RESPOSTA IMMUNOLÒGICA A LA VACUNA SARSCOV-2 EN PACIENTS AMB MALALTIA REUMÀTICA

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LLISTAT ABREVIATURES

AMPc: Adenosina monofosfat cíclica

AR: Artritis Reumatoide

APSO: Artritis Psoriàsica

CQ: Cloroquina

DNA: Àcid desoxiribonucleic

EA B27: Espondilitis Anquilosant B27

FAME: Fàrmac modificador de la malaltia

GCS: glucocorticoides

GMPc: Guanosina monofosfat cíclica

HCQ: Hidroxicloroquina

HTA: Hipertensió Arterial

IL: Interleucina

Ig: Immunoglobulina

JAK: Janus Cinasa

LES: Lupus Eritematós Sistèmic

MMF: Micofenolat Mofetil

MPOC: Malaltia Pulmonar Obstructiva Crònica

NETs: Trampes extracel·lulars de neutròfils

PCV13: Vacuna conjugada 13-valent

PDE4: Fosfodiesterasa 4

PPSV23: vacuna polisacàrid 23 valent

TNF: Factor de necrosi tumoral

RNA: Àcid ribonucleic

RNAm: àcid ribonucleic missatger

RTX: Rituximab

S. aureus: *Streptococcus aureus*

SLEDAI: *Systemic Lupus Erythematosus Disease Activity Measure*

TCZ: Tocilizumab

VHA: Virus Hepatitis A

VHB: Virus Hepatitis B

VGV: Vasculitis de Gran Vas

VHP: Virus Papil·loma Humà

HVZ: Herpes Varicel·la Zoster

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RESUM

La vacunació en pacients amb una malaltia reumatològica és particular. L'evidència existent fins al moment actual suggereix que aquests individus tenen menys probabilitat de respondre a les vacunes que la població sana. Tant els fàrmacs immunosupressors com l'alteració de la immunoregulació tant de la resposta innata com adaptativa causada per la pròpia malaltia podrien justificar aquesta situació.

Per això, l'objectiu del nostre estudi es avaluar la resposta a les vacunes mRNA contra la SARS-COV-2 a mig termini en pacients amb malalties autoimmunes reumatològiques.

La resposta immune ha estat analitzada mitjançant la determinació dels índex de seroconversió i els anticossos neutralitzants. En ambdós treballs publicats, els resultats obtinguts suggereixen que, independentment de la malaltia, els pacients que estan tractats amb fàrmacs biològics presenten una resposta vacunal menor que la resta d'individus. I aquesta resposta encara és menor si associen tractament combinat amb corticoides. També es va analitzar la resposta vacunal davant de diferents variants de les quals la resposta més alta de totes les variants va ser la wild-tipe, i la que va presentar una resposta més baixa va ser l'omicron.

La identificació de factors de pobra resposta podria tenir una aplicabilitat en pràctica clínica per identificar aquells pacients que poden precisar una estratègica de prevenció específica, modificant tant les guies de vacunació com l'aplicació de mesures específiques.

ABSTRACT

Vaccination in patients with rheumatologic disease is particular. The existing evidence so far suggests that these individuals are less likely to respond to vaccines than the healthy population. Both immunosuppressive drugs and the alteration of immunoregulation of both the innate and adaptive response caused by the disease itself could justify this situation.

Therefore, the objective of our study is to evaluate the medium-term response to mRNA vaccines against SARS-COV-2 in patients with autoimmune rheumatologic diseases.

The seroconversion index and neutralizing antibodies have been analyzed. In both published works, the results obtained suggest that, regardless of the disease, patients treated with biological drugs have a lower vaccine response than other individuals. This response is even lower if they are also on corticosteroid treatment. We also analyzed the vaccine response to different variants, finding that the highest response among all the variants was to the wild-type, and the lowest response was to omicron.

Identifying factors of poor response could have applicability in clinical practice to identify those patients who may require a specific prevention strategy.

