two markers (COVID spike-specific CD4+ and COVID spike-specific CD4+ and CD8+) were deemed less informative based on these findings (78).

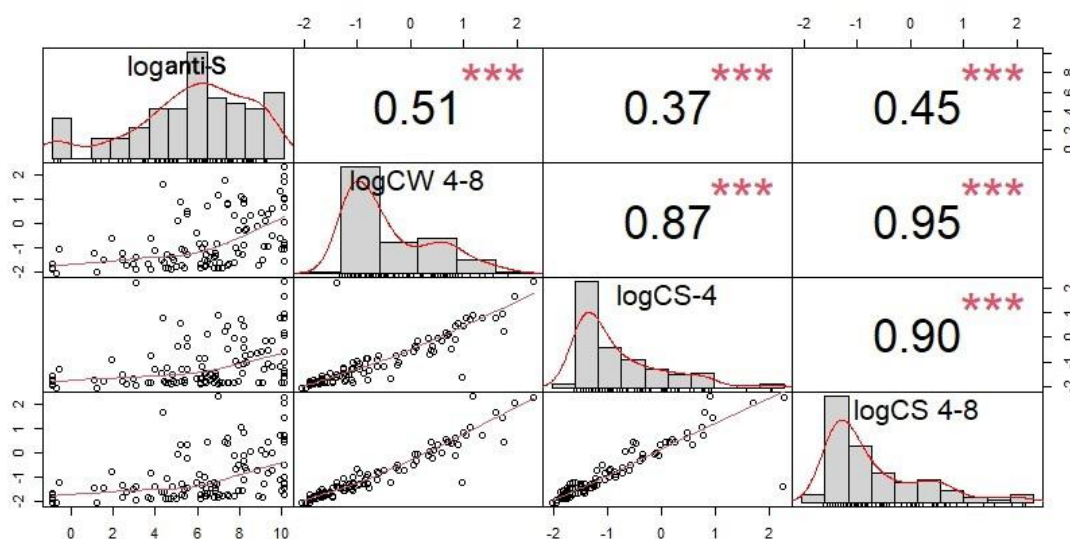


Figure 13. Assessment of associations between antibody-mediated and T-cell-specific immune responses. The upper right triangle shows squared correlation coefficients (R^2) between log-transformed antibody and T-cell immune parameters. COVID-19-specific CD4+ T-cell activation was measured by the CS-4 assay; combined CD4+/CD8+ activation by CS-4–8; and whole COVID virus-specific CD4+ and CD8+ T-cell response by CW 4-

8. Log anti-spike (S) denotes the logarithm of anti-spike antibody concentrations. **p < 0.001. Strong correlations among T-cell markers justified use of the combined whole COVID virus-specific CD4+ and CD8+ T-cell response (CW 4-8) in subsequent analyses (78).

Out of a total of 109 RA patients and 39 controls, 57 RA patients (52%) and 34 controls (87%) exhibited a positive COVID-specific T-cell response (Table 3). In healthy controls, the median level of IFN- γ (which reflects to the whole COVID virus-specific CD4+ and CD8+ T-cell response) was 0.60 IU/ml (0.30, 1.56), whereas RA patients showed a lower median response of 0.16 IU/ml (0.04, 0.94) (as shown in Table 3) (78).

In the entire study group, we observed that the COVID-specific T-cell mediated immune response varied with age. Older patients tended to have a diminished response, with an estimated decline of 2% per year (p<0.001) (Table 6). This initial immune response decrease occurs gradually, and the rate of decline is slower compared to the decline seen in anti-S antibodies, with a reduction of 6% each month (p=0.044) (Table 6). In comparison to natural SARS-CoV-2 infection, none of the vaccines showed significant effects on the COVID-specific T-cell response. Additionally, TNF- α inhibitors (N=51; p=0.7), IL-6 inhibitor (N=15; p>0.9), and JAK inhibitors (N=5; p=0.6) did not exhibit significant effects on the COVID-specific T-cell response (Table 6). However, anti-CD20 therapy was associated with a notable decrease in the COVID-specific T-cell response, on average by 65% (N=4; p=0.055) compared to patients who had not received anti-CD20 therapy but all other variables remain constant (Table 6) (78).

