Immunogenicity of therapeutics in inflammatory joint- and bowel diseases

Thesis by

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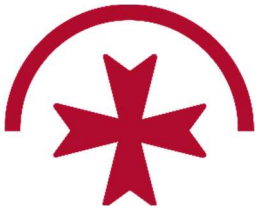

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Abbreviations

ACPA	Anti-citrullinated peptide antibodies
ADAb	Anti-drug antibodies
APC	Antigen-presenting cell
ASDAS	Ankylosing Spondylitis Disease Activity Score
AU/ml	Arbitrary units per millilitre
BAU/ml	Binding antibody unit per millilitre
bDMARD	Biologic Disease Modifying Anti-Rheumatic Drug
CRP	C-reactive protein
csDMARD	Conventional Synthetic Disease Modifying Anti-Rheumatic Drug
DAPSA	Disease Activity index for Psoriatic Arthritis
DAPSA28	Disease Activity index for Psoriatic Arthritis 28-joint count
DAS	Disease Activity Score
DAS28	Disease Activity Score 28-joint count
DMARD	Disease Modifying Anti-Rheumatic Drug
ESR	Erythrocyte sedimentation rate
EULAR	European Alliance of Associations for Rheumatology
F(ab)	Antigen-binding fragment
IMID	Immune-mediated inflammatory disease
Ln	Natural logarithm
MSIS	Norwegian Surveillance System for Communicable Diseases
NOR-DMARD	The Norwegian Antirheumatic Drug Registry
Nor-vaC	The Norwegian Study of Vaccine Response to COVID-19
PhGA	Physician global assessment
PtGA	Patient global assessment
RBD	Receptor binding domain
ROC	Receiver operating characteristic
SJC	Swollen joint count
SYSVAK	Norwegian Immunisation Registry
TCR	T-cell receptor
TDM	Therapeutic drug monitoring
TJC	Tender joint count

TNFi	Tumour necrosis factor inhibitor
tsDMARD	Targeted Synthetic Disease Modifying Anti-Rheumatic Drug

Summary of thesis

Immunogenicity refers to the capacity of a foreign substrate to trigger an immune response within the human body. This thesis explores the immunogenicity of two types of therapeutics, namely SARS-CoV-2 vaccines and the biologic drug adalimumab, in patients with inflammatory joint- and bowel diseases. Vaccines are designed to elicit a desired immune response, whereas immune responses against biologic drugs can potentially compromise the treatment effect.

Two of the studies included in this thesis were conducted during the first year of the COVID-19 vaccination programme. They describe the humoral (antibody) and cellular (T-cell) responses to the SARS-CoV-2 vaccines in patients with inflammatory joint- and bowel diseases who were using immunosuppressive drugs. This knowledge was important, as it was a concern whether the vaccines would be sufficiently effective in patients lacking a fully functional immune response. Further, it was crucial to assess the potential benefits of administering additional vaccine doses, especially in patients with reduced vaccine responses. There were also concerns if the immune responses elicited by the vaccines would aggravate autoimmunity, and trigger disease flares.

Of particular interest was the vaccine response in patients using the biologic drug rituximab, a CD20 B-cell inhibitor, as the B cells, which usually react to antigens such as vaccines, are depleted. Rituximab treated patients were at increased risk of severe COVID-19 before the vaccination programme. In the first paper, we explored the humoral and cellular vaccine responses following two and three vaccine doses in patients with rheumatoid arthritis on rituximab therapy. Patients with no or poor humoral response following the standard two vaccine doses received a third dose. We demonstrated a very poor humoral response after both two and three vaccine doses. The timing of rituximab infusions was a key factor for humoral vaccine response. We found that the interval between the last rituximab infusion and vaccination should be as long as possible and preferably more than nine months. Importantly, despite the lack of humoral response following two vaccine doses, 54% of patients had CD4+ T-cell responses and 74% of patients had CD8+ T-cell responses. Following a third vaccine dose, all patients had adequate CD4+ and CD8+ T-cell responses. The T-cell responses were independent of the humoral response. We concluded that a third vaccine dose was beneficial in patients on rituximab, as they might have to rely on their T-cell responses when encountering the virus. Rituximab treated patients were of special concern during the

pandemic, and the finding of beneficial effect of a third dose with T-cell responses that were comparable to healthy controls was reassuring for this large group of patients.

The second paper assessed the humoral response to two and three SARS-CoV-2 vaccine doses in patients with inflammatory joint- and bowel diseases using a range of immunosuppressive medications. This included tumour necrosis factor inhibitors (TNFi) in mono- and combination therapy, methotrexate, interleukin inhibitors, T-cell inhibitors and Janus Kinase (JAK) inhibitors. We found that most patients had humoral response following two vaccine doses, but their antibody levels were inferior compared to healthy controls. The lowest response rates and antibody levels were seen in patients treated with TNFi in combination therapy, JAK inhibitors or the T-cell inhibitor abatacept. The findings were consistent across diagnoses of inflammatory joint- and bowel diseases. In patients lacking a humoral response after two vaccine doses, a third dose was beneficial in 94% of patients. The findings in this paper supported that a third vaccine dose should be recommended as part of the primary SARS-CoV-2 vaccine series in patients treated with immunosuppressive drugs.

Safety of the SARS-CoV-2 vaccines in patients treated with immunosuppressive drugs was also an important concern, and this was explored in both papers. Patients generally reported less adverse events compared to healthy controls, and no serious adverse events or deaths occurred during the study period. After two vaccine doses, 50% of patients and 78% of healthy controls reported an adverse event. Following a third dose, 44% of patients reported an adverse event. There was a minor increase in patients reporting disease flares after the third vaccine dose. This was only in patients with inflammatory joint diseases, who were advised to pause their immunosuppressive medication when receiving the third vaccine dose.

The results showing that the SARS-CoV-2 vaccines were safe, the finding of lower antibody levels in patients treated with immunosuppressive drugs compared to healthy controls and that a third vaccine dose was valuable in those not responding to the first two doses, contributed to closing a knowledge gap at a time of severe pressure on the Norwegian health care system. In the autumn of 2021, all patients treated with immunosuppressive drugs in Norway were recommended a third vaccine dose as part of the SARS-CoV-2 vaccine prime series. Data from the two papers was part of the knowledge base for the Norwegian Institute of Public Health in deciding on this vaccine strategy.

The immunogenicity of biologic drugs, such as the TNFi adalimumab, can impact the treatment efficacy. Anti-drug antibodies have the potential to bind to and neutralise the drug

or affect the clearance of the drug, possibly resulting in lower serum drug levels. Therapeutic drug monitoring is a treatment strategy where the serum drug levels and anti-drug antibodies are measured and the results used to determine the treatment strategy. Based on knowledge of optimal therapeutic ranges and anti-drug antibodies, the drug dosage can be tailored. To implement therapeutic drug monitoring in clinical practice, it is essential to determine the optimal therapeutic ranges and assess the immunogenicity of the drugs.

Adalimumab is the world's top selling biologic drug, with indication across several diseases. The third paper describes the association between the serum drug level of adalimumab and treatment response in patients with inflammatory joint diseases as well as reporting the development of anti-drug antibodies. The aim was to find a therapeutic range for adalimumab to be used in therapeutic drug monitoring. We found that a serum drug level of 6 mg/L and above was associated with better treatment response and less drug discontinuation in patients with rheumatoid arthritis and psoriatic arthritis. In patients with spondyloarthritis, a therapeutic cut-off could not be determined, but with increasing serum adalimumab levels the chance of response to therapy increased. Already three months after initiating adalimumab treatment, 10% of the patients had developed anti-drug antibodies. This was related to less favourable treatment outcomes.

While biologic drugs have brought significant advancements in improving the health of patients with inflammatory joint- and bowel diseases, acknowledging the impact of their immunogenicity on treatment outcomes is crucial to ensure their safe and effective utilisation. Simultaneously, biologic drugs exert a profound influence on the immune response to vaccines. As a result, advances in understanding and managing immunogenicity will remain a pivotal factor in improving the utilisation of both biologic drugs and vaccines.

Norsk sammendrag

Immunogenisitet er evnen et kroppsfremmed stoff har til å aktivere immunforsvaret. Denne avhandlingen tar for seg immunogenisiteten til to behandlingstyper, SARS-CoV-2-vaksiner og det biologiske legemiddelet adalimumab. Vaksiner er designet for å fremkalle en immunrespons, mens immunrespons rettet mot biologiske legemidler potensielt kan kompromittere behandlingseffekten.

To av artiklene i denne avhandlingen ble skrevet i løpet av det første året COVID-19 vaksinasjonen pågikk i Norge. Artiklene beskriver den humorale (antistoff) og cellulære (T-celle) responsen på SARS-CoV-2 vaksinene hos pasienter med inflammatoriske ledd- og tarmsykdommer som bruker immundempende legemidler. Denne kunnskapen var viktig, ettersom pasienter behandlet med immundempende legemidler hadde høyere risiko for alvorlig COVID-19 enn friske før vaksinene kom. Det var en bekymring hvorvidt vaksinene ville være effektive nok hos pasienter som ikke har et fullt fungerende immunsystem. Hvis disse pasientene hadde dårlig effekt av de to første vaksinene, var det også viktig å finne ut om det ville det ha noen hensikt å gi flere doser. I tillegg lurte man på om vaksinene kunne forverre auto-immunitet, og gi sykdomsoppbluss.

Av spesiell interesse var vaksineresponsen hos pasienter med revmatoid artritt som brukte rituximab, en CD20 B-celle hemmer. Dette fordi B cellene, som vanligvis reagerer på antigener som vaksiner, mangler. Pasienter som brukte rituximab hadde økt risiko for alvorlig COVID-19 før vaksinene kom. I den første artikkelen undersøkte vi den humorale og cellulære vaksineresponsen etter to og tre vaksinedoser hos disse pasientene. Pasienter som ikke hadde antistoffrespons etter to vaksinedoser fikk en tredje vaksinedose. Vi fant at pasientene hadde svært dårlig antistoffrespons etter både to og tre vaksinedoser. Tidspunktet for siste rituximab infusjon var viktig. De pasientene som hadde antistoffrespons hadde lengre intervall mellom siste rituximab infusjon og første vaksinedose. Til tross for mangel på antistoffer etter to vaksinedoser, hadde 54% av pasientene CD4+ T-celle respons og 74% av pasientene CD8+ T-celle respons. Etter en tredje vaksinedose hadde alle pasientene god CD4+ og CD8+ T-celle respons. T-celle responsen var uavhengig av antistoffresponsen. Vi konkluderte med at en tredje vaksinedose var gunstig for disse pasientene, ettersom de på grunn av manglende antistoffrespons måtte stole på T-celle responsen i møte med SARS-CoV-2. Pasienter som brukte rituximab var en stor bekymring under pandemien, og kunnskap

om at de hadde T-celle responser som var like gode som friske kontroller var svært viktig for pasientgruppen og for de kliniske miljøene.

I den andre artikkelen undersøkte vi antistoffresponsen etter vaksinene hos pasienter med inflammatoriske ledd- og tarmsykdommer som brukte mange ulike immundempende legemidler. Dette inkluderte tumor nekrose faktor (TNF) -hemmere i mono- og kombinasjonsterapi, metotreksat, interleukin-hemmere, T-celle-hemmere og Janus Kinase (JAK)-hemmere. Vi fant at de fleste pasientene hadde antistoffrespons etter to vaksinedoser, men antistoffnivåene deres var lavere sammenlignet med friske kontroller. Lavest andel respondere og lavest antistoffnivå fant vi hos pasienter behandlet med TNF-hemmere i kombinasjonsterapi, JAK -hemmere eller abatacept. Funnene holdt seg på tvers av de ulike diagnosene. Som i den første artikkelen, fikk pasienter som manglet antistoffrespons etter to vaksinedoser en tredje dose. Dette gav en økning i antistoff-nivåene hos 84% av pasientene, kunnskap som var viktig for planlegging av videre vaksinestrategi. Funnene i denne artikkelen støttet at en tredje vaksinedose skulle anbefales som en del av SARS-CoV-2 grunnvaksinasjonen for disse pasientene.

Kunnskap om vaksinesikkerhet manglet også på dette tidspunktet av pandemien, og dette ble utforsket i begge artiklene. Pasienter rapporterte generelt mindre bivirkninger sammenlignet med friske kontroller, og ingen alvorlige bivirkninger eller dødsfall forekom i løpet av studieperioden. Etter to vaksinedoser rapporterte 50% av pasientene og 78% av friske kontroller bivirkninger, og etter en tredje dose rapporterte 44% av pasientene bivirkninger. Etter den tredje vaksinedosen var det en liten økning i pasienter som rapporterte sykdomsoppbluss. Dette var kun hos pasienter med inflammatoriske leddsykdommer, som hadde blitt rådet til å ta pause fra sine immundempende legemidler i forbindelse med den tredje vaksinedosen.

Det at vaksinene var trygge, at pasienter behandlet med immundempende legemidler hadde lavere antistoffnivåer enn friske og at en tredje vaksinedose var verdifull hos de som ikke responderte på de to første dosene, bidro til å tette et kunnskapshull i en tid med stor usikkerhet. Høsten 2021 ble alle pasienter som var behandlet med immundempende legemidler i Norge anbefalt en tredje vaksinedose som en del av SARS-CoV-2 grunnvaksinasjonen. Data fra de to første artiklene i denne avhandlingen var med å danne kunnskapsgrunnlaget da Folkehelseinstituttet besluttet vaksinestrategien.

Immunogenisiteten til biologiske legemidler, som TNF-hemmeren adalimumab, kan påvirke behandlingseffekten. Anti-legemiddelantistoffer har potensial til å binde seg til og nøytralisere legemidlet eller påvirke utskillelsen av det, noe som kan føre til lave serum-konsentrasjoner. Terapeutisk legemiddel monitorering er en behandlingsstrategi der serum-konsentrasjoner og anti-legemiddel antistoffer måles. Basert på kunnskap om optimale terapeutiske nivåer og anti-legemiddelantistoffer kan legemiddeldosen tilpasses. For å implementere terapeutisk legemiddel monitorering i klinisk praksis, er det essensielt å fastslå de optimale terapeutiske nivåene og vurdere legemiddelets immunogenisistet.

TNF-hemmeren adalimumab er verdens mest solgte biologiske legemiddel og har godkjenning ved mange ulike sykdommer. I den tredje artikkelen undersøkte vi sammenhengen mellom serumnivået av adalimumab og behandlingsrespons hos pasienter med inflammatoriske leddsykdommer, samt forekomsten av anti-legemiddelantistoffer. Målet var å finne et terapeutisk nivå for adalimumab for videre bruk i terapeutisk legemiddel monitorering. Vi fant at et serumnivå av adalimumab på 6 mg/L og over var assosiert med bedre behandlingsrespons og medikamentoverlevelse hos pasienter med revmatoid artritt og psoriasisartritt. Hos pasienter med spondyloartritt fant vi ikke et terapeutisk nivå, men med økende serumnivåer av adalimumab økte sjansen for respons på behandlingen. Allerede tre måneder etter oppstart av behandling med adalimumab hadde 10% av pasientene utviklet anti-legemiddelantistoffer. Dette var relatert til mindre gunstige behandlingsresultater.

Mens biologiske legemidler har før til betydelige fremskritt i behandlingen av pasienter med inflammatoriske ledd- og tarmsykdommer, er det viktig å vurdere deres immunogenisitet for effektiv og trygg bruk. Samtidig påvirker biologiske legemidler immunresponsen på vaksiner. Fremskritt i å forstå og håndtere immunogenisitet vil derfor være avgjørende for å forbedre bruken av både biologiske legemidler og vaksiner.

List of papers

I. **Humoral and cellular immune responses to two and three doses of SARS-CoV-2 vaccines in rituximab-treated patients with rheumatoid arthritis: a prospective, cohort study.**

Jyssum I[*], Kared H[*], Tran TT, Tveter AT, Provan SA, Sexton J, Jørgensen KK, Jahnsen J, Kro GB, Warren DJ, Vaage EB, Kvien TK, Nissen-Meyer LSH, Anderson AM, Grødeland G, Haavardsholm EA, Vaage JT, Mjaaland S, Syversen SW[**], Lund-Johansen F[**], Munthe LA[**], Goll GL[**].

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[*] Shared first authorship [**] Shared last authorship

II. **Immunogenicity and Safety of Standard and Third-Dose SARS–CoV-2 Vaccination in Patients Receiving Immunosuppressive Therapy.**

Syversen SW[*], **Jyssum I**[*], Tveter AT, Tran TT, Sexton J, Provan SA, Mjaaland S, Warren DJ, Kvien TK, Grødeland G, Nissen-Meyer LSH, Ricanek P, Chopra A, Andersson AM, Kro GB, Jahnsen J, Munthe LA, Haavardsholm EA, Vaage JT[**], Lund-Johansen F[**], Jørgensen KK[**], Goll GL[**].

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[*] Shared first authorship [**] Shared last authorship

III. **Adalimumab serum levels and anti-drug antibodies: associations to treatment response and drug survival in inflammatory joint diseases.**

Jyssum I, Gehin JE, Sexton J, Kristianslund EK, Hu Y, Warren DJ, Kvien TK, Haavardsholm EA, Syversen SW, Bolstad N, Goll GL

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Preface

Immunogenicity is the ability of a substance to trigger an adaptive immune response. The triggering substance might be a pathogen like virus or bacteria but could also be therapeutic agents like vaccines or a biologic drug. While the immunogenicity of vaccines is pivotal in their efficacy, it can reduce the therapeutic effect of biologic drugs.

The plan before the start of my PhD programme was to assess the immunogenicity and therapeutic ranges of biologic drugs with use of data from The Norwegian Antirheumatic Drug Registry (NOR-DMARD). However, in 2020 we were in the middle of the COVID-19 pandemic. This was a global health crisis, and hospital organisation and research projects were turned upside down. Early in my PhD period, in January 2021, the COVID-19 vaccination programme was about to start in Norway. Patients using immunosuppressive drugs were prioritised in the vaccination programme. There was an urgent need of knowledge on vaccines safety and efficacy in these patients. Researchers from across the globe swiftly mobilised to conduct research on COVID-19. Also in our research group, with competence on immunogenicity of biologic drugs, we redirected our focus to vaccine immunogenicity. The first 1.5 years of my PhD programme was consequently spent entirely working with vaccine immunogenicity, in the Norwegian Study of Vaccine Response to COVID-19 (Nor-vaC). When the pandemic became less intense, my work focus switched to investigating immunogenicity and therapeutic ranges of the tumour necrosis factor inhibitor adalimumab. Hence, the common theme of this thesis is the immunogenicity of therapeutics, herein both biologic drugs and SARS-CoV-2 vaccines.

The papers of this thesis are results of a strong research collaboration across clinical and laboratory specialities at the Division of Rheumatology and Research at Diakonhjemmet Hospital, Department of Gastroenterology at Akershus University Hospital, Department of Immunology at Oslo University Hospital, Department of Medical Biochemistry Oslo University Hospital and the Norwegian Institute of Public Health.

1 Background

1.1 The adaptive immune system

The immune system is usually divided into the adaptive and non-adaptive (innate) immune system. The non-adaptive immune system responds non-specifically to an antigen while the adaptive immune system refers to the targeted defence against an antigen. Two major characteristics of the adaptive immune system are an antigen specific response and immunological memory. This targeted defence includes both lymphocytes (cellular response) and antibodies (humoral response) (1). There are two main types of lymphocytes, the B and T cells. Both cell types are produced in the bone marrow from the same hematopoietic stem cell. The progenitor T cells migrate to the thymus for maturation, therefore the name thymus-dependent (T) cells, while B cells mature in the bone (B) marrow (1-4). The maturation and expansion of the cells are induced by antigen stimuli and the cytokine milieu. Maturation is the process of developing fully functional lymphocytes with antigen specific receptors. Mature cells are also called effector cells. The aim of lymphopoiesis, the production and maturation of lymphocytes, is to produce a varied array of B- and T-cell receptors on circulating cells (2-4). In this way the individual is able to mount an adaptive immune response against a broad spectrum of pathogens encountered throughout their lifetime (5). Proliferation is the rapid cell division that occurs when mature lymphocytes encounter their specific antigens. Clonal expansion is a type of proliferation, where a specific lymphocyte clone rapidly multiplies. This ensures an efficient production of antigen specific lymphocytes (2-4).

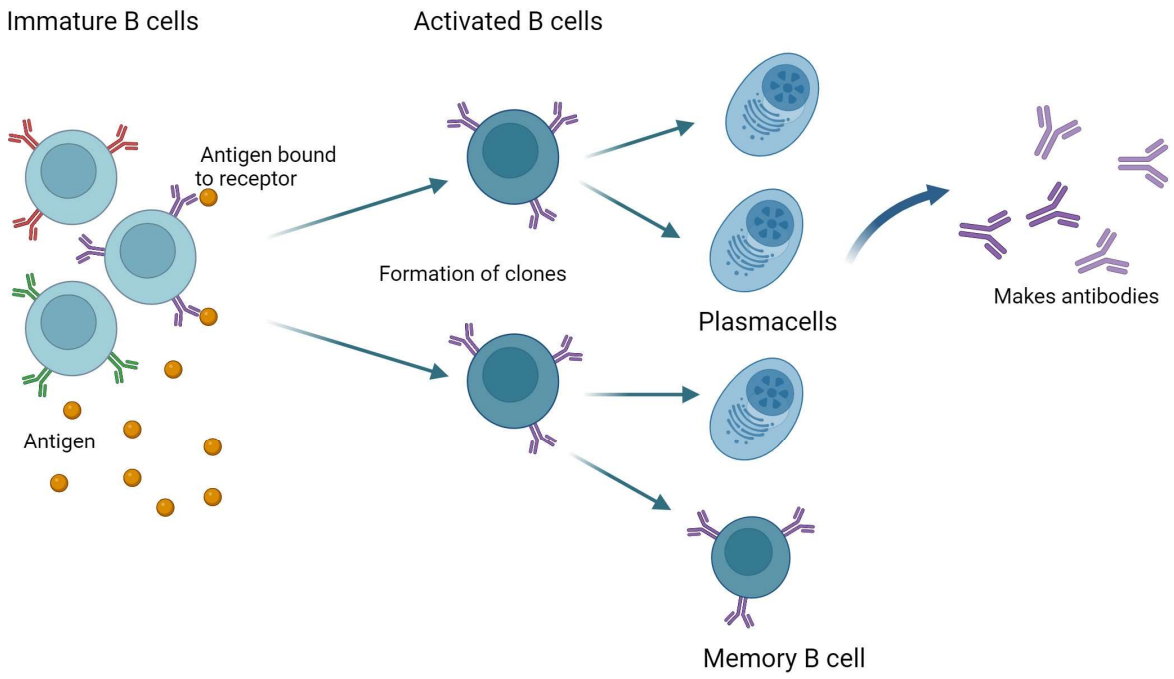
B cells

The maturation of naïve B cells to effector cells usually requires binding of an antigen to the B-cell receptor in addition to the interaction with a helper T cell (3). T cells bind to the antigen presented on a major histocompatibility complex (MHC) II molecule and cause activation of the B cell. Activated B cells may differentiate into memory B cells or plasma cells. Plasma cells are mature B cells that secrete vast amounts of antibodies with the same antigen specificity as the B-cell receptor. In this way, the antigen that activated the B cell becomes the target of the antibodies. Furthermore, some B cells undergo the process of affinity maturation, whereby antibodies with high affinity to the antigen are produced. There are different isotypes of antibodies, like IgG, IgM, IgA and IgE, with IgG being the most abundant isotype found in human blood and extracellular fluid (3). Antibodies can bind to and

neutralise antigens circulating in fluid, blood or presented on the surface of cells. A pathogen like a virus expresses different antigens on its surface and binding of antibodies will stop the virus from entering cells, and thereby hinder the virus replication (3). In addition, antibodies have other roles in the adaptive immune system, such as binding to and increasing clearance of the antigen, complement activation and antibody dependent cellular cytotoxicity (3). The high specificity and potential for large-scale production also make monoclonal antibodies attractive as therapeutic agents, by targeting specific proteins relevant for the disease process (6).

The activated B cells have a crucial role in presenting antigen fragments to T helper cells. This feature is important in activating and regulating the immune response (3). Further, specialised memory B cells can persist for a long period in the absence of the antigen they encountered, hence protecting against reinfection (5).

Figure 1. Maturation and expansion of B cells



Created with BioRender.com

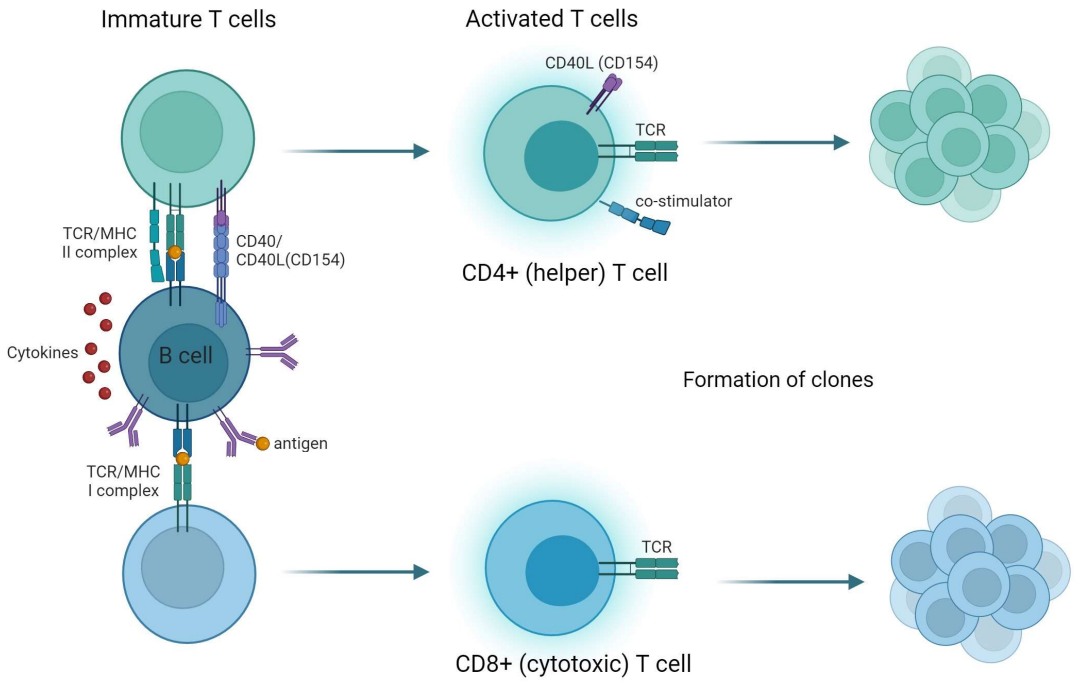
T cells

T cells have specific receptors on their surface, recognising antigens that are bound to HLA on an antigen presenting cell (APC) (B cell, macrophage or dendritic cell). The antigen binding, in addition to co-stimulation and secretion of interleukins (IL) (signalling molecules) from the APC, will induce maturation and clonal expansion, the production of many identical

cells sharing the same antigen specificity (4). The two main types of activated T cells are CD4+ and CD8+ T cells. CD4+ T cells are also called helper T cells. They activate and regulate other cells in the immune system, such as the B cells and CD8+ T cells, through cytokine release (4). CD8+ T cells are also called cytotoxic T cells. They induce cell death (apoptosis) of infected or abnormal cells by releasing cytotoxic molecules (4, 7).

Similar to B cells, some T cells also become long lived memory cells capable of swift reactivation upon encountering the same pathogen in the future (5).

Figure 2. Maturation and expansion of T cells



Created with BioRender.com, TCR=T-cell receptor; MHC= major histocompatibility complex; CD=cluster of differentiation

Surface molecules

Lymphocytes express different proteins or molecules on their surface when they are activated. This signals their engagement in the immune response and can be used to identify and characterise the activated cell (8). Activation markers include many cluster of differentiation (CD) molecules, major histocompatibility complex (MHC) II cell surface receptors (HLA-DR), and chemokine receptors (3, 4). In a laboratory setting, the expression of activation markers can be recognised by their specific binding to antibodies and can be detected by flow cytometry, assessing cell type and state of activation. An example is CD154/CD40 ligand which is an activation marker of CD4+ T cells (3). Not all CD molecules are activation

markers; CD20 is expressed on most stages of B-cell development, but not on the mature plasma cells (9). Other receptors, such as TNF receptors, also play a crucial role in the immune response as they are involved in signalling pathways that regulate inflammation (10).

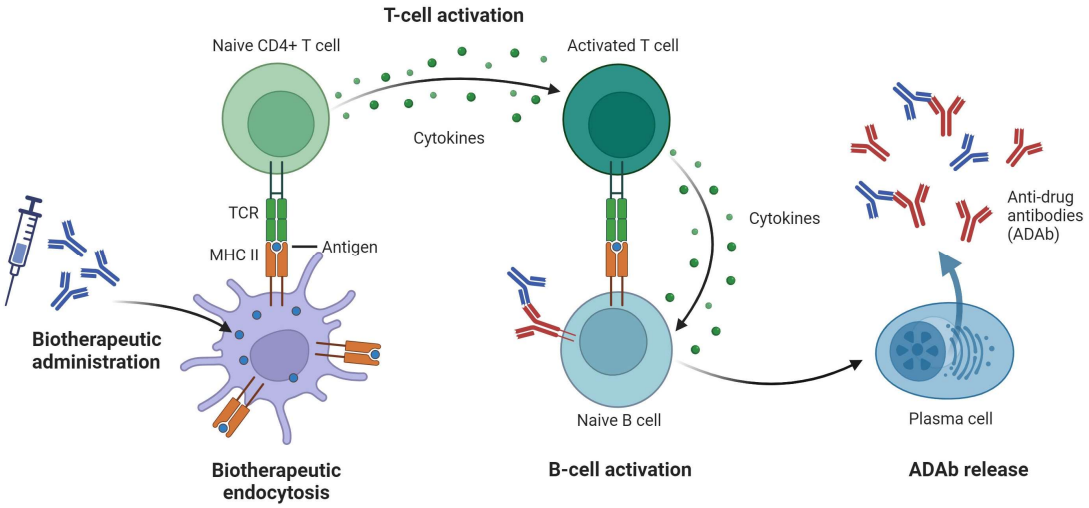
1.2 The concept of immunogenicity

Immunogenicity is defined as the ability of a foreign substrate to induce an immune response (1). The immune system can identify microbes, vaccines, therapeutic drugs and other biologic products as "non-self" and mount a defence response to neutralise or eliminate them (11, 12). For biologic drugs and vaccines, this immune response will impact the efficacy, safety and overall performance of the therapeutic substance (12, 13).

Depending on the therapeutic, immunogenicity can thus be a desirable or an undesirable feature. The immune response triggered by a pathogen like a virus or bacteria is important for the immediate protection during an ongoing infection and for future safeguards against subsequent encounters with the same pathogen. The perfect vaccine is highly immunogenic and triggers a strong adaptive immune response with formation of immunological memory that effectively can neutralise and eliminate the target pathogen when encountered later in life (14, 15).

According to the FDA, immunogenicity of biologic drugs is the "tendency to trigger an unwanted immune response against themselves" (16). Biologic drugs are complex molecules derived from living sources, human or non-human (17). These drugs are used to treat a wide range of medical conditions, including inflammatory joint- and bowel diseases. One important consideration when developing and using biologic drugs is their tendency to trigger the production of anti-drug antibodies (ADAb) (13). ADAb can reduce the therapeutic benefit of the drug (12). Neutralising ADAb binds to the drug and block the binding of the drug to its target, while non-neutralising ADAb binds to other regions of the drug and affect the clearance of the drug (18). In some cases, ADAb can lead to adverse events such as infusion reactions (19-21).

Figure 3: Anti-drug antibody formation



Adapted from BioRender. *"Anti-drug antibodies (ADA) Immunogenicity Assessment",* by BioRender (2020) Retrieved from https://app.biorender.com/biorender-templates/figures/likes/t-5f4d6f77d3e13800adf06dd8-anti-drug-antibodies-ada-immunogenicity-assessment TCR=T-cell receptor; MHC= major histocompatibility complex

The biologic drugs suppress the immune system, while the vaccines rely on a preserved immune response. Therefore, caution is warranted when vaccinating patients using immunosuppressive therapies (like biologic drugs), as the drugs may hamper vaccine efficacy or pose a potential risk. The latter being of particular concern when dealing with vaccines containing live attenuated viruses or bacteria. Different immunosuppressive drugs target different parts of the immune system and therefore have varied impact on the vaccine immunogenicity (22).

Factors that impact immunogenicity

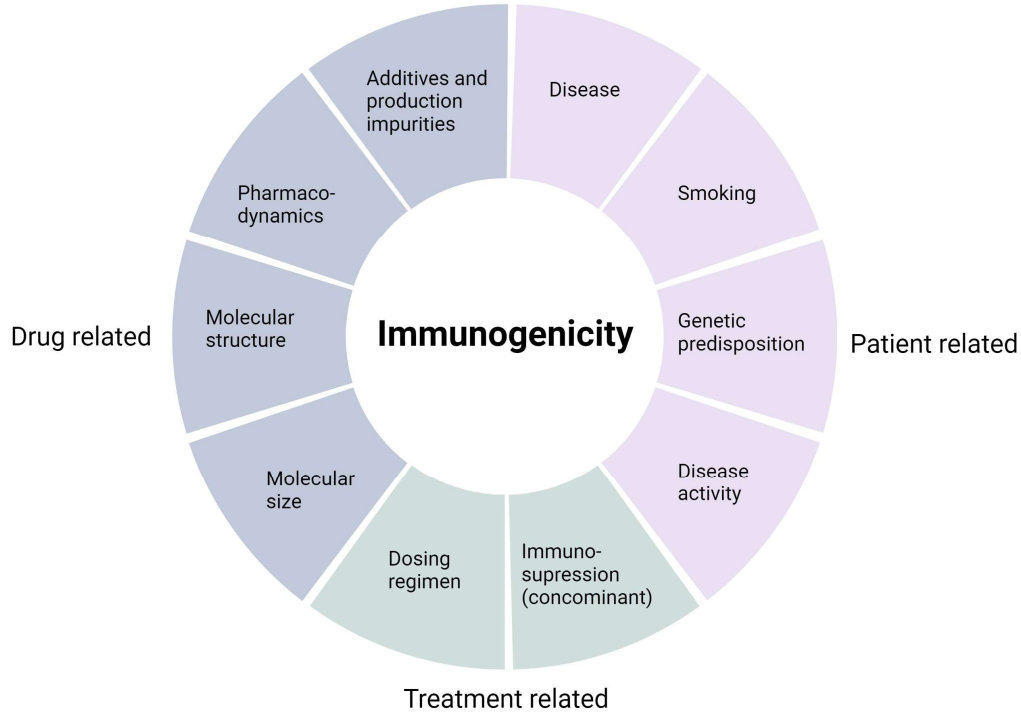
The immunogenicity of a specific agent can vary between patients, with some individuals producing stronger immune responses. Factors such as genetics, disease activity, smoking, type of disease, and baseline immunological status influence this variability (12, 23-26).

Factors in the substrate also impact the immunogenicity. For instance, larger and more complex molecules are possibly more easily targeted by the immune system (27). Foreign molecules are potential more immunogenic, e.g. drugs with sequences from other animals compared to drugs with only human sequences (12). Further, impurities from the production and manufacturing of the drugs as well as additives can make them more immunogenic (28).

In vaccines, the adjuvants are chosen especially for their ability to enhance the immunogenicity (14).

The immunogenicity of a treatment is also influenced by its dosing regimen, encompassing several key factors: the drug dosage, administration frequency, route of administration, formulation and treatment duration. Further, concomitant treatments may also impact the immunogenicity (29-31).

Figure 4. Factors that impact immunogenicity



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1.3 Inflammatory joint- and bowel diseases

Inflammatory joint- and bowel diseases are immune-mediated inflammatory diseases characterised by dysregulation of normal immunity and inflammation (32). They share some common pathogenic features and many of the same drugs are being used to treat the different diseases (33). This thesis will include rheumatoid arthritis, psoriatic arthritis, axial spondyloarthritis, ulcerative colitis and Crohn´s disease.

1.3.1 Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory joint disease, affecting 0.5-1% of the adult population, more common in females (34, 35). It can present at any age, but the incidence increases with age (36, 37). The typical presentation is symmetrical inflammation of joints (34). Rheumatoid arthritis is categorised as seropositive or seronegative by the presence of anti-citrullinated peptide antibodies (ACPA) and antibodies to the Fc fragment of IgG (Rheumatoid Factor) (34).

The inflammation is primarily located in the synovium of the joints and can cause bone and cartilage damage, possibly leading to joint deformation and destruction. In addition, extra-articular manifestations like pulmonary involvement, rheumatic nodules and vasculitis could be present, in addition to systemic comorbidities (34).

The pathogenesis of rheumatoid arthritis is complex and not fully understood. Genetic predisposition is prominent and a positive family history increases the risk of rheumatoid arthritis about 3-5 times (38). Environmental triggers (smoking, viral infections, periodontitis, microbiomes) can induce an activation of B and T cells and the production of autoantibodies like ACPA and Rheumatoid Factor. The link between autoantibody production, synovial inflammation and bone destruction is still not fully understood (34, 35). The later year's successful therapeutic agents have however highlighted some drivers of synovial inflammation in rheumatoid arthritis: cytokines like TNF and IL, in addition to B and T cells and the Janus kinase/signal transducers and activators of the transcription (JAK/STAT)-pathway (35).

1.3.2 Psoriatic arthritis

Psoriatic arthritis is a chronic inflammatory joint disease, affecting joints and entheses, in addition to the axial skeleton (39). The prevalence in the general population is 0.1-1% (40). Between 10 and 30% of patients with psoriasis have psoriatic arthritis (41). The typical onset of psoriatic arthritis is between 30 and 55 years of age, with an equal prevalence between females and males (39, 42).

It is a heterogeneous and complex disease, with joint and bone changes resembling features of both rheumatoid arthritis and spondyloarthritis. In addition to musculoskeletal involvement, it includes nail and skin disease, dactylitis, uveitis and osteitis, and it is associated with cardiovascular disease (39, 42).

The genetic predisposition is important also in psoriatic arthritis, with the risk considerably increasing with a first degree relative with psoriatic arthritis (43). Possibly environmental triggers include infections, dysbiosis of gut microbiomes and trauma (44). The pathogenesis is directed by a dysregulated immune response, with infiltration of immune cells into the skin and musculoskeletal tissues (45). Enthesitis has been proposed as the primary event in developing the disease (46). Both the TNF and IL-23/IL-17 axes are central in the pathogenesis and targeted therapeutic agents directed at these are used successfully in psoriatic arthritis (45).

1.3.3 Spondyloarthritis

The term spondyloarthritis encompasses several different diseases, classified as peripheral or axial (47). The following paragraphs focus on the diseases with primarily axial involvement, radiographic and non-radiographic axial spondyloarthritis. Axial spondyloarthritis affects up to 1% of the general population with a typical onset before 45 years. Radiographic axial spondyloarthritis is predominant in the male population, while the prevalence of non-radiographic axial spondyloarthritis is equal between females and males (48-50).

Axial spondyloarthritis is a chronic autoimmune disease affecting the spine joints, sacroiliac joints and soft surrounding tissue, such as tendons and ligaments. In severe cases, the inflammation can lead to fibrosis and calcification and further fusion of the spine, leading to loss of flexibility. Inflammatory bowel disease and uveitis are some of the associated extra articular manifestations of axial spondyloarthritis (47).

The pathogenesis is complex and not fully understood. There is a genetic component with the gene for HLA B27 being the strongest genetic contributor of a predisposition of axial spondyloarthritis, but it is not essential as only 5-6% of HLA B27 positive persons develop disease (47, 51). HLA B27 is thought to play a major role in the pathogenesis (51). Environmental factors such as infections, dysbiosis of gut microbioma and hormones can trigger the pathological pathways (47, 51). Further, abnormal lymphocyte activation and differentiation, the TNF and IL-23/17 axis are some of the mediators of inflammation (47, 51). Central in the pathogenesis of axial spondyloarthritis is also the formation of new bone in the ligaments and intervertebral joints, syndesmophyte formation (47). This is seen in contrast to the bone destruction that is dominant in peripheral joint involvement in axial spondyloarthritis, but also rheumatoid arthritis and psoriatic arthritis (34).

1.3.4 Inflammatory bowel disease

Inflammatory bowel diseases are chronic immune-mediated inflammatory diseases affecting the gastrointestinal (GI) tract. Crohn´s disease and ulcerative colitis are the main disease types. Crohn´s disease and ulcerative colitis affects up to 0.5% and 0.7% of the adult population, respectively, with the highest incidence in northern Europe and North America. Both diseases are most commonly diagnosed in young adults and affect females and males equally (52-54).

Crohn´s disease can cause inflammation in all parts of the GI tract, from mouth to anus, while ulcerative colitis occurs only in the colon and rectum. Both diseases are characterised by inflammation in the GI tract, with possible complications of structuring, fistulas and abscesses in Crohn´s disease and fulminant colitis in ulcerative colitis. Extra intestinal manifestations are common in inflammatory bowel diseases and include arthritis, uveitis, primary sclerosing cholangitis and erythema nodosum (55, 56).

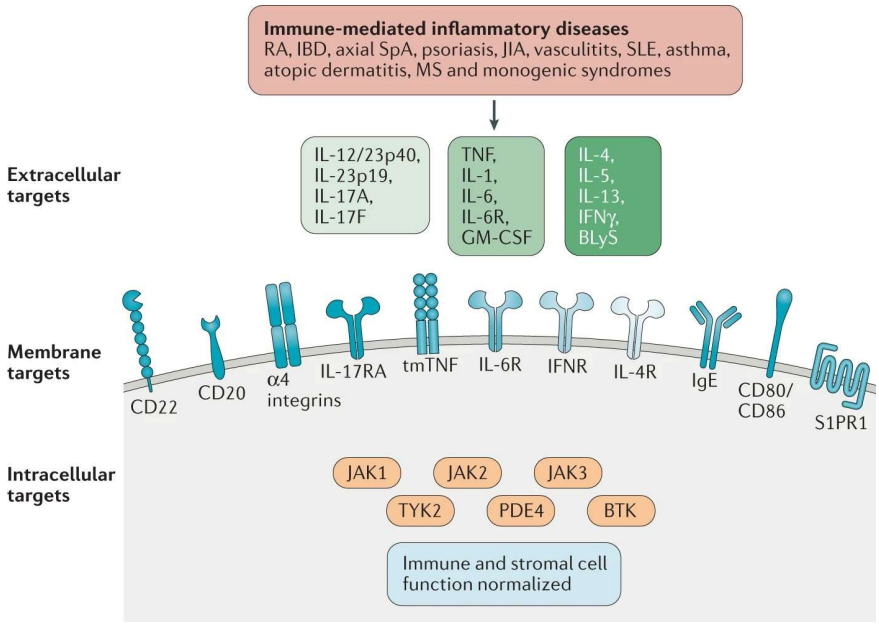
There is a strong genetic component in inflammatory bowel diseases with up to 20% of patients having an affected first degree relative (53). Both the TNF, IL-23/IL-17 and IL-12 axes are thought to play a major role in the pathogenesis. Neutrophils are recruited to the intestine, resulting in inflammation and tissue damage (54)

1.4 Disease modifying drugs

Many of the same therapeutic drugs are used across inflammatory joint- and bowel diseases (33). These drugs include tumour necrosis factor inhibitors (TNFi), non-TNFi biologic agents such as interleukin (IL) inhibitors and T- and B-cell inhibitors, metabolite inhibitors such as methotrexate, and targeted small molecule drugs such as JAK inhibitors (57-60). In addition, glucocorticoids are widely used (59).

In rheumatology, the Disease Modifying Anti-Rheumatic Drugs (DMARDs) are categorised as conventional synthetic (cs) DMARDs, biologic (b) DMARDs, and targeted synthetic (ts) DMARDs (59).