1. INTRODUCCIÓ

1.1 MALALTIES AUTOIMMUNES SISTÈMIQUES

1.1.1 ALTERACIÓ IMMUNE

Les malalties autoimmunes són un grup de condicions patològiques en les que el sistema immunitari desencadena respostes aberrants contra estructures sanes pròpies del cos (1). Malgrat que comparteixen mecanismes similars i s'engloben en una mateixa categoria, les manifestacions clíniques són molt diverses (des d'una simptomatologia aguda que amenaça la vida, fins a una alteració analítica lleu) (2). En condicions normals, l'organisme posseeix mecanismes per evitar l'autoagressió immunològica, el que es coneix com a tolerància i es defineix com l'estat de no reactivitat immunològica, resultant de mecanismes que tenen lloc tant a nivell central com perifèric durant el desenvolupament de limfòcits T i B (2,3). En un context patològic, existeix una ruptura d'aquesta tolerància i es desencadena una resposta contra teixits propis. A més, una vegada la resposta autoimmune s'ha iniciat, aquesta normalment s'autoperpetua, conduint a una lesió crònica o definitiva del teixit (3).

Per poder determinar que una malaltia inflamatòria és autoimmune es necessària la presència de limfòcits B o T autorreactius a la sang del pacient; la inducció de la malaltia quan es transfereixen aquestes cèl·lules o anticossos a animals d'experimentació; i/o la demostració de l'autorreactivitat en la localització de la lesió (per exemple, mitjançant la presència d' immunocomplexes, de citocines o d'infiltrats inflamatoris) (2,4,5).

Existeixen múltiples cèl·lules del sistema immunitari, tant innat com adaptatiu, que estan implicades en la patogènia d'aquestes malalties. Alguns dels mecanismes més destacats son els següents (2):

- Anticossos: en la malaltia poden alterar la funció cel·lular actuant tant d' agonistes com d'antagonistes); eliminen poblacions cel·lulars a través de la lisi mitjançada pel complement, de la citotoxicitat cel·lular anticòs dependent, de l'endocitosi o de la degradació per mitjà d'anticòs. També formen immunocomplexes que estimulen la cascada inflamatòria a través de l'activació del complement (6,7).

- Limfòcit T: els limfòcits T *helpers* (CD4+) estimulen els limfòcits B per la producció d'autoanticossos, mentre que els limfòcits T citotòxics (CD8+) lesionen i eliminen cèl·lules directament (8). Així doncs, l'etiopatogènia serà diferent en funció del tipus de limfòcit T que trobarem activat en cada malaltia, fet que pot comportar certes especificitats en cada una.

- Alteració de la tolerància: la generació de l'autorreactivitat representa la pèrdua de l'autotolerancia, un pas fonamental en la regulació de la immunitat, i que, com ja s'ha comentat anteriorment, en condicions d'absència de patologia evitaria la pròpia agressió del sistema immunitari (9,10).

- Genètica: la hipòtesi actual més acceptada és que la malaltia autoimmune es desenvolupa en pacients genèticament predisposats com a resposta a un desencadenant o noxa ambiental, tal i com succeeix en la majoria de patologies amb cert component de predisposició genètica (2). Específicament, les malalties sistèmiques autoimmunes es consideren dins les patologies multigèniques, és a dir, no dependents d'un sol gen, tot i que coneixem que l'alteració principal es troba en l'HLA i el complex major d'histocompatibilitat (CMH) que intervenen en la presentació d'autoantígens (11).

1.1.2 FÀRMACS IMMUNOMODULADORS

A Reumatologia s'utilitzen diversos fàrmacs pel control de les malalties sistèmiques que modulen el sistema immune o inhibeixen certes interleuquines o punts crítics de la cascada inflamatòria, esdevenint els pacients tractats, immunosuprimits, i per tant, amb un risc augmentat de patir certes infeccions. En els següents paràgrafs, comentarem les principals eines terapèutiques prescrites en el camp de les malalties autoimmunes del teixit connectiu.

Els glucocorticoides (GCS) representen la primera eina de tractament en aquestes malalties pel seu mecanisme d'acció ràpid i efectiu. El principal efecte antiinflamatori s'explica pel bloqueig dels canals de calci i sodi, que són els responsables de l'activació cel·lular i els receptors involucrats en les vies de senyalització intracel·lular (12). A més, també actuen sobre la funció dels neutròfils inhibint la migració, afectant l'adherència a l'endoteli vascular i disminuint l'activitat

bactericida; sobre el sistema monofagocític, provocant una monocitopènia perifèrica i alterant la seva funció; sobre els limfòcits produint una redistribució dels mateixos fora de la circulació (sobretot dels CD4+) i inhibint l'activació dels limfòcits T. Remarcar també que, a altes dosis, són capaços d'inhibir la producció d'immunoglobulines de les cèl·lules B (13).