Table 6. Results of the optimized regression analysis for whole COVID virus-specific CD4+ and CD8+ T-cell response (CW 4-8). In both cohorts, this parameter also exhibited a significant age-dependent decline, with an estimated 2% reduction per additional year of age (p<0.001). The CW 4-8 decreased by approximately 6% per month following vaccination (p=0.044). Among patients receiving anti-CD20 therapy, the expected T-cell response was 65% lower compared to the corresponding control group (p=0.055). ¹CI = Confidence Interval (78).

Characteristic	exp (Beta)	95% CI ¹	p-value
Sampling Interval	0.94	0.89, 1.00	0.044
Age	0.98	0.96, 0.99	<0.001
Targeted therapies			
anti-CD20	0.35	0.12, 1.01	0.055
IL-6 inhibitor	0.98	0.53, 1.79	>0.9
JAK inhibitor	1.25	0.48, 3.22	0.6
TNF- α inhibitor	0.94	0.63, 1.39	0.7

797

798 Figure 8B shows the expected curves depicting the CW 4-8 in four cases (S5-S8) after
799 BNT162b2 vaccination. Among these cases, S8 (75-year-old patient receiving rituximab
800 treatment) exhibited the lowest expected T-cell response curve, due to older age and
801 rituximab therapy.

802 Figure 14 illustrates that there was no correlation between CRP levels and IFN- γ levels
803 ($r=-0.110$), as well as between CRP levels and anti-S antibody levels ($r=0.097$). These
804 findings indicate that moderate systemic inflammation did not have significant impact on
805 the various immune responses against COVID-19. Furthermore, we studied the
806 relationship between the presence of anti-CCP, RF IgG, DAS28-CRP and the SARS-CoV-
807 2-specific immune responses. Anti-CCP and RF at baseline were categorized as
808 seropositive (anti-CCP: ≥ 20 CU; RF: ≥ 12 U/ml) or seronegative (anti-CCP: < 20 CU; RF:
809 < 12 U/ml). Initially, we individually examined the effects of these variables by adding
810 them into the established regression model. Notably, data from healthy volunteers were
811 excluded from this analysis. The regression analysis indicated that in the presence of anti-
812 CCP, the levels of anti-S antibodies increased substantially by 6.28 times ($p=0.00165$).
813 Similarly, patients with RF present were expected to have anti-S levels approximately
814 3.33 times higher than those without RF ($p=0.047$). However, when both factors were
815 included in the regression equation, only the effect of anti-CCP remained significant
816 ($p=0.01515$), with the multiplying effect essentially unchanged (6.06). This suggests that

anti-CCP is more directly associated with the humoral response compared to RF. Neither CRP nor DAS28-CRP exhibited any significant effect on anti-S antibody levels, and none of these factors had impact on the markers of T-cell mediated immune response (78).