Figure 5. Key targets for the management of immune-mediated inflammatory diseases



Reprinted from *Immune-mediated inflammatory disease therapeutics: past, present and future,* I. B. McInnes and E. M. Gravallese, Nature Reviews Immunology 2021. With permission from Rightslink. (33)

1.4.1 Biologic DMARDs

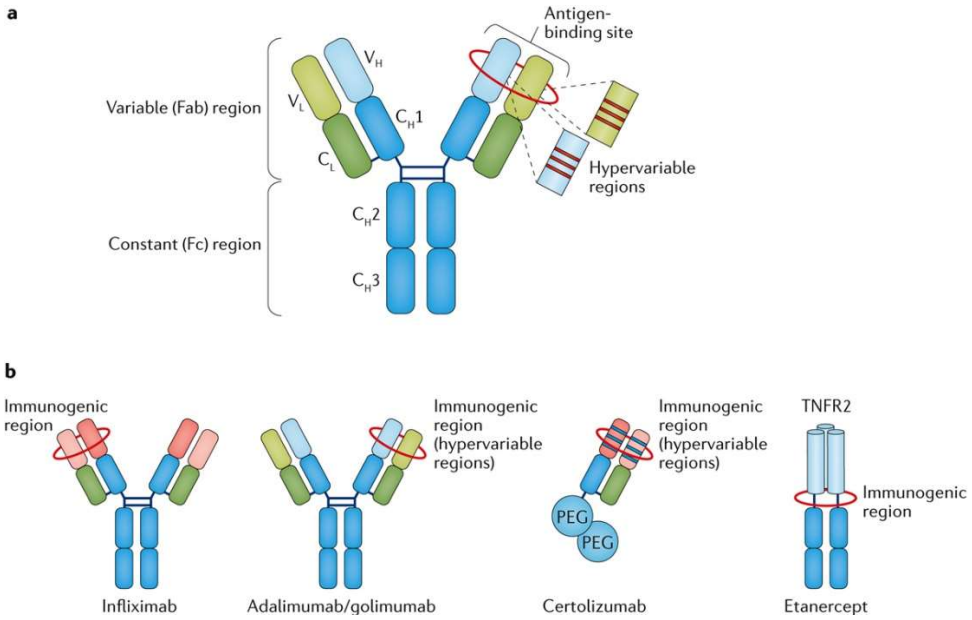
bDMARDs, including TNFi, B- and T-cell inhibitors and IL inhibitors, will be introduced in the following paragraphs.

Tumour necrosis factor alpha inhibitors

Tumour necrosis factor (TNF) is a cytokine produced by macrophages, lymphocytes and Natural Killer (NK) cells, and exists both as a soluble and transmembrane form (61). In normal homeostasis it regulates inflammation. TNF is considered a key factor in the pathogenesis of inflammatory joint- and bowel diseases and has become a target for treating these diseases (61). Tumour necrosis factor inhibitors (TNFi) are biologic drugs, monoclonal antibodies or fusion proteins, binding specifically to TNF and thereby blocking its inflammatory effects. TNFi are now widely used in many inflammatory autoimmune diseases in joint, bowel, skin and eyes (61). The development of TNFi has revolutionised the treatment possibilities of patients with inflammatory joint- and bowel diseases, making remission an achievable treatment goal and reducing long term disabilities (59).

Adalimumab is the most used TNFi on the market and one of the most used drugs worldwide (62). It is a fully human antibody, with no murine sequences (63), administered subcutaneously (s.c.).

Figure 6. Tumour necrosis factor inhibitor structure



Reprinted from *Immunogenicity and loss of response to TNF inhibitors: implications for rheumatoid arthritis treatment.* Kalden, J. R. & Schulze-Koops, H., Nat. Rev. Rheumatol 2017. With permission from Rightslink.

a | Schematic representation of an antibody molecule. The Fc region is responsible for the effector functions of the antibody, and the Fab region forms the antigen-binding site. Within the variable regions are small areas of hypervariability, which determine antigen specificity. **b** | Anti-TNF antibody constructs used in the treatment of rheumatoid arthritis. Infliximab is a chimeric monoclonal antibody with a murine variable region (shown in red) fused to a human Fcγ1 Ig. Adalimumab and golimumab are fully human monoclonal antibodies. Certolizumab is a humanized Fab' fragment bound to polyethylene glycol (PEG) molecules. Etanercept is a TNF receptor–Fcγ1 fusion protein. Potentially immunogenic areas within each antibody construct are indicated in red. Abbreviations: CH, constant heavy; CL, constant light; TNFR2, TNF receptor 2; VH, variable heavy; VL, variable light. (63)

Rituximab

Rituximab is a chimeric (containing murine sequences) monoclonal antibody designed to target the CD20 (Cluster of differentiation 20) receptor present on the surface of B cells (64). It is a treatment option in seropositive rheumatoid arthritis patients that have failed first line therapy, such as methotrexate and TNFi, or where these treatments are contraindicated (64). When rituximab binds to the CD20 receptor, it triggers the immune system to eliminate these B cells (64). The depletion of B cells through rituximab therapy effectively reduces the inflammation in patients with rheumatoid arthritis. However, it also compromises the body´s defence mechanism against pathogens (65, 66). CD20 is present during the entire B-cell maturation process, but not on stem cells and fully mature plasma cells. Consequently, depleting CD20-positive B cells should leave the protective immunologic memory from plasma cells unaffected (67). However, the short-lived plasma cell population will be substantially reduced as the B cell precursor are targeted by anti-CD20 (13).

Interleukin inhibitors and T cell inhibitor

In addition to TNFi and the B-cell inhibitor rituximab addressed in the sections above, other biologic drugs used in inflammatory joint- and bowel diseases include several IL inhibitors and a T-cell inhibitor. These are usually reserved for second- and third line treatment (59, 68).

IL inhibitors binds to IL receptors, thereby inhibiting inflammation and activation of immune cells. The repertoire of IL inhibitors is growing fast, with current used drugs targeting IL-6, IL-17, IL-12 and IL-23 (13, 69).

Abatacept (CTLA-4-IgG) selectively inhibits T cells by blocking the binding of the APC with the CD28+ expressed on T cell, thereby reducing inflammation and also activation of other immune cells (70).

1.4.2 Conventional synthetic DMARDs

Methotrexate is a metabolite inhibitor and folic acid analogue, inhibiting DNA and RNA and therefore cell division. It has had a major impact on treatment of rheumatoid arthritis the last 40 years and continues to be widely used (71). It is the first line treatment in rheumatoid arthritis and is also used in patients with psoriatic arthritis and inflammatory bowel diseases (59, 72, 73). If a patient needs an additional drug like a TNFi or rituximab, methotrexate is usually continued as it has additive therapeutic effect and reduces immunogenicity of biologic drugs (31).

Other csDMARDs commonly used in inflammatory joint- and bowel diseases include sulfasalazine, hydroxychloroquine, and azathioprine (59, 72).

1.4.3 Targeted synthetic DMARDs

JAK inhibitors function by inhibiting one or more of the Janus kinase enzymes (JAK1, JAK2, JAK3, TYK2), and thereby inhibiting cellular signal transduction (74). Uncontrolled JAK-STAT signalling is a key factor in autoimmune inflammatory conditions and JAK-dependent cytokines, like IL-6, play a central role in driving the pathology (74). JAK inhibitors have a limited but growing clinical use in inflammatory joint- and bowel diseases.

1.4.4 Biosimilars

When the patent of an originator drug expires, biosimilar products can be marketed. Biosimilars have the same amino acid sequence as the originator product, although they may display differences in 3D structure and variations in glycosylation patterns (75). Due to a complex production and natural variability, an exact replication of the originator product is

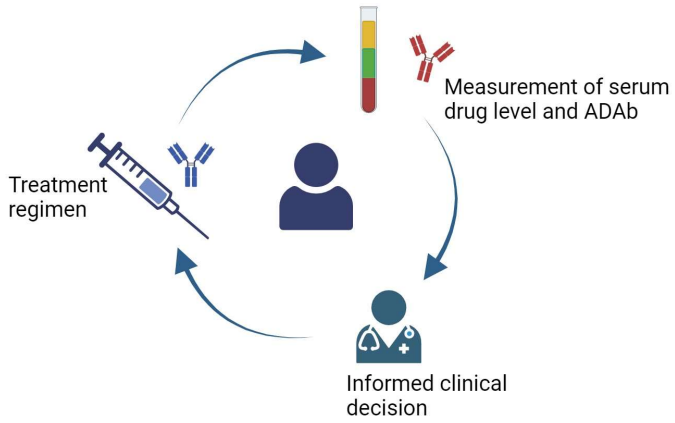
challenging. The biosimilar product is expected to have no meaningful differences from the originator in terms of biologic activity, safety, efficacy, and immunogenicity profile. The regulatory process for designating a product as biosimilar is strict, and includes immunogenicity assessment. However, limited clinical trials are required for regulatory approval of a biosimilar and there is a subsequent extension of indication (75, 76).

In Norway, a national tender system decides which biologic drug that is the first choice for the diverse diseases, with cost calculations being key factor for selection of drugs. The tender is renewed every second year (77). The introduction of biosimilar products has had a large impact on reducing the financial burden of health care budgets and could possibly make these drugs available in countries where prescription is restricted due to high costs (78).

1.5 Therapeutic drug monitoring

Therapeutic drug monitoring (TDM) is a treatment strategy used to optimise the efficacy of a drug, and can aid clinicians in treatment decisions. In TDM of biologic drugs, serum drug levels and anti-drug antibodies (ADAb) are measured, and dosage is adjusted based on knowledge of the drugs optimal therapeutic ranges. Switch of treatment if high levels of ADAb is detected also constitutes part of this treatment strategy (79, 80).

Figure 7. Concept of therapeutic drug monitoring



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The rationale behind TDM is a large variability in serum drug levels between patients and that there is an association between the serum drug levels and treatment response (79, 80). Further, ADAb can cause non-response, by binding to the drug and blocking the binding to its target

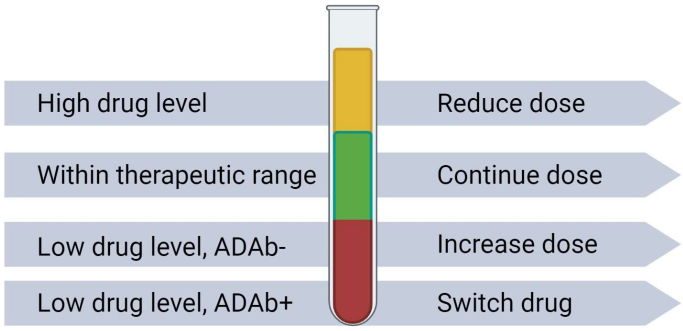
(neutralising ADAb) or affecting the clearance of the drug (non-neutralising ADAb). While low levels of ADAb may be temporary, elevated ADAb levels can impact the drug´s pharmacokinetics and lead to reduced serum drug levels (12). Patients with ADAb formation have poorer clinical outcomes. Assessing ADAb status can be decisive in understanding the reason for sub therapeutic drug levels and non-response, and has the potential to detect treatment failures prior to a clinical flare (21).

Clinical utility

TDM offers an opportunity to individualise the treatment for biologics, aiming for rapid remission and minimising loss of response and disease flares (81). Avoiding disease flares is crucial, as they can increase the risk of long-term disabilities due to joint damage and result in temporary or prolonged loss of work capacity for the patient (82, 83).

The use of TDM in clinical care can be *reactive* or *proactive*. Proactive TDM refers to scheduled assessments regardless of the clinical situation (79). The aim of proactive TDM is to tailor treatment to prevent treatment failures, improve safety and reduce costs associated with overtreatment (12). When TDM is utilised under specific circumstances, for instance a suspected disease flare, it is termed reactive (79). Reactive TDM seeks to understand the reasons for non-response and aid further treatment decisions (12).

Figure 8. Possible algorithm for proactive therapeutic drug monitoring



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The latest “EULAR points to consider” of TDM recommends reactive TDM to help identify loss of treatment response and to assist further treatment choices. Also, it states that TDM can aid tapering in patient with low disease activity or remission (79). Currently, proactive TDM is not recommended in clinical care (79). However, the NOR-DRUM B trial was the first RCT to show a benefit of proactive TDM (81). Patients treated with the intravenous (i.v.)

TNFi infliximab were randomised to either usual care or TDM. In the TDM-group, drug dosage was adjusted if the serum drug level of the patient were over or under the therapeutic range of infliximab. Patients were followed for 52 weeks and the primary endpoint was disease flare. Results from the study demonstrated 17.6% less disease flares in the TDM-group. For other TNFi there are limited data from RCTs, but observational studies have shown that there is a concentration-effect association for several of the drugs (84-86).

In order to use TDM as a tool in clinical care, the therapeutic ranges for the drugs must be determined and algorithms for dose adjustments must be developed. The recent "EULAR points to consider" of TDM stated that there is an association between serum drug levels and treatment effect, but that the lack of identified optimal ranges for the drugs limits the use in clinical care (87).

Anti-drug antibodies and serum drug levels of adalimumab

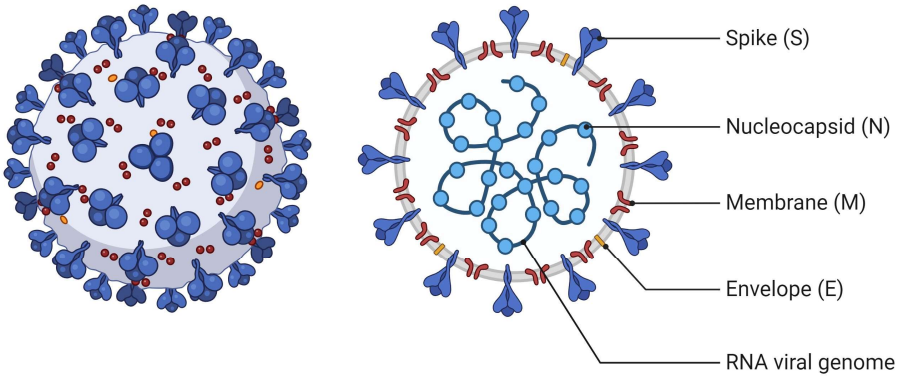
Trials assessing the use of TDM in adalimumab treatment are limited. However, previous studies conducted in Crohn´s disease have demonstrated benefit of TDM (88, 89). A concentration-effect relationship has been demonstrated for adalimumab in inflammatory joint diseases (80). The data existing before paper III suggested a therapeutic range of adalimumab between 4–12 mg/L for patients with rheumatoid arthritis and psoriatic arthritis (90-92). In patients with spondyloarthritis a therapeutic range has not yet been determined (93-96).

Adalimumab has a substantial immunogenic potential, both for the originator and biosimilar products (12, 97). Previous studies have found ADAb in 10–60% of patients treated with adalimumab, and that ADAb formation is associated with poorer treatment outcomes (18, 63, 98-101). Concomitant treatment with methotrexate is recommended in patients with rheumatoid arthritis on adalimumab treatment (59). Methotrexate can reduce ADAb formation and increase the serum adalimumab level (31, 100, 102-104). Other factors possibly related to TNFi immunogenicity are BMI, smoking and drug-holidays (24).

1.6 The COVID-19 pandemic and vaccines

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first discovered in China late 2019, and the World Health Organization (WHO) declared COVID-19 to be a pandemic in March 2020. As of August 2023, 6.7 million deaths and more than 769 million confirmed cases of COVID-19 have been reported globally (105).

Figure 9. SARS-CoV-2 structure



Adapted from BioRender *"Human Coronavirus Structure",* by Gu, J. (2020). Retrieved from https://app.biorender.com/biorender-templates/figures/all/t-5f21e902837656600b08fbe9d-human-coronavirus-structure

SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus. The RNA encodes structural proteins such as spike, nucleocapsid, membrane, and envelope proteins (106, 107) (Figure 9). The spike protein has been a key focus for vaccine development (108). It contains the receptor binding domain (RBD) that binds specifically on the angiotensin-converting enzyme 2 (ACE) receptor on human cells, an essential step for viral entry in host cells (109). The SARS-CoV-2 vaccines work by triggering an immune response against the spike protein, with antibodies to the RBD one of the components of the immune response (110). Consequently, antibodies to RBD have become a target for measuring the immune response to the vaccines (111, 112). Mutations to the spike protein have generated different variants of SARS-CoV-2, such as the delta and omicron (113).

Before the SARS-CoV-2 vaccines were developed, patients treated with immunosuppressive medications were identified to be at risk of severe COVID-19 disease, especially organ transplanted patients (114). In the population with inflammatory joint diseases, rituximab and general comorbidities were identified to put the patients at increased risk (65, 115, 116). Patients, health care providers and decision makers expressed great concerns about the potential outcomes of the COVID-19 pandemic for this group (117, 118).

Immunogenicity of SARS-CoV-2 vaccines

The SARS-CoV-2 vaccines were developed at an unpresented pace (15). Early 2021, the vaccination of health personnel, elderly and immunocompromised patients started. In addition to vector-vaccines, new mRNA vaccines were developed (119, 120). Patients treated with

immunosuppressive medications were not included in the trials preceding the approvals of the vaccines, raising concerns regarding the efficacy and safety of the vaccines within this particular patient group. Identifying patients at risk of poor vaccine response was important to prioritise further vaccine doses and for the update of infection control measures (117, 121).

Before paper I, the vaccine response after two doses in rituximab treated patients were described in mostly small cohorts with different diseases included, but they indicated a poor humoral response and that the T-cell response could be independent of the humoral response (122-126). Further, data prior to paper II suggested a reduced humoral response to two vaccine doses in patients with inflammatory joint- and bowel diseases treated with other drugs than rituximab, as compared to healthy controls (22, 122, 127).

Previous case-series on immunogenicity of a third vaccine dose indicated that, in terms of humoral response, rituximab treated patients had limited effect, but that patients treated with other immunosuppressive drugs could benefit from this (128-131). One case series in rituximab treated patients suggested a possible increase in cellular response after a third dose (128).

Safety of SARS-CoV-2 vaccines

Safety concerns of vaccines can contribute to vaccine hesitancy, possibly reducing the effect of the vaccine programmes (132). A few case series had reported risk of vaccine induced disease flares and increased autoimmunity in patients with various autoimmune diseases. This raised concerns of immune activation with the vaccine response and the possible triggering of concurrent disease related inflammation (22, 133, 134). Accordingly, the knowledge of safety of the initial two vaccines but also to repeated vaccination was crucial at this time.

2 Aims and research questions

General aim

The general aim of this thesis was to assess the immunogenicity of SARS-CoV-2 vaccines and the TNFi adalimumab as well as to identify a therapeutic range of adalimumab in patients with immune mediated inflammatory diseases.

Research questions

- What is the impact of immunosuppressive drugs treatments on the humoral immune response to SARS-CoV-2 vaccines in patients with inflammatory joint- and bowel diseases? (Paper I and II)
- What is the impact of rituximab treatment on the cellular immune response to SARS-CoV-2 vaccines in patients with rheumatoid arthritis? (Paper I)
- Are SARS-CoV-2 vaccines safe in patients treated with immunosuppressive drugs? (Paper I and II)
- What is the therapeutic target range of serum adalimumab in patients with inflammatory joint diseases? (Paper III)
- What is the occurrence and clinical impact of ADAb formation in patients with inflammatory joint diseases treated with adalimumab? (Paper III)

3 Materials and methods

Data from two prospective, observational cohort studies was used in this thesis: *The Norwegian Study of Vaccine Response to COVID-19* (Nor-vaC), and *The Norwegian Antirheumatic Drug Registry* (NOR-DMARD).

3.1 Nor-vaC (Paper I and II)

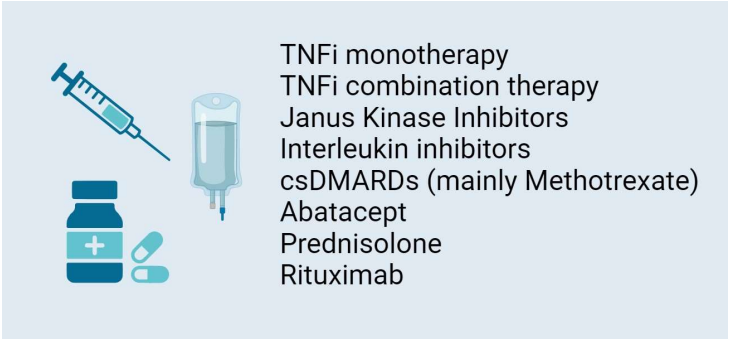
Nor-vaC is a prospective, observational study, following patients receiving SARS-CoV-2 vaccines while treated with immunosuppressive medication within rheumatology and gastroenterology.

3.1.1 Study design

Inclusion criteria and inclusion procedure

Adult patients (>18 years) treated with immunosuppressive medication and with a diagnosis of rheumatoid arthritis, psoriatic arthritis, spondyloarthritis, ulcerative colitis or Crohn´s disease were eligible for inclusion. In addition, patients with autoimmune hepatitis and patients with a liver transplant were included. The aim of the study is to assess response and safety of the SARS-CoV-2 vaccines. Participants were recruited from the Division of Rheumatology and Research at Diakonhjemmet Hospital and the Department of Gastroenterology at Akershus University Hospital in February 2021, before the initiation of the Norwegian Corona Vaccination programme. Health care workers from Diakonhjemmet Hospital, Akershus University Hospital and Oslo University Hospital were included as healthy controls. Approximately 2300 patients and 300 healthy controls are included in the study, and follow up is ongoing.

Figure 10. Medications included in Nor-vaC



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Vaccination procedures

All participants received standard SARS-CoV-2 vaccines through the Norwegian Corona vaccination programme. Three vaccine types were available for the first three vaccine doses: BNT162b2 (Comirnaty [BioNTech/Pfizer]), mRNA-1273 (Spikevax [Moderna]) and ChAdOx1 (Vaxzevria [AstraZeneca]). The two mRNA vaccines were given with an interval of 3–6 weeks between the two doses. The ChAdOx1 vaccine was withdrawn from the Norwegian Vaccination programme on the 11th of March, 2021, and everyone who had received one dose of this vaccine received one of the mRNA vaccines as the second dose.

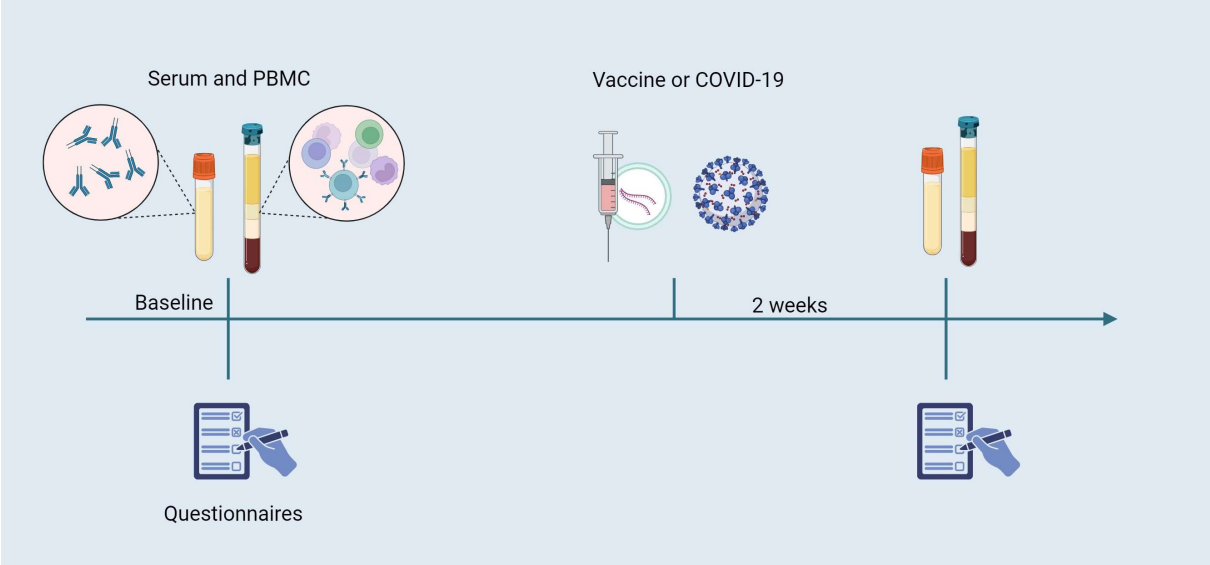
A subset of patients with no or weak humoral response (anti-RBD <100 arbitrary units per millilitre [AU/ml]) after two vaccine doses were recruited into a separate study (EudraCT database no. 2021-003618-37) and received a third vaccine dose during the summer of 2021, prior to the general recommendation for immunosuppressed patients. The cut-off for humoral response when selecting patients qualifying for a third vaccine dose was based on discussions within the Nor-vaC steering group, with specialists in immunology and with the National Institute of Public Health. It was based on knowledge available at that time and the wish to include not only non-responders (<70 AU/ml) but also weak responders (<100 AU/ml), in order to ascertain whether both these groups could mount a response after a third vaccine dose.

3.1.2 Data collection

Blood samples

Blood samples for antibody and cellular analyses were collected at baseline and 2–4 weeks after each SARS-CoV-2 vaccine dose and after COVID-19. All participants included donated serum for antibody assessments. A subset of 20 patients in each medication group, in addition to 20 healthy controls, were also asked to donate peripheral blood mononuclear cells (PBMC). The choice of 20 samples from each group was due to feasibility of conducting complex cellular analyses and previous experience of the laboratory experts.

Figure 11. Study design Nor-vaC



Created with BioRender.com, PBMC=peripheral blood mononuclear cells

Questionnaires

Participants were asked to complete questionnaires at baseline and 2–4 weeks after each vaccine dose. The questionnaires assessed:

- demographic data (diagnosis, age, sex, smoking status)
- medication information (current drugs used, dose, treatment duration)
- patient reported disease activity
- COVID-19 related questions (symptoms, test results, hospital admissions)
- pausing of medication at the time of vaccination
- adverse events after all vaccine doses

At Diakonhjemmet Hospital, data was collected through Nettskjema/Services for Sensitive Data (TSD) (University of Oslo, Oslo, Norway). At Akershus University Hospital, data was collected through Viedoc (Viedoc Technologies, Uppsala, Sweden).

Registry data

Vaccine information (date and type) was obtained from the Norwegian Immunisation Registry (SYSVAK) and positive tests for COVID-19 were collected from the Norwegian Surveillance System for Communicable Diseases (MSIS).

Data from medical records

For paper I, data on rituximab infusions, disease duration and previous medications was collected from the medical records at baseline. Disease activity (DAS28) was assessed by a physician 2–4 weeks after the second vaccine dose.

For paper II, disease activity scores (Disease activity score 28 joint count - Erythrocyte Sedimentation Rate [DAS28] in rheumatoid arthritis and psoriatic arthritis; Ankylosing Spondylitis Disease Activity Score [ASDAS] in spondyloarthritis; Harvey-Bradshaw Index in Crohn´s disease; Partial Mayo Scoring Index in ulcerative colitis) were collected at baseline for patients with inflammatory bowel diseases and from the last clinical visit within three months before receiving the first vaccine dose in inflammatory joint diseases.

3.1.3 Study populations

Participants (patients and healthy controls) with an available blood sample after two or three SARS-CoV-2 vaccines were included in paper I and II. Patients with a clinical diagnosis of rheumatoid arthritis using rituximab were included in paper I. Patients with a clinical diagnosis of rheumatoid arthritis, psoriatic arthritis, spondyloarthritis, ulcerative colitis or Crohn´s disease using all other medications than rituximab were included in paper II. In addition to healthy controls included in Nor-vaC, healthy controls and blood donors from Oslo University Hospital were included in the analyses in both papers.

3.1.4 Laboratory analyses

All assessments of vaccine immunogenicity for paper I and II were performed at the Department of Immunology at Oslo University Hospital.

Serological analyses and cut-offs

The serum samples were analysed with an in-house bead-based assay measuring IgG antibodies to the receptor binding domain (RBD) of the full-length spike protein. The assay measures antibodies to the Wuhan "wild-type" virus, the dominant variant at the time of paper I and II. Seroconversion was defined as anti-RBD ≥ 5 AU/ml. With use of the World Health Organization international standard for anti-RBD antibody, the assay showed a lower detection limit of 1 binding antibody unit per millilitre (BAU/ml) and an upper dynamic range of ~100 BAU/ml. For better quantification of antibodies, most samples for paper II were analysed using a second assay measuring the binding of ACE2 to RBDs from different SARS-CoV-2 variants, as an indicator for neutralising antibody activity. This assay had an upper dynamic range of 300–10 000 BAU/ml (111). Based on findings in healthy individuals, the

cut-off for response was pre-set to an anti-RBD level of 70 AU/ml. Calibration to the WHO international standard showed that 70 AU/ml corresponded to ~40 BAU/ml (111).

Cellular analyses

By flow cytometry, the cells size, granularity, expression of surface markers and intracellular response to the antigen stimuli can be detected and quantified. Briefly, living lymphocytes isolated from blood are stimulated with peptides from the pathogen of interest. This will induce a response in the cells, the cells start producing cytokines and express surface markers. Antibodies with different fluorescence attached can be added to identify the activated surface markers. In the flow cytometer each cell passes through a laser beam scattering the light, the colour and light feedback will determine the size, granularity, surface markers and cytokines produced in the cell. Gating is the procedure of choosing only one type of cells to be analysed, for instance if only interested in the CD4+ T cell´s expression of a specific surface marker (135).

In paper I, thawed Peripheral Blood Mononuclear Cells (PBMC) were stimulated with SARS-CoV-2 spike protein peptides from the wild type and delta variant. Antigen specific T cells were identified by the expression of the surface activation marker CD154 in combination with intracellular TNF in CD4+ T cells, and intracellular expression of IFN-γ or TNF in CD8+ T cells. All samples were analysed by flow cytometry and the results were given in percentage of cells responding.

3.1.5 Main outcomes

The main outcomes were patients with humoral and T-cell responses after two and three SARS-CoV-2 vaccine doses, and the change in antibody levels and T-cell responses after receipt of a third dose. Humoral response was defined as anti-RBD > 70AU/ml. Cellular response was defined as >0.01% responding CD4+ and CD8+ T cells. Additional outcomes were patients with adverse events after two and three vaccine doses.

3.2 NOR-DMARD (Paper III)

NOR-DMARD is a longitudinal multicentre observational study including patients with inflammatory joint diseases starting treatment with a biologic or targeted synthetic DMARD.

3.2.1 Study design

Adult patients (>18 years) with rheumatoid arthritis, psoriatic arthritis, spondyloarthritis, adult juvenile idiopathic arthritis or undifferentiated arthritis starting new treatment with a biologic or targeted synthetic DMARD are eligible for inclusion. Diagnoses are clinical and set by a physician. Patients are re-included if switching to another drug. Biosimilar switching (for instance between adalimumab originator and biosimilar GP2017) does not trigger a new inclusion. Since 2012, biobank sampling at the baseline and 3-months visits have been included. The two centres recruiting patients in addition to collecting biobank samples are Diakonhjemmet Hospital in Oslo and Lillehammer Hospital for Rheumatic Diseases. As of May 2023, 12 000 patients and 20 818 treatment courses have been included in NOR-DMARD and inclusion and follow-up is ongoing.

3.2.2 Data collection

Blood samples

Biobank samples (full blood, serum and urine) are collected at the baseline and the 3-months visit, and stored at -80 ºC. Biobank samples are collected when the patient is at the hospital for regular blood samples, regardless of where they are in the treatment cycle.

Clinical data

Demographic data is collected at the baseline visit and includes sex, co-morbidities, diagnostic data including immunological markers, disease duration, smoking, alcohol consumption, education, work status and marital status. Clinical data is collected at baseline, 3, 6, 9 and 12 months and thereafter every 12 months at visits at the outpatient clinic, either with a study nurse or a physician. Data from each visit is collected through Viedoc (Viedoc Technologies, Sweden).

3.2.3 Study population

Patients with a clinical diagnosis of rheumatoid arthritis, psoriatic arthritis or spondyloarthritis initiating treatment with adalimumab (originator or GP2017) with an available biobank sample were included in paper III. Patients who had terminated their adalimumab treatment before the 3-months visit were not included.

Biobank samples from the 3-months visit, in addition to clinical data from baseline, 3-months visit and the last visit registered were used in paper III.

3.2.4 Laboratory analyses

Biobank samples collected at the 3-months visit were stored at -80ºC. All assessments of adalimumab and ADAb were performed at the Department of Medical Biochemistry at Oslo University Hospital Radiumhospitalet. Both adalimumab and ADAb assays were fully automated on the AutoDELFIA immunoassay platform.

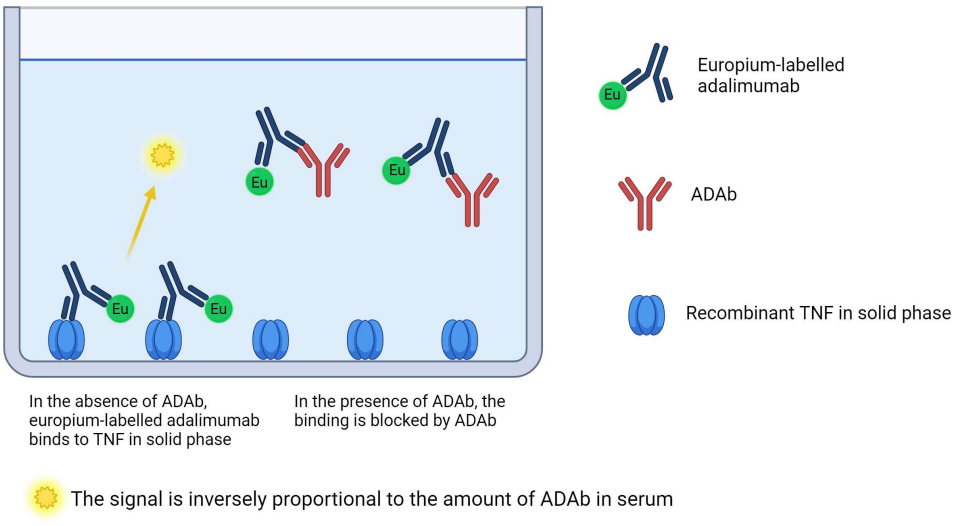
Analyses of serum adalimumab

The samples were analysed with a validated time-resolved fluorescence assay. Human recombinant TNF was the solid phase and the tracer was europium-labelled protein A. The assay measures only free/pharmacologically active drug, as the target molecule TNF is the capture molecule. Adalimumab already bound to its target or blocked by neutralising ADAb is not measured.

Analyses of anti-drug antibodies

Samples with adalimumab levels <3mg/L were further analysed with a drug sensitive assay measuring ADAb. Human recombinant TNF was the solid phase and europium-labelled adalimumab F(ab`)2 used as tracer protein. In the absence of ADAb, europium labelled adalimumab will bind to TNF in the solid phase. In the presence of ADAb, it will block the binding between europium-labelled adalimumab and TNF in the solid phase. ADAb levels ≥15µg/L were defined as positive, levels ≥50 µg/L were considered moderate and levels ≥120µg/L were considered high.

Figure 12. Anti-drug antibody assay



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3.2.5 Main outcomes

The main outcome was treatment response, defined as EULAR Good or Moderate response in rheumatoid arthritis and psoriatic arthritis and ASDAS Major or Clinically Important Improvement in spondyloarthritis.

Disease activity scores and criteria for treatment response in rheumatoid arthritis

Disease Activity Score 28 joint count - Erythrocyte Sedimentation Rate (DAS28-ESR) is the most commonly used disease activity measure in rheumatoid arthritis (136, 137). The score is a modified version of the original DAS and it is based on: tender joint count (TJC), swollen joint count (SJC), erythrocyte sedimentation rate (ESR) and patient global assessment (PtGA) indicated by a score of 0–10 on the visual analogue scale (VAS). It is calculated with the following formula:

$$DAS28\text{-}ESR = 0.56 * \sqrt{(TJC28)} + 0.28 * \sqrt{(SJC28)} + 0.70 * Ln(ESR) + 0.014 * PtGA$$

The DAS28 score is commonly used in clinical care and can classify patients in categories based on disease activity: remission < 2.6, low disease activity ≥ 2.6 – < 3.2, moderate disease activity ≥ 3.2 – <5.1 and high disease activity > 5.1. Criticism of DAS28 includes the lack of joint count of the feet, and that the subjectivity in scoring PtGA can affect the consistency of the score (136).

Table 1. DAS28 categories

Disease activity	DAS28 score
Remission	< 2.6
Low	≥ 2.6 – < 3.2
Moderate	≥ 3.2 – ≤ 5.1
High	> 5.1

DAS28 is also utilised in the EULAR (European Alliance of Associations for Rheumatology) response criteria, which take both the improvement in disease activity and the present DAS28 score into consideration (136). EULAR response is classified as good, moderate or non-response, as shown in table 2.

Table 2. EULAR response criteria

DAS28 score	DAS28 improvement		
	> 1.2	> 0.6 – ≤ 1.2	≤ 0.6
≤ 3.2	Good	Moderate	Non-response
> 3.2 – ≤ 5.1	Moderate	Moderate	Non-response
>5.1	Moderate	Non-response	Non-response

Disease activity scores and criteria for treatment response in psoriatic arthritis

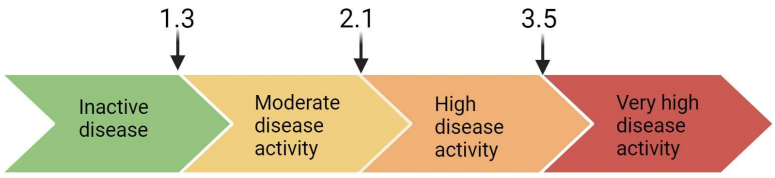
DAS28 and EULAR response criteria have been shown to be valid in psoriatic arthritis and are frequently used as outcome measures for psoriatic arthritis, also in clinical trials (138, 139). However, it does not take into consideration the presence of skin and nail manifestations or involvement of distal joints in the feet. There are composite scores developed for psoriatic arthritis, but they are not widely accepted. The modified DAPSA, DAPSA28, is developed for feasibility reasons as 68 joint count can be hard to obtain in the clinic (140, 141).

Disease activity scores and criteria for treatment response in spondyloarthritis

Ankylosing Spondylitis Disease Activity Score (ASDAS), developed in 2009, is commonly used to evaluate disease activity in spondyloarthritis (142). In addition to CRP, an objective measure of inflammation, it includes patient reported measures of back pain, duration of morning stiffness, peripheral pain/swelling and PtGA on a 10 cm VAS. It is calculated with the following formula:

ASDAS-CRP = 0.12 x Back Pain + 0.06 x Duration of Morning Stiffness + 0.11 x PtGA + 0.07 x Peripheral Pain/Swelling + 0.58 x Ln(CRP+1)

Figure 13. Cut-offs for ASDAS disease activity



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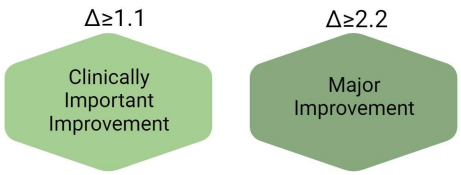
Three cut-offs are used to separate the ASDAS categories of disease activity: 1.3 between inactive and moderate, 2.1 between moderate and high and 3.5 between high and very high. ASDAS is utilised in the response criteria ASDAS improvement, which is classified as clinically important improvement (CII) (change of ≥1.1 unit) or major improvement (MI) (change of ≥ 2.0 unit). These cut-offs take only the change in ASDAS score into account, not the current ASDAS score (143).

Figure 14. Cut-offs for ASDAS improvement scores



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3.3 Statistics

Analyses were carried out in collaboration with a statistician, with the use of STATA (version 16), Graph Pad Prism (version 9) and R (version 3.4.4 and 4.0.3). Statistical analyses of T-cell responses were done using Graph Pad Prism (version 9) by Hassen Kared (shared first author on paper I).

3.3.1 Descriptive statistics

Demographic data, adverse events, humoral and cellular responses after vaccination, serum adalimumab levels and ADAb were summarised with descriptive statistics. Normal distributed variables are presented as mean with standard deviation (SD) and skewed variables as median with 25^{th} – 75^{th} percentile (interquartile range [IQR]). Comparisons of vaccine responses, adalimumab levels and ADAb occurrence were assessed with Mann-Whitney U test (skewed measures), Wilcoxon paired samples (signed rank) test (paired skewed measures), $\chi 2$ test (dichotomous measures) or independent samples t-test (normally distributed measures), as appropriate.

In paper I, Kruskal-Wallis tests for continuous variables and Fisher's exact tests for categorical variables were used to compare potential risk factors in groups of humoral vaccine response.

3.3.2 Multivariable analyses

Paper I and II

Comparisons of humoral response (anti-RBD >70AU/ml) between patients and controls (paper I and II) were done by logistic regression analyses with adjustments for age, sex and vaccine type. In paper II, three models were made with healthy controls as reference group: Model 1 compared to patients, Model 2 compared to the different diseases and Model 3 compared to the different medications.

Predictors of humoral response (anti-RBD >70AU/ml) to two vaccine doses (paper I and II) were assessed by multivariable logistic regression analyses. In paper I, the multivariable model was built by backward elimination, initially including variables from the univariable model with p-value <0.15. Age and sex were included in the final model. CD19+ cell count was not included in the model due to collinearity with time between last rituximab infusion and first vaccine dose. In paper II, all variables from the univariable analyses were included in

the final model, and rheumatoid arthritis, TNFi in monotherapy and two BNT162b2 vaccine doses were the reference groups.

Paper III

Associations between the suggested therapeutic cut-offs and treatment response were explored by logistic regression analyses. Analyses were adjusted for, age, sex, prior use of bDMARDs and concomitant use of methotrexate.

Possible factors associated with adalimumab level and ADAb formation were assessed with multivariable linear and logistic regression analyses. The independent variables were age, sex, prior use of bDMARDs, concomitant methotrexate use, adalimumab type (originator or GP2017) and baseline disease activity. As a surrogate for baseline disease activity across the three diagnoses, we used baseline ESR.

3.3.3 Therapeutic range statistics

Therapeutic range statistics were performed in paper III. Patients were divided in eight equal groups by adalimumab serum levels, resulting in a group size of approximately 21 for rheumatoid arthritis/psoriatic arthritis and 22 for spondyloarthritis. The therapeutic ranges were identified by visual determination of the concentration-effect relationship, pinpointing the cut-off in the analyses. The cut-offs were subsequently used in adjusted analyses (described in the section above) and in the analyses of time until drug discontinuation (Cox proportional hazard multivariable regression analyses).

ROC analyses

Receiver operating characteristic (ROC) analyses were also performed to assess the lower cut-off for adalimumab serum levels, by determining the optimal cut-off for discrimination between response and non-response. Youden Index was utilised to find the cut-off, maximising the sum of sensitivity and specificity (144).

Cox regression

In paper III, drug survival (time until drug discontinuation) was assessed with Kaplan Meyer curves and Cox proportional hazard multivariable regression analyses, adjusted for age, sex, prior use of bDMARDs and concomitant use of methotrexate. Patients were censored at their last visit if they discontinued treatment due to pregnancy, remission, or lacked information regarding the reason for drug discontinuation.

3.3.4 Missing data

In paper I and II, only the patients with an available blood sample after SARS-CoV-2 vaccination were included in the analyses. Hence, there was no imputation of missing data. Some patients did not respond to questionnaires regarding adverse events after vaccination. This was not imputed. Instead, sample size was reduced to the number of patients responding.

In paper III, missing data was handled by median imputation if disease activity components were missing, and by next observation carried backwards (6-month data) if the whole 3-months visit was missing.

3.4 Ethical aspects

All participants in Nor-vaC and NOR-DMARD provided a written informed consent. The studies followed the principles of the Declaration of Helsinki and were approved by an independent ethics committee (Regional Committees for Medical and Health Research Ethics South East Norway).

4 Summary of results

4.1 Paper I

Humoral and cellular immune responses to two and three doses of SARS-CoV-2 vaccines in rituximab-treated patients with rheumatoid arthritis: a prospective, cohort study.