Els antipalúdics (cloroquina i hidroxicloroquina) han demostrat àmpliament els seus beneficis com a tractament de manteniment en les malalties autoimmunes sistèmiques, sobretot en el lupus eritematós sistèmic o en les manifestacions articulars o cutànies associades a positivitat dels anticossos antinuclears (ANAs). Malgrat que el seu mecanisme immunomodulador no es coneix amb certesa, sembla que el seu principal efecte el presenta a través de la interrupció de l'activitat lisosomal, l'autofàgia, la presentació d'antígens i de les vies de senyalització (14). Aquests fàrmacs s'acumulen en els lisosomes, desestabilitzant-ne les membranes i alliberant-ne els enzims al citoplasma cel·lular, el que inhibeix l'activitat dels limfòcits (15).

L'azatioprina s'inclou en el grup dels immunosupressors sintètics. Per realitzar efecte, es converteix, a través de diversos processos enzimàtics, en diferents metabòlits des de la 6-mercaptopurina fins a la 6-tiofuanosina monofosfat, un anàleg citotòxic de les purines que s'incorpora a les molècules de DNA i RNA, inhibint el creixement i la divisió cel·lulars. D'aquesta manera, aquest fàrmac produeix el seu efecte antiinflamatori i immunosupressor bloquejant la proliferació dels limfòcits (16).

El micofenolat mofetil (MMF), també es un immunosupressor sintètic i inhibeix la formació del nucleòtid guanina. Aquest nucleòtid és especialment necessari per la formació de DNA i, per tant, per la replicació. Concretament, aquest fàrmac inhibeix l'enzim inosina monofosfat deshidrogenasa, responsable de sintetitzar la guanina. A diferència d'altres cèl·lules, els limfòcits tant T com B són altament sensibles a la interrupció d'aquest procés i, per tant, es consideren una diana relativament selectiva per aquest fàrmac sense provocar mielotoxicitat (16).

Del metotrexat, fins al moment actual, se'n desconeix la seva funció exacta. Inicialment, es va formular per inhibir la síntesi de purines i pirimidines necessàries per la formació del DNA i l'RNA i, per tant, la proliferació de cèl·lules. Malgrat que aquesta és la principal hipòtesi sobre l'actuació del metotrexat en la supressió de la resposta inflamatòria, també es plantegen altres possibilitats, com la inhibició de reaccions de transmetilació (requerides per algunes funcions cel·lulars), la disminució de poliamines o el desacoblament de l'òxid nítric sintasa. Així, aquest fàrmac regula la funció de gairebé tots els tipus de cèl·lules implicades en la inflamació (neutròfils, monòcits, limfòcits T i B, cèl·lules endotelials) (13,17).

La leflunomida es considera un inmunomodulador que regula el cicle cel·lular inhibint la biosíntesi del ribonucleòtid pirimidina de les cèl·lules limfocitàries en fase creixement durant la mitosi, pel que bloqueja la proliferació tant de limfòcits T com B (18).

L'apremilast és una petita molècula, orientada específicament a inhibir l'enzim fosfodiesterasa 4 (PDE4), que modula l'expressió de múltiples mediadors

proinflamtoris i antiinflamatoris. La PDE4 pertany a una família d'enzims que catalitzen la inactivació dels nucleòtids cíclics de l'adenosina monofosfat (AMPc) i guanosina monofosfat (GMPc) a 5'-AMP i 5'GMP respectivament, controlant d'aquest manera la concentració d'aquests nucleòtids i l'activitat que exerceixen sobre les cascades de senyalització intracel·lular (19, 20).

Un grup de fàrmacs utilitzats en reumatologia, són els inhibidors de la calcineurina, entre els que trobem la ciclosporina, que té com a principal diana l'RNA missatger (RNAm) responsable de l'activació d'alguns gens proinflamatoris, com el C-myc). També inhibeix la síntesi d'IL-2, IL-3, IL-4 i INFα (21). L'efecte combinat d'aquests canvis retarda la proliferació de limfòcits T. A més, també s'ha vist que la ciclosporina inhibeix l'acció de presentació d'antígens del complex major d'histocompatibilitat (MHC) de classe II dels macròfags (22).