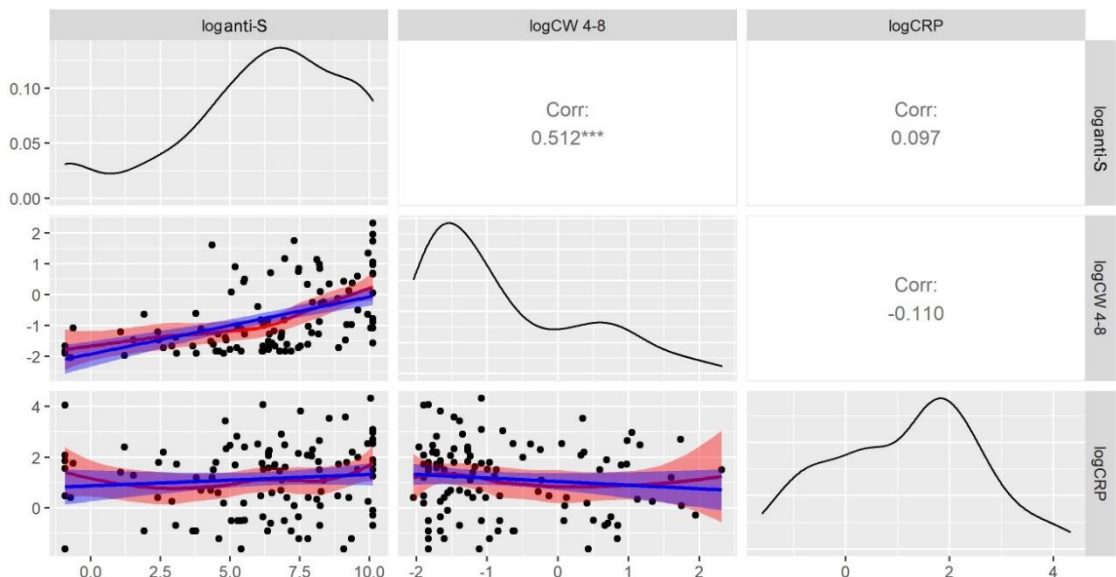


Figure 14. Associations between C-reactive protein (CRP), anti-spike (S) antibody levels and whole COVID virus-specific CD4+ and CD8+ T-cell response (CW 4-8). Lower panels show pairwise scatter plots, diagonals display variable distributions and upper panels indicate squared correlation coefficients (R^2). Log-transformed CRP levels showed no correlation with anti-S antibodies or CW 4–8 responses, suggesting that vaccine- or infection-induced immunity is independent of inflammatory activity in RA patients. ** $p < 0.001$ (78).

4.3.2 Follow-up study

At both sampling time points, RA patients exhibited significantly lower CW4–8 T-cell responses than healthy controls (Sampling 1: $p = 0.004$; Sampling 2: $p = 0.005$, Wilcoxon rank-sum test; Figure 10B). At follow-up (Sampling 2), the median (IQR) IFN- γ level reflecting the CW4–8 T-cell response was 0.72 IU/ml (0.52, 1.25) in healthy controls and 0.25 IU/ml (0.05, 1.28) in RA patients. Additionally, 43% (33 out of 76) of RA patients and 75% (9 out of 12) of healthy individuals demonstrated an increased CW4-8 T-cell response compared to baseline results (Figure 11). It is worth noting that a significant low correlation was noted between the level of anti-S antibodies and the CW4-8 T-cell response in each study group ($r=0.3891$, $p<0.0001$). Markers of SARS-CoV-2-specific T-

cell responses did not reveal a consistent pattern across study groups. However, among RA patients prior SARS-CoV-2 infection - but not COVID-19 vaccination - was associated with a significant 2.09-fold increase in the whole COVID virus-specific CD4+ and CD8+ T-cell response (95% CI: 1.30–3.36; $p < 0.01$) (77).

In Figure 12B, there is a comparison between the weekly deaths due to SARS-CoV-2 infection in Hungary from November 29, 2021, to November 28, 2022, and the median of CW4-8 T-cell response over time in different study groups. According to these findings, the number of weekly deaths attributed to SARS-CoV-2 infection decreased gradually over time in Hungary. However, there was not a significant change observed in the level of CW4-8 T-cell response within the study populations during this period (77).

5 Discussion

This study provides a comprehensive evaluation of SARS-CoV-2 induced humoral and cellular immune responses in RA, integrating observational and longitudinal findings to characterize both the magnitude and durability of immunity following SARS-CoV-2 infection and/or COVID-19 vaccination. By combining immunological measurements with clinical and therapeutic variables, these studies offer insight into impaired antiviral immunity in this immunosuppressed population and extend observations on vaccine responsiveness (77, 78).

Across both studies, RA patients consistently demonstrated significantly reduced anti-S antibody levels and diminished SARS-CoV-2-specific T-cell response compared with healthy controls. These findings are concordant with large cohort studies showing attenuated vaccine-induced immunity in patients with autoimmune rheumatic diseases, particularly those receiving bDMARDs or tsDMARDs. Reduced serological responses after COVID-19 vaccination have been repeatedly documented in RA and related conditions, supporting the concept that immune dysregulation and immunosuppressive therapy collectively contribute to impaired vaccine immunogenicity (53, 56, 90). The baseline study demonstrated that age, time since antigenic exposure, vaccine platform and targeted RA therapies independently influenced the magnitude of the humoral immune response. In line with prior reports, TNF- α inhibitors and IL-6 inhibitor significantly reduced anti-S antibody levels, while anti-CD20 therapy profoundly suppressed both humoral and cellular immunity. These observations are consistent with studies showing that TNF- α signaling is required for optimal germinal center formation and long-lived plasma cell survival, whereas B-cell depletion directly inhibits antibody production and compromises antigen presentation for T-cell priming (91, 92). The marked effect of anti-CD20 therapy observed in our studies can highlight the importance of tailored vaccination timing or alternative protective strategies in this patient subgroup.