The aim of this study was to assess the humoral and cellular responses to two and three SARS-CoV-2 vaccine doses, as well as vaccine safety, in patients with rheumatoid arthritis treated with rituximab.

87 patients (median age 60 years [IQR 55–67]; 69 [79%] female sex) treated with rituximab were included in the analyses, in addition to 1114 healthy controls (median age 43 years [IQR 32–55]; 854 [78%] female sex). Following two vaccine doses, 19 (22%) of 87 patients had a humoral response compared to 1096 (98%) of 1114 healthy controls. Patients with humoral response had longer time interval between the last rituximab infusion and the first vaccine dose (median 267 days [IQR 222–324]) and a higher number of recovered B cells (mean CD19 count 121 [SD 103]) compared to patients with no response (median days 107 [IQR 801–52] and mean CD19 count 6.5 [SD 17.3]). Factors associated with humoral response after two vaccine doses were the length of the interval between the last rituximab infusion and the first vaccine dose, and the vaccine type (mRNA-1273 compared to BNT162b2).

49 patients that were lacking antibodies after two vaccine doses received a third vaccine dose a median of 70 (IQR 49–104) days after the second dose. The third dose resulted in higher antibodies in 8 (16%) patients.

Cellular analyses were performed by flow cytometry in a subset of 19 and 12 patients after the second and the third vaccine dose, respectively. Following the second dose, 10 (53%) of 19 patients had CD4+ T-cell responses and 14 (74%) of 19 patients had CD8+ T-cell responses. After the third dose, all 12 patients assessed had CD4+ and CD8+ T-cell responses, and the responses corresponded to those observed in healthy controls. The T-cell responses were independent of the humoral responses, as none of the patients included in cellular analyses had a humoral response. Both following two and three vaccine doses, the T-cell responses to the two dominant virus types at the time (wild type and delta) correlated.

Following the second dose, 32 (48%) of 67 patients and 191 (78%) of 244 healthy controls reported adverse events. 17 (42%) of 45 patients reported adverse events after the third dose.

Among these, 5/37 (14%), 3/39 (8%), and 7/45 (16%) reported disease flares after the first, second, and third doses. Additionally, more patients reported an increase in bleeding and bruises following the third dose (7/45 [16%]) than after the second dose (2/39 [5%]). No serious adverse events or deaths occurred during the study period.

We concluded that rheumatoid arthritis patients treated with rituximab have poor humoral response to both two and three vaccine doses. However, the cellular responses were independent of the humoral response and a third vaccine dose induced T-cell responses in all patients. Additionally, both two and three vaccine doses were safe.

4.2 Paper II

Immunogenicity and Safety of Standard and Third-Dose SARS-CoV-2 Vaccination in Patients Receiving Immunosuppressive Therapy

The aim of this study was to assess the humoral response and safety following two and three SARS-CoV-2 vaccine doses in patients with inflammatory joint- and bowel diseases treated with a range of immunosuppressive therapies.

A total of 1647 patients were included in the analyses (566 with rheumatoid arthritis, 305 with spondyloarthritis, 295 with psoriatic arthritis, 280 with Crohn´s disease, and 195 with ulcerative colitis; median age 52 years [IQR 40–63]; 899 [55%] female sex), in addition to 1114 healthy controls (median age 43 years [IQR 32–55]; 854 [77%] female sex). Following two vaccine doses, 1493 (91%) of 1647 patients and 1096 (98%) of 1114 healthy controls had antibody levels ≥70AU/ml, and were considered humoral responders. The anti-RBD levels were lower in patients (median 619 AU/ml [IQR 192–4191]) than in healthy controls (3355 AU/ml [IQR 896–7849]). The lowest response rates, in addition to lowest antibody levels, were seen in patients on TNFi in combination therapy (86% responders; median antibody level 312 AU/ml [IQR 120–2178]), JAK inhibitors (78% responders; median antibody level 361 AU/ml [IQR 45–4204]) or abatacept (53% responders; median antibody level 70 AU/ml [IQR 38–138]).

Following two vaccine doses, younger age and vaccine type (mRNA-1273 compared to BNT162b) were positive predictors of humoral response. Further, treatment with TNFi in combination therapy, JAK inhibitors and abatacept were negative predictors of humoral

response, as compared to TNFi in monotherapy. Pausing of medication prior to vaccination was not a predictor of humoral response following two vaccine doses.

153 patients with weak or no humoral response following the second vaccine dose received a third dose. This included: 97 patients on TNFi in mono and combination therapy, 27 patients on methotrexate monotherapy, 11 patients on JAK inhibitors, 4 patients on abatacept and 11 patients on other medications. After the third dose, 129 (94%) of 153 patients had an observed increase in antibody levels, with a median change of 362 AU/ml (IQR 48–2501).
Adverse events were reported by 810 (50%) of 1516 patients and 191 (78%) of 244 healthy controls after the second dose, and the safety profile was comparable. Further, after the third dose, 70 (44%) of 159 patients reported adverse events. There were no new safety issues, except for a self-reported increase in disease flares in patients with inflammatory joint diseases (16% patients after the third dose compared to 6% patients after both the first and second dose).

We concluded that patients with inflammatory joint- and bowel diseases treated with immunosuppressive medications have attenuated humoral response to two SARS-CoV-2 vaccine doses. A third dose was safe and beneficial in non-responders.

4.3 Paper III

Adalimumab serum levels and anti-drug antibodies: associations to treatment response and drug survival in inflammatory joint diseases

The aim of this study was to assess the association between adalimumab serum levels and treatment response in patients with inflammatory joint diseases, with the intention to suggest a therapeutic range to be used for therapeutic drug monitoring of adalimumab. In addition, we aimed to assess the occurrence and impact of ADAb formation.

We included 340 patients (97 rheumatoid arthritis, 69 psoriatic arthritis, 174 spondyloarthritis; mean age 46 years [SD 14]; 181 [53%] female sex), all initiating adalimumab in a standard dose and with available biobank samples at the 3-months visit. Concomitant treatment with methotrexate was used in 121 (36%) of 340 patients, most of them with a diagnosis of rheumatoid arthritis. At the 3-months visit, the median adalimumab level was 7.3 mg/L (IQR 4.0–10.3), and 33 (10%) patients had developed ADAb. Both findings were comparable across diagnoses.

Based on concentration-effect analyses, patients with rheumatoid arthritis and psoriatic arthritis with serum adalimumab ≥ 6 mg/L were more likely to have response to treatment. This was also consistent in the logistic regression analyses with adjustment for potential confounders (age, sex, previous use of bDMARDs and concomitant methotrexate use). In spondyloarthritis, a cut-off could not be suggested, but we found increasing response rates with increasing serum adalimumab. This was also consistent when adjusting for potential confounders.

Factors associated with ADAb formation were previous use of one or more bDMARD, no co-medication with methotrexate, and originator adalimumab compared to biosimilar GP2017. There were no differences in treatment outcomes between adalimumab versions.

We concluded that there was a concentration-effect association for all diagnoses and that the suggested lower cut-off for adalimumab was 6 mg/L in patients with rheumatoid arthritis and psoriatic arthritis. The large variation in serum drug levels between patients on the same standard adalimumab dosage, along with the notable proportion of patients developing ADAb already at three months underscores the need for additional research into the potential benefits of TDM of adalimumab.

5 Discussion

5.1 Discussion of methodology

In this section I will discuss methodological aspects and possible sources of bias in the papers and the attempts to avoid them.

5.1.1 Study design

Both Nor-vaC and NOR-DMARD are observational studies. The strengths of observational studies lie in the *external validity*; the studies reflect "real life" settings and conditions. Inclusion criteria are often less strict than in randomised controlled trials (RCTs), allowing a diverse range of participants. The external validity refers to the generalisability of the results, if the findings hold true beyond the context of the studies (145). The main contributors to the external validity in the papers in this thesis are the use of real-life data from large, unselected cohorts. Both Nor-vaC and NOR-DMARD have broad inclusion and few exclusion criteria. These are meant to capture a wide range of patients, with different levels of disease activity and comorbidities. Participating in the studies does not influence the choice of treatment given and there is no protocol guiding the change of therapy.

Additional strengths of Nor-vaC are the inclusion of healthy controls, multiple assessments of vaccine responses and safety data, and the baseline sampling of antibodies and T cells. Further, the large sample size of paper I and II is a strength.

Other strengths of NOR-DMARD include the standardised follow up of all patients, with visits at pre-specified time points. In this way, patients with high and low disease activity are followed the same way. Further, analyses of ADAb and drug levels were done retrospectively, both limiting confounding by indication. The modest sample size of paper III may reduce the external validity of the study by reduced statistical power and limited subgroup analyses.

The main weakness of both Nor-vaC and NOR-DMARD, as in other similar observational studies, is loss to follow up.

A general limitation to observational studies is the risk of *bias*, also called s*ystematic errors.* A study can be biased in the selection of patients (*selection bias*), the way the variables are measured (*information bias*) or by *confounding factors* (146). In addition, *random errors,* or the statistical variation*,* are the unpredictable and unavoidable variation in measurements or observations. Such errors will be neutralised if the study population becomes infinitely large (147). In large studies, where random errors are minimised, the main concern is bias (146).

5.1.2 Data collection

Information bias is error in the collection of data or measurements, or missing data. This affects accuracy and reliability and can result in a discrepancy between the measured and the true value (146). In the following sections, potential sources of information bias will be addressed.

Questionnaires

Diagnoses and medications are self-reported in paper I and II, and this may introduce bias. For paper I, diagnoses and medications were checked in all patients with reference to the medical records. For paper II, it was done in a subset of patients. We found reassuringly few mistakes. However, we cannot rule out that some patients are categorised incorrectly in paper II, but the large number of patients included serve to increase the precision.

Recall bias is when participants inaccurately recall previous events or exposures (146). In paper I and II, adverse events were assessed by questionnaires 2–4 weeks after each vaccine dose. Patients with a high burden of symptoms of adverse events may be more likely to recall these than patients with more subtle symptoms. Also, many patients with inflammatory joint- and bowel diseases have daily symptoms resembling the usual adverse events after SARS-CoV-2 vaccination, like fatigue, pain and general feeling of sickness. However, underreporting of mild adverse events does not alter the conclusion that the vaccines are safe in this population.

COVID-19 and impact on vaccine responses

In paper I and II, patients with previous COVID-19 could be a source of information bias, as this probably would influence the interpretation of the antibody level and T-cell reactivity. We measured pre-vaccination antibodies to SARS-CoV-2 but we did not exclude participants showing the presence of antibodies pre-vaccination from our analyses. They constituted a very low number, and therefore we do not suspect this to have influenced the results. In paper I, one patient had positive anti-RBD and anti-nucleocapsid pre-vaccination, this patient had a history of COVID-19 and received one vaccine dose only. None of the patients included in cellular analyses had previous COVID-19. In paper II, 45 (3%) of 1647 patients had pre-vaccination anti-RBD levels above 5 AU/ml, of whom six had levels above 70 AU/ml. Of these six patients, three had known, recent COVID-19 and received one vaccine dose only as per the national vaccination programme. Based on this we can estimate that 3 (0.2%) 1647 of patients most likely had experienced a “silent” COVID-19 prior to vaccination. The rate of

COVID-19 in Norway until the spring of 2021 was low. By the date of our last blood sampling for paper II (the 11th of June, 2021) 2.6% of the Norwegian population was reported to have had COVID-19, supporting our findings that a low number of patients had "silent" COVID-19 prior to vaccination (148).

Timing of samples

For paper I and II, we asked participants to donate blood 2–4 weeks following vaccination. However, we had drop-in service at the laboratory and the participants could therefore come at other times. This might influence the interpretation of the results, but we carefully assessed time of vaccination vs time of blood sampling, based on SYSVAK data. The rise in antibody levels following vaccination is not immediate (149), and in addition, antibodies decay over time (150). In paper II, we excluded participants with < 5 days between vaccination and blood sampling. We based this decision on discussion with collaborating specialist in immunology and on current knowledge of vaccine responses at the time. We cannot exclude that participants especially exposed to and worried about low antibody levels came earlier than recommended and therefore were excluded from analyses, but in paper II this amounted to only two patients after the second and two patients after the third vaccine dose. The timing of blood sampling was satisfactory in patients both in paper I (median 16 days after the second and 23 days after the third dose) and in paper II (median 20 days after the second dose and 23 days after the third dose). Participants who were included for cellular analyses (paper I) had a specific appointment for blood sampling and the timing was therefore within the preferred interval.

For paper III, we used non-trough samples. Trough samples are the gold standard in measuring drug concentrations, as they are taken just before the next infusion/injection and therefore reflect the lowest serum level in the individual between two dosing cycles. In s.c. TNFi, the drug is injected by the patient at home and for feasibility reasons sampling is done when the patient comes to the hospital for a visit (151). For adalimumab and other TNFi administered s.c., the serum levels are quite stable through an injection cycle (151-153). However, we cannot exclude that non-trough sampling has contributed to some of the variability in serum drug levels seen in paper III and the use of non-trough samples to suggest a therapeutic range must be considered when comparing our results to other studies. There is also a possibility that sampling just after adalimumab injection could have led to false low ADAb occurrence, as samples with adalimumab > 3mg/L were not analysed for ADAb.

Missing data

Missing data can be a source of information bias. In paper I and II, we had some missing data on questionnaires asking for adverse events after vaccination. In paper I, 23% of patients did not respond following the second and 15% of patients did not respond following the third vaccine dose. In paper II, 7% of patients did not respond to questionnaires after the second vaccine dose, but all patients responded after the third dose. Missing data was not imputed. Patients with mild or no adverse events might have been less likely to respond to questionnaires, as discussed in section *"Questionnaires"*. In addition, if a patient had a serious adverse event leading to hospital admission or even death, this could have been a source of bias. Reassuringly, we do have data from the Norwegian Cause of Death Registry and the Norwegian Patient Registry confirming that no patients died or were hospitalised due to serious adverse events following vaccination (154).

In paper III, 10 patients had the entire 3-months visit missing, and nine and eight patients had missing one disease activity component at baseline or at the 3-months visit, respectively. Missing 3-months visits were imputed with last observation carried backwards (6-months visit), as imputation of the baseline visit was considered too strict. Missing disease activity components were handled with median imputation. Both median imputation and last observation carried backwards reduces variability and assumes stability of the variables over time (155). Other ways of handling missing data could have been utilised, like multiple imputation by chained equations (156). However, given the minimal amount of missing data, we opted for the methods mentioned above. Additionally, sensitivity analyses excluding patients with missing data demonstrated no alteration in the observed associations. At visits subsequent to the 3-months visit, we had more missing data. Especially during the COVID-19 pandemic, all routine visits were performed digitally and only patients with suspected disease flares were seen at the hospital. Preferably, we would have used longitudinal response data, but this might have been a source of bias in the study with the large amount of missing data. Therefore, we chose to use only 3-months visit data to assess treatment response.

5.1.3 Study populations

Systematic errors, such as *selection bias*, can impact the representativeness of the study population. If the participants included in a study do not reflect the real population, the exposure and outcome association may differ between the participants and nonparticipants (146). This can reduce the external validity of the study. In the following sections potential sources of bias in the study populations will be addressed.

Patient inclusion

For paper I and II, patients with an available blood sample following vaccination were included. Of 92 patients treated with rituximab that were included in Nor-vaC, 87 were included in paper I. Further, of 2178 patients included in Nor-vaC at that time, 1647 were included in paper II. Fragility, fear of infections, and knowledge of their individual antibody levels are some factors that might have influenced the probability of a patient venturing out to donate blood.

There is a chance that the most fragile patients did not come to the hospital for blood sampling, or that they did not participate in the study at all. Fragile patients might be older with more comorbidities and at higher risk at low vaccine responses. They might also be more worried of the COVID-19 transmission pressure and therefore not willing to come to the hospital for blood sampling. In paper I, the humoral vaccine responses were very low, and we do not anticipate that a different selection of patients would change this. The vaccine responses were poorer than what could be explained by age alone. In the adjusted analyses, age was not a significant contributor to humoral response. Nevertheless, age was associated with cellular responses, yet all the patients exhibited robust responses after receiving a third dose.

Patients included in Nor-vaC (paper I and II) were informed of the result of their individual antibody tests. Patients more worried of poor vaccine responses might be more interested in joining the study and also in taking the recommended blood samples in the study. At the time of the vaccination programme when patients had received two vaccine doses, the study-adherence was high. Among the patients with inflammatory joint disease who signed an informed consent, 85% came for at least one and 79% came for at least two blood samples. 15% of patients signed an informed consent but did not take any blood samples, among them 5 patients using rituximab. The age and gender distributions were similar in patients with and without blood samples available, both in paper I and II.

For paper III, patients with an available biobank sample at 3-months visit were included. This means that patients who terminated adalimumab before the 3-months visit, or for other reasons did not donate blood, were not included. By January 2021, 1221 patients (all diagnoses) who had started adalimumab treatment were included in NOR-DMARD. 383 patients had an available biobank sample at three months. The remaining patients had either terminated adalimumab or not provided a biobank sample. In NOR-DMARD, the drug survival rate among adalimumab (RA) patients at three months was around 88%. Early

termination of adalimumab can have various reasons, but could be related to non-response due to low serum drug levels and ADAb development (94, 100). Not including patients with early termination of adalimumab could underestimate the occurrence and negative impact of ADAb. Also, loss to follow up can be associated with low serum drug levels, for instance if the patients are non-adherent. Non-adherence would affect the results, and make the study population less representative. Biobank samples, however, are collected concurrently with routine blood tests during TNFi therapy, increasing the chance of sampling at random. The challenge of collecting complete biobank samples is common to longitudinal registry studies. In NOR-DMARD, 21% of patients treated with adalimumab who had a 3 month visit also provided biobank samples. Missing biobank samples is therefore a limitation to paper III. However, our study PI has not detected any systematic pattern in the groups of patients donating biobank samples, the impression is rather that this is quite random (personal communications).

Patient categorisation

If a participant is categorised incorrectly, for instance put into the wrong diagnosis category, it can influence the representativeness of the patient population (146). In RCTs, strict classification criteria are used. In the studies in this thesis, a clinical diagnosis was used. We cannot rule out that some patients should have belonged to another disease category. However, the diagnoses are given by physicians and we argue that they reflect real life, and likely reflect the populations where our findings may be applied to clinical care.

In the analyses in paper I and III, patients were stratified by methotrexate use. Methotrexate is recommended as co-medication both for adalimumab and rituximab in rheumatoid arthritis, as it has an additive effect and reduces immunogenicity. Patients without methotrexate are possibly a selected group, with lower disease activity and perhaps not tolerating methotrexate. On the other hand, patients with higher disease activity and more aggressive joint disease are possibly more likely to receive methotrexate, and perhaps more likely to respond to treatment, as high baseline disease activity has been shown to be a positive predictor of treatment response in rheumatoid arthritis (157, 158). However, sensitivity analyses in relation to paper III showed that baseline disease activity did not differ between rheumatoid arthritis patients with or without methotrexate. Co-medication with methotrexate was adjusted for both in paper I and paper III.

5.1.4 Laboratory analyses

The handling of blood samples and the laboratory analyses in paper I-III could have introduced *random errors.* The use of validated, automated assays and trained personnel minimise the impact of random errors, and a considerably large number of participants reduces the effect of such errors on the results.

Antibodies, including therapeutic antibodies like adalimumab, are stable in serum. Therefore we do not suspect handling of the samples in terms of freezing and thawing to have a major impact on the results.

The assay for SARS-CoV-2 antibodies (paper I and II) was calibrated according to the World Health Organization international standard, and to the Roche Elecsys anti-SARS-CoV-2 S assay (111).

The cellular samples (paper I) were handled in a standardised way to minimise errors. Reactivity in the cells was tested with use of CytoStim^{TM} (general stimulation). Patients with less than 30 % of live cells and/or no response to CytoStim^{TM} were excluded from the analyses due to the poor recovery and quality of the samples. In order to avoid batch effects, activated VeriCells^{TM} were used as internal control.

The adalimumab and ADAb assays (paper III) were calibrated against the respective pharmaceutical compound and an in-house developed monoclonal antibody, respectively. The assays have been used in routine care since 2014.

5.1.5 Main outcomes

The main outcomes in the studies were response to SARS-CoV-2 vaccines (paper I and II) and treatment response to adalimumab (paper III).

Cut-offs for humoral vaccine response

The use of cut-offs for humoral response and for indication of a third vaccine dose in paper I and II is susceptible to the introduction of *misclassification bias*. If the cut-off is inaccurate or wrong, a patient can be classified as responder when truly being a non-responder and vice versa. This may affect the validity of the findings. During the first year of SARS-CoV-2 vaccination, little was known of the cut-offs and whether the suggested cut-offs corresponded to clinically meaningful protection, initially to the original virus variant, but later also to different variants of concern. Additionally, different assays were used in laboratories around the world, making comparisons between studies and cut-offs difficult. The cut-off for

response (>70AU/ml) used in paper I and II were based on samples from healthy donors, where > 98% had levels > 70AU/ml after two vaccine doses. Later, a micro-neutralisation assay showed that 200 BAU/ml was the lower cut-off for detection of neutralising antibodies, and that the antibody levels to wild-type RBD correlated to the binding and neutralisation of multiple later variants (111).

Disease activity measures

In paper III, the outcome measure is treatment response. Definitions of treatment response are presented in section 3.2.5. Response criteria are based on disease activity measurements, and in rheumatology, some of these are highly subjective. Examples of this are patient global and BASDAI components. A large number of factors, individual to different patients, influences the measures. We cannot exclude that these subjective measures have an impact on the concentration-effect analyses. However, the disease activity measures used are validated and commonly used across studies (143, 159).

We have used DAS28 as disease activity measure in psoriatic arthritis paper III. Evaluating disease activity could also be accomplished through a psoriatic arthritis specific disease activity measure including 68 joint count and extra articular manifestations. However, for feasibility reasons, only 28 joints were assessed in NOR-DMARD, thus preventing the calculation of DAPSA. We performed sensitivity analyses using DAPSA28 in the psoriatic arthritis group and the results were comparable to the results from the DAS28 analyses.

5.1.6 Statistical considerations

Regression analyses

Unmeasured variables, *confounders*, may be related to both the exposure and outcome and thereby alter the association. Confounders are challenging to control in observational studies as they may be unknown or unmeasured. Confounders can be adjusted for in the analyses, if they are measured. Stratifying or use of multivariable regression analyses are ways of adjusting for potential confounders (146).

The goal of regression analyses is to understand and estimate the relationship between the outcome/response variable and one or more variables/covariates (160). It can be used to form predictions, assess causality and summarise data. The choice of variables included and the presentation of the results can differ with the aim of the analysis. Limitations to regression analyses may include the choice or availability of the variables to include or the way models are presented. Knowledge of how the variables are related to each other is important in the

interpretation of presented estimates. Furthermore, caution is warranted when interpreting the precise estimates and p-values (161).

In paper I and II, humoral vaccine responses were assessed by multivariable models. The aim was to compare patients and healthy controls with adjustments for potential confounders. We selected the co-variates prior to performing the analyses. There may still be unknown or unmeasured confounders. Further, models for prediction of humoral vaccine response were made in both papers. In paper I and II, data on comorbidities was not available and therefore not included in the model. Comorbidities are important when assessing COVID-19 outcomes, but we do not suspect different comorbidities to have a major impact on vaccine immunogenicity. Rituximab is not the first drug of choice in rheumatoid arthritis patients, and patients treated with rituximab may have comorbidities that make them unsuitable for other biologic drugs. Nevertheless, it is unlikely that the absence of humoral response in the majority of rituximab treated patients can be attributed to confounding factors such as comorbidities.

In paper III, the aim of the regression analyses was to explore if the estimates for the proposed therapeutic cut-off were consistent after adjusting for potential confounders. We did not have data on smoking and body mass index (BMI) and consequently could not adjust for this.

Strategies to identify therapeutic ranges of adalimumab (Paper III)

To elucidate a therapeutic level for adalimumab, the serum drug range was divided in 8 equal sized groups and the response and remission rates were assessed in each group. The selection of 8 groups was pragmatic. Opting for a higher number of groups (e.g., 10) resulted in a scarcity of patients within each group, whereas choosing fewer groups (e.g., 6) posed challenges in establishing a cut-off. The choice of segmenting the patients according to drug level and visualising the treatment response was also made to provide a depiction of what happens to disease activity after reaching the suggested serum adalimumab cut-off.

The suggested cut-offs were further tested with adjustment for potential cofounders in multivariable logistic regression analyses and cox proportional hazard multivariable regression analyses. To test the cut-off in the same population as the one used to obtain it may be a limitation to this study, with possible issues of multiple testing. Multiple testing increases the likelihood of a false-positive result (type 1 error). Preferably we would have tested the suggested cut-offs in another population, but this was not feasible. Further concerns regarding the cox analyses included the data on discontinuation. Seven patients were recorded as having

discontinued adalimumab treatment, but lacked data on the reason for discontinuation. These patients were censored at their last registered visit, potentially introducing a source of information bias.

Additional ROC analyses were performed after the review process of paper III, supporting the results from the therapeutic range statistics. This approach, however, also has some limitations, including the weighting of sensitivity and specificity by use of the Youden index or other methods. ROC analyses alone do not provide guidance on the optimal threshold. The choice of threshold depends on the clinical context and should be determined based on factors like the severity of the disease and the consequences of misclassification (144, 162). Setting a lower cut-off means that more patients are seen as likely to respond to treatment, but it might also include patients without response (false positives). On the other hand, setting a higher cut-off makes it more certain that patients with serum drug levels above the cut-off have response to treatment, but it might also exclude those who have response to treatment with lower serum drug levels (false negatives).

In paper III, rheumatoid arthritis and psoriatic arthritis were combined in the analyses to achieve adequate statistical size. The use of DAS28 in psoriatic arthritis is already discussed in section 5.1.5. Sensitivity analyses of the therapeutic cut-off yielded comparable results in the two groups, both with the use of EULAR response and DAPSA28 response. Concerns regarding the subgroup analyses revolved around limited sample sizes and potential issues linked to multiple testing.

5.2 Discussion of main results

In this section, I will discuss and interpret the main results according to the specific research questions of this thesis, and compare them to other studies. I will also elucidate clinical implications of the papers.

5.2.1 Humoral response to SARS-CoV-2 vaccines

Two vaccine doses

In rheumatoid arthritis patients treated with rituximab, we found that very few patients had humoral response to two SARS-CoV-2 vaccine doses. Previous studies yielded similar findings as ours (122, 163). Also in line with previous studies, we found that time between the last rituximab infusion and the first vaccine dose was associated with humoral response

following the second vaccine dose, with a median interval of nine months in patients with humoral response (163).

In patients with inflammatory joint- and bowel diseases treated with the other immunosuppressive drugs, we found that most patients had a humoral response to two vaccine doses, but that the antibody levels were considerably lower than in healthy controls. Other studies preceding ours had a limited number of participants, and the data was somewhat conflicting (22, 127, 163-165). In paper II, more than 1000 patients treated with TNFi were included, constituting the largest TNFi cohort at that time. Reduced vaccine immunogenicity, both in response rates and antibody levels, was seen in patients treated with TNFi in combination with methotrexate or azathioprine. As described in section 1.5, these drugs reduce the immunogenicity of TNFi and may also have the same effect on vaccine immunogenicity. Further, both the humoral response rates and antibody levels were low in patients treated with abatacept and JAK inhibitors, but these groups were small and thus our findings must be interpreted with caution. Previous studies in patients on abatacept and JAK inhibitors were also small, with between 8 and 16 included patients (122). However, two studies from 2022 have later confirmed our findings, demonstrating reduced humoral responses in patients treated with JAK inhibitors and abatacept (166, 167).

Age was associated with humoral response to two vaccine doses in paper II, in line with previous studies in healthy subjects (168). In paper I, we did not find the same association, possibly due to the low number of patients with humoral response.

Both in paper I and paper II, we found that vaccination with mRNA-1273, as compared with BNT162b2, was associated with humoral response to two vaccine doses. This had previously been shown in healthy subjects, indicating that mRNA-1273 is more immunogenic than BNT162b2 (169). This difference is probably related to the different amount of mRNA used in the respective vaccines, 30 µg in BNT162b2 and 100 µg in mRNA-1273 (119, 120).

Three vaccine doses

Prior to paper I and II, only case reports had been published on the humoral response after three SARS-CoV-2 vaccine doses in patients treated with immunosuppressive drugs. These reports indicated a marginal additional benefit among patients treated with rituximab, and a moderate additional benefit in patients treated with other immunosuppressive drugs (128-131, 170). We found that in rituximab treated rheumatoid arthritis patients without response to two vaccine doses, a third dose did not improve the humoral response in the majority of patients.

It is plausible that the humoral response in rituximab treated patients, independent of the number of vaccines, will emerge only after B-cell repopulation (171, 172). In patients treated with other immunosuppressive drugs not responding to the first two vaccine doses, 94% improved their humoral response following a third dose. A later Nor-vaC paper, that also included patients responding to the first two vaccine doses, showed that the third dose closed the gap in antibody levels between patients and healthy controls (173).

The implications of poor humoral response were an issue of discussion through the first years of the COVID-19 vaccination, and are still not fully determined. Studies preceding paper I and II, had shown a correlation between the levels of antibodies and the protection from breakthrough COVID-19 (174). Further, higher antibody levels are needed for protection from novel virus strains that are emerging faster than the SARS-CoV-2 vaccines are updated (175, 176). A recent submitted paper from Nor-vaC found that patients with the highest antibody levels had lower risk of COVID-19 (154).

The findings of reduced humoral vaccine response after two vaccine doses, and that a third dose improved the response in most patients, supported a third dose as part of the primary SARS-CoV-2 vaccine series in patients treated with immunosuppressive drugs. This knowledge was important in planning further vaccine strategies, also as vaccines were a limited resource at this time.

Data from paper I clarified the important point that humoral and cellular vaccine responses were unrelated, and that patients lacking a humoral response were still protected against severe disease. Also, these data aided clinicians in timing of rituximab infusions and vaccination to optimise the vaccine response. If possible, the interval between rituximab infusions and vaccination should be at least nine months. In this way, the B cells have the possibility to regenerate, increasing the chance of humoral vaccine response.

5.2.2 Cellular response to SARS-CoV-2 vaccines

The interaction between the humoral and cellular vaccine responses in rituximab treated rheumatoid arthritis patients was not elucidated before paper I was published. Other studies were in general very small and did not discriminate between CD4+ and CD8+ T-cell responses. Our assay provides a more detailed view of the vaccine responses than the widely used IGRA test. CD4+ T cells are mostly engaged in the humoral response, such as activation of B cells, while CD8+ T cells eliminates virus infected cells (3, 4).

Despite a lack of humoral response after two vaccine doses, we found that 53% of the patients had CD4+ and 78% of patients had CD8+ T-cell responses. Previous studies in rituximab treated patients with different rheumatic diseases found that 20-58% of patients had IFNγ-secreting SARS-CoV-2-specific T cells following two vaccine doses, with half of them lacking a humoral response (124-126). We found that all patients analysed following a third vaccine dose had CD4+ and CD8+ T-cell responses, despite the lack of humoral response both following the second and third vaccine dose. One previous case series found that 9/10 patients had IFNγ-secreting SARS-CoV-2-specific T cells both following the second and third vaccine dose, and that the third dose might improve the T-cell responses (128).

Previous studies had shown lower severity of COVID-19 in otherwise healthy patients if they had early and robust SARS-CoV-2 specific T-cell responses, and that in the absence of humoral response, the cellular immunity contributed to protection (177-180). In COVID-19 patients with haematological malignancies, some of them also treated with anti-CD20 therapy, robust CD8+ T-cell responses were associated with improved survival (181). A previous study demonstrated that memory T cells to SARS-CoV-1 are long-lived (182). Recently submitted data from Nor-vaC indicate that rituximab treated patients acquire normal memory T-cell immunity, both after multiple vaccination and breakthrough COVID-19 (paper submitted).

The findings in paper I of robust T-cell responses despite the lack of humoral response in rituximab treated rheumatoid arthritis patients were very reassuring at this time of the pandemic. The data supported a third dose as part of the primary SARS-CoV-2 vaccine series in these patients, as they might have to rely on their cellular responses alone. Many rituximab treated patients shielded through most of the pandemic due to fear of severe COVID-19, as the anticipation was that they would lack humoral response. The awareness of their robust cellular responses following a third dose offered these patients the hope for a return to normalcy in their lives. Later data from Nor-vaC also showed that none of the rituximab treated patients died from COVID-19 post-vaccination, supporting the importance of T-cell immunity when counteracting the virus (154). Paper I was in a review article referred to as one of three most notable papers on the subject of COVID-19 vaccination in individuals with inflammatory rheumatic diseases in 2022 (172).

5.2.3 Safety of SARS-CoV-2 vaccines

Data on SARS-CoV-2 vaccine safety in patients with inflammatory joint- and bowel diseases was scarce before the publication of paper I and II (121). The findings in these two papers

were overall very reassuring, as patients reported lower frequency of adverse events compared to healthy controls and there were no serious adverse events. Patients and healthy controls reported the same type of adverse events. The findings of lower occurrence of adverse events in patients than in healthy controls could be due to the age differences in the two groups, as patients were older. More adverse events have previous been reported with younger age (119). However, there might also be an association between adverse events and humoral vaccine response. In that case, the immunosuppressive medication used could reduce both the adverse events and immunogenicity of the vaccines.

In paper I, more patients reported an increase in bleeding and bruises after the third than the second dose. The sample size was small and these findings should be interpreted with caution. There have been case reports of thrombocytopenia following vaccination (183). However, we cannot conclude that this was the case for the patients in paper I, as none of them were admitted to the hospital with suspicion of bleeding following vaccination (154).

There had been concerns if the SARS-CoV-2 vaccines could aggravate autoimmune diseases but we concluded that the vaccines were safe (22, 133, 134, 184). Both in paper I and II, 16% of patients reported a disease flare after the third vaccine dose. This was only reported in patients with inflammatory joint diseases who had been advised to pause their immunosuppressive medications one week before through two weeks after the third dose. We suspect this to impact on the disease flare reports. All patients were followed in our clinic and we are not aware of any changes in medication due to the reported disease flares after vaccination. Later evidence has supported that there is no increased risk of disease flares following vaccination (185, 186).

Overall, we concluded that both two and three vaccine doses were safe in the patients with inflammatory joint- and bowel diseases treated with immunosuppressive drugs. This information was very useful for clinicians informing patients about vaccine recommendations. Being able to report safety findings were key to avoid vaccine hesitancy among these patients. Safety data was considered when deciding the subsequent vaccine strategy with a third vaccine dose as part of the SARS-CoV-2 prime series in patients treated with immunosuppressive drugs.

5.2.4 Therapeutic range of adalimumab

We found a high variability in serum adalimumab in patients on the same standard dose, and a concentration-effect relationship both in rheumatoid arthritis/psoriatic arthritis and spondyloarthritis.

Rheumatoid arthritis/psoriatic arthritis patients with serum adalimumab levels ≥ 6.0 mg/L were more likely to respond to treatment and had lower risk of drug discontinuation. This finding is in line with previous studies (90-92). Some patients with lower adalimumab levels also responded to treatment. This may be due to differences in disease phenotypes and disease activity, or potentially indicate spontaneous remission where the drug may not be necessary. Patients with adalimumab ≥ 12 mg/L had less response to treatment. Finding an upper cut-off is challenging with assessment of treatment efficacy alone. Other factors such as adverse events and drug costs should be taken into consideration, these factors were not accounted for in our study. The assay used in our analyses measures free drug (as most TNFi assays). High adalimumab levels in a patient not responsive to treatment may also indicate a different disease modality, where the pro-inflammatory effect of TNF is less important. In that case, the patient is not responsive to TNFi treatment, and therefore has higher levels of free TNFi in the serum. Switching treatment may thus be indicated. Further, as discussed in section 5.1.5, the disease activity measures used are partly subjective, and with the limited number of patients in the groups, the suggestion of an upper therapeutic cut-off was pragmatic.

In patients with spondyloarthritis, we found a clear concentration-effect relationship, but the concentration-effect curve did not plateau as seen in the rheumatoid arthritis/psoriatic arthritis group. Other studies have also been unable to identify a therapeutic cut-off in spondyloarthritis (93-96). We found that patients with serum adalimumab < 1.5 mg/L had the lowest response rates and drug survival, indicating that patients at least should have above this level. But we also saw that the highest response rate was seen in the group with serum adalimumab > 11.5 mg/L, suggesting that some patients benefit from high serum levels. The reasons for the difficulties of finding a cut-off, also seen in the ROC analyses, may be several and possibly include interpatient variability in cut-off for therapeutic response. However, it is noteworthy that the spondyloarthritis population differs from the other diagnoses in this regard. This population is heterogeneous and the disease activity outcome ASDAS is mainly based on patient reported measures, making objective assessments in this group challenging. The spondyloarthritis population also differs from rheumatoid arthritis and psoriatic arthritis with regard to disease mechanism. TNF may have a different role in the pathogenesis of

spondyloarthritis compared to rheumatoid arthritis, and spondyloarthritis patients may therefore need less or more TNFi to achieve treatment response (187). The NOR-DRUM trial showed a group effect of TDM of i.v. infliximab in patients with spondyloarthritis, indicating that serum drug level is of consequence also in this patient group (81).

We do believe that the large variability in serum adalimumab levels in patients on the same standard dose, in addition to the findings of better response rates and drug survival in patients with higher drug levels, support the need for individualising drug dosage regiments. The utility of TDM for s.c. TNFi needs to be further investigated in clinical trials, including cost-effect analyses.

5.2.5 Immunogenicity of adalimumab

We found that after three months of treatment, 10% of patients had developed ADAb. This finding was consistent across the three diagnoses. With different assays, sampling time and populations, previous studies have reported an ADAb occurrence of 10-60% (18, 63, 98, 99). The assay used in our work is drug sensitive, meaning that the capacity to detect ADAb is constrained when there is a circulating drug present. The assay detects only ADAb that binds to and blocks the TNF binding capacity of adalimumab. Drug tolerant assays can detect ADAb in the presence of a drug. The clinical relevance of drug tolerant assays is unclear, as they measure free ADAb. The clinical impact of ADAb mainly depends on their neutralisation or reduction of active drug to sub therapeutic levels (12).

Our finding of similar occurrence of ADAb across the three diagnoses is in contrast to the NOR-DRUM-A trial (24). In this RCT, testing the effectiveness of TDM in patients initiating the i.v. TNFi infliximab, patients with spondyloarthritis had lover ADAb occurrence compared to rheumatoid arthritis and psoriatic arthritis patients. The differences seen between the NOR-DRUM-A trial and our study might be due to the induction phase of i.v. infliximab, where patients with spondyloarthritis receive higher doses than patients with rheumatoid arthritis. In adalimumab, the dosage is the same across the three diagnoses, with no induction phase. In line with previous studies, we found that patients with ADAb formation had poorer response rates and drug survival than patients without ADAb formation (18, 102).

Factors associated with ADAb formation were previous use of one or more bDMARD, no methotrexate co-medication and adalimumab type. Co-medication with methotrexate has previously been shown favourable in rheumatoid arthritis patients treated with adalimumab

(31). This is suggested to be partly due to its effect on the pharmacokinetics, with increased serum drug levels and reduced ADAb formation (12, 31, 90, 188).

We found higher occurrence of ADAb formation in originator than biosimilar (GP2017) adalimumab. Previous studies have demonstrated slight variations in immunogenicity between originator and biosimilar products. However, treatment outcomes remain comparable, consistent with findings in our study, and the differences were not regarded clinically significant (97, 189). In Norway, a national annual tender system decides the order of biologic drug to use in inflammatory joint- and bowel diseases (77). Adalimumab was the first choice in two consecutive periods of two years each, first originator and then biosimilar (GP2017) drug. Patients starting originator adalimumab before this period (when only originator was available) are possibly a different population. They might have longer disease duration, and tried more medications before initiating adalimumab. In sensitivity analyses without this population, the differences between the drug types diminished. This could be due to the differences in the population, as discussed above, or the reduction of sample size in the sensitivity analyses. As the phase III studies of both originator and biosimilar compounds are done on a highly selected population, we argue that post marketing surveillance of immunogenicity in real life populations such as the NOR-DMARD cohort is important.

6 Conclusions

6.1 Answers to research questions

With reference to the specific research questions presented in section 2, we drew the following conclusions:

- Immunosuppressive drugs reduce the humoral response to the initial two SARS-CoV-2 vaccine doses in patients with inflammatory joint- and bowel diseases. A third dose given to non-responding patients increased the humoral response, except in patients using rituximab. (Paper I and II)

- Rheumatoid arthritis patients treated with rituximab have CD4+ and CD8+ T-cell responses to SARS-CoV-2 vaccines, independent of the humoral response. A third vaccine dose induced T-cell responses in all patients comparable to healthy controls, underlining the importance of a third dose in rituximab treated patients. (Paper I)

- Two and three SARS-CoV-2 vaccine doses are safe in patients with inflammatory joint- and bowel diseases treated with immunosuppressive drugs. (Paper I and II)

- In rheumatoid arthritis and psoriatic arthritis, the suggested lower cut-off for the therapeutic range of serum adalimumab is 6 mg/L. In spondyloarthritis, a cut-off was not identified, but increasing response rates are seen with increasing serum adalimumab. (Paper III)

- 10% of patients across diagnoses developed ADAb to adalimumab after three months of treatment, which was associated with poorer treatment outcomes, both in terms of response and drug survival. (Paper III)

6.2 Further research and future perspectives

6.2.1 Vaccine immunogenicity

As mentioned in the discussion, section 5.2, the findings in paper I and II were useful in the critical situation of the ongoing pandemic. Subsequent papers from Nor-vaC have elucidated some of the questions raised during the course of the pandemic.

The paper *"The persistence of anti-Spike antibodies following two SARS-CoV-2 vaccine doses in patients on immunosuppressive therapy compared to healthy controls—a prospective cohort study"* showed that following the second vaccine dose, patients on a range of immunosuppressive drugs lost their antibodies more rapidly than healthy controls. This, in addition to the initial lower antibody levels, made a large amount of patients fall under the anticipated positive cut-off for antibodies (150). The paper *"Immunogenicity and safety of a three-dose SARS-CoV-2 vaccination strategy in patients with immune-mediated inflammatory diseases on immunosuppressive therapy"* assessed the third vaccine dose in all patients, not only those lacking response after the second dose. Here, we showed that the third vaccine dose closed the gap in antibody levels between patients and healthy controls (173). The paper *"Four SARS-CoV-2 vaccine doses or hybrid immunity in patients on immunosuppressive therapies: a Norwegian cohort study"* showed that patients with hybrid immunity (COVID-19 + three vaccine doses) had higher antibody levels than patients with four vaccine doses (190). Further research (submitted manuscript) show that patients with higher antibody levels have lower risk of breakthrough infection. In addition, the vaccinated patients infected in the omicron area had a good prognosis (154).

Ongoing research from the Nor-vaC project group includes longitudinal T-cell responses in patients with inflammatory joint- and bowel diseases treated with TNFi, in depth characterisation of the B and T-cell response in rituximab treated rheumatoid arthritis patients, and neutralising antibodies and cellular responses following the fifth vaccine dose with different boosters.

Moving forward, we hope to be able to answer some of the remaining research questions:

- What is the long-term immunity to SARS-CoV-2 vaccines/infection?
- Whom to boost and when to do it?
- What is the safety of repeated vaccines?
- Who are the weak responders despite multiple vaccine doses and why?

6.2.2 Therapeutic drug monitoring

TDM has the potential to enhance patient care by tailoring treatments to improve clinical outcomes. Possible future developments of TDM include incorporation of dashboard systems or clinical support tools, facilitating truly personalised dosing (191, 192). Moreover, advancements in rapid testing methods (even at point of care) can make TDM more accessible, allowing the clinician to assess drug levels and make necessary dose adjustments during outpatient clinic visits. Home-test solutions that enable the patient to collect blood-samples from finger pricks have been developed, and open novel possibilities of TDM use in clinical care. Patients can conveniently send blood samples to the laboratory, and the result will be readily available during their clinical appointment (191, 193). Extension of indications for TNFi across diagnoses, and the dosing of TNFi in a one-dose-fits all manner, results in under- but also overexposure to the drugs. TDM assisted tapering of TNFi can contribute to reduce unnecessary high drug doses, with the advantages of reducing drug costs and avoiding possible adverse events (194).