A major contribution of the longitudinal study is the demonstration that, despite persistently lower immune responses, RA patients exhibited a significant increase in anti-S antibody levels over time. This finding contrasts with early pandemic studies reporting rapid antibody waning after vaccination or natural infection (74, 93) and suggests that repeated antigenic exposure - through booster vaccination, SARS-CoV-2 infection following vaccination or asymptomatic viral circulation - can partially overcome

immunosuppression-related deficits. Similar longitudinal increases in antibody titers after booster doses have been reported in immunocompromised populations (94, 95).

In contrast to humoral immunity, SARS-CoV-2-specific T-cell response remained relatively stable over time in both RA patients and healthy controls, despite being quantitatively reduced in the RA group. This observation aligns with evidence that T-cellular immunity is more durable and less sensitive to viral mutations than antibody responses (96, 97). Preservation of T-cell reactivity across SARS-CoV-2 variants, including Omicron, has been demonstrated even in individuals with reduced neutralizing antibody titers, supporting a central role for cellular immunity in protection against severe COVID-19. The relevance of the SARS-CoV-2-specific T-cell response was further supported by our previous study (98), which evaluated the prognostic value of CW 4-8 and SARS-CoV-2-specific humoral immunity in critically ill COVID-19 patients requiring venovenous extracorporeal membrane oxygenation (VV-ECMO). In that cohort, CW4-8 T-cell responses were significantly higher in survivors than in non-survivors. The differential sensitivity of humoral and cellular immune responses to immunomodulatory therapies observed in this work may have clinically relevant implications. While TNF- α and IL-6 blockade predominantly affected antibody production, anti-CD20 therapy impaired both immune compartments. These findings support emerging stratified vaccination strategies and are consistent with real-world effectiveness data showing higher infection rates but preserved protection against severe disease in patients with intact T-cell responses (99, 100).

COVID-19 exhibits pronounced clinical heterogeneity, with manifestations ranging from asymptomatic infection to severe viral pneumonia, ARDS, multiorgan dysfunction and mortality. Emerging evidence indicates that disease severity is primarily modulated by host-intrinsic factors, including immune and inflammatory responses (Figure 15). Large-scale epidemiological studies have consistently identified specific host risk factors that predispose individuals to severe COVID-19 outcomes. Among the most robust risk factors are malignancy and obesity, both of which are associated with profound immune dysregulation, chronic inflammation and impaired antiviral defense mechanisms (36, 37). These observations underscore the central role of host immune competence in shaping clinical outcomes and provide a broader context for the present findings, in which immunosuppression and immune modifying therapies in RA similarly contribute to

altered SARS-CoV-2-specific immune responses and increased vulnerability to severe disease.