Current treatment recommendations endorse the use of reactive but not proactive TDM in clinical care (87). These recommendations were published before the NOR-DRUM-B trial, showing benefit of proactive TDM in the maintenance phase of i.v. infliximab (81). To be able to implement TDM in clinical care, more research is needed in establishing therapeutic ranges and algorithms for adjusting drug dosages. Paper III and other studies assessing the therapeutic range of TNFi add on to this knowledge. Further, RCTs are needed to explore the effect of TDM as a treatment strategy (195).

Funded by EU's Horizon Europe, the REMEDY center will coordinate the "Rheumatoid Arthritis Therapeutic Drug Monitoring Trial" (RA-DRUM). RA-DRUM is a multicentre, multinational, RCT that aims to assess if proactive TDM is superior to usual dosing in order to achieve sustained disease control without disease flares in patients with rheumatoid arthritis treated with s.c. TNFi. I will continue to pursue immunogenicity of TNFi working as a national coordinator/post doc in RA-DRUM.

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8 Papers

Paper I

Humoral and cellular immune responses to two and three doses of SARS-CoV-2 vaccines in rituximab-treated patients with rheumatoid arthritis: a prospective, cohort study

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Summary

Background In rituximab-treated patients with rheumatoid arthritis, humoral and cellular immune responses after two or three doses of SARS-CoV-2 vaccines are not well characterised. We aimed to address this knowledge gap.

Methods This prospective, cohort study (Nor-vaC) was done at two hospitals in Norway. For this sub-study, we enrolled patients with rheumatoid arthritis on rituximab treatment and healthy controls who received SARS-CoV-2 vaccines according to the Norwegian national vaccination programme. Patients with insufficient serological responses to two doses (antibody to the receptor-binding domain [RBD] of the SARS-CoV-2 spike protein concentration <100 arbitrary units [AU]/mL) were allotted a third vaccine dose. Antibodies to the RBD of the SARS-CoV-2 spike protein were measured in serum 2–4 weeks after the second and third doses. Vaccine-elicited T-cell responses were assessed in vitro using blood samples taken before and 7–10 days after the second dose and 3 weeks after the third dose from a subset of patients by stimulating cryopreserved peripheral blood mononuclear cells with spike protein peptides. The main outcomes were the proportions of participants with serological responses (anti-RBD antibody concentrations of ≥70 AU/mL) and T-cell responses to spike peptides following two and three doses of SARS-CoV-2 vaccines. The study is registered at ClinicalTrials.gov, NCT04798625, and is ongoing.

Findings Between Feb 9, 2021, and May 27, 2021, 90 patients were enrolled, 87 of whom donated serum and were included in our analyses (69 [79·3%] women and 18 [20·7%] men). 1114 healthy controls were included (854 [76·7%] women and 260 [23·3%] men). 49 patients were allotted a third vaccine dose. 19 (21·8%) of 87 patients, compared with 1096 (98·4%) of 1114 healthy controls, had a serological response after two doses (p<0·0001). Time since last rituximab infusion (median 267 days [IQR 222–324] in responders *vs* 107 days [80–152] in non-responders) and vaccine type (mRNA-1273 *vs* BNT162b2) were significantly associated with serological response (adjusting for age and sex). After two doses, 10 (53%) of 19 patients had $CD4^+$ T-cell responses and 14 (74%) had $CD8^+$ T-cell responses. A third vaccine dose induced serological responses in eight (16·3%) of 49 patients, but induced $CD4^+$ and $CD8^+$ T-cell responses in all patients assessed (n=12), including responses to the SARS-CoV-2 delta variant (B.1.617.2). Adverse events were reported in 32 (48%) of 67 patients and in 191 (78%) of 244 healthy controls after two doses, with the frequency not increasing after the third dose. There were no serious adverse events or deaths.

Interpretation This study provides important insight into the divergent humoral and cellular responses to two and three doses of SARS-CoV-2 vaccines in rituximab-treated patients with rheumatoid arthritis. A third vaccine dose given 6–9 months after a rituximab infusion might not induce a serological response, but could be considered to boost the cellular immune response.

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Introduction

SARS-CoV-2 vaccines have proven efficient and safe in the general population,[1,2] but a good vaccine response depends on a functional immune system that includes concerted B-cell and T-cell responses. Immunosuppressive medications, and particularly rituximab, an anti-CD20 B-cell-depleting therapy, are known to impair the immunogenicity of influenza and pneumococcal vaccines.[3] Patients with rheumatoid arthritis on rituximab therapy have been reported to be at increased risk of severe outcomes from COVID-19,[4–7] and it is crucially important to evaluate their response to SARS-CoV-2 vaccination. Observational data in small cohorts of patients with rheumatoid arthritis have

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Research in context

Evidence before this study

We searched PubMed for studies published in English between Jan 1, 2020, and Sept 29, 2021, using different combinations of the search terms, "Rheumatoid arthritis", "vaccination", "SARS-CoV-2", "COVID-19", "rituximab", and "response". Previous observational studies on vaccine responses in patients with rheumatoid arthritis were generally small, but indicated that rituximab impairs serological responses to vaccines, including SARS-CoV-2 vaccines. Sparse information exists on T-cell responses to SARS-CoV-2 vaccines and no data exist on three-dose SARS-CoV-2 vaccination in rituximab-treated patients with rheumatoid arthritis.

Added value of this study

In this cohort of 87 patients with rheumatoid arthritis on rituximab treatment, only 19 (21·8%), compared with 1096 (98·4%) of 1114 healthy controls, had a serological response after two vaccine doses. Time between the last rituximab infusion and the first vaccine dose was significantly associated with vaccine response, with a median interval of about 9 months in responders. Cellular immune responses were present in more than half of patients after two doses. A third vaccine dose given to patients with insufficient serological responses to two doses was safe and elicited a robust T-cell response in all patients tested, despite inducing serological responses in only a small proportion of patients.

Implications of all the available evidence

If possible, patients should be vaccinated against COVID-19 before the initiation of rituximab therapy. For an optimal response, the interval between rituximab infusion and vaccination should be as long as possible, preferably at least 9 months. In rituximab-treated patients with rheumatoid arthritis, a cellular immune response might be present after vaccination in the absence of anti-SARS-CoV-2 antibodies. A third vaccine dose given 6–9 months after a rituximab infusion might not induce a serological response but could be considered to boost the cellular immune response. The clinical significance of the cellular immune response in the absence of virus-specific antibodies remains to be elucidated. Alternative anti-rheumatic therapies might be considered in individual patients if repeated rituximab infusions preclude the development of protective anti-SARS-CoV-2 antibodies.

indicated that rituximab impairs serological SARS-CoV-2 vaccine responses.[8–11] Previous reports have suggested that T cells are necessary for protection against severe COVID-19 in settings of low antibody titres,[12] for rapid and efficient resolution of COVID-19[13] and for protection against fatal outcomes in patients treated with anti-CD20 therapies for haematological malignancies.[14] To date, sparse data exist regarding cellular responses to SARS-CoV-2 vaccines in rituximab-treated patients with rheumatoid arthritis.[11,15] In the absence of a normal serological response, cellular immunity is of crucial interest in this patient group.

The utility of a third vaccine dose in immunocompromised patients, and in the general population, is an urgent question in the global medical community and for policy makers.[16,17] Whether patients with B-cell depletion who do not serologically respond to two vaccine doses will benefit from a third dose is unclear. A case series on rituximab-treated patients indicated limited benefit from a third dose.[18]

We therefore aimed to assess humoral and cellular responses and adverse events following two doses and three doses of SARS-CoV-2 vaccines in patients with rheumatoid arthritis treated with rituximab.

Methods

Study design and participants

Nor-vaC is an ongoing, longitudinal, prospective, cohort study being conducted at two Norwegian hospitals with large specialist clinics: the Division of Rheumatology and Research at Diakonhjemmet Hospital, Oslo, and the Department of Gastroenterology at Akershus University Hospital, Oslo. Eligibility criteria are presented in the appendix (p 2). Eligible patients identified by hospital records received an invitation to participate in the study on Feb 15, 2021, before initiation of the national vaccination programme. This analysis includes rituximab-treated patients with rheumatoid arthritis. Healthy controls were blood donors and health-care workers from collaborating hospitals (Diakonhjemmet Hospital, Akershus University Hospital, and Oslo University Hospital) in Oslo, Norway. The study was approved by an independent ethics committee (Regional Committees for Medical and Health Research Ethics South East; reference numbers 235424, 135924, and 204104) and by appropriate institutional review boards. All patients and healthy controls provided written informed consent.

See Online for appendix

Procedures

All participants received SARS-CoV-2 vaccines according to the Norwegian national vaccination programme. Three SARS-CoV-2 vaccines were available: BNT162b2 (Pfizer–BioNtech), mRNA-1273 (Moderna), and ChAdOx1 nCoV-19 (AstraZeneca). The two mRNA vaccines were given with an interval of 3–6 weeks between the two doses. The ChAdOx1 nCoV-19 vaccine was withdrawn from the Norwegian vaccination programme on March 11, 2021, and all people who had received one dose of this vaccine received one of the mRNA vaccines as the second dose. The vaccines were administered to participants following a priority list given by the Norwegian Institute of Public Health. According to

the programme, people who had recovered from COVID-19 received one vaccine dose only. During the conduct of this study, patients with concentrations of antibodies against the receptor-binding domain (RBD) of SARS-CoV-2 of less than 100 arbitrary units (AU)/mL after two vaccine doses were recruited into a separate study (EudraCT number 2021–003618–37) and allotted a third vaccine dose in July–August, 2021. Patients receiving a third dose were asked to pause their concomitant disease-modifying antirheumatic drug (DMARD) treatment 1 week before until 2 weeks after vaccination.

Informed consent forms and questionnaires were collected through the Services for Sensitive Data platform at the University of Oslo, Oslo, Norway. At baseline and approximately 14 days after the first, second, and third vaccine doses, participating patients were asked to complete questionnaires regarding: demographic data (eg, diagnosis, age, sex, weight, height, and smoking status); medication use; patient-reported disease activity; COVID-19-related questions (ie, symptoms, test results, and hospitalisation); pausing of medication at the time of vaccination; and adverse events after all doses. The date of the last rituximab infusion, the total number of rituximab infusions, disease duration, rituximab treatment duration, co-medications, and number of previous DMARDs were obtained from medical records by investigators at baseline. Disease activity (disease activity score in 28 joints, patient global assessment, and physician global assessment) was assessed 2–4 weeks after the second vaccine dose by investigators. Information about vaccination dates and vaccine types was obtained from the Norwegian Immunisation Registry, SYSVAK by investigators.[19] Information regarding patients testing positive for COVID-19 before and during the study period was obtained from the Norwegian Surveillance System for Communicable Diseases by investigators.[20] For 868 healthy controls, only information on vaccine date and type, sex, and age were collected. 246 controls (health-care workers at Diakonhjemmet Hospital and Akershus University Hospital) additionally answered detailed questionnaires on demographic data and adverse events at baseline and 14 days after each vaccine dose.

Antibodies to the full-length spike protein and the RBD of SARS-CoV-2 were measured 2–4 weeks after the second vaccine dose and 2–4 weeks after the third dose by use of an in-house bead-based method (appendix pp 3–4).[21] We defined antibody concentrations higher than the second percentile of those from healthy individuals vaccinated with two doses, corresponding to concentrations of 70 AU/mL or more, as response.[22] Concentrations of less than 5 AU/mL were defined as no response and concentrations of 5–69 AU/mL were defined as weak response. Calibration to the WHO international standard showed that 70 AU/mL corresponds to approximately 40 binding antibody units per mL.

Before the first vaccine dose, a subset of patients (n=20) and controls (n=20) were asked to provide blood samples for cellular analysis before and 7–10 days after the second vaccine dose. The number was based on the feasibility of conducting complex cellular analyses and the previous experience of the researchers conducting them. 12 of 20 patients were recipients of a third dose and additionally donated blood for cellular analyses 3 weeks after the third dose. Thawed peripheral blood mononuclear cells were stimulated with SARS-CoV-2 PepTivator spike protein peptides (Miltenyi Biotec; Bergisch Gladbach, Germany) of the wild-type or delta variant (B.1.617.2), which consisted of 15-mer sequences with 11 amino acids overlap covering the immunodominant parts of the spike protein, in the presence of costimulatory antibodies against CD28 and CD49d (0·5 µg/mL for both; BD Biosciences; Franklin Lakes, NJ, USA) and Brefeldin A (10 µg/mL; MilliporeSigma; Burlington, MA, USA). SARS-CoV-2-specific T cells were identified by dual expression of tumour necrosis factor (TNF) and CD40-L (CD154) for $CD4^+$ T cells and by single or dual intracellular expression of interferon-γ (IFNγ) and TNF for $CD8^+$ T cells. All samples were acquired on an Attune NxT (Thermofischer; Waltham, MA, USA) flow cytometer and analysed by use of FlowJo software (version 10). For a detailed description of the methodology regarding T cells, please see the appendix (pp 5–6).

For the **Services for Sensitive Data platform** see https://www.uio.no/tjenester/it/forskning/sensitiv/

Objectives and outcomes

The two main objectives of this study were to assess (1) humoral and T-cell responses to two doses and three doses of SARS-CoV-2 vaccines in patients with rheumatoid arthritis on rituximab therapy compared with healthy controls and (2) changes in humoral and T-cell responses after a third vaccine dose given to patients with insufficent serological responses (anti-RBD <100 AU/mL) to two doses. Other objectives were to assess the safety of two-dose and three-dose vaccination and to identify predictors of serological response in patients.

The outcomes were: the proportions of participants with serological responses (anti-RBD antibody concentrations of >70 AU/mL) and T-cell responses to spike peptides following two and three doses of SARS-CoV-2 vaccines; the change in concentrations of anti-RBD antibodies and T-cell responses to spike peptides after the third dose; adverse events; and predictors of serological responses to two-dose and three-dose vaccination.

Statistical analysis

A formal sample size calculation was not done and all eligible patients willing to participate were included. Demographic data, adverse events, and serological responses were summarised by use of descriptive statistics. Comparisons of serological response between patients and controls were done by logistic regression.

Adjustments were made for sex, age, and vaccine type. Comparison between pre-vaccination and post-vaccination samples in patients receiving a third vaccine dose was done by a Wilcoxon paired samples test. GraphPad Prism paired analysis and the Wilcoxon matched pairs signed rank test were used to compare the frequencies of antigen-specific T cells. Comparisons of potential risk factors between response groups were done by Kruskal–Wallis tests for continuous variables and Fisher's exact tests for categorical variables. To assess predictors of serological response to vaccine doses, univariable and multivariable logistic regression analyses were done. Relevant variables were chosen by the investigators after a review of the existing literature. For multivariable model building, all factors with p values of less than 0·15 from univariable analyses, age, and sex were included. The final model was obtained with significant variables only by backward elimination of the least significant variable. Spearman correlation tests were used to compare T-cell responses versus age and the time since last rituximab infusion, to compare T-cell responses to wild-type spike protein versus delta spike protein, and to compare specific responses of $CD8^+$ T cells and $CD4^+$ T cells. All tests were two-sided and done at the 0·05 significance level. Analyses were done using Stata (version 16), GraphPad Prism (version 9), and R (version 3.4.4). The study is registered at ClinicalTrials.gov, NCT04798625.

	Patients receiving at least two doses (n=87)	Patients receiving third dose (n=49)	Healthy controls receiving two doses (n=1114)
Age, years	60 (55–67)	62 (56–67)	43 (32–55)
Sex			
Female	69 (79·3%)	43 (87·8%)	854 (76·7%)
Male	18 (20·7%)	6 (12·2%)	260 (23·3%)
Body-mass index, kg/m²	25 (23–29)	25 (22–28)	..
Current smoker*	11 (12·6%)	7 (14·3%)	0
Vaccines			
Two doses of BNT162b2	63 (72·4%)	39 (79·6%)	625 (56·1%)
Two doses of mRNA-1273	21 (24·1%)	8 (16·3%)	246 (22·1%)
BNT162b2 plus mRNA-1273	0	0	2 (0·2%)
ChAdOx1 nCoV-19 plus BNT162b2 or mRNA-1273	0	0	241 (21·6%)
SARS-CoV-2 infection plus BNT162b2 or mRNA-1273*	3 (3·4%)	2 (4·1%)	0
Rituximab monotherapy	31 (35·6%)	16 (32·7%)	..
Prednisolone use	14 (16·1%)	5 (10·2%)	..
Dose of prednisolone, mg/day	5 (1)	5 (2)	..
Methotrexate use	42 (48·3%)	22 (44·9%)	..
Dose of methotrexate, mg/week	15 (6)	14 (6)	..
Duration of rituximab therapy, years	6 (3–9)	6 (3–9)	..
Number of rituximab infusions	9 (3–15)	11 (4–16)	..
Number of previous DMARDs	5 (3–7)	5 (3–6)	..
$CD19^+$ B cell count†‡, cells per µL	28·9 (67·4)	9·7 (20·7)	..
C-reactive protein concentration†§, mg/L	3·8 (5·0)	3·3 (4·5)	..
Erythrocyte sedimentation rate†§, mm/h	11·5 (9·5)	9·7 (5·7)	..
DAS28†¶	2·4 (1·1)	2·1 (0·8)	..
Time between rituximab and first vaccine dose, days	140 (87–224)	100 (74–147)	..

Data are median (IQR), n (%), or mean (SD). DAS28=disease activity score in 28 joints. DMARDs=disease-modifying antirheumatic drugs. *Available data only on health-care workers at Diakonhjemmet Hospital and Akershus University Hospital. †Assessments done after the second dose. ‡Data available for 58 patients receiving at least two doses and 40 patients receiving a third dose. §Data available for 66 patients receiving at least two doses and 40 patients receiving a third dose. ¶Data available for 65 patients receiving at least two doses and 39 patients receiving a third dose.

Table 1: **Baseline characteristics**

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between Feb 9, 2021, and May 27, 2021, 90 patients with rheumatoid arthritis being treated with rituximab were enrolled, 87 of whom (median age 60 years [IQR 55–67]; 69 [79·3%] women and 18 [20·7%] men) donated serum at a median of 16 days (IQR 12–21) after the second vaccine dose and were included in our analyses (table 1). In addition, control samples from 1114 healthy health-care providers and blood donors (median age 43 years [IQR 32–55]; 854 [76·7%] women and 260 [23·3%] men) were included. 56 (64·4%) of 87 patients used a conventional systemic DMARD concomitantly: methotrexate (n=42), leflunomide (n=9), sulfasalazine (n=4), or hydroxychloroquine (n=1). 14 (16·1%) patients used prednisolone as co-medication, all of whom took a dose of less than 10 mg/day. Most patients were either vaccinated with two doses of BNT162b2 (63 [72·4%]) or mRNA1273 (21 [24·1%]); three patients had had COVID-19 before vaccination and received only one vaccine dose (table 1). No patients developed COVID-19 after two-dose or three-dose vaccination.

19 (21·8%) of 87 patients, compared with 1096 (98·4%) of 1114 healthy controls, had a serological response after two doses (p<0·0001; table 2). After two doses, 14 (16·1%) patients and 14 (1·3%) controls had a weak response, and 54 (62·1%) patients and four (0·4%) controls had no response (table 2; figure 1A). The median time between the last rituximab infusion and the first vaccine dose was significantly longer in responders than in patients with a weak response or no response (table 3; figure 1B). Univariable logistic regression identified the interval between the last rituximab infusion and the first vaccine dose (per 100 days), $CD19^+$ cell count, and vaccine type (mRNA-1273 compared with BNT162b2) to be significantly associated with humoral response after two doses (appendix p 8). In the multivariable logistic regression model, the interval between the last rituximab

	Healthy controls receiving two doses (n=1114)	Patients receiving at least two doses (n=87)	Patients receiving third dose (n=49)
No response*	4 (0·4%)	54 (62·1%)	29 (59·2%)
Weak response*	14 (1·3%)	14 (16·1%)	12 (24·5%)
Response*	1096 (98·4%)	19 (21·8%)	8 (16·3%)
Anti-RBD antibody titre, AU/mL	257 (198–327)	3 (2–34)	3 (2–18)

Data are n (%) or median (IQR). AU=arbitrary units. RBD=receptor-binding domain. *Anti-RBD antibody concentrations of less than 5 AU/mL defined no response, of 5–69 AU/mL defined weak response, and of 70 AU/mL or more defined response.

Table 2: **Serological response to two and three vaccine doses in patients and healthy controls**

infusion and the first vaccine dose (per 100 days) and vaccine type (mRNA-1273 compared with BNT162b2) were significantly associated with serological response when adjusted for age and sex (appendix p 8).

49 patients (median age 62 years [IQR 56–67]; 43 [87·8%] women and six [12·2%] men) with insufficent serological responses (<100 AU/mL) to two doses were allotted a third vaccine dose at a median of 70 days (IQR 49–104) after the second vaccine dose. In these patients, median anti-RBD antibody concentrations were 2 AU/mL (IQR 2–3) after the second dose and 3 AU/mL (2–18) after the third dose (figure 1A, C). Comparison between anti-RBD antibody concentrations in samples after the second dose and samples after the third dose showed a median change of 0·96 AU/mL (IQR 0·05–27·38; p<0·0001). Eight (16·3%) of 49 patients had a serological response after the third dose, with a median interval between the last rituximab infusion and the third dose of 250 days (IQR 206–265; table 2; figure 1C, D; appendix p 7). Two patients who had initially received one vaccine dose because they had a history of previous COVID-19, and later received their second dose with inclusion in this group, did not develop a serological response. No significant associations between the investigated factors and serological response after the third dose were found in a multivariable regression analysis (appendix p 8), possibly due to the low number of patients with a response (n=8).

T-cell responses were analysed in 19 of 20 invited patients after the second vaccine dose. 12 of these 19 patients were allotted a third vaccine dose and provided blood samples for T-cell response assessment after the third dose. After two doses, 10 (53%) of 19 patients had SARS-CoV-2 wild-type-specific $CD4^+$ T-cell responses and 14 (74%) had SARS-CoV-2 wild-type-specific $CD8^+$ T-cell responses (figure 2A; appendix p 6). The patients without anti-spike protein $CD8^+$ T-cell responses (five [26%]) also did not have detectable anti-spike protein $CD4^+$ T cells. Time since the last rituximab infusion was not correlated with T-cell response (data not shown). T-cell responses were detected in all vaccinated healthy donors (n=20)

	No response* (n=54)	Weak response* (n=14)	Response* (n=19)	p value†
Age				
≤30 years	2 (4%)	0	1 (5%)	0·10
31–65 years	30 (56%)	12 (86%)	15 (79%)	..
>65 years	22 (41%)	2 (14%)	3 (16%)	..
Body-mass index, kg/m²	25 (22–28)	26 (24–28)	27 (23–31)	0·47
Sex				
Female	45 (83%)	9 (64%)	15 (79%)	0·26
Male	9 (17%)	5 (36%)	4 (21%)	..
Current smoker	6 (11%)	1 (7%)	4 (21%)	0·47
Co-medication with DMARDs‡	34 (63%)	10 (71%)	12 (63%)	0·90
Number of previous DMARDs	4 (2–6)	5 (3–7)	5 (3–7)	0·62
Number of rituximab infusions	11 (4–16)	5 (2–14)	9 (6–13)	0·44
$CD19^+$ B cell count§, cells per µL	6·5 (17·3)	48·5 (95·2)	121·0 (103·3)	<0·0001
Erythrocyte sedimentation rate, mm/h	11·2 (7·5)	8·3 (6·2)	15·1 (14·7)	0·45
C-reactive protein concentration, mg/L	4·2 (5·9)	2·2 (1·7)	4·2 (4·0)	0·33
DAS28	2·3 (0·9)	2·1 (1·1)	2·9 (1·5)	0·13
Time between rituximab and first vaccine dose, days	107 (80–152)	137 (61–233)	267 (222–324)	<0·0001
Vaccines				
SARS-CoV-2 infection plus BNT162b2 or mRNA-1273	0	2 (14%)	1 (5%)	0·016
Two doses of BNT162b2	44 (81%)	9 (64%)	10 (53%)	..
Two doses of mRNA-1273	10 (19%)	3 (21%)	8 (42%)	..

Data are n/N (%), n (%), median (IQR), or mean (SD). AU=arbitrary units. DAS28=disease activity score in 28 joints. DMARDs=disease-modifying antirheumatic drugs. RBD=receptor-binding domain. *Anti-RBD antibody concentrations of less than 5 AU/mL defined no response, of 5–69 AU/mL defined weak response, and of 70 AU/mL or more defined response. †p values correspond to comparisons of categories across response groups using Kruskal–Wallis tests for continuous variables and Fisher's exact tests for categorical variables. ‡Includes methotrexate, leflunomide, sulfasalazine, and hydroxychloroquine. §Five patients received rituximab between having their second dose and donating blood for $CD19^+$ B cell count measurement and are not included here.

Table 3: **Baseline factors according to response to two vaccine doses in patients**

after their second vaccine dose, with response magnitudes similar to those seen in patients (figure 2A). The reduced T-cell responsiveness to the vaccine in patients versus controls could not directly be explained by the regimen of immunosuppressive drugs (rituximab monotherapy or rituximab combined with conventional synthetic DMARDs) because the activation induced by polyclonal stimulation of the T-cell receptor (with Cytostim) was similar between patients and controls, indicating normal functional responses (data not shown). After the third dose, all 12 patients had detectable anti-wild-type spike protein $CD4^+$ and $CD8^+$ T-cell responses, including five patients who did not have T-cell responses after the second dose (figure 2A).

To evaluate the potential of vaccines to induce a cross-protection against currently circulating viral strains, we

extended the T-cell analysis, challenging peripheral blood mononuclear cells from vaccinated patients with spike peptides derived from the SARS-CoV-2 delta variant (B.1.617.2). The magnitude of T-cell responses to the delta variant spike protein correlated with the magnitude of responses towards wild-type spike protein for both $CD4^+$ and $CD8^+$ T-cell responses after the second and third dose (figure 2B). Combined anti-spike protein T-cell responses directed against wild-type and delta SARS-CoV-2 spike peptides are shown in figure 2C. The positive correlation between $CD4^+$ T-cell responses and $CD8^+$ T-cell responses (Spearman r=0·6401; p<0·001) suggested that the vaccine elicited concerted T-cell immunity. Patient age negatively correlated with the number of anti-spike protein $CD4^+$ T cells (figure 2D).

After two doses, adverse events were reported in 32 (48%) of 67 patients and in 191 (78%) of 244 healthy controls (figure 3; appendix p 9). 19 (42%) of 45 patients receiving a third dose reported an adverse event (figure 3; appendix p 9). For patients who received a third dose, the numbers of adverse events were similar after the second dose and after the third dose, with the exception of bleeding and bruises, which were more frequently reported after the third dose (seven [16%] of 45 patients) than after the second

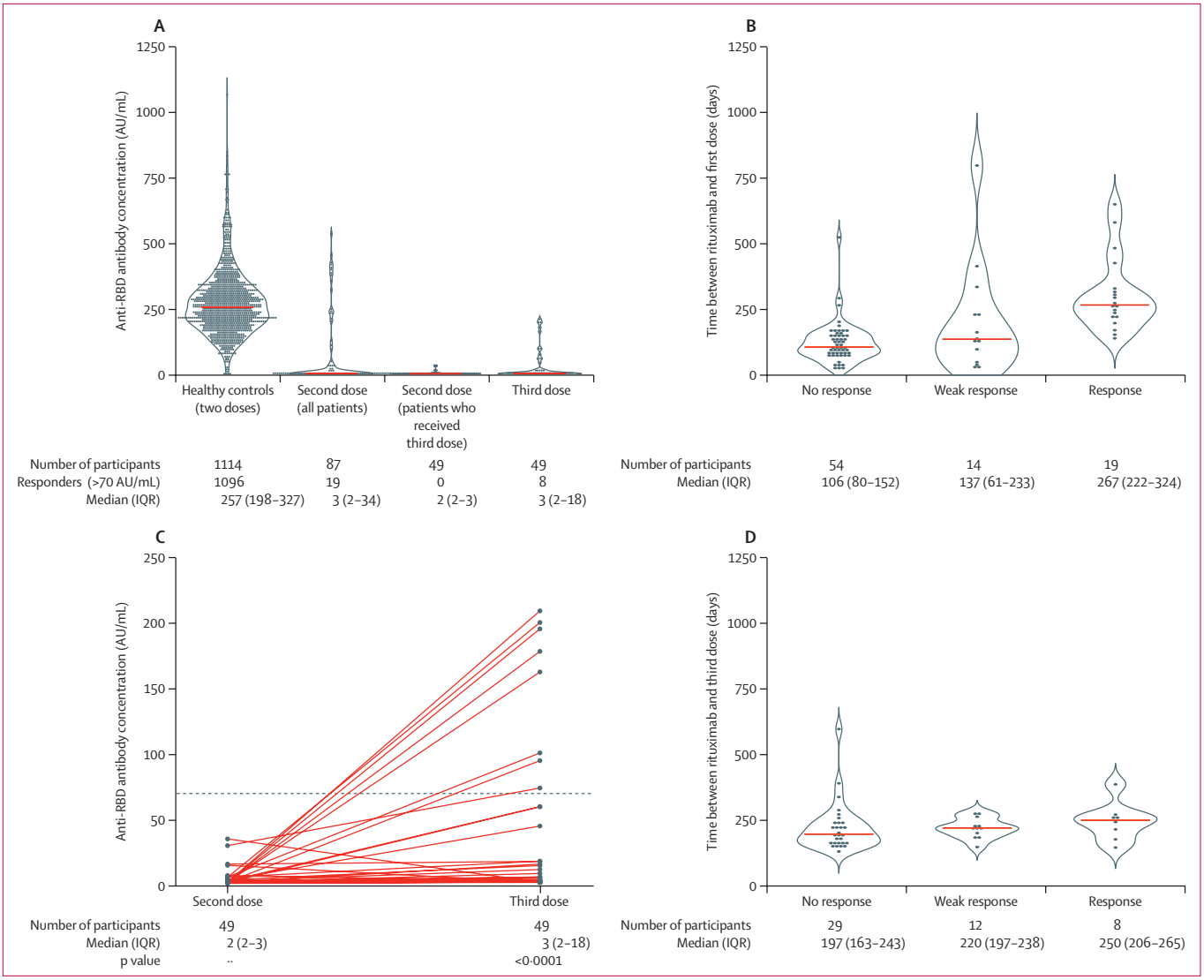

Figure 1: **Humoral response to two and three vaccine doses**
(A) Anti-RBD antibody concentrations in controls, patients who had received at least two doses, patients who had received two doses and would later receive a third, and patients who had received three doses. The violin illustrates the kernel probability density and the orange line indicates the median. Dots denote individual patients. (B) Time between last rituximab infusion and first vaccine dose according to response status in all patients after their second vaccine dose. The violin illustrates the kernel probability density and the orange line indicates the median. Dots denote individual patients. (C) Anti-RBD antibody concentrations after the second and third doses. Solid lines connect patients' two samples (circles). The horizontal dotted line indicates the cutoff for positivity (70 AU/mL). (D) Time between the last rituximab infusion and anti-RBD response after the third vaccine dose. AU=arbitrary units. RBD=receptor-binding domain.

dose (two [5%] of 39 patients; appendix p 9). Among patients who received a third dose, five (14%) of 37, three (8%) of 39, and seven (16%) of 45 reported disease flares after the first, second, or third doses, respectively (appendix p 9). No serious adverse events were reported and there were no deaths during the study period.

Discussion

To our knowledge, this large observational study is the first to report on the immunogenicity and safety of two and three doses of SARS-CoV-2 vaccines in rituximab-treated patients with rheumatoid arthritis. After two doses, only 21·8% of patients, compared with 98·4% of healthy

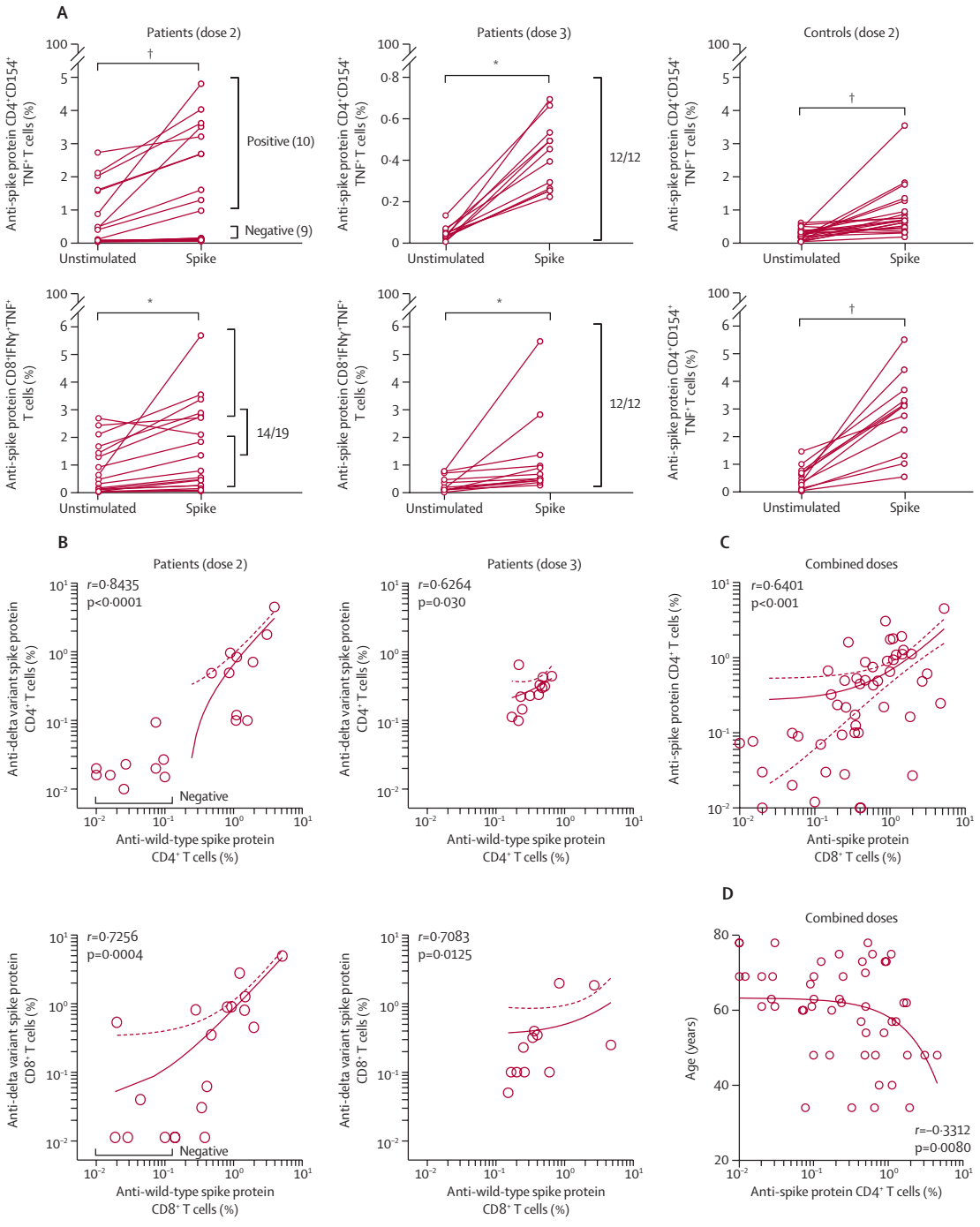

Figure 2: **T-cell responses after two and three vaccine doses**
(A) Anti-wild-type spike protein-specific T-cell responses in patients after two and three doses and in healthy controls after two doses. $CD4^+$ T-cell responses and $CD8^+$ T-cell responses are shown for all unstimulated and stimulated pairs. The p values from Wilcoxon matched pairs signed rank tests are shown, with * indicating p<0·001 and † indicating p<0·0001. Patients with a response (positive) and patients without a response (negative) are indicated. (B) Analysis of T-cell responses directed against wild-type and delta variant SARS-CoV-2 spike peptides in patients after two and three doses (Spearman correlation). Solid lines show simple linear regression of correlation and dotted lines represent 95% CIs. (C) Percentage of anti-spike protein $CD4^+$ T cells versus anti-spike protein $CD8^+$ T cells in patient responders to wild-type and delta variant spike peptides using combined data of the second and third doses. Spearman correlation is shown. (D) Percentage of anti-spike protein $CD4^+$ T cells versus age in patient responders to wild-type and delta variant spike peptides using combined data of the second and third doses. Spearman correlation is shown. See the appendix (p 5) for supplementary data for gating and controls.

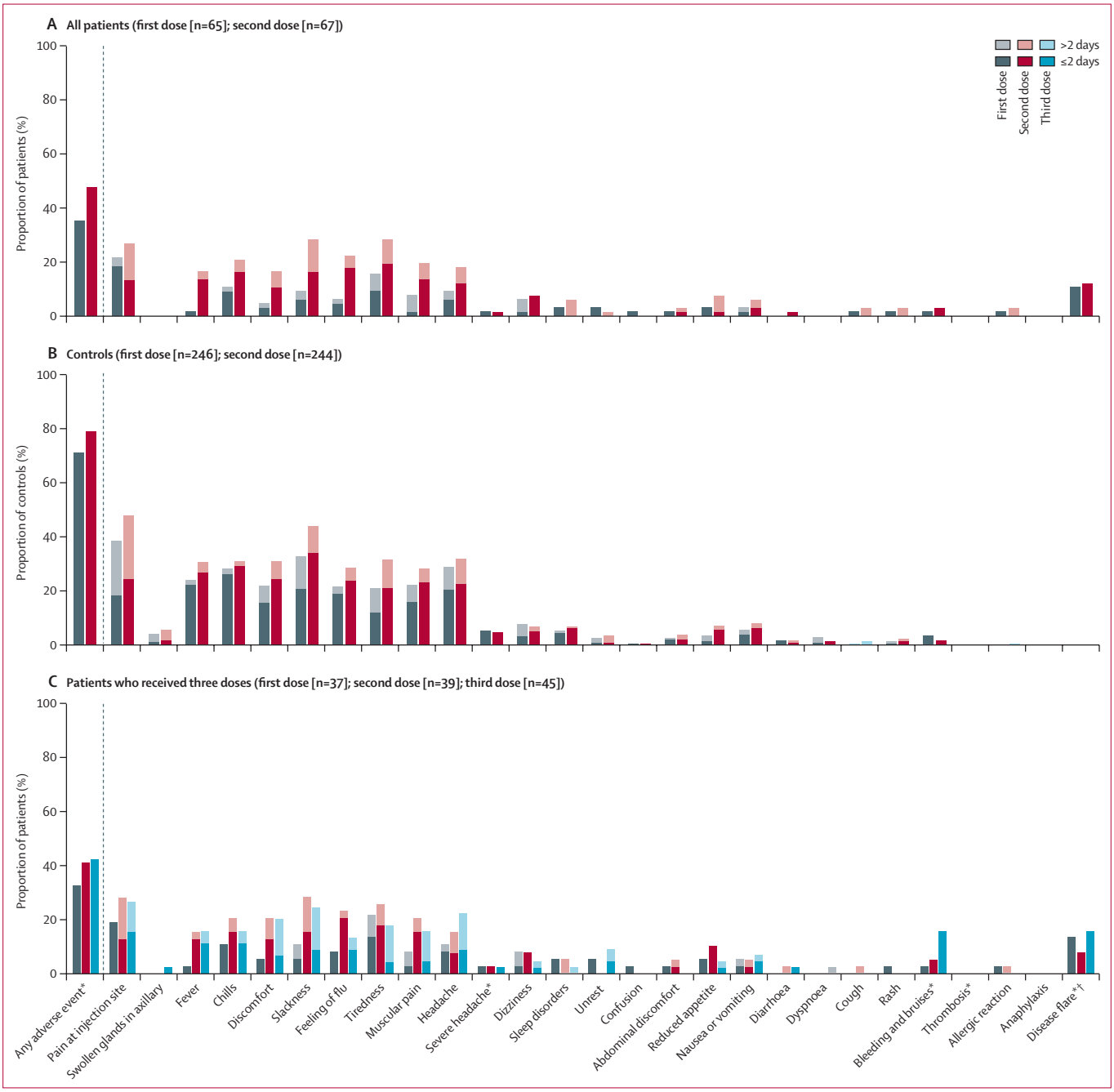

Figure 3: **Adverse events following two or three vaccine doses in patients and controls**
(A) All patients. (B) Controls. (C) Patients who received three vaccine doses. Adverse events were reported for all patients and a subset (n=246) of healthy controls (health-care workers at Diakonhjemmet Hospital and Akershus University Hospital, Oslo, Norway). *Duration not measured. †No patients were hospitalised due to disease flares after vaccination.

controls, developed a humoral response. We found that, despite these severely attenuated humoral responses and the absence of $CD19^+$ B cells, $CD8^+$ T-cell responses were present in 74% of rituximab-treated patients after two doses and in all patients after three doses. T-cell responses to wild-type spike peptides correlated with those seen towards the delta variant spike peptides, showing that the vaccine also elicited immunity to this variant. Both the standard two-dose regimen and the third dose were safe in terms of patient-reported adverse events.

To date, this study is the largest to combine sensitive measurements of humoral and cellular immunity with a description of adverse events after two doses of SARS-CoV-2 vaccines in patients with rheumatoid arthritis treated with rituximab.

Previous studies have shown a positive correlation between the concentrations of neutralising antibodies and protection from symptomatic COVID-19.[23,24] However, serological responses decay with time after vaccination.[25] By contrast, SARS-CoV T-cell memory is long-lasting and was found 17 years post-infection.[26] A study in rhesus macaques showed that SARS-CoV-2-specific T-cell immune responses contributed to protection when antibody responses were low,[12] bridging insufficient humoral immunity. $CD4^+$ T cells and $CD8^+$ T cells counteract viral infections by producing effector cytokines, such as IFNγ and TNF, and by exerting cytotoxic activity against virus-infected cells. Early and robust SARS-CoV-2-specific T-cell responses were associated with lower severity of COVID-19 in otherwise healthy patients.[13] Robust $CD8^+$ T-cell responses were also associated with improved survival in patients with COVID-19 and haematological malignancies, including patients on anti-CD20 therapies,[14] underlining the importance of T-cell immunity in patients with impaired B cells.

We found that 53% of patients had $CD4^+$ T-cell responses and 74% had $CD8^+$ T-cell responses after two vaccine doses. These findings are in line with a study of rituximab-treated patients with various rheumatic diseases (IgG4-related disease, connective tissue diseases, vasculitis, and rheumatoid arthritis), which found that 26 (58%) of 45 patients had detectable IFNγ-secreting SARS-CoV-2-specific T cells and 14 (54%) of 26 did not have a serological response;[9] however, this study did not discriminate between $CD4^+$ and $CD8^+$ T cells. In our study, fewer patients had $CD4^+$ T-cell responses, which are required for optimal B-cell responses, than $CD8^+$ T-cell responses after two vaccine doses.

In patients with insufficient serological responses to two vaccine doses, we found that only a few patients mounted a serological response after a third dose. By contrast, the third dose induced anti-spike protein $CD4^+$ and $CD8^+$ T cells in all patients tested, regardless of humoral responses. The coordinated development of helper and cytotoxic T-cell responses might constitute protective immunity against future infections by SARS-CoV-2 and its variants. Our results suggest that the third dose enables robust T-cell immunity in patients with rheumatoid arthritis treated with rituximab, potentially improving protection in this patient group.

Our multivariable analyses show that the time since last rituximab infusion was significantly associated with serological response to two SARS-CoV-2 vaccine doses, with responders having a median interval of about 9 months between their last rituximab infusion and their first vaccine dose. This finding supports those found in a study by Furer and colleagues[10] and observational data[11] from smaller cohorts showing that the seroconversion rate in patients treated with rituximab increased from 20% to 50% when the interval between rituximab and SARS-CoV-2 vaccination increased from 6 months to 12 months. $CD19^+$ cell count was also associated with serological response to two doses in univariable logistic regression analyses. This result indicates that $CD19^+$ cell counts could be used as a surrogate measure for B-cell function when timing vaccinations. Vaccination with mRNA-1273, as compared with BNT162b2, was significantly associated with serological response to two vaccine doses. This finding is in line with previous findings of higher humoral immunogenicity to mRNA-1273 compared with BNT162b2 in healthy participants.[27]

Both two and three vaccine doses were safe with respect to patient-reported adverse events, with no serious adverse events being reported. Numerically, patients reported fewer adverse events than healthy controls. This result could be due to the younger age of healthy controls compared with patients,[1,2] although we cannot rule out an association between adverse events and humoral response in which immunosuppressive medication reduces side-effects from, and the immunogenicity of, SARS-CoV-2 vaccines. More patients reported bleeding and bruises after the third dose than after the second dose, but the sample size was small and the current results on adverse events should be interpreted with caution.

The strengths of this study include: the broad inclusion criteria, with all rituximab-treated patients receiving a personal invitation, which increase the generalisability of our findings; close follow-up, including an assessment of adverse events; and the broad assessment of vaccine response—both humoral and cellular—to two and three vaccine doses.

This study also has some limitations. First, the patients were older (median 60 years) than the healthy controls (median 43 years), which might interfere with the comparability of results. The difference in serological response, however, was greater than what can be explained by age alone,[28,29] and we adjusted for age in the analyses. Second, the number of included patients was too low to draw definite conclusions regarding safety, but our data on the safety of three vaccine doses in immunocompromised patients with insufficient responses to two doses are reassuring. Third, for feasibility reasons, only 12 patients had T-cell assessments after the third dose. However, patients chosen for T-cell analyses were randomly selected before the first dose, and our findings were consistent across all patients tested. Finally, only patients were offered a third dose; hence, patient response after a third dose could not be compared with healthy controls.

Rituximab-treated patients with rheumatoid arthritis are at risk of severe COVID-19,[4,7] and are in particular need of protection by vaccination. In terms of serological responses, our data suggest that a prolonged interval between the last rituximab infusion and vaccination (>9 months) could be beneficial. Most rituximab-treated patients did not have serological responses to two or three vaccine doses, but did have T-cell responses and few adverse events upon receiving a third dose. Further studies are needed to assess the clinical protection provided by a cellular response in the absence of anti-SARS-CoV-2 antibodies, but our results raise the possibility that patients on regular rituximab infusions might rely on cellular immunity alone. This study supports the provision of three-dose vaccination to patients with rituximab-treated rheumatoid arthritis to help protect this clinically vulnerable group from COVID-19, informing patients, health-care providers, and decision makers on the optimal vaccination strategy.

Contributors

All authors critically revised the manuscript and approved the final submitted version, and take responsibility for the completeness and accuracy of the data and analyses. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. IJ, HK, GLG, SWS, ATT, FL-J, LAM, and JS accessed and verified the underlying data. IJ, GLG, SWS, KKJ, FL-J, LAM, and JTV conceived and designed the study. GLG, SWS, KKJ, FL-J, LAM, ATT, SAP, and IJ oversaw the implementation of the study. GLG, SWS, SAP, KKJ, ATT, and IJ collected the data. IJ, HK, GLG, SWS, FL-J, LAM, JS, ATT, and SAP interpreted the data and drafted the manuscript. FL-J developed the assay used for serological assessment. FL-J, EBV, and TTT did the serological analysis. HK, SM, and LAM did the T-cell analysis. JS was the study statistician. ATT, DJW, TKK, EAH, SM, GG, GBK, and JJ contributed to study conception and design. L-SHN-M and AMA contributed to data collection.

Declaration of interests

KKJ reports speakers bureaus from Roche and BMS and advisory board participation for Celltrion and Norgine. JJ reports grants from Abbvie, Pharmacosmos, and Ferring; consulting fees from Abbvie, Boerhinger Ingelheim, BMS, Celltrion, Ferring, Glihead, Janssen Cilag, MSD, Napp Pharma, Novartis, Orion Pharma, Pfeizer, Pharmacosmos, Takeda, Sandoz, and Unimedic Pharma; and speakers bureaus from Abbvie, Astro Pharma, Boerhinger Ingelheim, BMS, Celltrion, Ferring, Glihead, Hikma, Janssen Cilag, Meda, MSD, Napp Pharma, Novartis, Oriuon Pharma, Pfeizer, Pharmacosmos, Roche, Takeda, and Sandoz. TKK reports grants from AbbVie, Amgen, BMS, MSD, Novartis, Pfizer, and UCB; consulting fees from AbbVie, Amgen, Biogen, Celltrion, Eli Lilly, Gilead, Mylan, Novartis, Pfizer, Sandoz, and Sanofi; speakers bureaus from Amgen, Celltrion, Egis, Evapharma, Ewopharma, Hikma, Oktal, Sandoz, and Sanofi; and participation on a data safety monitoring board for AbbVie. LAM reports funding from the KG Jebsen foundation; support for infrastructure and biobanking from the University of Oslo and Oslo University Hospital; grants from the Coalition of Epidemic Preparedness Innovations (CEPI); and speakers bureaus from Novartis and Cellgene. GG reports consulting fees from the Norwegian System of Compensation to Patients and AstraZeneca, and speakers bureaus from Bayer, Sanofi Pasteur, and Thermo Fisher. JTV reports grant from the CEPI. FL-J reports grants from the CEPI and the South-Eastern Norway Regional Health Authority. GLG reports funding from the Karin Fossum foundation, Diakonhjemmet Hospital, Oslo University Hospital, Akershus University Hospital, the Dr Trygve Gydtfeldt og frues Foundation, and the South-Eastern Norway Regional Health Authority; consulting fees from AbbVie and Pfizer; speakers fees from AbbVie, Pfizer, Sandoz, Orion Pharma, Novartis, and UCB; and advisory board participation for Pfizer and AbbVie. All other authors declare no competing interests.

Data sharing

A deidentified patient dataset and the protocol can be made available to researchers upon reasonable request after we have published all data on our predefined research objectives. The data will only be made available after submission of a project plan outlining the reason for the request and any proposed analyses, and will have to be approved by the Nor-vaC steering group. Project proposals can be submitted to the corresponding author (ingrid.jyssum@gmail.com). Data sharing will have to follow appropriate regulations.

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Supplementary appendix

Supplement to Jyssum I, Kared H, Tran T et al **Humoral and cellular immune responses to standard and third dose SARS-CoV-2 vaccination in rituximab treated rheumatoid arthritis patients – a prospective cohort study**

Table of Contents

Section 1. Inclusion and exclusion criteria

Main Inclusion Criteria	• An established clinical diagnosis of one of the following immune-mediated diseases: rheumatoid arthritis (RA), spondyloarthritis (SpA), psoriatic arthritis (PsA), ulcerative colitis (UC,) Crohn's disease (CD), autoimmune hepatitis (AIH) or patients who have undergone a liver transplantation • On treatment with relevant immunosuppressive and/or immunomodulating medication * • Adult patients (≥ 18 years) • Patient intends to obtain vaccination against COVID-19 during the next 6 months
Main exclusion criterion	• Allergy or intolerance to elements of the COVID-19 vaccines

*Relevant immunosuppressive medication

Medication group	**Included medications**
Tumour necrosis factor inhibitor	Infliximab, etanercept, golimumab, adalimumab, certolizumab
Janus kinases inhibitor	Tofacitinib, baricitinib, upadacitinib, filgotinib
Tumour necrosis factor inhibitor combination	+ methotrexate, azathioprine, sulfasalazine or leflunomide
Methotrexate	
Azathioprine	
Tocilizumab	
Abatacept	
Sulfazalazine	
Hydroxyclorokine	
Vedolizumab	
Ustekinumab	
Secukinumab	
Leflunomide	
High dose prednisolone (≥15mg)	
Rituximab	
Risankizumab	
6-mercaptopurine	
Tacrolimus	
Mycophenoloate mofetil	

Section 2. Methods description serology

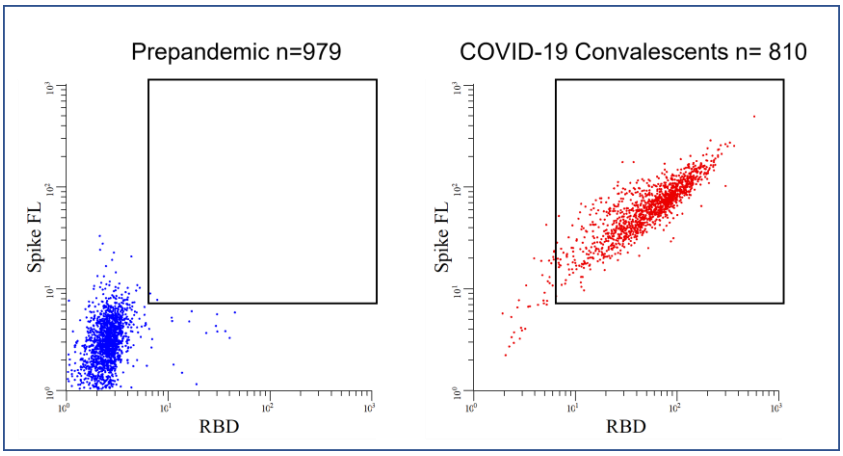
Expression and biotinylation of virus proteins: Bacterially expressed nucleocapsid from SARS-CoV2 was purchased from Prospec Bio (www.prospecbio.com) Plasmids encoding SARS-CoV2 RBD and full-length spike were obtained from Florian Krammer and Ian McLellan, respectively.[1,2] The sequence of the HexaPro plasmid from McLellan was used as basis for custom-made constructs encoding Spike proteins from the alfa, beta and gamma variants (ordered from Genscript). His-tagged virus proteins were expressed in Expi293F cells using protocols recommended by the manufacturer. Proteins were purified on HisTrap columns using standard protocols, and then by size exclusion chromatography (Superdex 200 increase columns) using phosphate-buffered saline (PBS) as running buffer. Purified recombinant viral proteins were solubilized in PBS and biotinylated chemically with sulfo-NHS-LC-biotin (sulfo-NHS-LC-biotin, Proteochem, USA) at a biotin to protein ratio of 1:1. Free biotin was removed with G50 sephadex spin columns.

Bead-based arrays with virus proteins. A multiplexed bead-based flow cytometry assay, referred to as microsphere affinity proteomics (MAP), was adapted for detection of SARS-CoV2 antibodies.[3,4] Amine-functionalized polymer beads (Bangs Laboratories, IN, USA) were suspended at 10% solids in PBS with 1% Tween 20 (PBT) in PCR-plates (Axygen) and dyed successively with serially diluted Cy5-NHS (Lumiprobe), Bodipy-NHS (Lumiprobe) and Pacific Blue-NHS (Thermo) to generate a 108-plex (6 x 6 x 3). The starting concentrations were 300ng/ml, dilutions were 1:2·2, and incubation time 10–15 min with constant agitation on an Eppendorf MixMate. Each step was followed by three wash steps in Phosphate-Buffered Saline Tween20 (PBT). Dyed beads were next incubated successively with biotin-LC-NHS (sulfo-NHS-LC-biotin, Proteochem, USA) and Neutravidin (Thermo Fisher). Dyed and Neutravidin-coupled beads were washed five times in PBT and incubated with PBT containing biotinylated virus proteins (100ug/ml) for 30 min. Beads with different colour-codes and proteins were washed and then pooled in assay buffer to generate bead-based arrays. The assay buffer was PBT containing 1% Bovine serum albumin (BSA), 0·1% sodium azide, 10ug/ml D-Biotin and 10ug/ml Neutravidin. A production lot yields eight sub-arrays, each with the same content of proteins. Ten colour-codes corresponded to different virus proteins, while two were used as reference for background binding of IgG to Neutravidin beads. The eight sub-arrays have bar codes that can be discriminated by flow cytometry to allow sample multiplexing. They were distributed into positions A1, A2, B1, B2 in two 384 well plates prefilled with assay buffer. These served as stock plates and were kept at 4–8 ℃.

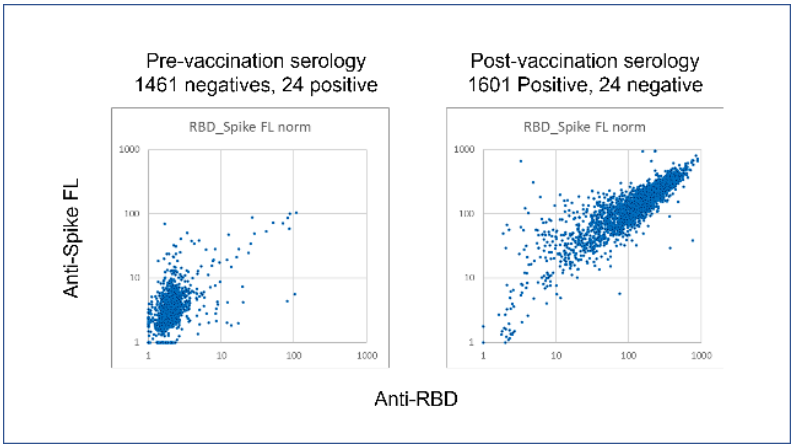
Preparation of serum: Serum (100ul) was transferred from standard vacuum blood sampling tubes into 384 well serum stock plates using a Tecan Robot. A 384-head CyBio SELMA robot was used to transfer 10 µl of serum into a 384 well plate prefilled with 90ul assay buffer plus. The buffer composition is the same as assay buffer described above except that the neutravidin concentration is ten-fold higher (100ug/ml) to neutralize neutravidin-reactive antibodies. The plates were typically kept for a week at 4–8℃ before use. Serum remaining in the original plates was stored at -20℃.

Data analysis: Raw flow cytometry data were analysed using WinList. The median R-Phycoerythrin fluorescence intensity (MFI) for each bead subset was exported to a spreadsheet. Further analysis was done in Microsoft Excel. The MFI values measured for beads with viral proteins were divided by those of beads with Neutravidin only. The relative MFI values are hereafter referred to as arbitrary units (AU/ml).

Validation of method



Results obtained with 979 pre-pandemic sera and 810 samples from COVID-19 convalescents show a false positive rate of 0·3% and a sensitivity of 95% using a double cut-off for anti-RBD and anti-Spike. The x- and y-axes show relative median fluorescence intensity of beads coupled with recombinant full-length Spike (y) or the receptor-binding domain (RBD).

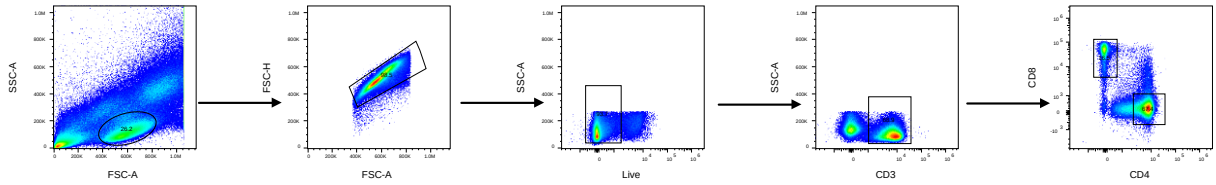


The method was further validated in healthy controls (health care professionals) pre- and post - vaccination. These data showed that only 24 of 1461 tested healthy controls (1·6%) were positive prior to vaccination. This is slightly lower than the average sero-prevalence in Oslo Jan-March 2021. After vaccination 1601 out of 1625 were positive (98·5%)

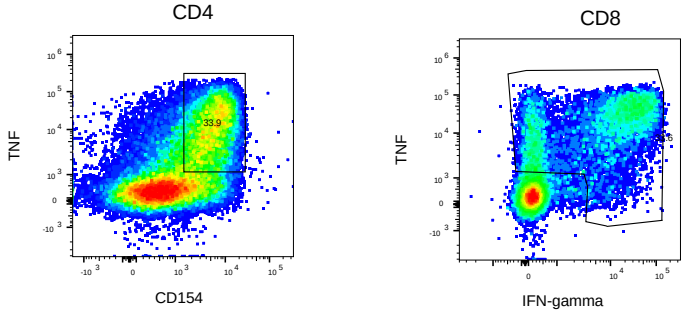
Section 3. Methods description T cells

Peripheral Blood Mononuclear Cells (PBMCs) were thawed in warm RPMI-medium supplemented with 10 % of FBS and incubated 15 minutes at room temperature with DNASE (StemCells) to remove dead cells. Cell counts were obtained with a Countess Automated Cell Counter (Thermo Fisher Scientific) and each sample was resuspended in a serum-free cell culture medium TexMACS to a density of 5×10^6 cells per mL in 96-well round-bottom plates and rested 4 hours in a humidified incubator at 37 °C and 5% CO2. After overnight stimulation with overlapping peptides, cells were collected, washed and stained for 10 min at room temperature with Infrared live dead cell markers (Thermofischer). After one wash in FACS buffer, surface staining was performed for 20 min at room temperature with antibodies directed against: CD3-BV605, CD4-PECY5, CD8-PERCP5.5 and 4-1BB-PE in FACS buffer. Cells were washed twice in FACS buffer, fixed and permeabilized for 30 min at 4 °C (Foxp3/Transcription Factor buffer) and washed once in 1× permeabilization buffer before staining for intra-cellular IFNγ-A647, TNF-PECY7, and CD40L-BV510 during one hour at room temperature. Cells were then washed twice and resuspended in 1% paraformaldehyde in PBS before data acquisition. Activated Veri-Cells Cytokine PBMCs were used as internal control (Biolegend).

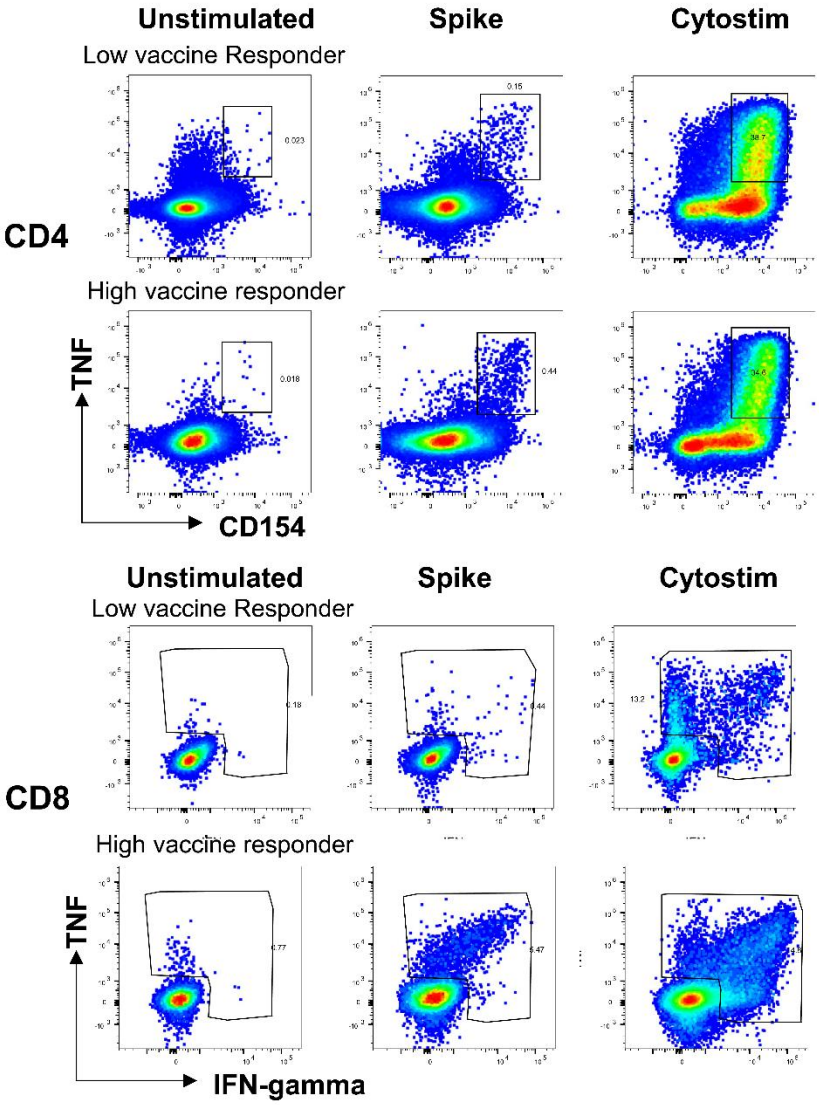
The gating strategy is depicted below: Lymphocytes were identified through their discrimination by the size and granularity according to Forward Scatter (FSC) and Side Scatter (SSC) respectively. Doublets were excluded from analysis to include only single cells in the further analysis (FSC-A versus FSC-H). Dead cells were identified by the staining with Amine-based fixable Live/ Dead indicators prior antibodies staining and fixation. From single cells live lymphocytes, T cells were segregated by their expression of CD3 before the identification of further subsets such as CD4 and CD8 T cells. Patients with less than 30 % of live cells and/ or no response to Cytostim were excluded from the analysis due to the poor recovery and quality of the samples.



Antigen-specific T cells were identified by the expression of CD154 and TNF or IFN-γ and TNF for CD4 and CD8 T cells respectively. In order to avoid batch effects, we used activated Veri-cells as internal control (see below). The lyophilized cells were reconstituted and stained with an identical panel, except for the Live/ Dead marker.



Representative dot plots of CD4 and CD8 T cells from vaccinated rituximab-treated patients are shown in the Figure below. Top: gated $CD4^+$ anti-Spike T cells are activated and co-express TNF and CD154; bottom: Gated $CD8^+$ anti-Spike T cells express TNF/IFNγ. Unstimulated, SARS-CoV-2 Spike peptide stimulated and Cytostim stimulated cells are shown.


Unstimulated
Spike
Cytostim
Low vaccine Responder
CD4
High vaccine responder
TNF
CD154
Unstimulated
Spike
Cytostim
Low vaccine Responder
CD8
High vaccine responder
TNF
IFN-gamma

Section 4. Supplementary tables

Supplementary table 1: Anti-RBD response to the third dose, by the response to the second dose

	Third dose response			
	No response*	Weak response*	Response*	AU/ml (IQR)
Second dose response				
No response*	26 (63%)	10 (24%)	5 (12%)	3 (2–12)
Weak response*	3 (38%)	2 (25%)	3 (38%)	18 (3–81)

Numbers are n (%), except SARS-CoV-2 antibody levels who are median AU/ml (IQR)
AU/ml=Arbitrary Units per milliliter; IQR=inter quartile range
*No response, anti-RBD<5AU/mL; Weak response, anti-RBD 5–70AU/mL; Response, anti-RBD>70AU/mL

Supplementary table 2: Predictors of serological response following standard vaccination

	Odds ratio (95% CI) [Univariable]	p-value [Univariable]	Odds ratio (95% CI) [Multivariable]	p-value [Multivariable]
Age (years)	0·96 (0·92–1·01)	0·091	0·96 (0·91–1·01)	0·13
Female sex	0·97 (0·27–3·48)	0·97	0·93 (0·2–4·22)	0·92
BMI (kg/m^2)	1·05 (0·96–1·15)	0·28	··	··
Current smoker	2·32 (0·58–9·23)	0·22	··	··
Co-medication with a DMARD *	0·94 (0·32–2·75)	0·90	··	··
Number of past DMARDs*	1·06 (0·86–1·30)	0·56	··	··
Number of RTX infusions	0·99 (0·91–1·07)	0·77	··	··
CD19$^+$ cell count †	1·02 (1·0–1·03)	0·026	··	··
ESR	1·05 (0·99–1·11)	0·11	·	·
CRP	1·02 (0·91–1·14)	0·71	··	··
DAS28	1·61 (0·96–2·70)	0·065	··	··
mRNA-1273 vaccine	3·81 (1·26–11·52)	0·016	9·12 (2·15–38·62)	0·0022
Time since RTX (per 100 days)	2·37 (1·45–3·89)	0·0005	2·97 (1·67–5·29)	0·0002

BMI=Body Mass Index; CD19$^+$ cells= B-lymphocytes expressing CD19 (Cluster of Differentiation 19); CRP= C-reactive protein; DAS28=disease activity score with 28 joint count; DMARDs = Disease Modifying Antirheumatic Drugs; ESR=Erythrocyte sedimentation rate; RTX=rituximab;

* Includes methotrexate, leflunomide, sulfasalazine, hydroxychloroquine † CD19 cell count was significantly associated with response in univariable analyses, but not included in multivariable analyses due collinearity with time between rituximab and first vaccine dose.

Relevant variables were chosen by the investigators after a review of the existing literature. For multivariable model building, all factors with a p-value of less than 0·15 from univariable analyses as well as age and sex were included. The final model was obtained with significant variables only by backward elimination of the least significant variable.

Supplementary table 3: Adverse events

	All patients						Controls						3rd dose patients								
	1st dose			2nd dose			1st dose			2nd dose			1st dose			2nd dose			3rd dose		
	(n=65)			(n=67)			(n=246)			(n=244)			(n=37)			(n=39)			(n=45)		
	<2 days	≥2 days	Total	<2 days	≥2 days	Total	<2 days	≥2 days	Total	<2 days	≥2 days	Total	<2 days	≥2 days	Total	<2 days	≥2 days	Total	<2 days	≥2 days	Total
	n	n	n (%)	n	n	n (%)	n	n	n (%)	n	n	n (%)	n	n	n (%)	n	n	n (%)	n	n	n (%)
Any adverse event			23 (35%)			32 (48%)			174 (71%)			191 (78%)			12 (32%)			16 (41%)			19 (42%)
Fever	1	0	1 (2%)	9	2	11 (16%)	54	4	58 (24%)	65	9	74 (30%)	1	0	1 (3%)	5	1	6 (15%)	5	2	7 (16%)
Chills	6	1	7 (11%)	11	3	14 (21%)	64	5	69 (280%)	71	4	75 (31%)	4	0	4 (11%)	6	2	8 (21%)	5	2	7 (16%)
Discomfort	2	1	3 (5%)	7	4	11 (16%)	38	15	53 (22%)	59	16	75 (31%)	2	0	2 (5%)	5	3	8 (21%)	3	6	9 (20%)
Slackness	4	2	6 (9%)	11	8	19 (28%)	51	29	80 (33%)	82	24	106 (43%)	2	2	4 (11%)	6	5	11 (28%)	4	7	11 (24%)
Feeling of flu	3	1	4 (6%)	12	3	15 (22%)	46	6	52 (21%)	58	11	69 (28%)	3	0	3 (8%)	8	1	9 (23%)	4	2	6 (13%)
Tiredness	6	4	10 (15%)	13	6	19 (28%)	29	22	51 (21%)	51	25	76 (31%)	5	3	8 (22%)	7	3	10 (26%)	2	6	8 (18%)
Pain at injection site	12	2	14 (22%)	9	9	18 (27%)	45	49	94 (38%)	59	57	116 (48%)	7	0	7 (19%)	5	6	11 (28%)	7	5	12 (27%)
Swollen glands in axillary	0	0	0 (0%)	0	0	0 (0%)	3	7	10 (4%)	4	9	13 (5%)	0	0	0 (0%)	0	0	0 (0%)	1	0	1 (2%)
Headache	4	2	6 (9)	8	4	12 (18%)	50	20	70 (29%)	55	22	77 (32%)	3	1	4 (11%)	3	3	6 (15%)	4	6	10 (22%)
Dizziness	1	3	4 (6%)	5	0	5 (8%)	8	11	19 (8%)	12	4	16 (7%)	1	2	3 (8%)	3	0	3 (8%)	1	1	2 (4%)
Abdominal discomfort	1	0	1 (2%)	1	1	2 (3%)	5	1	6 (2%)	5	4	9 (4%)	1	0	1 (3%)	1	1	2 (5%)	0	0	0 (0%)
Reduced appetite	2	0	2 (3%)	1	4	5 (8%)	3	5	8 (3%)	14	3	17 (7%)	2	0	2 (5%)	0	4	4 (10%)	1	1	2 (4%)
Nausea/vomiting	1	1	2 (3%)	2	2	4 (6%)	9	4	13 (5%)	15	4	19 (8%)	1	1	2 (5%)	1	1	2 (5%)	2	1	3 (7%)
Diarea	0	0	0 (0%)	0	1	1 (2%)	4	0	4 (2%)	2	2	4 (2%)	0	0	0 (0%)	0	1	1 (3%)	1	0	1 (2%)
Dyspnoea	0	0	0 (0%)	0	0	0 (0%)	2	5	7 (3%)	2	1	3 (1%)	0	0	0 (0%)	0	0	0 (0%)	0	1	1 (2%)
Cough	0	1	1 (2%)	0	2	2 (3%)	0	1	1 (0%)	2	1	3 (1%)	0	0	0 (0%)	0	1	1 (3%)	0	0	0 (0%)
Muscular pain	1	4	5 (8%)	9	4	13 (19%)	39	15	54 (22%)	56	12	68 (28%)	1	2	3 (8%)	6	2	8 (21%)	2	5	7 (16%)
Rash	1	0	1 (2%)	0	2	2 (3%)	1	2	3 (2%)	3	2	5 (2%)	1	0	1 (3%)	0	0	0 (0%)	0	0	0 (0%)
Sleep disorders	2	0	2 (3%)	0	4	4 (6%)	11	2	13 (5%)	15	1	16 (7%)	2	0	2 (5%)	0	2	2 (5%)	0	1	1 (2%)
Unrest	2	0	2 (3%)	0	1	1 (2%)	2	4	6 (2%)	2	6	8 (3%)	2	0	2 (5%)	0	0	0 (0%)	2	2	4 (9%)
Confusion	1	0	1 (2%)	0	0	0 (0%)	1	0	1 (0·4%)	1	0	1 (0·4%)	1	0	1 (3%)	0	0	0 (0%)	0	0	0 (0%)
Allergic reaction	1	0	1 (2%)	0	2	2 (3%)	0	0	0 (0%)	1	0	1 (0·4%)	1	0	1 (3%)	0	1	1 (3%)	0	0	0 (0%)
Anaphylaxis	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)
Bleeding/bruises			1 (2%)			2 (3%)			8 (3%)			4 (2%)			1 (3%)			2 (5%)			7 (16%)
Thrombosis			0 (0%)			0 (0%)			0 (0%)			0 (0%)			0 (0%)			0 (0%)			0 (0%)
Severe headache			1 (2%)			1 (2%)			13 (5%)			11 (5%)			1 (3%)			1 (3%)			1 (2%)
Disease flare*			7 (11%)			8 (12%)									5 (14%)			3 (8%)			7 (16%)

*No patients were hospitalized due to disease flare after vaccinations

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Paper II

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Immunogenicity and Safety of Standard and Third-Dose SARS–CoV-2 Vaccination in Patients Receiving Immunosuppressive Therapy

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Objective. Immunogenicity and safety following receipt of the standard SARS–CoV-2 vaccination regimen in patients with immune-mediated inflammatory diseases (IMIDs) are poorly characterized, and data after receipt of the third vaccine dose are lacking. The aim of the study was to evaluate serologic responses and adverse events following the standard 2-dose regimen and a third dose of SARS–CoV-2 vaccine in IMID patients receiving immunosuppressive therapy.

Methods. Adult patients receiving immunosuppressive therapy for rheumatoid arthritis, spondyloarthritis, psoriatic arthritis, Crohn's disease, or ulcerative colitis, as well as healthy adult controls, who received the standard 2-dose SARS–CoV-2 vaccination regimen were included in this prospective observational study. Analyses of antibodies to the receptor-binding domain (RBD) of the SARS–CoV-2 spike protein were performed prior to and 2–4 weeks after vaccination. Patients with a weak serologic response, defined as an IgG antibody titer of ≤100 arbitrary units per milliliter (AU/ml) against the receptor-binding domain of the full-length SARS–Cov-2 spike protein, were allotted a third vaccine dose.

Results. A total of 1,505 patients (91%) and 1,096 healthy controls (98%) had a serologic response to the standard regimen ($P < 0.001$). Anti-RBD antibody levels were lower in patients (median 619 AU/ml interquartile range [IQR] 192–4,191) than in controls (median 3,355 AU/ml [IQR 896–7,849]) ($P < 0.001$). The proportion of responders was lowest among patients receiving tumor necrosis factor inhibitor combination therapy, JAK inhibitors, or abatacept. Younger age and receipt of messenger RNA–1273 vaccine were predictors of serologic response. Of 153 patients who had a weak response to the standard regimen and received a third dose, 129 (84%) became responders. The vaccine safety profile among patients and controls was comparable.

Conclusion. IMID patients had an attenuated response to the standard vaccination regimen as compared to healthy controls. A third vaccine dose was safe and resulted in serologic response in most patients. These data facilitate identification of patient groups at risk of an attenuated vaccine response, and they support administering a third vaccine dose to IMID patients with a weak serologic response to the standard regimen.

INTRODUCTION

The ongoing COVID-19 pandemic is a global health emergency. Vaccines are important in resolving this crisis, having been proven to be efficacious and safe in the general population (1–4). Vaccines, however, rely on a functional immune system. Patients with immune-mediated inflammatory disease (IMID), including inflammatory joint and bowel diseases, have impaired immune

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systems due to treatment with immunosuppressive medications. There is a concern that immune responses to SARS–CoV-2 vaccines are attenuated in this large patient population, which is also at risk of severe COVID-19 (5,6). Patients with IMIDs were prioritized for vaccination to mitigate their COVID-19 risk, but because they were excluded from initial vaccine trials, there is a paucity of data on the efficacy and safety of SARS–CoV-2 vaccines in this population (1,2,7), as well as concerns regarding the risk of disease flares (5,8).

Rheumatoid arthritis (RA), spondyloarthritis (SpA), psoriatic arthritis (PsA), Crohn's disease (CD), and ulcerative colitis (UC) are different IMIDs, but they share several key features and are treated with many of the same immunosuppressive medications, such as tumor necrosis factor inhibitors (TNFi), non-TNFi biologics, metabolite inhibitors, and targeted small molecule drugs (9). It is important to identify which patients are at risk of a reduced vaccine response, due to either immunosuppression or underlying disease, yet it is still unclear whether the serologic response to vaccine among IMID patients should be monitored. In addition, no consensus currently exists on whether it would be beneficial to delay specific treatments in patients receiving vaccination (7). Observational studies of response to SARS–CoV-2 vaccine among IMID patients have been published recently, but they have generally involved few patients within each medication group (5,10–15).

The utility of 3 or more SARS–CoV-2 vaccine doses in immunosuppressed patients, as well as in the general population, is an urgent question in the global medical community and for policy makers (16,17). Findings of a recent study suggested that immunocompromised recipients of a solid organ transplant benefited from a third vaccine dose (18). Apart from a study of a third dose of vaccine in rituximab-treated RA patients, only a case report and small studies (involving 33 or 17 participants) have been published regarding the immunogenicity and safety of a third dose in IMID patients who were receiving other therapies and had no response to the 2-dose vaccination regimen (19–24). The prospective, observational Norwegian Study of Vaccine Response to COVID-19 (Nor-vaC) includes patients with any of 5 different IMIDs who are receiving any approved immunosuppressive medication. In this study, we evaluate the immunogenicity and safety of the standard 2-dose SARS–CoV-2 vaccination regimen in these groups and examine the response to a third vaccine dose in patients with a weak serologic response to the standard regimen.

PATIENTS AND METHODS

Participants, setting, and study design. Nor-vaC is an ongoing longitudinal observational study conducted at 2 Norwegian IMID referral centers: the Division of Rheumatology at Diakonhjemmet Hospital and the Department of Gastroenterology at Akershus University Hospital. Adult patients (age ≥18 years) with RA, SpA, PsA, UC, or CD who used any of the immunosuppressive medications of interest (Supplementary Materials, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42153) and intended to receive a SARS–CoV-2 vaccine were consecutively recruited into the study. All patients identified by hospital records as eligible for enrollment, based on a diagnosis of an IMID of interest, received an invitation to participate in the study prior to the initiation of the national vaccination program in February 2021. Healthy controls were either volunteer health care workers from Diakonhjemmet Hospital, Akershus University Hospital, and Oslo University Hospital or blood donors from Oslo University Hospital. In the present analyses, we included patients and healthy controls who provided blood specimens for serologic testing 2–4 weeks after receiving the second vaccine dose (Supplementary Figure 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42153). Patients with COVID-19 diagnosed before the second dose received only 1 dose of the standard vaccination regimen and were also included in the study.

Patients receiving CD20-depleting therapy were not included in the present analyses (Supplementary Figure 1). The study (ClinicalTrials.gov identifier: NCT04798625) was approved by an independent ethics committee (Regional Committees for Medical Research Ethics South East Norway, reference numbers 235424, 135924, and 204104) and by appropriate institutional review boards. All participants provided written informed consent.

During the Nor-vaC study, patients with a weak serologic response >3 weeks after completing the standard 2-dose regimen were recruited into a separate intervention study (EudraCT database no. 2021-003618-37) and allotted a third vaccine dose in July–August 2021. The cutoff for a weak serologic response (i.e., an IgG antibody level of ≤100 arbitrary units per milliliter [AU/ml] against the receptor-binding domain [RBD] of the full-length SARS–Cov-2 spike protein) when selecting patients qualifying for a third vaccine dose was based on discussions within the study group and with the Norwegian Institute of Public Health, with the aim of including not only patients with no response

Drs. Syversen and Jyssum contributed equally to this work. Drs. Vaage, Lund-Johansen, Jørgensen, and Goll contributed equally to this work.

A deidentified patient data set can be made available to researchers upon reasonable request. The data will only be made available after submission of a project plan outlining the reason for the request and any proposed analyses, and it will have to be approved by the Nor-vaC steering group. Project proposals can be submitted to the corresponding author. Data sharing will have to follow appropriate regulations.

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(i.e., an antibody level of <70 AU/ml) but also those with an impaired response (i.e., an antibody level of ≤100 AU). In the present observational study, the serologic response following receipt of a third dose is reported for 153 such patients. Those with inflammatory joint diseases (i.e., RA, SpA, and PsA), but not those with inflammatory bowel diseases (IBDs) (i.e., CD and UC), were asked to pause their medication from 1 week before through 2 weeks after receipt of the third vaccine dose.

Exposures. All patients and controls received SARS–CoV-2 vaccines according to the Norwegian national vaccination program, administered by the Norwegian Institute of Public Health. Three SARS–CoV-2 vaccine types were available: ChAdOx1 and the messenger RNA (mRNA) vaccines BNT162b2 and mRNA-1273. The 2 mRNA vaccines were given with an interval of 3–6 weeks between the 2 doses. ChAdOx1 was withdrawn from the Norwegian national vaccination program in March 2021, and all persons who had received 1 dose of this vaccine received one of the mRNA vaccines as the second dose. According to the program, persons with COVID-19 diagnosed before the second dose received only 1 dose of the standard vaccination regimen.

Table 1. Baseline characteristics of IMID patients and healthy controls who received a standard 2-dose SARS–CoV-2 vaccination regimen and IMID patients who received a third dose*

Characteristic	Patients		Healthy controls (n = 1,114)
	Overall (n = 1,647)	Third-dose recipients (n = 153)	
Age, median years (IQR)	52 (40–63)	57 (46–67)	43 (32–55)
Sex			
Female	899 (55)	80 (52)	854 (77)
Male	748 (45)	73 (48)	260 (23)
CRP level, median mg/dl (IQR)	1 (1–3)	1 (1–4)	No data
BMI, median kg/m^2 (IQR)	26 (23–29)	26 (24–29)	No data
IMID			
Joint			
Rheumatoid arthritis	566 (34)	52 (34)	NA
Psoriatic arthritis	295 (18)	21 (14)	NA
Spondyloarthritis	305 (19)	16 (10)	NA
Bowel			
Ulcerative colitis	195 (12)	17 (11)	NA
Crohn's disease	280 (17)	47 (31)	NA
Medication			
TNFi†			
Monotherapy	696 (42)	46 (30)	NA
Combination therapy	386 (23)	52 (34)	NA
Methotrexate	348 (21)	27 (18)	NA
Vedolizumab	55 (3)	7 (5)	NA
JAK inhibitor	50 (3)	11 (7)	NA
Ustekinumab	34 (2)	3 (2)	NA
Tocilizumab	32 (2)	2 (1)	NA
Abatacept	15 (1)	4 (3)	NA
Secukinumab	13 (1)	1 (1)	NA
Other‡	18 (1)	0	NA
Prednisolone comedication			
Overall	71 (4)	16 (10)	NA
Dose ≤7.5 mg	61/71 (86)	13/16 (81)	NA
Vaccine related§			
BNT162b2 regimen, 2 doses	1,152 (70)	131 (86)	625 (56)
mRNA-1273 regimen, 2 doses	401 (24)	14 (9)	246 (22)
Combination regimen, 2 doses	71 (4)	4 (3)	243 (22)
COVID-19 and 1 of any mRNA vaccine	23 (1)	4 (3)	0

* Except where indicated otherwise, values are no. (%) of patients or controls. IMID = immune-mediated inflammatory disease; IQR = interquartile range; CRP = C-reactive protein; BMI = body mass index; NA = not applicable.
† Monotherapy consisted of infliximab, etanercept, adalimumab, golimumab, or certolizumab pegol. Combination therapy consisted of methotrexate, sulfasalazine, leflunomide, or azathioprine, in addition to any tumor necrosis factor inhibitor (TNFi).
‡ Data are for sulfasalazine, leflunomide, azathioprine, risankizumab, and prednisolone monotherapy, each of which was received by <10 patients.
§ BNT162b2 and mRNA-1273 are messenger RNA (mRNA) vaccines. Combination regimen was defined as ChAdOx1 (first dose) + BNT162b2 or mRNA-1273 (second dose) or as BNT162b2 + mRNA-1273 in any sequence.

Assessments. Patients and controls were asked to provide serum samples prior to the first vaccine dose and 2–4 weeks after the second and third vaccine doses, respectively. Assessments of immunogenicity were performed at the Department of Immunology at Oslo University Hospital. The samples were first screened for antibodies to RBD at the full-length spike protein by using an in-house bead-based method, with seroconversion defined as an anti-RBD antibody level ≥5 AU (25,26). Measurement of the World Health Organization international standard for anti-RBD antibody showed that the screening assay has a lower detection limit of 1 binding antibody unit per milliliter (BAU/ml) and an upper dynamic range of ~100 BAU/ml. For quantification of antibody levels, most patient samples and a representative selection of control samples (Supplementary Table 1) were thereafter analyzed using a second assay, with a dynamic range of 300–10,000 BAU (25). In this assay, effects of sera on binding of angiotensin-converting enzyme 2 to RBDs from SARS–CoV-2 variants were measured as a proxy for neutralizing antibody activity (25).

The cutoff for response was preset to an anti-RBD antibody level of 70 AU/ml, based on results obtained from healthy individuals, of whom 98% had levels >70 AU/ml after receipt of 2 vaccine doses (27). Moreover, calibration to the World Health Organization international standard showed that 70 AU/ml corresponds to ~40 BAU/ml. Using a SARS–CoV-2 (Wuhan) microneutralization assay, we have determined that 200 BAU/ml is the lower threshold for detection of neutralizing antibodies (28).