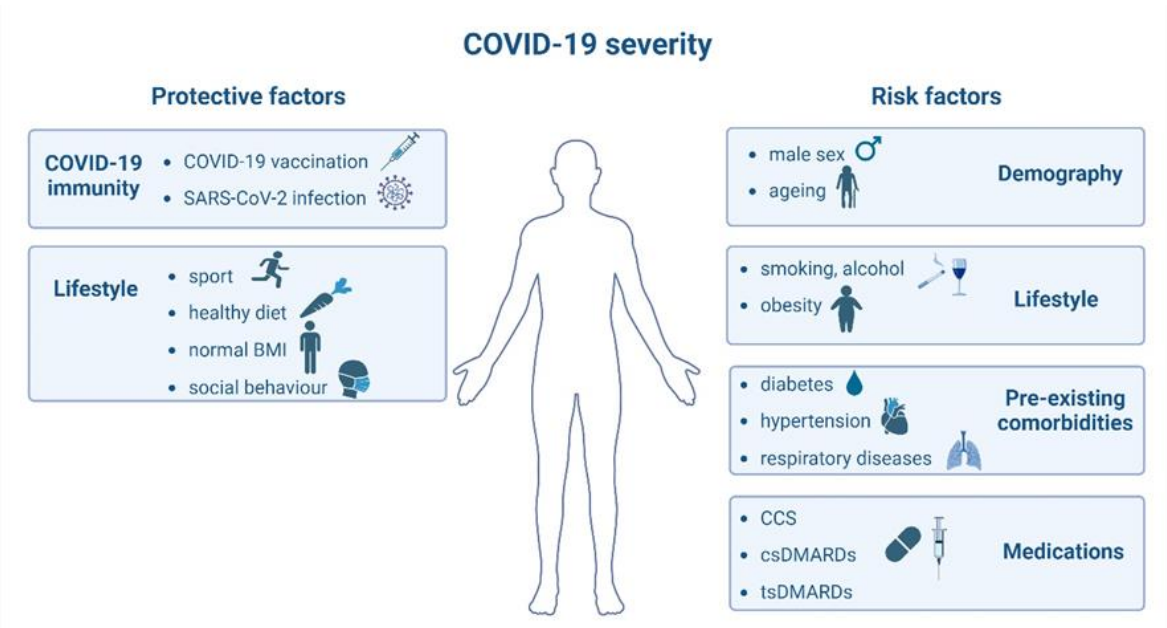


Figure 15. Determinants of COVID-19 severity: protective and risk factors. BMI (Body Mass Index), CCS (Corticosteroids), csDMARDs (conventional synthetic disease-modifying antirheumatic drugs), tsDMARDs (targeted synthetic disease-modifying antirheumatic drugs) are highlighted as key variables influencing disease outcomes (77). This figure was generated using BioRender.com.

COVID-19-related mortality in RA patients may be partially linked to dysregulated inflammatory responses (101). Severe COVID-19 is characterized by hyperinflammation and excessive cytokine release, including elevated IL-6, TNF- α and IL-1 β levels (64, 102). As RA is defined by chronic systemic cytokine activation (15), pre-existing immune dysregulation may predispose patients to an enhanced inflammatory response following SARS-CoV-2 infection. The convergence of baseline inflammation and virus-induced hyperinflammation may therefore contribute to increased disease severity and mortality in this population, consistent with large-scale epidemiological analyses identifying inflammatory comorbidities as risk factors for adverse COVID-19 outcomes (36).

RA patients and HCWs each represent high-risk groups during the COVID-19 pandemic, driven by different but interconnected determinants of vulnerability (70, 101). RA patients exhibit immune dysregulation and frequently receive immunosuppressive therapies,

predisposing them to impaired antiviral immunity and increased susceptibility to severe outcomes (15, 36, 101). In contrast, HCWs experience sustained occupational exposure to SARS-CoV-2, particularly during periods of high viral circulation (70). Within healthcare settings, these populations form a bidirectional transmission interface, in which immunocompromised patients may serve as prolonged viral reservoirs and infected HCWs may transmit infection during asymptomatic or presymptomatic phases (103). Moreover, parallel observations in HCWs demonstrating robust vaccine-induced immunity, waning antibody levels over time and the impact of booster immunization further emphasize the importance of adaptive vaccination strategies in both immunosuppressed and highly exposed populations (74).

At a population level, the observed escalation of humoral immunity over time was associated with a decline in COVID-19-related mortality, suggesting that cumulative immune exposure contributes to reduced disease severity. This observation is consistent with epidemiological data indicating that hybrid immunity - arising from vaccination combined with infection - confers superior and more durable protection than either alone (104, 105). The coexistence of sustained T-cell immunity, increasing antibody titers and widespread viral circulation may have contributed to the shift toward milder clinical outcomes in later phases of the pandemic.

Several limitations should be acknowledged. Neutralizing activity and variant-specific antibody responses were not directly evaluated and subgroup analyses were limited by small sample sizes for certain therapies. Furthermore, the potential influence of obesity and other comorbidities on SARS-CoV-2-specific immune responses was not examined. As these factors may independently affect immune function and vaccine-induced antibody responses, their potential confounding effects cannot be excluded. Anti-CD20 therapy was associated with the strongest reduction in SARS-CoV-2-specific antibody levels. In the four rituximab-treated RA patients, the interval between rituximab treatment and COVID-19 vaccination was 3.5 ± 0.87 months (mean $\pm$ SD), which should be considered when interpreting the observed reduction in the humoral immune response. However, given the very small number of patients receiving anti-CD20 therapy (n=4), this finding should be interpreted with caution and requires confirmation in larger cohorts. There was also a notable difference in age and gender distribution between the healthy control and RA groups. In particular, the healthy controls were substantially younger than

the RA patients, and increasing age was independently associated with lower anti-S antibody levels. Although age was included as a covariate in the multivariable analyses, residual confounding related to the substantial age imbalance cannot be excluded. Therefore, physiological immunosenescence may have contributed to the lower humoral immune response observed in the RA cohort, and the observed differences cannot be attributed only to RA or its treatment. Nonetheless, the agreement between cross-sectional and longitudinal findings, together with consistency with international studies, supports the overall validity of the conclusions.

In summary, these findings indicate that SARS-CoV-2-specific immune responses in RA patients are decreased and dynamically modulated over time, with age, immunosuppressive therapy and repeated antigenic exposure playing important roles. While humoral immunity is quantitatively reduced, it can increase over time through COVID-19 vaccination and SARS-CoV-2 infection, whereas cellular immune response remains relatively stable and may provide sustained protection against severe disease. Given the shared and bidirectional risk between RA patients and HCWs, these findings underscore the need for individualized vaccination strategies and long-term, risk-adapted management in patients with autoimmune inflammatory diseases.

6 Conclusions

The major conclusions of our study are:

- SARS-CoV-2-specific immune responses are strongly influenced by host-related factors, prior infection, vaccination status and immunosuppressive therapy.
- RA patients exhibit reduced SARS-CoV-2-specific humoral and cellular immune responses compared with healthy individuals.
- Diminished SARS-CoV-2-specific immune responses in RA are associated with increasing age, longer time since antigenic exposure and use of immunosuppressive treatments.
- TNF- α inhibitors and IL-6 inhibitor predominantly impair SARS-CoV-2-specific humoral immune responses, whereas anti-CD20 therapy was associated with reduced humoral and cellular immune responses, although this observation requires confirmation in larger cohorts.
- Longitudinal analyses indicate that SARS-CoV-2-specific humoral immunity in RA remains adaptable over time, while SARS-CoV-2-specific T-cell response is relatively stable.
- Data from HCWs confirm that mRNA-based COVID-19 vaccination is safe and highly immunogenic.
- Prior SARS-CoV-2 infection enhances COVID-19 vaccine-induced immune responses and booster vaccination increases antibody levels and is associated with milder clinical disease among HCWs.
- These findings support individualized, therapy-aware COVID-19 vaccination and immune monitoring strategies, particularly for patients receiving immunosuppressive treatments, to optimize immune protection and reduce the risk of severe disease.

7 Summary

COVID-19 shows marked clinical heterogeneity driven by host-related factors, highlighting autoimmune patients and HCWs as key populations for immune studies. This study evaluated SARS-CoV-2-specific humoral and cell-mediated immune responses, along with their host-, and therapy-associated determinants, in RA patients, healthy control subjects and HCWs after SARS-CoV-2 infection and COVID-19 immunisation.

RA patients exhibited reduced anti-S antibody levels and SARS-CoV-2-specific T-cell responses compared with healthy individuals, with diminished immunity associated with older age, longer time since COVID-19 vaccination or SARS-CoV-2 infection and immunosuppressive therapies. TNF- α inhibitors and IL-6 inhibitor primarily reduced humoral immunity, while anti-CD20 therapy impaired both humoral and cellular responses, whereas MTX and low-dose GCS showed no consistent independent effect. In HCWs, mRNA-based COVID-19 vaccination was safe and highly immunogenic; prior SARS-CoV-2 infection markedly enhanced antibody responses and booster vaccination increased antibody levels and was associated with milder disease despite limited protection against Omicron infection. Longitudinal analyses revealed that humoral immune responses in RA patients increased over time, whereas SARS-CoV-2-specific T-cell response remained relatively stable. In parallel, the co-circulation of multiple SARS-CoV-2 variants, potentially associated with attenuated clinical manifestations, may have contributed to the comparatively low incidence of severe COVID-19 in the general population.

In summary, this thesis suggests that SARS-CoV-2-specific immune responses in RA are influenced by host-related factors, prior infection, vaccination and immunosuppressive therapy and may remain adaptable over time despite evidence of impairment. When considered alongside data from HCWs, these findings are consistent with the safety and effectiveness of mRNA COVID-19 vaccination and highlight the potential value of individualized, risk-adapted vaccination strategies in these subgroups.

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