Table 2. Serologic response to the standard 2-dose SARS–CoV-2 vaccination regimen among healthy controls and among IMID patients overall and by clinical and demographic characteristic*

Population, characteristic	Response, proportion (%)	OR (95% CI)	*P*	Anti-RBD IgG level, median AU/ml (IQR)
Healthy controls	1,096/1,114 (98)	1	–	3,355 (896–7,849)
Patients, characteristic				
Overall	1,504/1,647 (91)	0.19 (0.11–0.32)	<0.001	619 (192–4,191)
IMID				
Joint				
Rheumatoid arthritis	503/566 (89)	0.16 (0.08–0.29)	<0.001	548 (194–4,311)
Psoriatic arthritis	286/295 (97)	0.19 (0.09–0.41)	<0.001	652 (215–4,501)
Spondyloarthritis	271/305 (89)	0.17 (0.08–0.36)	<0.001	689 (225–3,893)
Bowel				
Ulcerative colitis	184/195 (94)	0.13 (0.06–0.26)	<0.001	1,403 (219–5,940)
Crohn's disease	255/280 (91)	0.19 (0.08–0.45)	<0.001	409 (155–2,262)
Medication				
TNFi†				
Monotherapy	664/696 (95)	0.3 (0.15–0.57)	<0.001	726 (225–4,293)
Combination therapy	332/386 (86)	0.08 (0.04–0.15)	<0.001	312 (120–2,178)
Methotrexate	317/348 (91)	0.2 (0.09–0.42)	<0.001	709 (206–4,670)
Vedolizumab	52/55 (95)	0.31 (0.08–1.21)	0.091	2,415 (412–10,177)
JAK inhibitor	39/50 (78)	0.05 (0.02–0.12)	<0.001	361 (45–4,204)
Tocilizumab	32/32 (100)	–	–	956 (356–4,578)
Ustekinumab	32/34 (94)	0.19 (0.04–0.99)	0.049	3,286 (281–8,097)
Abatacept	8/15 (53)	0.01 (0–0.04)	<0.001	70 (38–138)
Secukinumab	11/13 (85)	0.2 (0.03–1.25)	0.086	1,165 (276–1,456)
Other‡	16/18 (89)	–	–	2,907 (391–8,981)
Vaccine related§				
BNT162b2 regimen, 2 doses	1,026/1,152 (89)	–	–	408 (170–2,205)
mRNA-1273 regimen, 2 doses	391/401 (98)	–	–	2,308 (377–8,812)
Combination regimen, 2 doses	65/71 (92)	–	–	699 (272–4,253)
COVID-19 and 1 of any mRNA vaccine	22/23 (96)	–	–	6,969 (878–10,768)
Other				
Age, years				
<30	169/176 (96)	–	–	2,247 (418–7,536)
30–65	1,070/1,155 (93)	–	–	667 (192–4,175)
>65	265/316 (84)	–	–	329 (155–1,838)
Female sex	826/899 (92)	–	–	682 (197–4,639)
Current smoker	143/157 (91)	–	–	446 (168–1,809)

* Response was defined as an IgG antibody level of ≥70 AU/ml against the receptor-binding domain (RBD) of SARS–CoV-2 spike protein, and it was evaluated using logistic regression analysis (adjusted for age, sex, and vaccine type), with healthy controls as the reference group. OR = odds ratio; 95% CI = 95% confidence interval; AU = arbitrary units (see Table 1 for other definitions).
† Monotherapy consisted of infliximab, etanercept, adalimumab, golimumab, or certolizumab pegol. Combination therapy consisted of methotrexate, sulfasalazine, leflunomide, or azathioprine, in addition to any TNFi.
‡ Data are for sulfasalazine, leflunomide, azathioprine, risankizumab, and prednisolone monotherapy, each of which was received by <10 patients.
§ BNT162b2 and mRNA-1273 are mRNA vaccines. Combination regimen was defined as ChAdOx1 (first dose) + BNT162b2 or mRNA-1273 (second dose) or as BNT162b2 + mRNA-1273 in any sequence.

The Norwegian Immunization Registry and Norwegian Surveillance System for Communicable Diseases provided information on the date of vaccination, the type of vaccine received, and, when applicable, the date of COVID-19 (29,30). Additionally, information regarding COVID-19 was also obtained from patient questionnaires.

Electronic data collection at Diakonhjemmet Hospital was conducted using the Services for Sensitive Data platform (University of Oslo), and by Viedoc, version 4 (Viedoc Technologies), at Akershus University Hospital. Demographic data were collected at baseline only, while data on medication use, patient-reported disease activity, and responses to COVID-19–related questions were also collected during follow-up. For healthy controls, age and sex were recorded. Disease activity scores (i.e., the Disease Activity Score in 28 joints [DAS28] for patients with RA and patients with PsA, the Ankylosing Spondylitis Disease Activity Score for patients with SpA, the Harvey-Bradshaw Index for CD, and the Partial Mayo Scoring Index for patients with UC) (31–34) were obtained at the baseline visit for patients with IBD and retrieved from the medical records for patients with inflammatory joint disease (i.e., from a clinic visit within 3 months before or after receipt of the first vaccine dose). Adverse events were reported ~14 days after receipt of the first, second, and third doses in all patients and in a subset (n = 245) of the healthy controls (i.e., health care workers from Diakonhjemmet Hospital and Akershus University Hospital).

Objectives and outcomes. The 2 main objectives of this study were 1) to assess humoral responses to standard SARS–CoV-2 vaccination in IMID patients receiving immunosuppressive therapy as compared to that in healthy controls, and 2) to assess changes in humoral responses after a third vaccine dose given to IMID patients with weak serologic responses to standard vaccination. Other objectives were to assess the safety of the standard regimen and the third dose and to identify predictors of serologic response in patients. The main end points were 1) the proportion of participants with a serologic response (i.e., an anti-RBD antibody level >70 AU/ml) and the anti-RBD antibody level following the standard regimen and third dose and 2) the change in levels of anti-RBD antibody after receipt of the third dose. Other end points included adverse events and predictors of the serologic response to the standard regimen and the third dose.

Statistical analysis. Demographic data, adverse events, and serologic response according to medication group were summarized using descriptive statistics. Comparisons of the serologic response between patients and controls were performed by logistic regression. Adjustments were made for sex, age, and vaccine type. Comparisons of anti-RBD antibody level between patients and healthy controls were performed using the Mann-Whitney U test. Prevaccination and postvaccination samples collected from patients receiving a third dose were compared by the Wilcoxon's signed rank test for paired samples. There were no missing data for the main variables. Predictors of response among patients were assessed by univariable and multivariable logistic regression. All tests were 2-sided, and *P* values of less than 0.05 were considered statistically significant. All analyses were performed using R, release 4.0.3.

RESULTS

Patient and control characteristics. Between February 2, 2021, and June 11, 2021, a total of 2,178 patients were included in the Nor-vaC study. A total of 1,647 eligible patients (566 with RA, 305 with SpA, 295 with PsA, 280 with CD, and 195 with UC; median age 52 years [interquartile range (IQR) 40–63]; female sex, 899 [55%]) and 1,114 healthy controls (median age 43 years [IQR 32–55]; female sex, 854 [77%]) underwent serologic testing after receipt of the standard 2-dose vaccination regimen and were included in the present analyses. Patient disposition is summarized in Supplementary Figure 1, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42153. Baseline characteristics of patients and controls are shown in Table 1 and Supplementary Tables 1 and 2, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42153. The most common immunosuppressive medications were TNFi (n = 1,082 patients) and methotrexate monotherapy (n = 348). Seventy percent of patients and 56% of controls received BNT162b2 for doses 1 and 2. In total, 23 patients (1%) had COVID-19 before the second dose and received only the first of 2 doses in the standard vaccination regimen. Controls were included in this study only if they had received 2 vaccine doses and had no signs or symptoms consistent with clinical COVID-19.

Humoral response to the standard regimen. A total of 1,628 patients (98.8%) receiving immunosuppressive therapy and 1,110 healthy controls (99.6%) had detectable antibodies to SARS–CoV-2 (level, >5 AU/ml) after receiving the standard 2-dose vaccination regimen (Supplementary Figures 1A and B, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42153). In this population, 1,493 patients (91%) as compared to 1,096 healthy controls (98%) had anti-RBD antibody levels ≥70 AU/ml and were considered serologic responders ($P < 0.001$) (Table 2 and Supplementary Figures 1A and 1B, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42153). Response was detected in ≥90% of patients receiving methotrexate, TNFi monotherapy, ustekinumab, tocilizumab, or vedolizumab, in 80–90% of patients receiving TNFi combination therapy or secukinumab, and in ≤80% receiving JAK inhibitors (78%) or abatacept (53%) (Table 2). To obtain more precise information about antibody levels, samples were reanalyzed using a quantitative assay (Supplementary Figures 1C and D, available at http://onlinelibrary.wiley.com/doi/10.1002/art.42153). Patients had

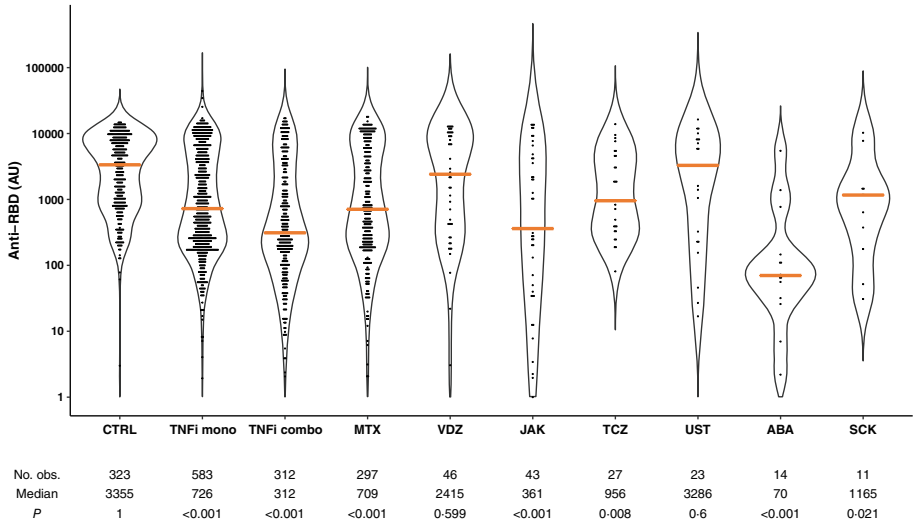

Figure 1. Violin plots of probability densities, smoothed by a kernel density estimator, of IgG antibody levels against the receptor-binding domain of SARS–CoV-2 spike protein (anti-RBD) after the standard 2-dose SARS–CoV-2 vaccination regimen among healthy controls (CTRL) and among patients with immune-mediated inflammatory disease (IMID) stratified by immunosuppressive therapy. Points denote participants, and solid orange lines show group medians. *P* values show comparisons to CTRL and were calculated by Mann-Whitney U test. TNFi mono = tumor necrosis factor inhibitor monotherapy; TNFi combo = TNFi combination therapy; MTX = methotrexate; VDZ = vedolizumab; TCZ = tocilizumab; UST = ustekinumab; ABA = abatacept; SCK = secukinumab. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.42153/abstract.

significantly lower levels of anti-RBD antibody as compared to healthy controls (median 619 AU/ml [IQR 192–4,191] and 3,355 AU/ml [IQR 896–7,849]) (Figure 1).

Predictors of response. Age (odds ratio [OR] 0.96, 95% confidence interval [95% CI] 0.94–0.98]) and vaccination with mRNA-1273 as compared to BNT162b2 (OR 4.45, 95% CI 1.66–11.92) were identified as predictors of a serologic response following receipt of the standard 2-dose vaccination regimen (Table 3). A total of 98% of patients receiving mRNA-1273 as compared to 89% receiving BNT162b2 were responders, with median anti-RBD antibody levels of 2,308 AU/ml (IQR 377–8,812) and 408 AU/ml (IQR 170–2,205), respectively. Patients receiving TNFi combination therapy (OR 0.27, 95% CI 0.14–0.52), JAK inhibitors (OR 0.18, 95% CI 0.05–0.64), or abatacept (OR 0.01, 95% CI 0.01–0.13) were less likely to have a response following receipt of the standard regimen, compared to patients receiving TNFi monotherapy (Table 3). Pausing treatment did not improve vaccine response (Table 3). The same predictors (i.e., age, mRNA-1273 receipt, and comedication use) were identified in a subanalysis of patients receiving TNFi monotherapy or combination therapy (Supplementary Table 3, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42153).

Response to a third vaccine dose. A total of 153 patients (median age 57 years [IQR 46–67]; 80 female patients [52%]) with weak responses to the standard 2-dose regimen (anti-RBD antibody levels ≤100 AU/ml) were allotted a third vaccine dose a median of 70 days (IQR 56–90) after the second vaccine dose. An increase in antibody levels was observed in 129 (94%) of 153 patients ($P < 0.001$), with a median change of 362 AU/ml (IQR 48–2,501) (Figure 2). Median antibody levels were 45 AU/ml (IQR 17–105) and 544 AU/ml (IQR 143–4,543) before and 2–4 weeks after receipt of the third vaccine dose, respectively (Figure 2). Percentages of responders, stratified by therapy, were as follows: 89% (41 of 46) among TNFi monotherapy recipients, 84% (44 of 52) among TNFi combination therapy recipients, 75% (21 of 28) among methotrexate recipients, 63% (7 of 11) among JAK inhibitor recipients, and 100% (4 of 4) among abatacept recipients. Except for age, no predictors of response to the third vaccine dose were identified (Supplementary Table 4, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42153).

Adverse events. Among recipients of the standard 2-dose vaccination regimen, adverse events were reported in 810 (50%) of 1,516 patients and 191 (78%) of 244 healthy controls, with a comparable safety profile (Figure 3 and Supplementary Table 5, available on the *Arthritis & Rheumatology* website at http://onlinelibrary.wiley.com/doi/10.1002/art.42153). Following receipt of the third dose, 70 patients (44%) reported adverse events; no new safety issues emerged, except for an increase in disease flares, which were reported by 26 patients (16%), all of whom had inflammatory joint disease. After receipt of the first and second doses, disease flare was reported by 78 patients (6%) and 88 patients (6%), respectively.

Table 3. Univariable and multivariable analyses to determine predictors of a serologic response among IMID patients after receipt of the standard 2-dose SARS–CoV-2 vaccination regimen*

Potential predictor	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Demographic				
Age, years	0.96 (0.95–0.98)	<0.001	0.95 (0.93–0.97)	<0.001
Male sex	0.92 (0.62–1.37)	0.68	0.70 (0.41–1.22)	0.199
IMID				
Joint				
Rheumatoid arthritis	1	–	1	–
Spondyloarthritis	1.53 (0.83–2.69)	0.16	0.39 (0.14–1.09)	0.066
Psoriatic arthritis	1.89 (0.99–3.63)	0.05	1.436 (0.47–3.91)	0.562
Bowel				
Crohn's disease	1.36 (0.81–2.28)	0.242	0.34 (0.13–0.89)	0.026
Ulcerative colitis	2.22 (1.11–4.45)	0.021	0.54 (0.18–1.58)	0.25
Medication				
TNFi†				
Monotherapy	1	–	1	–
Combination therapy	0.38 (0.23–0.64)	<0.001	0.27 (0.14–0.52)	<0.001
Methotrexate	0.61 (0.34–1.09)	0.089	0.36 (0.13–1.04)	0.286
Vedolizumab	1 (0.29–3.49)	0.998	1.17 (0.28–4.93)	0.824
JAK inhibitor	0.21 (0.09–0.49)	<0.001	0.18 (0.05–0.64)	0.007
Tocilizumab‡	Not done	0.978	Not done	0.983
Ustekinumab	0.92 (0.2–4.17)	0.917	0.36 (0.13–8.06)	0.528
Abatacept	0.02 (0.01–0.10)	<0.001	0.01 (0–0.013)	<0.001
Secukinumab	0.35 (0.04–3.11)	0.334	0.1 (0.01–1.21	0.064
Prednisolone	0.27 (0.14–0.51)	<0.001	0.41 (0.13–1.24)	0.106
Vaccine related§				
BNT162b2 regimen, 2 doses	1	–	1	–
mRNA-1273 regimen, 2 doses	5.06 (2.29–11.18)	<0.001	4.45 (1.66–11.92)	0.002
Combination regimen, 2 doses	1.11 (0.46–2.69)	0.814	0.72 (0.24–2.12)	0.54
COVID-19 and 1 of any mRNA vaccine§	–	0.977	–	0.995
Other				
IBD or IJD duration	1 (0.98–1.02)	0.945	1.01 (0.99–1.04)	0.389
CRP level	0.97 (0.96–0.99)	0.01	0.97 (0.95–1.0)	0.018
BMI	1.01 (0.98–1.05)	0.474	1.03 (0.98–1.08)	0.292
Pause in medication¶	1.8 (0.81–4.03)	0.142	1.59 (0.5–5.07)	0.428

* Response was defined as an IgG antibody level of ≥70 AU/ml against the RBD of SARS–CoV-2 spike protein. IBD = inflammatory bowel disease; IJD = inflammatory joint disease (see Table 2 for other definitions).
† Monotherapy consisted of infliximab, etanercept, adalimumab, golimumab, or certolizumab pegol. Combination therapy consisted of methotrexate, sulfasalazine, leflunomide, or azathioprine.
‡ Because of the low number of tocilizumab recipients, analysis was not performed.
§ BNT162b2 and mRNA-1273 are mRNA vaccines. Combination regimen was defined as ChAdOx1 (first dose) + BNT162b2 or mRNA-1273 (second dose) or as BNT162b2 + mRNA-1273 in any sequence.
¶ Patient-reported pause in medication from 1 week before through 2 weeks after receipt of a vaccine dose.

DISCUSSION

This study, the largest to date on response to the standard 2-dose SARS–CoV-2 vaccination regimen in IMID patients receiving immunosuppressive therapy, demonstrated that the percentage of responders and the anti-RBD antibody level were lower in 1,647 patients as compared to 1,114 healthy controls. Adverse reactions were comparable in the 2 groups. Among patients with a weak serologic response after the standard 2-dose regimen, the third dose was safe and resulted in a response in most recipients.

The study provides detailed information regarding the impact of commonly used immunosuppressive drugs for inflammatory joint diseases and IBDs on the serologic response to SARS–CoV-2 vaccines. A difference among the medications was shown, with the lowest proportion of responders observed among recipients of abatacept (50%), JAK inhibitors (78%), TNFi used in combination with methotrexate or azathioprine (86%), and sekukinumab (88%), suggesting a rationale for postvaccination serologic monitoring in patients using these medications. Prior studies regarding the effect of abatacept and JAK inhibitors on the immunogenicity of SARS–CoV-2 vaccines differ in their conclusions, which may be due to the limited number of patients they evaluated (n = 8–16) (11,13,35). Data regarding the effect of TNFi on the immunogenicity of SARS–CoV-2 vaccines have also been conflicting (5,10–13,35). The Nor-vaC study included >1,000 TNFi recipients, roughly the same total number previously described across several smaller studies (35). In the present study, attenuated immunogenicity was mainly seen in TNFi recipients receiving combination therapy with azathioprine or methotrexate. These synthetic

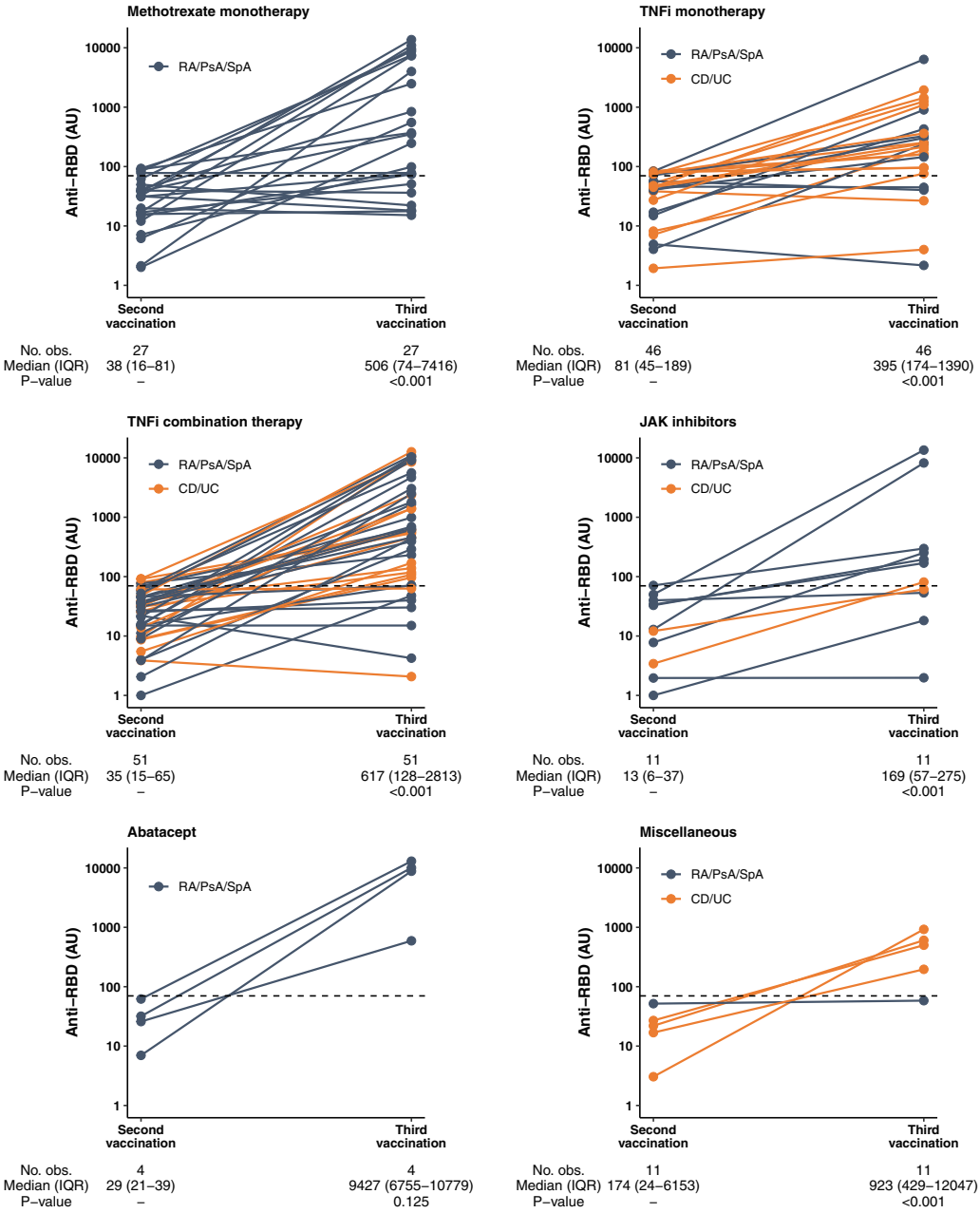

Figure 2. Anti-RBD levels after receipt of a third SARS–CoV-2 vaccine dose among IMID patients with a weak response to the standard 2-dose vaccination regimen. Levels were measured 2–4 weeks after the second and third vaccine doses. Horizontal dotted lines indicate the serologic response cutoff (70 arbitrary units per milliliter [AU/ml]). Orange dots and lines indicate anti-RBD levels in individual patients with inflammatory bowel disease; blue dots and lines indicate levels in individual patients with inflammatory joint disease. *P* values were calculated by Wilcoxon paired test. RA = rheumatoid arthritis; PsA = psoriatic arthritis; SpA = spondyloarthritis; obs. = observations; IQR = interquartile range; CD = Crohn's disease; UC = ulcerative colitis; miscellaneous = vedolizumab, ustekinumab, tocilizumab, secukinumab, or azathioprine (see Figure 1 for other definitions).

drugs are known to reduce antidrug antibody responses to the TNF inhibitor itself, and it is reasonable to assume similar effects on vaccine immunogenicity (36).

Despite the relatively high response rates in most medication groups, the median anti-RBD antibody levels were significantly lower among patients, compared to healthy controls. There is increasing evidence that antibody levels correlate to the degree of clinical protection against breakthrough COVID-19 (37) and that anti-RBD antibody levels correlate to SARS–CoV-2 neutralization levels, with higher levels needed for neutralizing novel virus strains (28,38). As antibody levels decay over time, it seems likely that patients who attain a weak antibody response after vaccination will

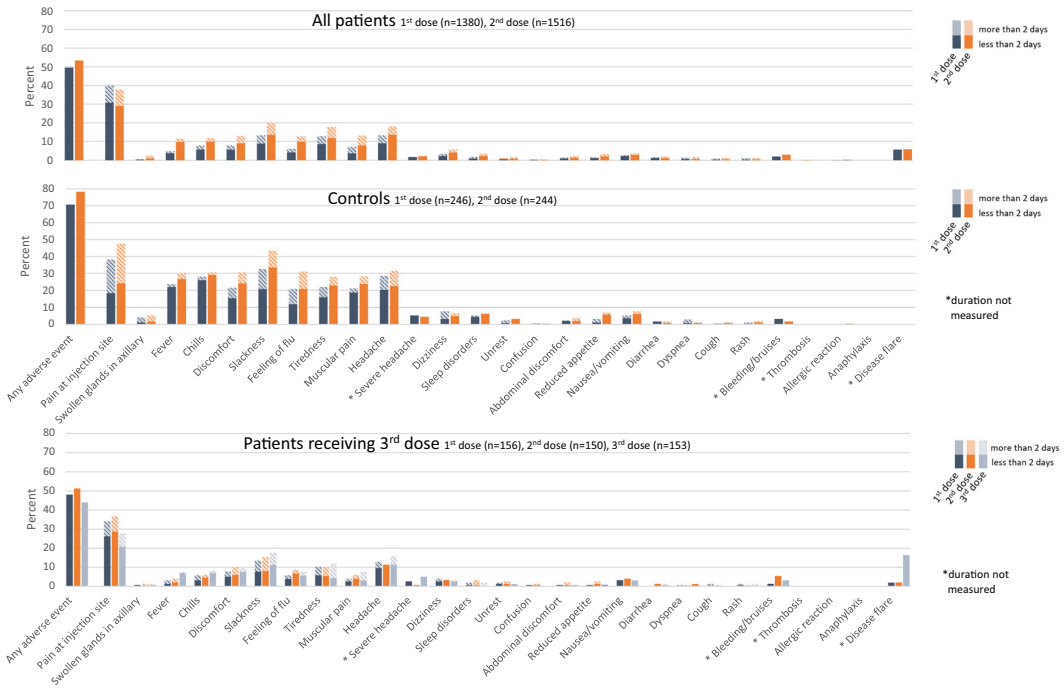

Figure 3. Type and duration of adverse events reported after doses 1 (blue bars) and 2 (orange bars) of SARS–CoV-2 vaccine among patients with immune-mediated inflammatory disease (IMID) and healthy controls and after dose 3 (gray bars) among IMID patients who had a weak serologic response (defined as <70 arbitrary units per milliliter) to doses 1 and 2. Adverse events were reported for all patients and a subset of 246 healthy controls described in Patients and Methods. Color figure can be viewed in the online issue, which is available at http://onlinelibrary.wiley.com/doi/10.1002/art.42153/abstract.

have a less durable response (39). Patients with a weak response may also have developed less robust immunologic memory responses (40). Further studies are needed to elucidate whether IMID patients receiving immunosuppressive therapy lose their protective immunity more quickly than the general population.

In addition to medication type, lower age and receipt of mRNA-1273 were predictors of a serologic response. Prior studies have suggested that mRNA-1273 may be more immunogenic than BNT162b2 in healthy subjects (41). To our knowledge, this is the first study presenting findings on the immunogenicity of different vaccine types in IMID patients. Subanalyses in TNFi recipients showed similar results.

In the 153 patients receiving a third vaccine dose, a response was induced in the majority of patients. The effectiveness of additional vaccine doses for immunocompromised patients, as well as the utility of booster shots for healthy people, is now being debated in the scientific community (16). Prior data on the immunogenicity of 3 SARS–CoV-2 vaccine doses in IMID patients who were receiving immunosuppressive drugs other than rituximab and had no response to the standard 2-dose vaccination regimen consist of case series and small studies (n = 33 and n = 17) and indicated a moderate additional humoral response following receipt of the third dose (19,23,24). The present data show a clear benefit in terms of serologic response, while the frequency and profile of reported adverse events were comparable to those observed after receipt of the standard 2-dose regimen. We did not find that pausing medication benefited vaccine immunogenicity. The humoral response to the third dose was comparable in patients with inflammatory joint diseases, for whom a pause in medication was recommended, and in patients with IBDs, who did not receive this recommendation. Further, self-reported pausing of medication was not associated with a humoral response to the standard vaccination regimen. These results must be interpreted with caution, however.

There are limited data on the safety of SARS–CoV-2 vaccines in IMID patients (13,42). This study supports that these vaccines are safe in an immunosuppressed population, and it demonstrates that the frequency of reported adverse events was lower among IMID patients than among controls, with the same range of adverse events reported in both groups. This finding suggests that immunosuppressive medication might reduce the frequency of adverse events due to SARS–CoV-2 vaccines and might also reduce the vaccines' immunogenicity. A major concern has been whether the mRNA SARS–CoV-2 vaccines may cross-react with human proteins and aggravate autoimmunity (43). The Nor-vaC results are reassuring in this regard, as hardly any patients reported a disease flare after receiving the standard 2-dose vaccination regimen. However, we found a clear increase in disease flares among inflammatory joint disease

patients following receipt of the third dose. This was not seen in patients with IBDs. Among patients with inflammatory joint diseases, the increase may have been due to the recommended pause in medication from 1 week before through 2 weeks after receipt of the third dose.

Strengths of this study include the prospective study design, the broad inclusion criteria, the well-characterized population of patients, and the large sample sizes of patients and controls. A further strength is that the study population was drawn from both gastroenterology and rheumatology settings, enabling assessment of patients across a range of diseases who are being treated with the same medical compounds.

This study has some limitations. First, we did not measure cellular immune responses. The adaptive immune response to SARS–CoV-2 depends not only on virus-specific antibodies but also on T cell–mediated responses (44). Further studies are needed to determine if the serologic responses are predictive of protection against severe disease. Second, some medication groups included a low number of patients. Third, controls or patients with a normal antibody response to the standard 2-dose vaccination regimen were not given a third dose; hence, we could not evaluate the response to and safety of a third dose in these groups. Fourth, the patients were generally older than the controls, raising the possibility of biased results. However, we have corrected for age in all analyses comparing patients and controls. Fifth, full data on comorbidity were not available. Sixth, we cannot exclude the possibility that some of the participants may have had a subclinical SARS–CoV-2 infection. However, the rate of SARS–CoV-2 infection in Norway during the relevant period was very low.

The proportion of responders and the anti-RBD antibody levels were lower among IMID patients as compared to controls following receipt of the standard vaccination regimen. These data facilitate identification of patient groups who are at risk of an attenuated vaccine response and therefore should be considered for postvaccination serologic monitoring. Receipt of a third vaccine dose by patients with a weak response was safe and resulted in a response in most. These results will aid health care systems in the planning and implementation of SARS–CoV-2 vaccine programs aimed at IMID patients treated with immunosuppressive medication and will aid clinical decision-making regarding revaccinations and tailoring of medication to keep this vulnerable population protected against severe COVID-19.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revised in critically for important intellectual content, and all authors approved the final version to be published. Dr. Goll had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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Analysis and interpretation of data. Syversen, Jyssum, Tveter, Sexton, Provan, Mjaaland, Grødeland, Kro, Jahnsen, Munthe, Vaage, Lund-Johansen, Jørgensen, Goll.

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Supplementary materials
Syversen SW, Jyssum I, Tveter AT et al. **Immunogenicity and Safety of Standard and Third Dose SARS-CoV-2 Vaccination in Patients on Immunosuppressive Therapy**

Table of Contents

Supplementary Appendix Inclusion and Exclusion Criteria

Inclusion Criteria	• An established clinical diagnosis of one of the following immune-mediated diseases: rheumatoid arthritis (RA), spondyloarthritis (SpA), psoriatic arthritis (PsA), ulcerative colitis (UC), and Crohn's disease (CD) • On treatment with relevant immunosuppressive and/or immunomodulating medication* • Adult patients (≥ 18 years) • Patient intends to obtain vaccination against COVID-19 during the next six months
Exclusion Criterion	• Allergy or intolerance to elements of the COVID-19 vaccines

*Relevant immunosuppressive medication

Medication group	**Included medications**
Tumor necrosis factor inhibitor	Infliximab, etanercept, golimumab, adalimumab, certolizumab pegol
Janus kinases inhibitor	Tofacitinib, baricitinib, upadacitinib, filgotinib
Tumor necrosis factor inhibitor in combination	+ methotrexate, azathioprine, sulfasalazine or leflunomide
Methotrexate	
Azathioprine	
Tocilizumab	
Abatacept	
Sulfasalazine	
Vedolizumab	
Ustekinumab	
Secukinumab	
Leflunomide	
High dose prednisolone (≥15mg)	
Rituximab	
Risankizumab	
6-mercaptopurine	

Supplementary Methods Assessments of SARS-CoV2 Antibodies

Description of Methods:

Expression and biotinylation of virus proteins: Bacterially expressed nucleocapsid from SARS-CoV2 was purchased from Prospec Bio (www.prospecbio.com). Plasmids encoding SARS-CoV2 RBD and full-length spike were obtained from Florian Krammer and Ian McLellan, respectively.[1,2] The sequence of the HexaPro plasmid from McLellan was used as basis for custom-made constructs encoding Spike proteins from the alfa, beta and gamma variants (ordered from Genscript). His-tagged virus proteins were expressed in Expi293F cells using protocols recommended by the manufacturer. Proteins were purified on HisTrap columns using standard protocols, and then by size exclusion chromatography (Superdex 200 increase columns) using phosphate-buffered saline (PBS) as running buffer. Purified recombinant viral proteins were solubilized in PBS and biotinylated chemically with sulfo-NHS-LC-biotin (sulfo-NHS-LC-biotin, Proteochem, USA) at a biotin to protein ratio of 1:1. Free biotin was removed with G50 sephadex spin columns.

Bead-based arrays with virus proteins. A multiplexed bead-based flow cytometry assay, referred to as microsphere affinity proteomics (MAP), was adapted for detection of SARS-CoV2 antibodies.[3,4] Amine-functionalized polymer beads (Bangs Laboratories, IN, USA) were suspended at 10% solids in PBS with 1% Tween 20 (PBT) in PCR-plates (Axygen) and dyed successively with serially diluted Cy5-NHS (Lumiprobe), Bodipy-NHS (Lumiprobe) and Pacific Blue-NHS (Thermo) to generate a 108-

plex (6 x 6 x 3). The starting concentrations were 300ng/ml, dilutions were 1:2.2, and incubation time 10–15 min with constant agitation on an Eppendorf MixMate. Each step was followed by three wash steps in Phosphate-Buffered Saline Tween20 (PBT). Dyed beads were next incubated successively with biotin-LC-NHS (sulfo-NHS-LC-biotin, Proteochem, USA) and Neutravidin (Thermo Fisher). Dyed and Neutravidin-coupled beads were washed five times in PBT and incubated with PBT containing biotinylated virus proteins (100ug/ml) for 30 min. Beads with different color-codes and proteins were washed and then pooled in assay buffer to generate bead-based arrays. The assay buffer was PBT containing 1% Bovine serum albumin, 0. 1% sodium azide, 10ug/ml D-Biotin and 10ug/ml Neutravidin. A production lot yields eight sub-arrays, each with the same content of proteins. Ten color-codes corresponded to different virus proteins, while two were used as reference for background binding of IgG to Neutravidin beads. The eight sub-arrays have bar codes that can be discriminated by flow cytometry to allow sample multiplexing. They were distributed into positions A1, A2, B1, B2 in two 384 well plates prefilled with assay buffer. These served as stock plates and were kept at 4–8 C.

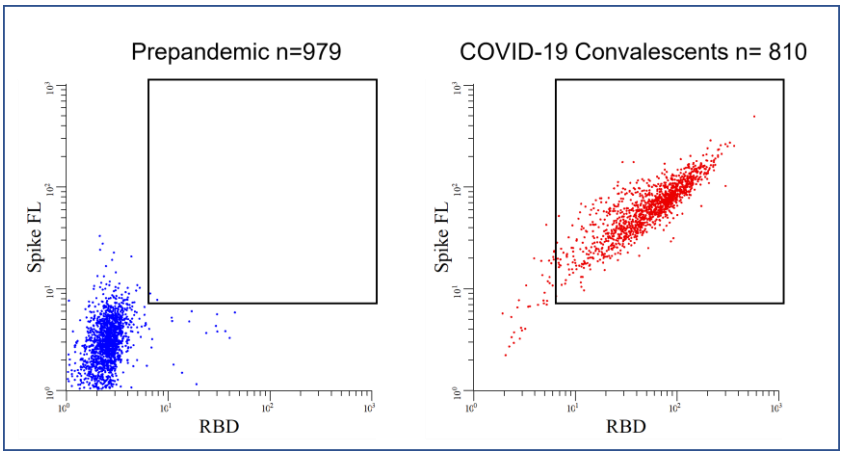
Preparation of serum: Serum (100ul) was transferred from standard vacuum blood sampling tubes into 384 well serum stock plates using a Tecan Robot. A 384-head CyBio SELMA robot was used to transfer 10 µl of serum into a 384 well plate prefilled with 90ul assay buffer plus. The buffer composition is the same as assay buffer described above except that the neutravidin concentration is ten-fold higher (100ug/ml) to neutralize neutravidin-reactive antibodies. The plates were typically kept for a week at 4–8C before use. Serum remaining in the original plates was stored at -20C.

Array-based measurement: The SELMA robot was used to transfer 3ul of beads and 10ul of diluted serum into the wells of 384 wells plate prefilled with 90 µl assay buffer. The plates were agitated on an Eppendorf MixMate at 1800rpm for one hour. The contents of each plate were next distributed into two plates using the SELMA robot. Both were centrifuged at 500 x g for 1 min to pellet the beads. For detection of IgG, the beads were washed twice in PBT and labelled for 30 min with R-Phycoerythrin-conjugated Goat-anti-Human IgG Fc (Jackson Immunoresearch, stock diluted 1:600 in assay buffer). Beads used for ACE2-Spike interaction measurement were not washed. Digoxigenin-labelled recombinant ACE2 (30µl, 300ng/ml) was added to the beads, and the plate was agitated for 40 min. The beads were washed twice in PBT and labelled with monoclonal anti-Digoxin (Jackson Immunoresearch) conjugated in-house to R-Phycoerythrin (1µg/ml) for 30 min at constant agitation. After labelling with secondary antibodies, the beads were washed twice. The contents of the two 384 well plates were then pooled into a 96 well deep well plate prefilled with PBT containing 1% BSA using the Zephyr Robot. The beads were next analyzed with an Attune Next Flow cytometer equipped with four lasers (violet, blue, yellow and red) and a harvesting unit for microwell plates). The analysis time averaged 60 minutes per plate.

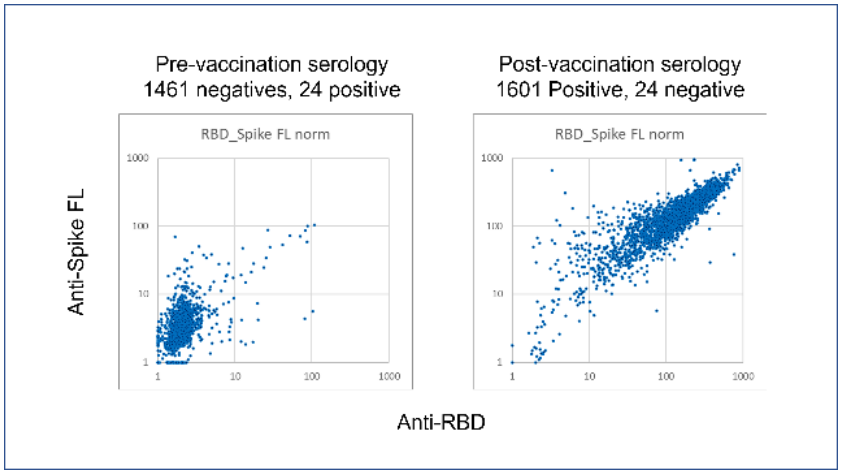
Data analysis: Raw flow cytometry data were analyzed using WinList. The median R-Phycoerythrin fluorescence intensity (MFI) for each bead subset was exported to a spreadsheet. Further analysis was done in Microsoft Excel. The MFI values measured for beads with viral proteins were divided by those of beads with Neutravidin only. The relative MFI values are hereafter referred to as arbitrary units (AU/ml).

Validation of Methods

Validation of initial method

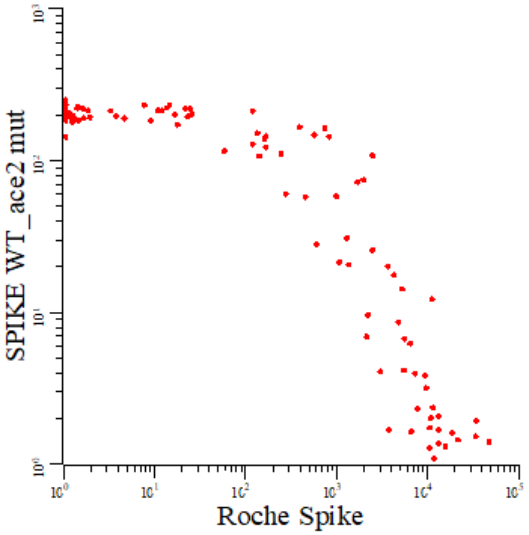


Results obtained with 979 pre-pandemic sera and 810 samples from COVID-19 convalescents show a false positive rate of 0. 3% and a sensitivity of 95% using a double cut-off for anti-RBD and anti-Spike. The x- and y-axes show relative median fluorescence intensity of beads coupled with recombinant full-length Spike (y) or the receptor-binding domain (RBD).

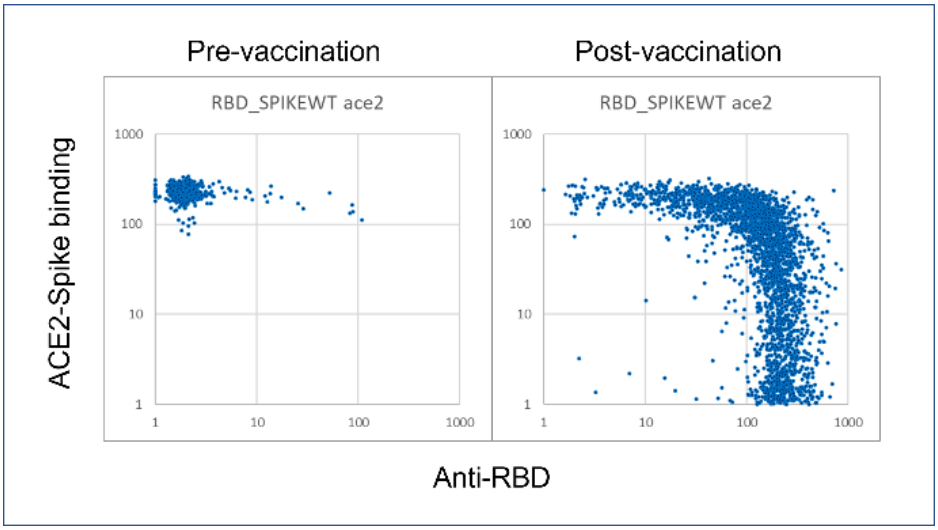


The method was further validated in the healthy controls (health care professionals) pre- and post - vaccination. These data showed that only 24 of 1461 tested healthy controls (1.6%) were positive prior to vaccination. This is slightly lower than the average sero-prevalence in Oslo Jan-March 2021. After vaccination 1601 out of 1625 were positive (98.5%)

<u>Validation of ACE2-Spike interaction assay for quantitative measurement of antibodies</u>



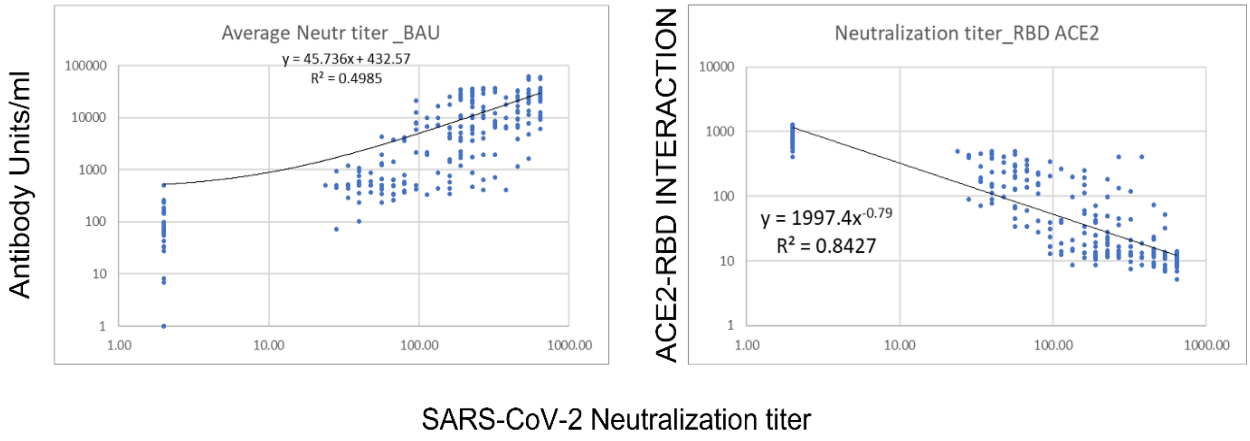
The dot plot shows Binding Antibody Units (BAU, x-axis) measured using the Roche Elecsys anti-spike assay and fluorescence from ACE2-binding to Spike on the y-axis. The results show that the ACE2-Spike interaction assay has a dynamic range between 1000 to 10.000 BAU.



The method was further validated in healthy controls pre- and post-vaccination. The two dot plots show measurement of healthy controls samples taken before (1559) or after vaccination (1616) respectively. The dynamic range for the ACE2 signal (y-axis) starts approximately at 100 AU RBD (x-axis). Thus, IgG-and ACE2- signals yield high resolution for low

and high antibody levels, respectively.

The dot plots below show SARS-CoV2 virus neutralization titer (x-axis). The y-axis is AU/ml (left) and ACE2-RBD interaction (right). The results display a positive correlation between AU/ml and virus neutralization titer, and an inverse correlation between ACE2-RBD interaction and neutralization titer. Virus neutralization is observed in samples that have AU >200/ml.
The method used for virus neutralization is described in a recent publication.[5]



Supplementary Table 1 Baseline Characteristics Healthy Controls

	All healthy controls n=1114	Healthy controls with samples analyzed by the ACE2-Spike interaction assay[a] n=323
Demographics		
Age, years (median, IQR)	43 (32-55)	44 (33-56)
Female sex, n (%)	854 (77)	241 (75)
Male sex, n (%)	260 (23)	82 (25)
Vaccines		
BNT162b2 x2, n (%)	625 (56)	162 (50)
mRNA-1273 x2, n (%)	246 (22)	71 (22)
Combination of vaccines[b], n (%)	243 (22)	90 (28)

[a]Assay measures the inhibitory effect of serum on binding of ACE2 to Spike, which is a surrogate for neutralizing antibodies. Described in detail in eAppendix 2
[b]Combination of vaccines: ChAdOx1 + BNT162b2/mRNA-1273 or BNT162b2 + mRNA-1273

Supplementary Table 2 Baseline Characteristics Diseases and Therapies

	Patients n=1647
Diseases n (%)	
Rheumatoid arthritis	*566 (35)*
Disease Activity Score 28 Joints, median (IQR), n=225	2.1 (1.4-3.1)
Disease duration yrs, median (IQR), n=543	9.2 (4.1-17.2)
Psoriatic arthritis	*295 (18)*
Disease Activity Score 28 Joints, median (IQR), n=87	2.0 (1.4-2.6)
Disease duration yrs, median (IQR), n=272	9.0 (4.5-17.5)
Spondyloarthritis	305 (19)
Ankylosing Spondylitis Disease Activity Score, median (IQR), n=178	1.1 (0.7-1.7)
Disease duration yrs, median (IQR), n=290	9.9 (4.8-19.3)
Ulcerative colitis	195 (12)
Partial Mayo Score, median (IQR), n=195	0 (0-1)
Disease duration yrs, median (IQR), n=195	8.5 (5.1-15.4)
Crohn's disease	280 (17)
Harvey-Bradshaw Index, median (IQR), n=279	2 (0-4)
Disease duration yrs, median (IQR), n=280	12.6 (5.3-20.3)
Medication n (%)	
Tumor necrosis factor inhibitor, monotherapy*	696 (42)
Years on therapy	<1 yr 98 (14%), 1-5 yrs 270 (40%), >5 yrs 302 (46%)
Tumor necrosis factor inhibitor combination therapy†	386 (23)
Years on therapy	<1 yr 48 (13%), 1-5 yrs 140 (37%), >5 yrs 186 (50%)
Methotrexate	348 (21)
Years on therapy	<1 yr 50 (15%), 1-5 yrs128 (38%), >5 yrs 163 (48%)
Vedolizumab	55 (3)
Years on therapy	<1 yr 3 (6%), 1-5 yrs 35 (73%), >5 yrs 10 (21%)
Monotherapy, n (%)	53 (96)
Janus kinases inhibitor	50 (3)
Years on therapy	<1 yr 12 (28%), 1-5 yrs 20 (47%), >5 yrs 11 (25%)
Monotherapy, n (%)	35 (70)
Ustekinumab	34 (3)
Years on therapy	<1 yr 10 (37%), 1-5 yrs 13 (48%), >5 yrs 4 (14%)
Monotherapy, n (%)	31 (91)
Tocilizumab	32 (3)
Years on therapy	<1 yr 3 (10%), 1-5 yrs 14 (48%), >5 yrs 12 (42%)
Monotherapy, n (%)	24 (75)
Abatacept	15 (1)
Years on therapy	<1 yr 1 (7%), 1-5 yrs 7 (46%), >5 yrs 7 (46%)
Monotherapy, n (%)	8 (53)
Secukinumab	13 (1)
Years on therapy	<1 yr 2 (18%), 1-5 yrs 5 (46%), >5 yrs 4 (36%)
Monotherapy, n (%)	10 (77)

Other‡	18 (1)
Years on therapy	<1 yr 4 (23%), 1-5 yrs 8 (46%), >5 yrs 6 (31%)

IQR= Inter quartile range.
*Tumor necrosis factor inhibitors: infliximab, etanercept, adalimumab, golimumab, certolizumab pegol. †Combination therapy: methotrexate, sulfasalazine, leflunomide, azathioprine.
‡Drugs with less than 10 patients included: sulfasalazine, leflunomide, azathioprine, risankizumab, prednisolone monotherapy.

Supplementary Table 3 Predictors of Response Following Standard Vaccination in Patients on TNF inhibitors

	Univariable analysis		**Multivariable analysis**	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Demographics				
Age in years	0.96 (0.94,0.98)	<0.001	0.95 (0.93,0.97)	<0.001
Male sex	0.85 (0.48,1.51)	0.574	0.69 (0.36,1.32)	0.25
Diagnoses				
Rheumatoid arthritis	1.00 (-)	-	1.00 (-)	-
Spondyloarthritis	1 (0.39,2.57)	0.998	0.38 (0.12,1.17)	0.085
Psoriatic arthritis	1.73 (0.5,6)	0.38	1.3 (0.34,4.9)	0.692
Crohn's disease	1.04 (0.45,2.41)	0.923	0.39 (0.13,1.14)	0.079
Ulcerative colitis	1.57 (0.57,4.32)	0.373	0.6 (0.17,2.09)	0.415
Medication				
Tumor necrosis factor inhibitor monotherapy*	1.00 (-)	-	1.00 (-)	-
Tumor necrosis factor inhibitor combination therapy†	0.35 (0.19,0.63)	<0.001	0.28 (0.15,0.55)	<0.001
Prednisolone therapy	0.21 (0.06,0.72)	0.011	0.26 (0.06,1.13)	0.067
Vaccines				
BNT162b2 x 2	1.00 (-)	-	1.00 (-)	-
mRNA-1273 x 2	8.95 (2.09,38.38)	0.003	8.64 (1.96,38.18)	0.004
Combination of vaccines§	1.94 (0.44,8.58)	0.375	1.33 (0.28,6.29)	0.711
COVID-19 infection and any mRNA vaccine¶	NA		NA	
Other factors				
Pause in medication‱	1.35 (0.4,4.63)	0.623	1.31 (0.33,5.29)	0.697

OR=Odds ratio, CI=Confidence interval
*Tumor necrosis factor inhibitors: Infliximab, etanercept, adalimumab, golimumab, certolizumab pegol. †Combination therapy: methotrexate, sulfasalazine, leflunomide, azathioprine. ‡Analyses not applicable due to low number of included patients. §Combination of vaccines: ChAdOx1+ BNT162b2/mRNA-1273 or BNT162b2 + mRNA-1273. ¶BNT162b2 or mRNA-1273. ‱Pause in medication prior to and after vaccination

Supplementary Table 4 Predictors of Response Following Third Dose Vaccination

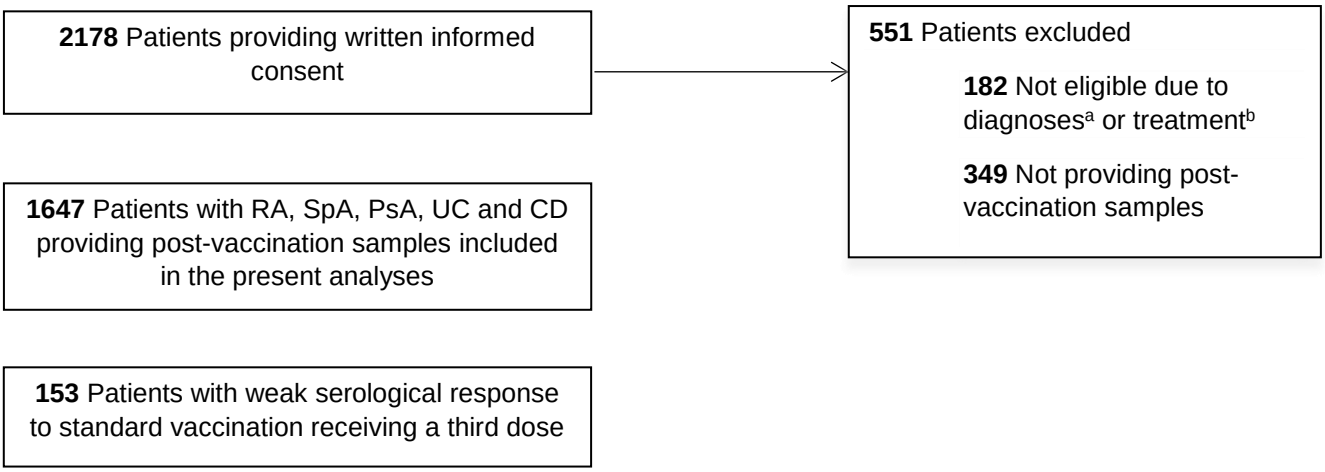
	Univariable analysis		**Multivariable analysis**	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Demographics				
Age in years	1.01 (0.98–1.04)	0.361	1.05 (1–1.09)	0.032
Male gender	1.06 (0.44–2.59)	0.893	0.91 (0.28–2.96)	0.871
Diagnoses				
Rheumatoid arthritis	1	..	1	..
Spondyloarthritis	0.63 (0.18–2.22)	0.462	0.37 (0.05–2.87)	0.33
Psoriatic arthritis	1.57 (0.38–6.5)	0.524	1.89 (0.35–10.35)	0.453
Crohn's disease	3.84 (0.97–15.15)	0.05	5.11 (0.21–125.13)	0.307
Ulcerative colitis	1.96 (0.38–10.24)	0.413	2.25 (0.09–54.52)	0.611
Medication				
Tumor necrosis factor inhibitor[a], monotherapy	1	..	1	..
Tumor necrosis factor inhibitor, combination therapy[b]	0.67 (0.2–2.26)	0.51	0.68 (0.14–3.29)	0.628
Methotrexate	0.42 (0.11–1.57)	0.186	0.26 (0.03–2.08)	0.195
Vedolizumab[^]	..	..	..	..
Janus kinases inhibitor	0.21 (0.04–1)	0.046	0.21 (0.02–1.84)	0.15
Tocilizumab[^]	..	..	..	..
Ustekinumab[^]	..	..	..	..
Azathioprine[^]	..	..	..	..
Abatacept[^]	..	..	..	..
Vaccines				
BNT162b2 x 2	1	..	1	..
mRNA-1273 x 2	2.6 (0.31–21.82)	0.369	1.84 (0.18–18.38)	0.598

OR=Odds ratio, CI=Confidence interval
[a]Tumor necrosis factor inhibitors: Infliximab, etanercept, adalimumab, golimumab, certolizumab pegol. [b]Combination therapy: methotrexate, sulfasalazine, leflunomide. [^]Analyses not applicable due to low number of included patients

Supplementary Table 5 Adverse Events

	All patients						Controls						Patients receiving a third dose								
	1st dose (n=1380)			2nd dose (n=1516)			1st dose (n=246)			2nd dose (n=244)			1st dose (n=155)			2nd dose (n=149)			3rd dose (n=159)		
	<2 days n	**≥2 days** n	**Total** n (%)	**<2 days** n	**≥2 days** n	**Total** n (%)	**<2 days** n	**≥2 days** n	**Total** n (%)	**<2 days** n	**≥2 days** n	**Total** n (%)	**<2 days** n	**≥2 days** n	**Total** n (%)	**<2 days** n	**≥2 days** n	**Total** n (%)	**<2 days** n	**≥2 days** n	**Total** n (%)
Any adverse event			684 (50%)			810 (53%)			174 (71%)			191 (78%)			75 (48%)			77 (52%)			70 (44%)
Fever	53	13	76 (6%)	149	24	173 (11%)	54	4	58 (24%)	65	9	74 (30%)	2	3	5 (3%)	2	3	5 (3%)	11	1	12 (8%)
Chills	80	28	108 (8%)	152	26	178 (12%)	64	5	69 (28%)	71	4	75 (31%)	5	4	9 (6%)	6	2	8 (5%)	11	2	13 (8%)
Discomfort	78	31	109 (8%)	138	60	198 (13%)	38	15	53 (22%)	59	16	75 (31%)	8	4	12 (8%)	8	5	13 (9%)	12	3	15 (9%)
Slackness	123	61	184 (13%)	206	102	308 (20%)	51	29	80 (33%)	82	24	106 (43%)	12	9	21 (14%)	12	10	22 (15%)	18	10	28 (18%)
Feeling of flu	59	25	84 (6%)	150	43	193 (13%)	46	6	52 (21%)	58	11	69 (28%)	6	3	9 (6%)	10	3	13 (9%)	9	3	12 (8%)
Tiredness	119	57	176 (13%)	180	90	270 (18%)	29	22	51 (21%)	51	25	76 (31%)	9	7	16 (10%)	9	6	15 (10%)	7	12	19 (12%)
Pain at injection site	426	124	550 (40%)	440	133	573 (38%)	45	49	94 (38%)	59	57	116 (48%)	41	12	53 (34%)	43	12	55 (37%)	33	11	44 (28%)
Swollen glands in axilla	6	1	7 (1%)	14	22	36 (2%)	3	7	10 (4%)	4	9	13 (5%)	1	0	1 (1%)	0	1	1 (1%)	1	1	2 (1%)
Headache	125	58	183 (13%)	207	67	274 (18%)	50	20	70 (29%)	55	22	77 (32%)	15	5	20 (13%)	12	4	16 (11%)	18	7	25 (16%)
Dizziness	32	13	45 (3%)	63	24	87 (6%)	8	11	19 (8%)	12	4	16 (7%)	4	2	6 (4%)	5	0	5 (3%)	4	1	5 (3%)
Abdominal discomfort	13	7	20 (1%)	20	13	33 (2%)	5	1	6 (2%)	5	4	9 (4%)	1	0	1 (1%)	1	2	3 (2%)	1	0	1 (1%)
Reduced appetite	15	6	21 (1%)	32	18	50 (3%)	3	5	8 (3%)	14	3	17 (7%)	1	0	1 (1%)	2	2	4 (3%)	1	1	2 (1%)
Nausea/vomiting	31	5	36 (3%)	42	12	54 (4%)	9	4	13 (5%)	15	4	19 (8%)	5	0	5 (3%)	5	1	6 (4%)	5	0	5 (3%)
Diarrhea	16	5	21 (2%)	17	13	30 (2%)	4	0	4 (2%)	2	2	4 (2%)	0	0	0 (0%)	1	1	2 (1%)	1	1	2 (1%)
Dyspnea	9	10	19 (1%)	9	16	25 (2%)	2	5	7 (3%)	2	1	3 (1%)	0	1	1 (1%)	0	1	1 (1%)	0	2	2 (1%)
Cough	5	7	12 (1%)	8	10	18 (1%)	0	1	1 (0.4%)	2	1	3 (1%)	0	2	2 (1%)	0	1	1 (1%)	0	0	0 (0%)
Muscular pain	51	46	97 (7%)	121	79	200 (13%)	39	15	54 (22%)	56	12	68 (28%)	4	2	6 (4%)	6	3	9 (6%)	5	7	12 (8%)
Rash	5	9	14 (1.0%)	6	12	18 (1%)	1	2	3 (2%)	3	2	5 (2%)	1	1	2 (1%)	0	1	1 (1%)	0	2	2 (1%)
Sleep disorders	12	12	24 (1.7%)	35	18	53 (4%)	11	2	13 (5%)	15	1	16 (7%)	1	2	3 (2%)	1	4	5 (3%)	0	3	3 (2%)
Unrest	6	6	12 (0.9%)	12	11	23 (2%)	2	4	6 (2%)	2	6	8 (3%)	2	1	3 (2%)	2	2	4 (3%)	0	2	2 (1%)
Confusion	3	0	3 (0.2%)	4	3	7 (1%)	1	0	1 (0.4%)	1	0	1 (0.4%)	1	0	1 (1%)	1	1	2 (1%)	0	0	0 (0%)
Allergic reaction	1	1	2 (0.1%)	2	1	3 (0.2%)	0	0	0 (0%)	1	0	1 (0.4%)	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)
Anaphylaxis	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)	0	0	0 (0%)
Bleeding/bruises			27 (2%)			44 (3%)			8 (3%)			4 (2%)			2 (1%)			8 (5%)			5 (3%)
Thrombosis			1 (0.1%)			0 (0%)			0 (0%)			0 (0%)			0 (0%)			0 (0%)			0 (0%)
Severe headache			23 (2%)			32 (2%)			13 (5%)			11 (5%)			4 (3%)			1 (1%)			8 (5%)
Disease flare			78 (6%)			88 (6%)									3 (2%)			4 (3%)			26 (16%)

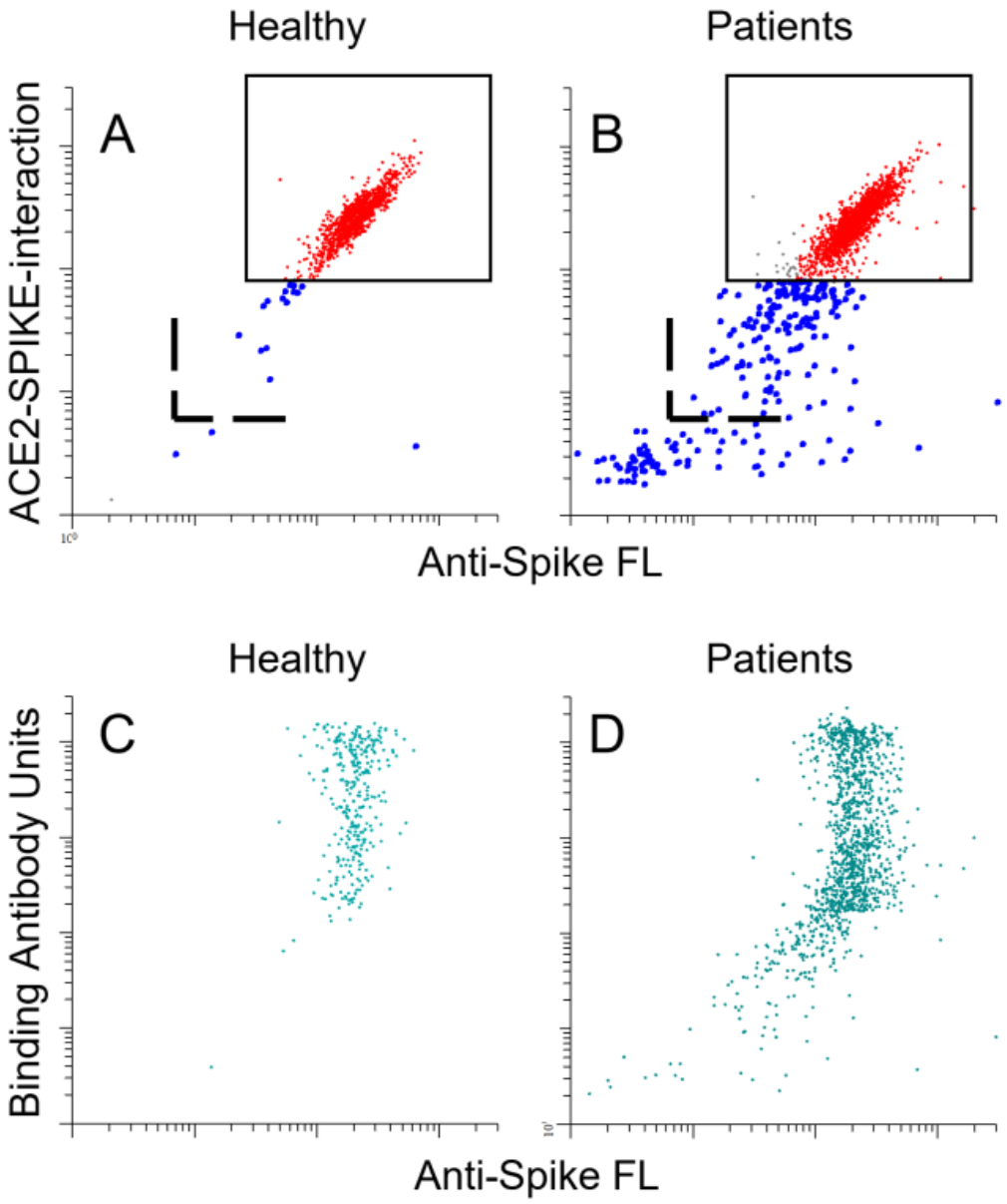
Supplementary Figure 1 Patient disposition



RA= Rheumatoid arthritis. SpA=Spondyloarthritis. PsA=Psoriatic arthritis. UC=Ulcerative colitis. CD= Crohn's disease
[a]Liver transplanted patients (n=39) and patients with autoimmune hepatitis (n=58)
[b] Patients on CD-20 depleting therapy (n= 85)

Supplementary Figure 2 a-d Measurements of anti-Spike antibodies in patients and healthy controls

a-b) The dot plots show measurement of IgG antibodies to full length (FL) Spike from SARS-CoV-2 (x-axis) and the receptor-binding domain (RBD) in healthy controls and patients on immunosuppressive therapy (as described in Methods). Red dots correspond to individuals defined as responders (RBD median fluorescence intensity MFI ≥70 AU/ml). C-d) Anti-Spike IgG versus Binding Antibody Units measured in sera from healthy individuals (n=323) and patients (n=1442) (as described in Methods). ACE=Angiotensin converting enzyme



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Paper III

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Original Article

Basic science

Adalimumab serum levels and anti-drug antibodies: associations to treatment response and drug survival in inflammatory joint diseases

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Abstract

Objectives: To explore associations between serum adalimumab level, treatment response and drug survival in order to identify optimal drug levels for therapeutic drug monitoring of adalimumab. Also, to assess the occurrence and risk factors of anti-drug antibody (ADAb) formation.

Methods: Non-trough adalimumab and ADAb levels were measured by automated fluorescence assays in serum collected after 3 months of adalimumab treatment in patients with RA, PsA or axial SpA (axSpA) included in the observational NOR-DMARD study. Treatment response was evaluated after 3 months and drug survival was evaluated during long-term follow-up.

Results: In 340 patients (97 RA, 69 PsA, 174 axSpA), the median adalimumab level was 7.3 mg/l (interquartile range 4.0–10.3). A total of 33 (10%) patients developed ADAbs. Findings were comparable across diagnoses. In RA and PsA, adalimumab levels ≥6.0 mg/l were associated with treatment response [odds ratio (OR) 2.2 (95% CI 1.0, 4.4)] and improved drug survival [hazard ratio 0.49 (95% CI 0.27, 0.80)]. In axSpA, a therapeutic level could not be identified, but higher adalimumab levels were associated with response. Factors associated with ADAb formation were previous bDMARD use, no methotrexate comedication and the use of adalimumab originator compared with GP2017.

Conclusion: Higher adalimumab levels were associated with a better response and improved drug survival for all diagnoses, with a suggested lower threshold of 6.0 mg/l for RA/PsA. This finding, the large variability in drug levels among patients receiving standard adalimumab dose and the high proportion of patients developing ADAbs encourages further investigations into the potential role of therapeutic drug monitoring of adalimumab.

Keywords: adalimumab, serum drug level, inflammatory joint disease, anti-drug antibodies, TNF inhibitors.

Rheumatology key messages

- Higher adalimumab levels were associated with treatment response and improved drug survival across diagnoses.
- The indicated lower threshold of adalimumab was 6.0 mg/l in RA/PsA.
- ADAbs were found in 10% of patients, more commonly without methotrexate comedication, reducing treatment effect.

Introduction

TNF-α inhibitors (TNFis) and other biologic drugs have, together with novel treatment strategies such as treat to target, contributed to a revolution in the treatment of inflammatory joint diseases [1]. For patients with RA, PsA and axial SpA (axSpA), remission or inactive disease is now a realistic treatment goal. Nevertheless, despite current therapies and treatment strategies, a large proportion of patients do not respond sufficiently to therapy and approximately half of patients lose efficacy over time [2, 3]. Failure to maintain disease control has a major impact on quality of life and increases the risk of joint destruction for patients with peripheral arthritis. Loss of efficacy can be caused by underexposure to drugs, with or without development of anti-drug antibodies (ADAbs) [4–6]. Therapeutic drug monitoring (TDM), individualized dosing based on assessment of drug levels and ADAbs, is one strategy suggested to improve the effectiveness of TNFis [7]. TDM provides an opportunity to minimize both under- and overexposure to drugs. While underexposure can lead to loss of efficacy, overexposure increases costs and may predispose

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patients to adverse events [8]. In addition, timely identification of ADAbs enables early adjustment of treatment, possibly preventing a clinical flare [9]. In order to develop TDM algorithms, therapeutic ranges for the drug in question must be identified [10]. Recently published EULAR points to consider stated that one important barrier to using TDM in clinical care is the current lack of identified therapeutic ranges for most TNFis [10].

Adalimumab, a fully human monoclonal antibody, is the most commonly used TNFi worldwide in the treatment of several immune-mediated inflammatory diseases, including inflammatory joint diseases [11, 12]. While prior data suggest that adalimumab levels of 4–12 mg/l are associated with treatment response in RA and PsA patients [13–18], less is known regarding optimal serum adalimumab levels in axSpA [19–21].

Adalimumab has a high immunogenic potential, both in originator and biosimilar products, with ADAb development in 10–60% of patients, depending on the diagnosis and assay used for detection [6, 22]. Except for co-medication with methotrexate, which reduces the occurrence of ADAbs, little is known regarding factors associated with ADAb formation to adalimumab [23–25].

The main aim of this study was to explore associations between serum adalimumab levels, treatment response and drug survival in patients with inflammatory joint diseases, with the intention of identifying a therapeutic level. This would allow the development of TDM algorithms for adalimumab that can be validated in prospective clinical trials. Additionally, we aimed to explore the occurrence, risk factors and clinical implications of neutralizing ADAbs.

Methods

Study population

The Norwegian Antirheumatic Drug Registry (NOR-DMARD) (clinicaltrials.gov: NCT01581294) is a longitudinal multicentre observational study including adult patients with inflammatory joint diseases initiating therapy with biologic DMARDs (bDMARDs) [26, 27]. Clinical data are registered at baseline, 3, 6, 9 and 12 months and thereafter every 6 months. Biobank samples are collected at baseline and after 3 months.

In the current study we included patients with a clinical diagnosis of RA, PsA or axSpA with an available serum sample collected 3 months after initiating adalimumab. For assessment of treatment response, clinical data from baseline and 3 months were used. Information from the last registered follow-up visit was used in the assessment of drug survival.

All patients started adalimumab in a dose of 40 mg every second week. Patients were started on originator adalimumab up to 1 February 2020 and on the biosimilar GP2017 thereafter, based on a national annual tender system for biologic drugs in Norway [28].

The study was approved by an independent ethics committee (Regional Committees for Medical and Health Research Ethics South East; reference number 2011/1339) and all participants provided written informed consent.

Treatment response

In RA and PsA patients, disease activity was measured by the 28-joint Disease Activity Score with ESR (DAS28-ESR). Remission was defined as DAS28-ESR <2.6 and treatment response as EULAR good or moderate response [29, 30]. In patients with PsA, sensitivity analyses were performed using the 28-joint Disease Activity Score for Psoriatic Arthritis (DAPSA28), with DAPSA28 improvement $\geq 50\%$ defined as treatment response [31, 32]. In axSpA patients, disease activity was measured by Ankylosing Spondylitis Disease Activity Score with CRP (ASDAS-CRP). Inactive disease was defined as ASDAS-CRP <1.3, treatment response as ASDAS major improvement (MI; change ≥ 2.0 units) or clinically important improvement (CII; change ≥ 1.1 units) [33]. ESR was used as a generic surrogate for disease activity.

Measurements of serum adalimumab levels and ADAbs

Non-trough serum samples stored at $-80°C$ were analysed using a validated time-resolved fluorescence assay. The solid phase protein is human recombinant TNF and the tracer protein is europium-labelled protein A. Serum samples with adalimumab levels <3.0 mg/l were analysed with a drug-sensitive in-house fluorescence assay measuring neutralizing ADAbs, with human recombinant TNF as the solid phase protein and europium-labelled adalimumab $F(ab')_2$ as the tracer protein. ADAb levels ≥ 15 µg/l were defined as positive, with levels ≥ 50 µg/l considered moderate or high [9]. Both assays are fully automated on the AutoDELFIA immunoassay platform (PerkinElmer, Waltham, MA, USA).

Statistical analyses

Baseline characteristics were summarized with descriptive statistics. Comparisons of adalimumab levels and ADAb occurrence between treatment response and inactive disease/remission groups were analysed with the Mann–Whitney U test, χ^2 test or independent samples t-test, as appropriate. Explorative concentration–effect analyses were used to suggest a possible therapeutic level, dividing the drug level range into segments with ≈21 and 22 patients in each (separately for RA/PsA and axSpA). Associations between the suggested therapeutic cut-offs and treatment response were further analysed by multivariable logistic regression, with response being the dependent variable and the independent variables being adalimumab cut-off, age, sex, prior use of a bDMARD (yes/no) and concomitant use of methotrexate. Sensitivity analyses using receiver operating characteristics (ROC) analyses were also performed to find the lower adalimumab cut-off by use of the Youden Index, maximizing the sum of sensitivity and specificity [34]. Drug survival was assessed using Kaplan–Meier curves and Cox proportional hazards regression analysis (adjusted for the same covariates as above). Patients who discontinued treatment due to remission, pregnancy or with missing information regarding the reason for drug discontinuation were censored at their last registered visit. Possible factors associated with adalimumab levels and ADAb formation were assessed with linear and logistic regression, respectively, with the independent variables being age, sex, prior use of a bDMARD (yes/no), concomitant methotrexate use, adalimumab type (originator or GP2017) and baseline disease activity. All tests were two-sided and performed at a 0.05 significance level.

The same outcomes were used in RA and PsA, and to increase the power they were handled as one disease group throughout the analyses. Subgroup analyses were performed.

Missing disease activity components were handled with median imputation and missing whole visits at 3 months were handled by next observation (6-month data) carried backwards.

All analyses were done in Stata 16.1 (StataCorp, College Station, TX, USA) and GraphPad Prism 9.4.1 (GraphPad Software, San Diego, CA, USA).

Results

Study population and baseline characteristics

A total of 340 patients [97 RA, 69 PsA, 174 axSpA; mean age 46 years (S.D. 14); 181 (53%) female) starting adalimumab between 6 June 2012 and 27 October 2021 were eligible for inclusion in the present analyses (Supplementary Fig. S1, available at *Rheumatology* online). Originator adalimumab was used by 212 (62%) and GP2017 by 128 (38%) patients. Methotrexate co-medication was used by 121 patients (36%), mostly [108/121 (89%)] in patients with a diagnosis of RA or PsA (Table 1).

Adalimumab serum levels, treatment response and drug survival

The median adalimumab level at 3 months was 7.3 mg/l [interquartile range (IQR) 4.0–10.3], with comparable results across diagnoses (Fig. 1A). In RA/PsA patients, the adalimumab levels were not significantly different in patients with EULAR good or moderate response compared with non-responders [7.7 mg/l (IQR 5.0–10.3) *vs* 5.9 mg/l (IQR 1.9–10.2), $P = 0.095$] (Fig. 1B). In contrast, axSpA patients with ASDAS-CRP improvement (MI or CII) had higher adalimumab levels than patients without ASDAS-CRP improvement [7.9 mg/l (IQR 5.4–10.8) *vs* 6.6 mg/l (IQR 2.8–9.6), $P = 0.014$] (Fig 1B). Those in EULAR remission/ASDAS inactive disease had higher adalimumab levels than the remaining patients, both for RA/PsA [7.8 mg/l (IQR 5.1–10.5) *vs* 5.8 mg/l (IQR 1.2–9.0), $P = 0.014$] and axSpA [8.7 mg/l (IQR 6.1–10.9) *vs* 5.4 mg/l (IQR 2.2–8.6), $P < 0.0001$]) (Fig. 1C).

Based on explorative concentration–effect analyses in RA/PsA, the highest rates of response and remission were found in patients with adalimumab levels between 6.0 and 12.0 mg/l (Fig. 2A and 2B). ROC analyses supported this cut-off [6.0 mg/l (95% CI 1.3, 10.7)] with an area under the curve of 0.610 and a sensitivity of 68% and specificity of 55% (Supplementary Fig. S2A, available at *Rheumatology* online). Adalimumab levels ≥6.0 mg/l were associated with treatment response and improved drug survival, and this was consistent when adjusting for possible confounders [odds ratio (OR) for response 2.2 (95% CI 1.0, 4.4) (Supplementary Table S1, available at *Rheumatology* online) and hazard ratio (HR) 0.49 (95% CI 0.28, 0.85)] (Fig. 2C). Drug levels ≥12.0 mg/l were associated with a lower rate of response [OR 0.28 (95% CI 0.87, 0.93)] compared with levels between 6.0 and 12.0 mg/l. Separate graphs for RA and PsA and sensitivity analyses using DAPSA28 in PsA are shown in Supplementary Fig. S3, available at *Rheumatology* online.

Despite a concentration–effect relationship, we could not identify a therapeutic range for axSpA (Fig. 3A and 3B and Supplementary Fig. S2B, available at *Rheumatology* online), as the likelihood for both ASDAS response and inactive disease increased with increasing adalimumab [OR 1.2 (95% CI 1.02, 1.3) and OR 1.4 (95% CI 1.2, 1.7) per adalimumab level group increase, respectively]. The lowest response rate was seen in patients with adalimumab levels <1.5 mg/l [OR 0.2 (95% CI 0.08, 0.7) as compared with ≥1.5 mg/l]. Drug survival curves in axSpA patients for different drug-level groups are shown in Fig. 3C.

Occurrence of ADAbs, treatment response and drug survival

At 3 months, 33 (10%) patients had developed ADAbs, [10 (10%) RA, 8 (12%) PsA and 15 (9%) axSpA patients], with a median ADAb level of 103 µg/l (IQR 60–182.5). Of patients with ADAb formation, 26/33 (79%) had moderate or high levels. The proportion with response to treatment was higher in patients without ADAb formation [178 (59%)] than in patients

Table 1. Baseline characteristics

Characteristics	Total ($N = 340$)	RA ($n = 97$)	axSpA ($n = 174$)	PsA ($n = 69$)
Age, years, mean (SD)	46 (14)	53 (14)	40 (12)	51 (13)
Female, *n* (%)	181 (53)	74 (76)	72 (41)	35 (51)
Current smoker, *n* (%)	28/232 (12)	6/56 (11)	17/135 (13)	5/41 (12)
Disease duration, years, median (IQR) years[a]	4.7 (1.0–11.9)	5.5 (1.2–11.3)	3.8 (0.8–12.6)	4.4 (1.2–12.2)
Previous use of bDMARDs, *n* (%)	112/333 (34)	30/96 (31)	61/170 (36)	21/67 (31)
RF positivity, *n* (%)		49/93 (53)		
Anti-CCP positivity, *n* (%)		65/96 (68)		
HLA-B27 positivity, *n* (%)			136/164 (83)	
Peripheral involvement, *n* (%)			52/173 (30)	
Adalimumab, *n* (%)				
Originator	212 (62)	58 (60)	113 (65)	41 (59)
GP2017	128 (38)	39 (40)	61 (35)	28 (41)
Co-medication, *n* (%)				
Methotrexate[b]	121 (36)	68 (70)	13 (7)	40 (59)
Sulfasalazine	10 (3)	5 (5)	4 (2)	1 (1)
Hydroxychloroquine	2 (0.5)	2 (2)		
Leflunomide	6 (2)	4 (4)		2 (3)
Prednisolone[c]	44 (14)	34 (35)	4 (2)	6 (9)
Disease activity				
DAS28-ESR, mean (S.D.)		3.7 (1.5)		3.22 (1.3)
ASDAS-CRP, mean (S.D.)			2.57 (1.0)	
DAPSA28-CRP, mean (S.D.)				17.1 (11.2)
Patient-reported global pain (VAS scale), median (IQR)	44 (23–64)	38 (16–58)	45 (25–66)	43 (26–60)

VAS: visual analogue scale.
[a] Available data in 180 patients.
[b] Median dose of methotrexate per week: 20 mg (IQR 10–20).
[c] Median prednisolone dose per day: 8.75 mg (IQR 5–15).

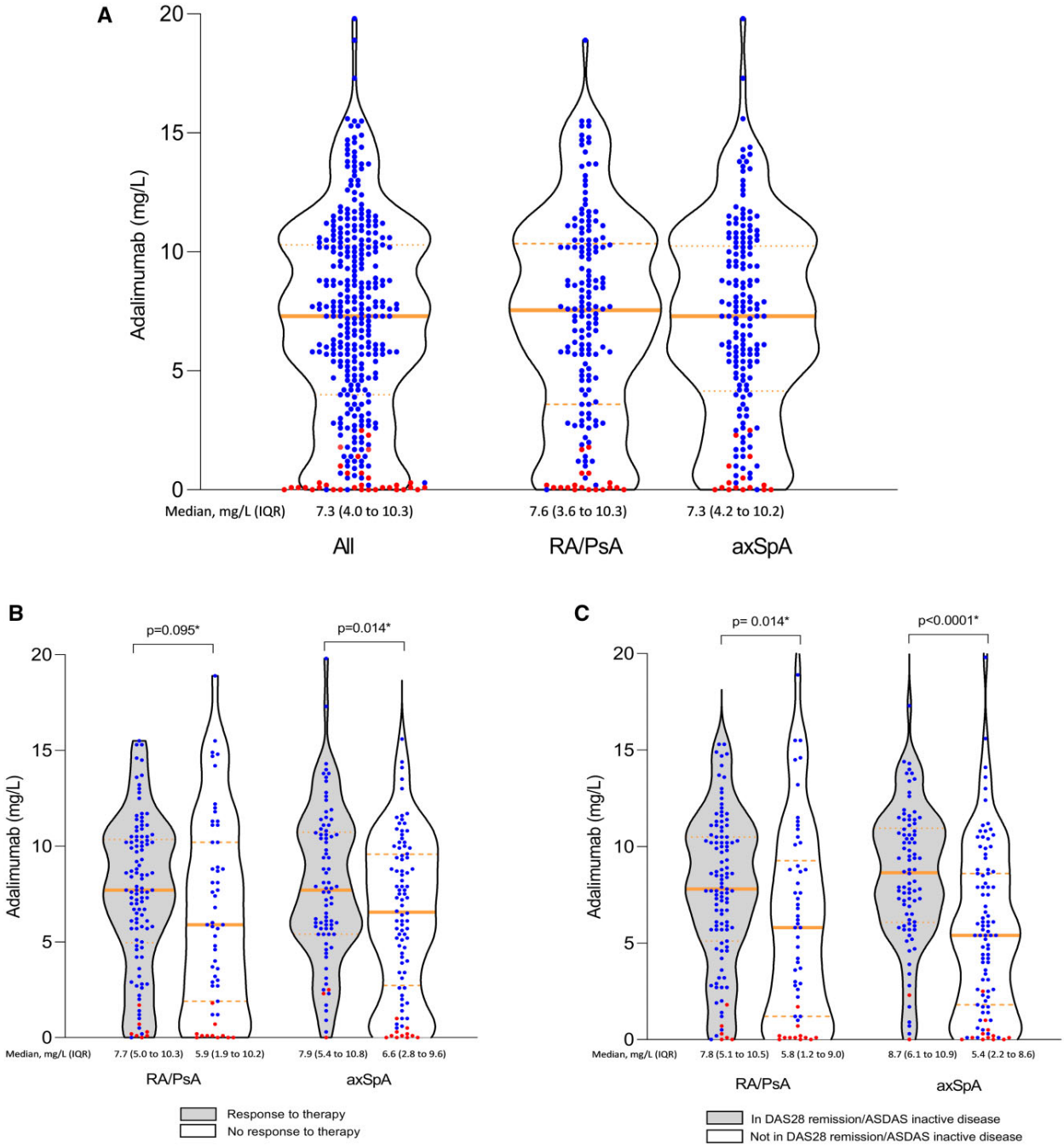

Figure 1. Serum adalimumab levels by diagnosis, treatment response and remission/inactive disease at 3 months. Violin plot showing the probability density of the data at different values, smoothed by a kernel density estimator. Each data point is a participant, the solid orange line shows the group median and red dots are participants with ADAb formation. **(A)** Adalimumab levels for all participants and by diagnosis. (**B** and **C**) Adalimumab levels by (**B**) response to treatment (EULAR good and moderate response and ASDAS major and clinically important improvement) and (**C**) DAS28 remission/ASDAS inactive disease at 3 months. *Mann-Whitney *U* test

with ADAb formation [10 (31%)], with an OR of 2.3 (95% CI 1.04, 5.3). Further, patients with ADAb formation had a higher rate of drug discontinuation [HR 3.3 (95% CI 2.0, 5.3)] (Supplementary Fig S4, available at *Rheumatology* online).

Factors associated with adalimumab levels and ADAb formation

RA/PsA patients on concomitant treatment with methotrexate had significantly higher adalimumab levels compared with patients without co-medication [8.4 mg/l (IQR 5.5–11.0) *vs* 5.8 mg/l (IQR 1.2–8.7), $P = 0.0002$] and had less ADAb formation [5/108 (5%) *vs* 13/58 (22%), $P < 0.001$] (Table 2 and Supplementary Table S2, available at *Rheumatology* online). Further, patients without methotrexate co-medication had a higher drug discontinuation rate [HR 1.9 (95% CI 1.1, 3.3)].

Patients treated with adalimumab originator had lower serum drug levels [6.4 mg/l (IQR 3.1–9.9) *vs* 8.3 mg/l (IQR 5.6–11.0), $P = 0.0004$] and a higher rate of ADAb

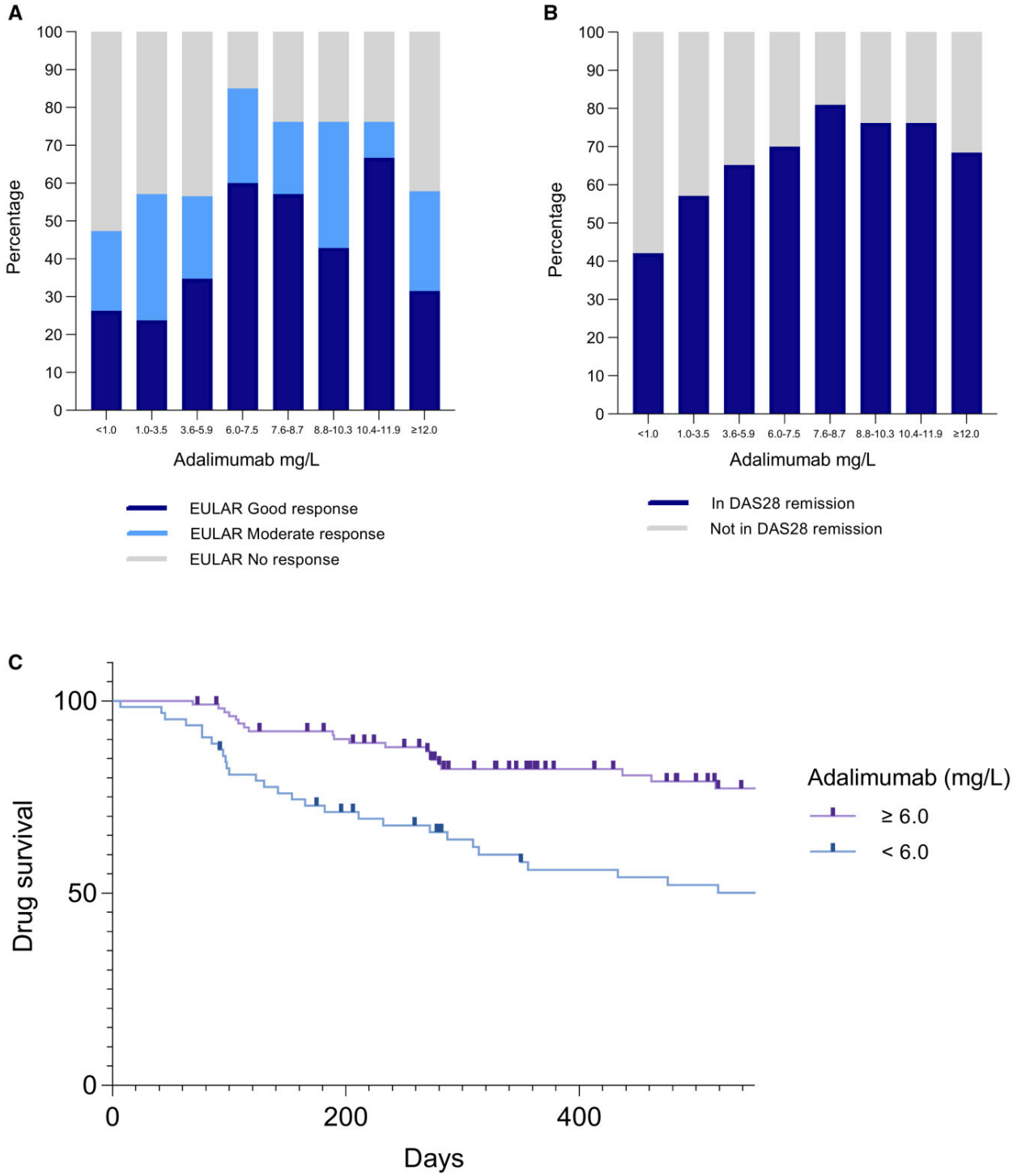

Figure 2. Treatment response, remission and drug survival in RA and PsA patients. **(A** and **B)** Drug-level range is divided into groups with ≈21 patients in each. Percent distribution of **(A)** EULAR response and **(B)** DAS28 remission in RA/PsA patients at 3 months according to adalimumab level. **(C)** Kaplan–Meier curve for 1.5 years drug survival stratified by adalimumab level at 3 months. Comparing RA/PsA patients with adalimumab ≥6 mg/l *vs* <6 mg/l, there was a significant difference in the survival estimates; $P = 0.0003$ (logrank)

formation [27/212 (13%) *vs* 6/128 (5%), $P = 0.015$] compared with patients treated with GP2017 (Fig. 4A). The between-group differences in serum drug levels and ADAb formation were consistent in multivariable regression analyses [β coefficient −1.45 (95% CI −2.4, −0.53) and OR 2.6 (95% CI 1.0, 6.7), with GP2017 as the reference group] and across diagnostic groups (Table 2 and Supplementary Table S2, available at *Rheumatology* online). There was no statistically significant difference in response to treatment or drug survival between adalimumab originator and GP2017 (Supplementary Table S3, available at *Rheumatology* online and Fig. 4B).

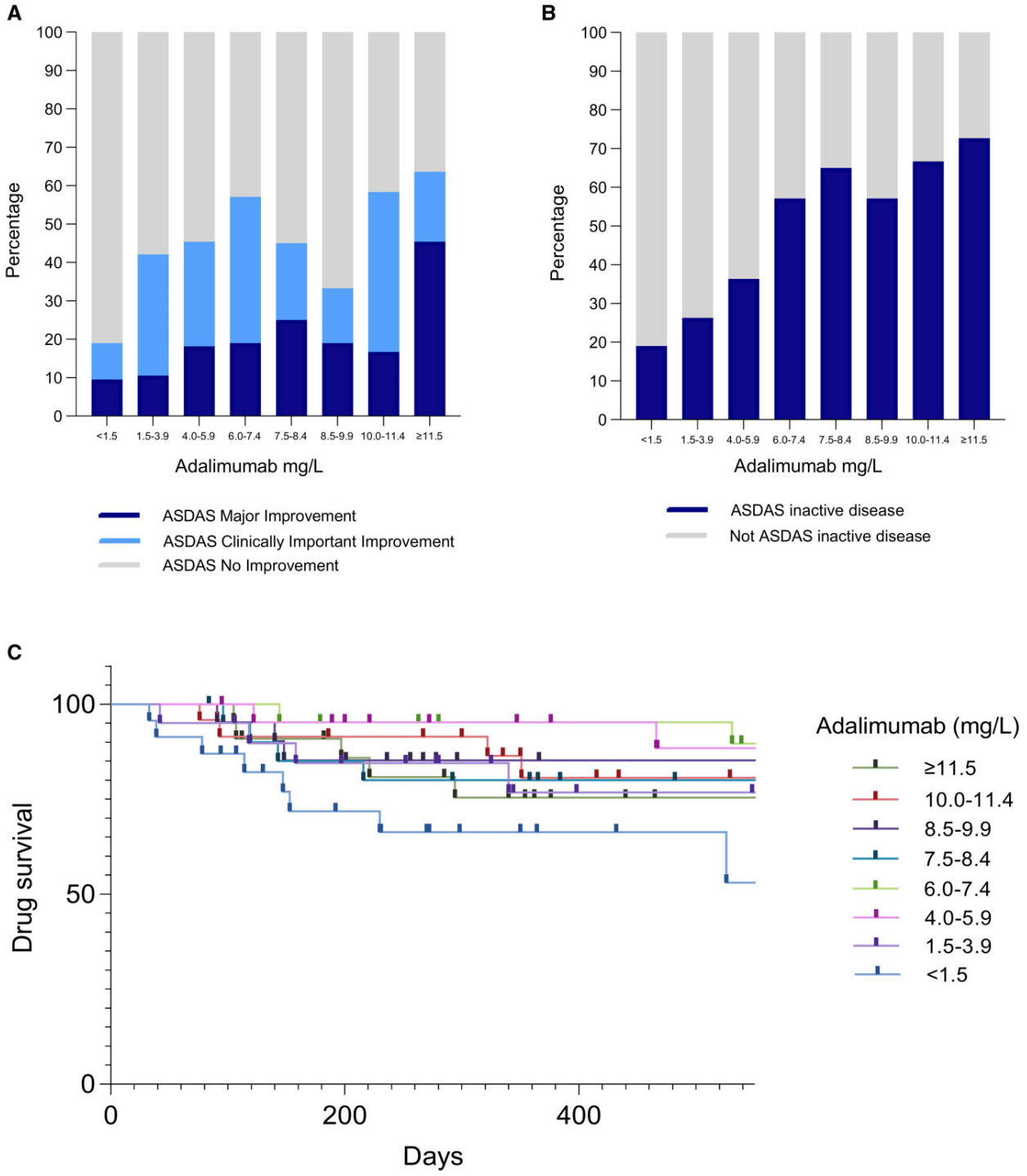

Figure 3. Treatment response, inactive disease and drug survival in axSpA patients. **(A** and **B)** Drug-level range is divided into groups with ≈22 patients in each. Percent distribution of **(A)** ASDAS improvement and **(B)** ASDAS inactive disease in axSpA patients at 3 months according to adalimumab level. **(C)** Kaplan–Meier curve for 1.5 years drug survival stratified by adalimumab level at 3 months. There was no significant difference in the survival estimates; $P = 0.082$ (logrank)

Age, sex and baseline DASs were not associated with drug level or ADAb formation (Table 2 and Supplementary Table S2, available at *Rheumatology* online).

Discussion

This observational study is the first to explore associations between adalimumab serum levels and clinical outcomes across all adult inflammatory joint diseases. The current results suggest 6.0 mg/l as a lower therapeutic level for adalimumab in RA/PsA patients. These findings can contribute to the development of TDM algorithms for adalimumab that could be further tested in clinical trials.

In patients with axSpA, higher adalimumab levels were associated with response to treatment, but a specific therapeutic level could not be identified.

Table 2. Factors associated with ADAb formation

Factors	Univariable OR (95% CI)	*P*-value	Multivariable OR (95% CI)	*P*-value
Age	1.0 (0.98, 1.0)	0.51	1.0 (0.98, 1.04)	0.52
Female sex	1.8 (0.87, 4.0)	0.11	2.1 (0.94, 4.6)	0.071
Previous use of one or more bDMARDs	2.6 (1.3, 5.4)	**0.009**	2.8 (1.3, 5.9)	**0.009**
Methotrexate co-medication	0.37 (0.15, 0.93)	**0.034**	0.31 (0.12, 0.82)	**0.018**
Adalimumab originator	3.0 (1.2, 7.4)	**0.020**	2.7 (1.1, 6.8)	**0.039**
ESR at baseline	1.0 (0.98, 1.02)	0.68	1.01 (0.98, 1.03)	0.69

Significant results are in bold.

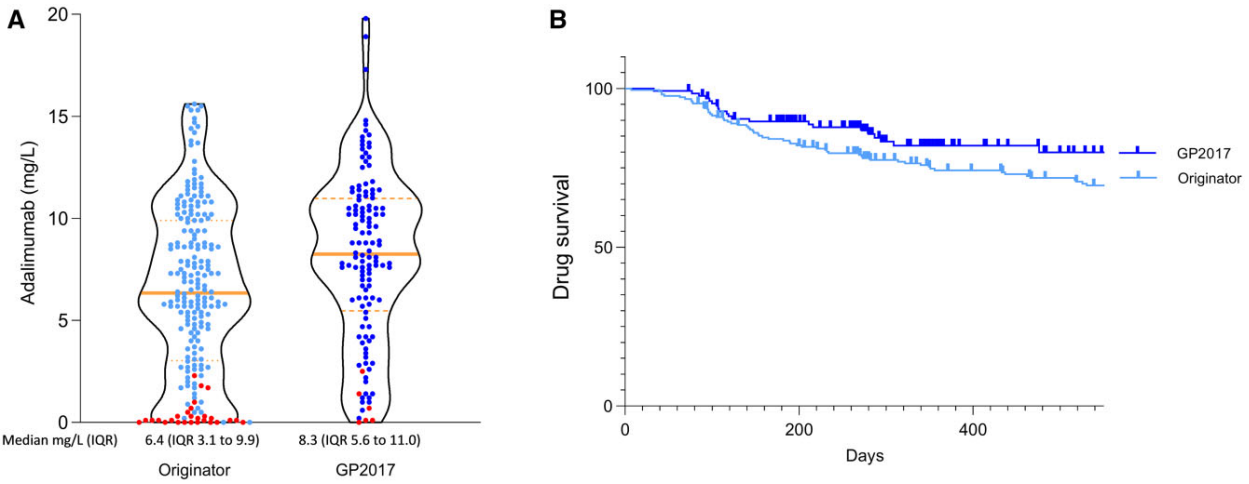

Figure 4. Serum drug level and drug survival by originator and GP2017 adalimumab. **(A)** Violin plot showing the probability density of the data at different values, smoothed by a kernel density estimator. Each data point is a participant and the solid orange line shows the group median. Red dots are participants with ADAb formation. **(B)** Kaplan–Meier curve for 1.5 years drug survival stratified by originator and GP2017 adalimumab. There was no significant difference in the survival estimates; $P = 0.16$ (logrank)

We found a large variability in adalimumab levels despite all patients receiving the same drug dose and a significant proportion of patients developing neutralizing ADAbs already at 3 months follow-up.

RA and PsA patients with serum drug levels ≥6.0 mg/l were more likely to respond to treatment at 3 months and had a lower risk of drug discontinuation. This finding corresponds to findings in previous studies where the suggested lower therapeutic level varied between 4 and 7 mg/l [13–18]. We suggest 6.0 mg/l as a lower therapeutic level for adalimumab, although we acknowledge that some individual patients will respond to therapy with lower levels depending on, for example, disease activity and disease phenotype. Based on our findings and prior data, 12.0 mg/l could be a possible upper limit for a therapeutic range [13, 15, 16, 18]. An upper limit of the therapeutic range is challenging to detect for TNFis, as it should take both risk of adverse events and drug costs into account. Despite these variables not being available in the present study, our results suggested 12.0 mg/l as a pragmatic upper limit for the therapeutic range, as adalimumab levels ≥12.0 mg/l were associated with lower response rates. However, as our assay measured free drug (as with most TNFi assays), high adalimumab levels in a patient not responsive to treatment may also indicate a different disease modality (where the pro-inflammatory effect of TNF is less important) not responsive to TNFi treatment and therefore with more free TNFi in the serum.

To our knowledge, this is the largest study to date examining the optimal adalimumab level in axSpA patients. Previous studies have diverging results [19, 20, 21, 35]. We could not identify a therapeutic range for axSpA, as the response rates increased with increasing adalimumab levels. However, Kaplan–Meier curves indicated that patients with adalimumab <1.5 mg/l had poorer drug survival. The lowest response rates were also found in this group, suggesting that patients should have levels at least >1.5 mg/l. Further, in patients with adalimumab >11.5 mg/l we saw a trend of more ASDAS major improvement, suggesting that some patients may benefit from very high levels. The outcome measures used in this study and other clinical trials within rheumatology are largely subjective, and factors like fibromyalgia could impact the measurements, making data on dose and response in these patients difficult to interpret. Also, the clinical axSpA diagnoses encompassed patients both with and without peripheral joint involvement, making for a somewhat heterogeneous population. These issues may account for the difficulty in determining therapeutic intervals in axSpA. The optimal therapeutic range may vary between patients due to individual differences in disease phenotype and disease activity. Future possibilities such as objective disease activity measurements, pharmacokinetic modelling and dashboard systems may enable individualized TDM [36].

In this study, 10% of patients had detectable neutralizing ADAbs after 3 months of adalimumab treatment, using a

drug-sensitive inhibition assay measuring clinically relevant neutralizing ADAbs [6]. Most of them had levels >50 µg/l, considered clinically relevant and rarely transient [9]. Previous studies have suggested an occurrence of ADAb formation of 10–60%, dependent of the study population and assay [6, 10, 24, 37, 38]. In line with previous studies, patients with ADAb formation were less likely to respond to treatment in addition to having a higher risk of drug discontinuation [24, 38]. We found a comparable occurrence of ADAbs in axSpA as in RA/PsA patients. This is in contrast to the Norwegian Drug Monitoring Study A (NOR-DRUM-A; NCT03074656), a randomised controlled trial that tested the effectiveness of TDM when initiating infliximab treatment, where the risk of ADAb formation was lower in axSpA than RA/PsA patients [39]. One reason for this difference could be that the infliximab dose is lower in RA patients than in axSpA, while for adalimumab the dosage is the same for all diagnoses. In the NOR-DRUM-A trial, underexposure to drug over time or drug holidays were found to be a risk factor for subsequent ADAb development [39].

Patients with RA and PsA on concomitant treatment with methotrexate had higher adalimumab levels and a lower occurrence of ADAb formation, in addition to a lower rate of drug discontinuation. Methotrexate has demonstrated a favourable effect on the pharmacokinetics of adalimumab in previous studies [6, 13, 25, 40, 41] and is recommended to optimize the treatment effect of adalimumab in RA. The number of axSpA patients on methotrexate co-medication was too low in our cohort to assess any impact on ADAb formation and drug survival in this disease group.

Patients treated with adalimumab originator had lower serum drug levels and more frequent ADAb formation compared with GP2017. These results could not be explained by differences in baseline characteristics. Importantly, when assessing treatment effect and drug survival, there were no differences between adalimumab originator and GP2017. On seeking approval, a biosimilar product must submit immunogenicity assessments equivalent to the originator, with some difference in immunogenicity deemed acceptable [22]. Our findings are in line with the P17-301 trial, preceding the European Medicines Agency (EMA) approval of biosimilar GP2017, reporting slightly higher serum drug levels in GP2017-treated psoriasis patients through the whole study period [42]. Further, a difference in non-neutralizing ADAbs (45.1% in adalimumab originator, 35.8% in GP2017) was found, but when assessing neutralizing ADAbs only, the differences vanished. Safety and efficacy were equivalent and the differences in drug levels and ADAb frequency were regarded as clinically irrelevant [22, 42, 43]. A wide range of factors may affect detection of ADAb (assay design, time of sampling, population tested). Hence, pre-approval testing may not detect clinically meaningful differences in immunogenicity. The EMA, World Health Organization and US Food and Drug Administration guidelines recommend consideration of immunogenicity in pharmacovigilance for biologic agents. Here we report ADAb rates in a clinically meaningful context and perform subgroup analyses in three different arthritis groups. Our independent analyses revealed that while there was a significant difference in ADAb formation between the two versions of adalimumab, we did not observe any significant differences in their impact on clinical outcomes. These findings could alleviate some of the apprehensions surrounding the distinct immunogenicity of originator and biosimilar adalimumab.

The strengths of this study are the inclusion of three diagnoses, with a large group of axSpA patients, and real-life data collected 3 months after initiating adalimumab treatment, making it relevant for regular clinical care.

This study has some limitations. First, to ensure adequate statistical power, patients with RA and PsA were combined in the analysis. While we acknowledge that DAS28-ESR might not the optimal outcome measure for PsA, the conducted sensitivity analyses using the modified DAPSA, DAPSA28, yielded comparable results. Second, information on body weight or BMI, as well as adverse events, was lacking in this cohort and this information is relevant to serum drug levels. Obesity, with a BMI >30 kg/m^2, has been shown to be associated with lower serum drug levels of adalimumab and etanercept and may therefore contribute to the observed variations in serum drug levels [40]. Third, the serum drug levels are non-trough, and the timing of the last injection was not registered. However, drug levels are reasonably stable through an injection cycle for adalimumab and other TNFis administered subcutaneously [44, 45]. This is reassuring as non-trough sampling is feasible in a clinical setting, while trough levels for subcutaneous drugs are difficult to obtain [40]. Finally, due to a lack of data, we were unable to explore the associations between smoking, drug holidays and adherence on serum drug levels and ADAb formation.

Conclusions

Higher adalimumab levels were associated with a better response and improved drug survival for all diagnoses, with 6.0 mg/l suggested as a lower therapeutic threshold in RA and PsA. As early as 3 months, 10% of patients had developed neutralizing ADAbs and these were associated with a lack of response to treatment and reduced drug survival. The considerable variability in drug levels in patients on the same standard dose, the correlation between these and treatment effectiveness and the high proportion of patients developing ADAbs all underscore the need for additional research to investigate the potential role of TDM in adalimumab treatment regimens. The present study will contribute to the development of TDM algorithms for adalimumab in patients with inflammatory joint diseases.

Supplementary material

Supplementary material is available at *Rheumatology* online.

Data availability

A de-identified patient data set can be made available to researchers upon reasonable request. The data will only be made available after submission of a project plan outlining the reason for the request and any proposed analyses and will have to be approved by the NOR-DMARD steering group. Project proposals can be submitted to the corresponding author. Data sharing will have to follow appropriate regulations.

Authors' contributions

All authors critically revised the report and approved the final submitted version and take responsibility for the completeness and accuracy of the data and analyses. All authors had full

access to all the data in the study and made the final decision to submit the manuscript for publication. I.J., G.L.G., S.W.S., N.B., J.E.G. and J.S. verified the underlying data, interpreted the data and drafted the report. I.J., G.L.G., S.W.S., N.B. and J.E.G. conceived and designed the study. D.J.W. developed all assay reagents and established the drug-level assay. I.J., N.B. and J.E.G. performed the drug-level and ADAb analyses. E.A.H., T.K.K. and E.K.K. contributed to the study conception and design. Y.H. contributed to data collection.

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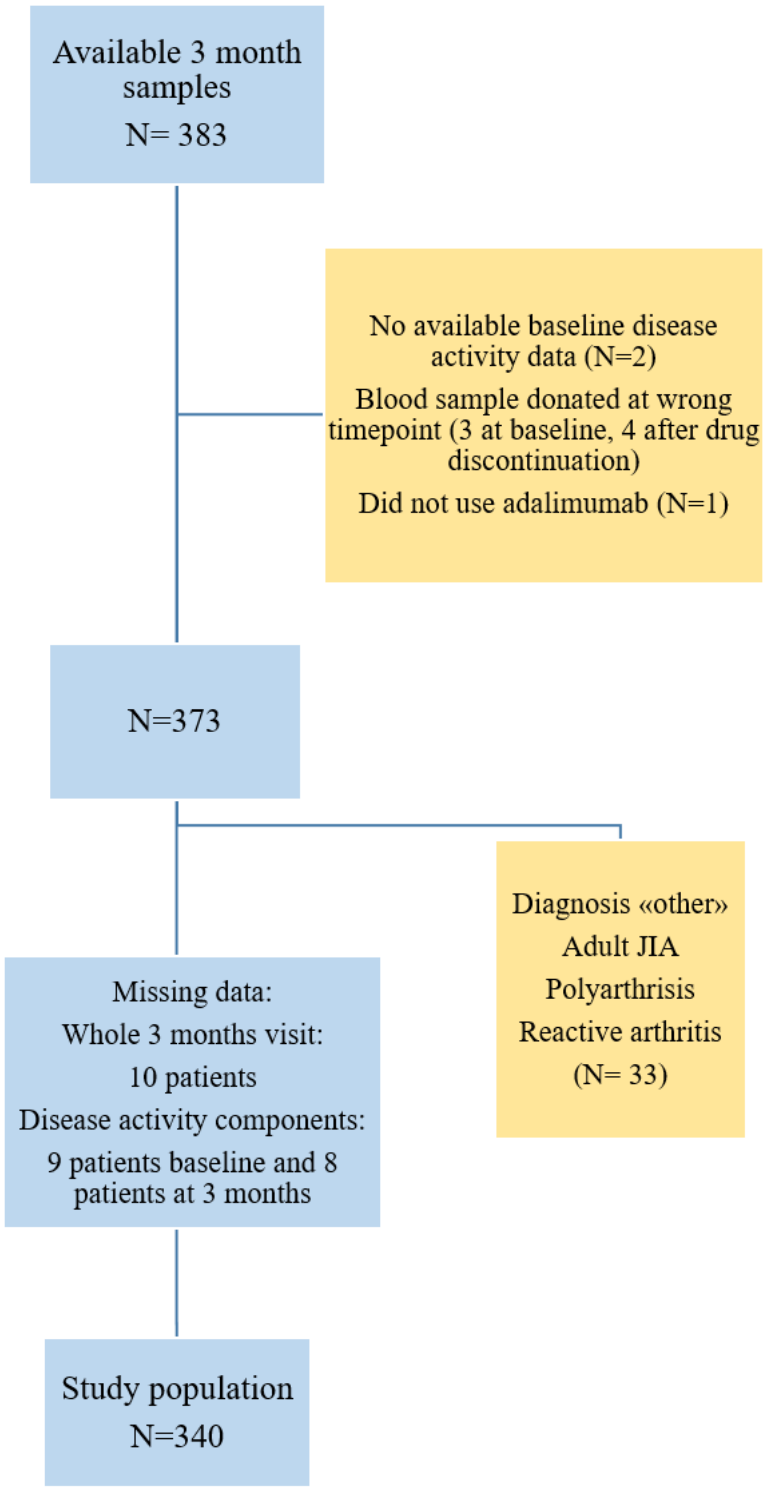
Supplementary file

Supplement to Jyssum I, et. al. **Adalimumab serum levels and anti-drug antibodies: associations to treatment response and drug survival in inflammatory joint diseases**

Table of Contents

Section 1. Supplementary Figures

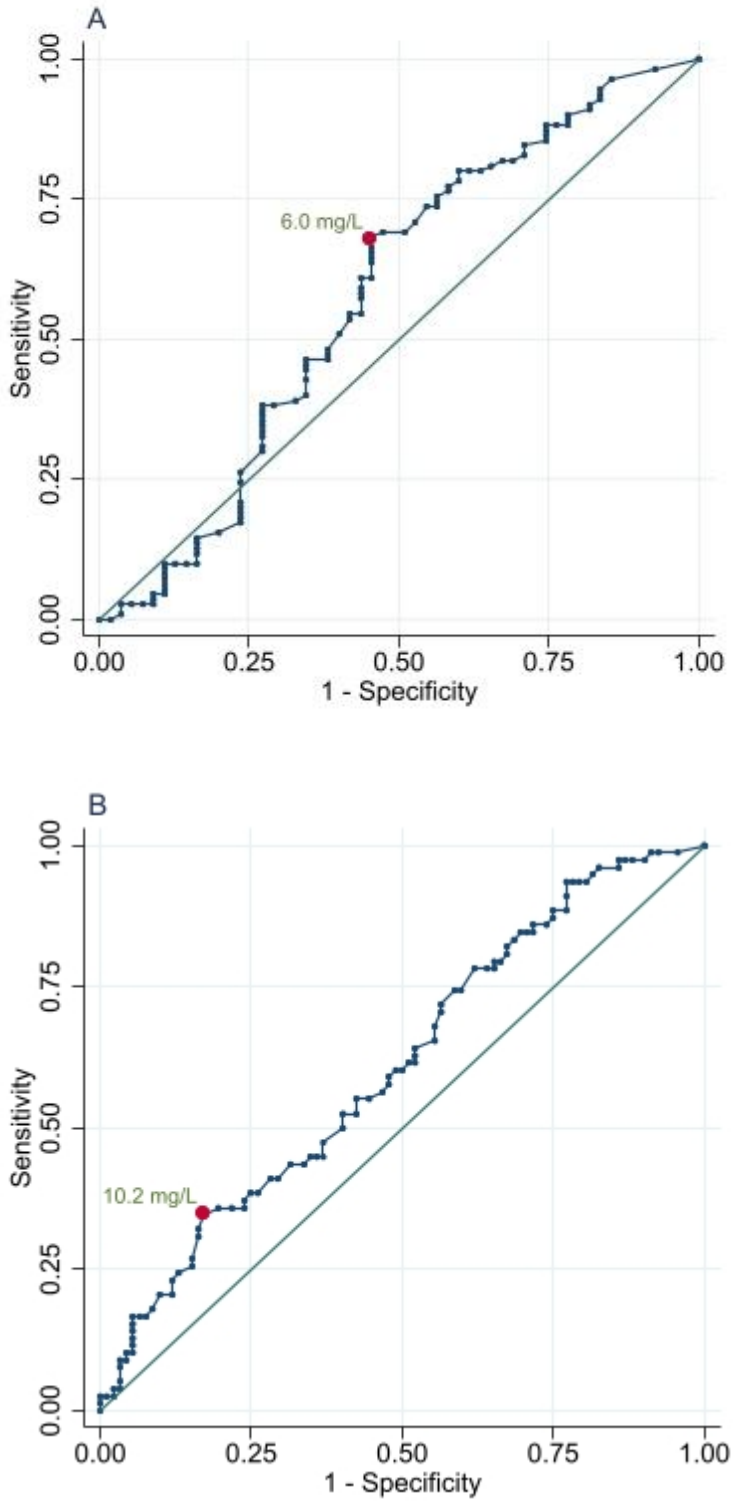
Supplementary Figure S1. Flow chart for included patients



[Legend Supplementary Figure S1]

Patients in yellow boxes are excluded.

Supplementary Figure S2. Cut-offs for adalimumab serum concentrations for best discrimination of patients with and without response to treatment.



[Legend Supplementary Figure S2]

Receiver operating characteristics curves with AUC to identify cut-offs for adalimumab serum concentrations for best discrimination of patients with and without response to treatment.

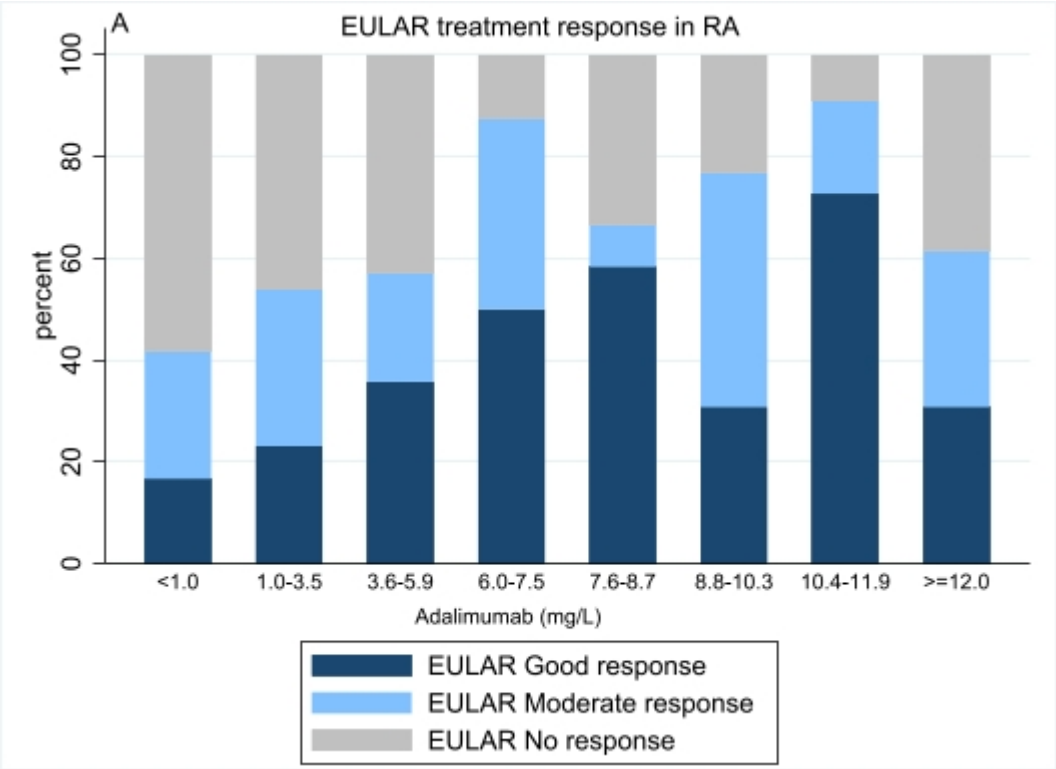
Predictive values were obtained from univariate regression analyses. The optimal cut-off point was defined by use of Youden-Index, maximizing the sum of sensitivity and specificity.

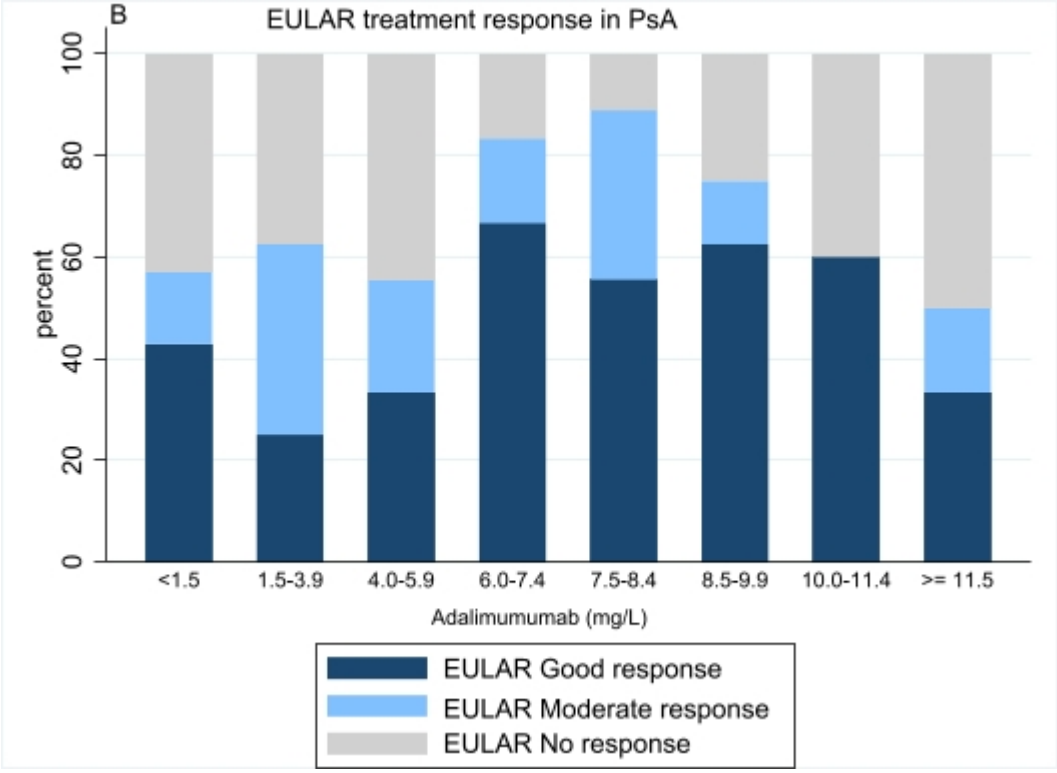
A)RA and PsA patients. Eular good and moderate response vs no response. AUC is 0.610 with the estimated optimal adalimumab cut-off at 6.0 mg/L (95% CI 1.3-10.7) with a sensitivity of 68 % and specificity of 55%.

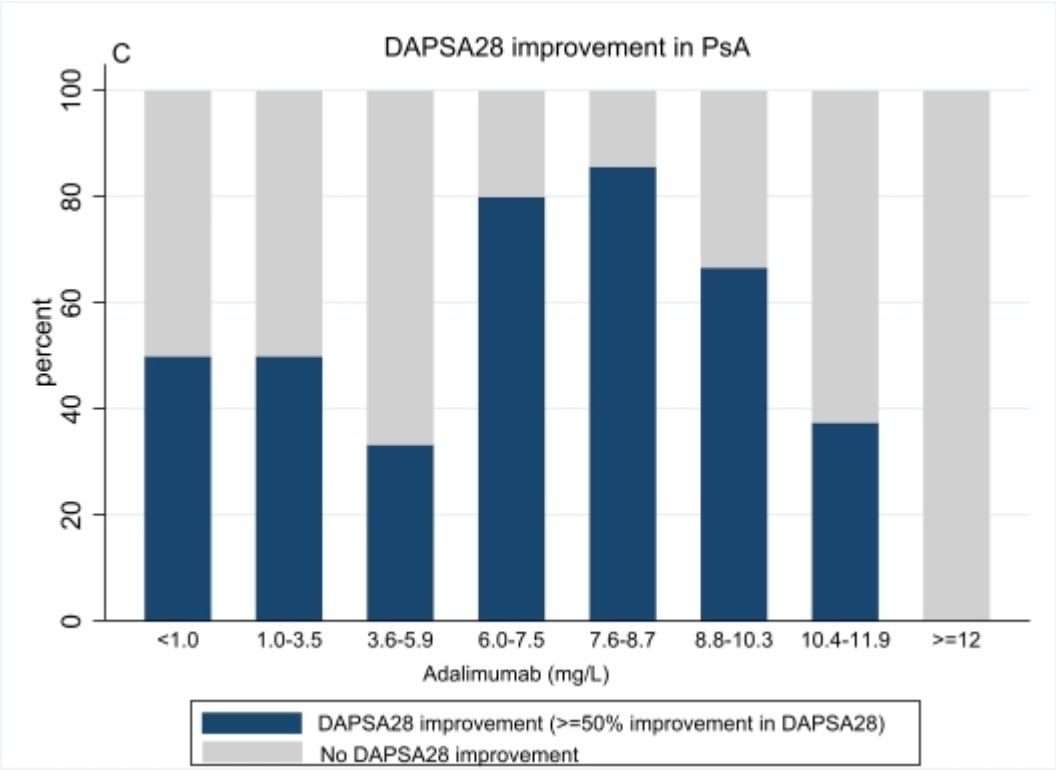
B) axSpA patients. ASDAS Major and Clinically Important Improvement vs no improvement. AUC is 0.609 with the estimated optimal cut-off at 10.2mg/L (95% CI 3.8-16.5) with a sensitivity of 35% and a specificity of 83%.

AUC, area under the curve; CI, 95% confidence interval.

Supplementary Figure S3. Treatment response in RA and PsA



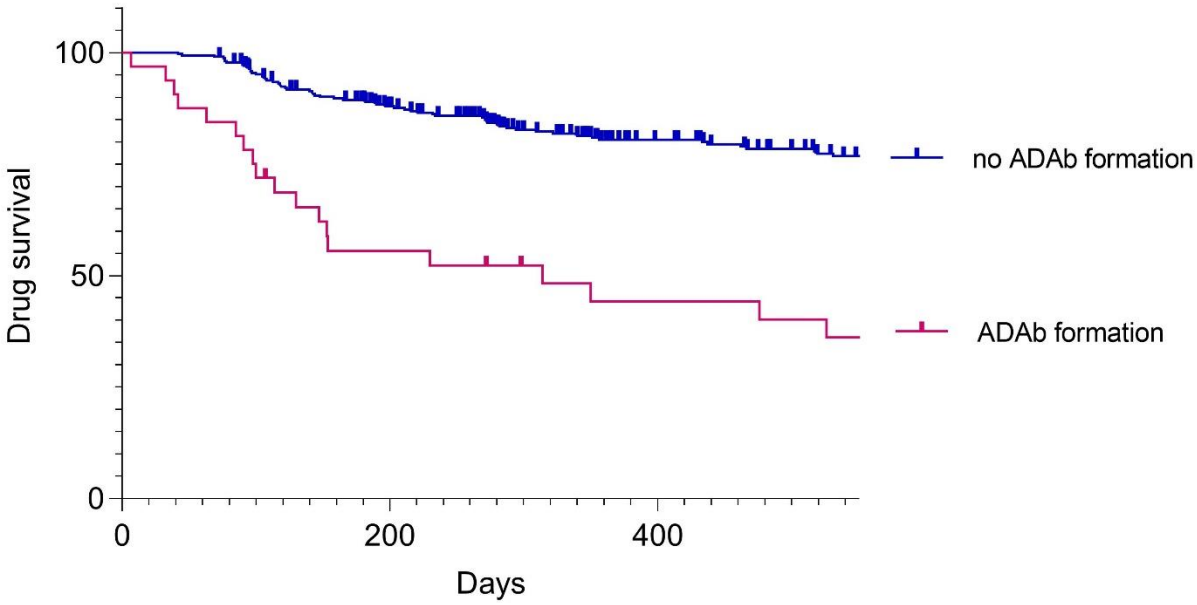




[Legend Supplementary Figure S3]

A) and B) Percent distribution of EULAR response in A) RA and B) PsA patients by adalimumab level. C) Percent distribution of DAPSA28 improvement in PsA patients by adalimumab level.

Supplementary Figure S4. Drug survival by anti-drug antibody (ADAb) formation



[Legend Supplementary Figure S4]

Kaplan Meier curve for 1.5 years drug survival stratified by ADAb formation at 3 months. There was a significant difference in the survival estimates, P<0.0001 (log-rank).

Section 2. Supplementary Tables

Supplementary Table S1. Uni- and multivariable logistic regression model of response to treatment in RA and PsA

	Univariable OR (95% CI)	p-value	Multivariable OR (95% CI)	p-value
Adalimumab ≥ 6mg/L	2.5 (1.3-4.9)	0.007	2.2 (1.0-4.4)	0.038
Age	0.9 (0.95-1.0)	0.178	0.9 (0.7-1.0)	0.262
Female sex	1.5 (0.8-2.9)	0.246	1.5 (0.7-3.0)	0.314
Previous use of one or more bDMARD	0.4 (0.2-0.7)	0.005	0.4 (0.2-0.9)	0.023
Methotrexate comedication	1.1 (0.6-2.1)	0.818	0.9 (0.4-1.8)	0.994
bDMARD= biologic disease-modifying anti-rheumatic drug, CI= confidence interval Response defined as EULAR good or moderate response				

Supplementary Table S2. Factors associated with higher adalimumab level

	Univariable β (95% CI)	P-value	Multivariable β (95% CI)	p-value
Age	-0.015 (-0.047, 0.017)	0.35	-0.020 (-0.52, -0.013)	0.24
Female sex	0.15 (-0.76, 1.05)	0.75	0.022 (-0.89, 0.93)	0.96
Previous use of one or more bDMARD	-1.46 (-2.4, -0.50)	**0.003**	-1.29 (-2.25, -0.34)	**0.008**
Methotrexate comedication	1.47 (0.54, 2.40)	**0.002**	1.56 (0.60, 2.51)	**0.001**
Adalimumab originator	-1.64 (-2.5, -0.73)	**0.000**	-1.45 (-2.37, -0.52)	**0.002**
ESR at baseline	0.0055 (-0.021, 0.032)	0.69	0.0045 (-0.21, 0.031)	0.74
bDMARDs= biologic disease-modifying anti-rheumatic drugs, β = beta (regression coefficient), ESR= Erytrocyte Sedimentation Rate, OR=Odds Ratio, CI= Confidence Interval				

Supplementary Table S3. Demographics adalimumab-originator/GP2017

	Originator (n=212)	GP2017 (n=128)	P-value
Diagnosis, no (%)			
RA	58 (27)	39 (30)	
PsA	41 (20)	28 (22)	
axSpA	113 (53)	61 (48)	
Serum drug level, median (IQR)	6.4 (IQR 3.1-9.9)	8.25 (IQR 5.6-10.95)	0.0004 [a]
By diagnosis			
RA	6.3 (3.0-10.0)	8.8 (4.2-11.4)	
PsA	7.1 (2.8-9.8)	7.8 (6.1-10.5)	
axSpA	6.2 (3.1-9.9)	8.1 (5.7-11.1)	
ADAb formation, no (%)	27 (13)	6 (5)	0.015 [b]
By diagnosis			
RA	8 (14)	2 (5)	
PsA	7 (17)	1 (4)	
axSpA	12 (11)	3 (5)	
Age, median years (IQR)	46 (35-57)	46 (32-58)	0.58 [a]
Smoker (current), no (%) [c]	16/140 (11)	12/92 (13)	0.71 [b]
Methotrexate use, no (%) [d]	71 (34)	50 (39)	0.30 [b]
Disease duration at treatment start, median years (IQR) [e]	4.5 (1.2-12.1)	4.9 (0.9-11.8)	0.78 [a]
Previous use of one or more bDMARD, no (%) [f]	75/206 (36)	37/127 (29)	0.17 [b]
Baseline disease activity, mean (SD)			
RA/PsA	3.5 (1.4)	3.5 (1.5)	0.53 [g]
axSpA	2.6 (1.0)	2.6 (1.1)	0.81 [g]
Change in disease activity from baseline to 3 months, mean (SD)			
RA/PsA	1.1 (1.3)	1.3 (1.2)	0.13 [g]
axSpA	1.0 (0.99)	1.2 (1.1)	0.41 [g]
Response to treatment [h] **at 3 months, no (%)**			
RA/PsA	60/98 (61)	50/67 (75)	0.073 [b]
axSpA	51/111 (46)	27/59 (46)	0.98 [b]

[a] Mann-Whitney U test
[b] $\chi 2$ test
[c] available data in 232 patients
[d] No difference in dosage of Methotrexate (median 20mg/week in both groups)
[e] available data in 180 patients
[f] available data in 333 patients
[g] t-test
[h] Response to treatment defined as EULAR good or moderate response in RA/PsA and Major Improvement or Clinically Important Improvement in axSpA
GP2017= Sandoz company code for its adalimumab biosimilar product. RA=rheumatoid arthritis, axSpA=spondyloarthritis, PsA=psoriatic arthritis