Entre la teràpia biològica trobem els inhibidors de factor de creixement tumoral alfa (anti-TNFα), àmpliament utilitzats en les malalties autoimmunes de diferents especialitats. El TNFα és una citocina inflamatòria del sistema immune innat produïda per macròfags i monòcits durant la resposta inflamatòria aguda, i és responsable de múltiples vies de senyalització que condueixen a la necrosi o apoptosi cel·lular (23). Els fàrmacs bloquegen el receptor del TNF tipus I i tipus II, presents en totes les cèl·lules excepte els eritròcits, inhibint així el cicle inflamatori que en depèn (24). Entre aquests fàrmacs hi trobem l'adalimumab, l'etanercept, el certolizumab, el golimumab i l'infliximab (25).

La interleucina 6 (IL6) és una de les principals citocines proinflamatòries. En les

malalties autoimmunes hi participen els inflamosomes, que provoquen la sobreproducció d'IL-1b i IL-18, les quals al seu torn estimulen la producció de citocines pro-inflamatòries com l'IL-6 i el TNFα per part de diverses cèl·lules incloses els monòcits, els macròfags, els limfòcits T i B i els fibroblastes (26). Els fàrmacs anti-IL6 com el tocilizumab (TCZ) o el sarilumab produeixen un efecte antinflamatori inhibint aquesta molècula.

Una altra de les citocines diana dels tractaments biològics és la IL-17, coneguda principalment per la seva capacitat d'iniciar una resposta inflamatòria potent que inclou la inducció de factors de granulopoiesis i quimiocines específiques de neutròfils (CXCL1, CXCL2, CXCL5, CXCL8), mediadors de la resposta de fase aguda (IL-6), citocines proinflamatòries/ressortives òssies (TNF, IL-1β i RANKL), i metal·loproteinases de la matriu (27). Els objectius de la IL-17 inclouen principalment cèl·lules epitelials, endotelials i altres cèl·lules estromals com fibroblasts, osteoblasts, condròcits i cèl·lules sinovials (27). Són inhibidors d'aquesta citocina el secukinumab, ixekinumab i el bimekizuamb (28).

Finalment, la IL-23 presenta una estreta relació amb la IL-17. La IL-23 és una citocina pro inflamatòria secretada per cèl·lules dendrítiques i macròfags que pertany a la família de la IL-12 (29). Té efectes proinflamatoris i és una citocina clau i reguladora implicada en les respostes immunitàries protectores contra patògens, estimulant la diferenciació i proliferació dels efectors, com les cèl·lules Th17 (30). Un nombre creixent d'estudis han evidenciat que la IL-23 està implicada en la patogènesi de les malalties autoimmunes. S'ha demostrat que està present de manera incrementada en pacients amb LES, AR i l'artritis psoriàsica així com en els

teixit cutani de la psoriasi com en el sinovial de l'AR (31). El Guselkumab, Ustekinumab i el Risankizumab són anticossos diana contra aquesta citocina (28).

El Belimumab és un anticòs humanitzat aprovat pel LES. Aquest s'uneix específicament a BLyS un factor de supervivència dels limfòcits que ha demostrat estar implicat en la fisiopatogènia d'aquesta malaltia (32). Per tant es tracta d'un inhibidor molt més selectiu que els immunosupressors sintètics tradicionals, tot i que acaba afectant les funcions dels limfòcits (32,33).

Posteriorment a la pandèmia de COVID, s'ha aprovat un segon biològic pel LES, l'Anifrolumab, un inhibidor d'INF tipus I. Aquest fàrmac internalitza el receptor d'interferó alfa 1, induint així el bloqueig de la senyalització de la via STAT 1/2 activada per l'interferó de tipus I. A més de bloquejar aquesta citocina, l'anifrolumab també inhibeix parcialment les molècules coestimuladores i la producció d'altres citocines per part de les cèl·lules plasmocitoides dendrítiques (34).

El rituximab (RTX), malgrat la manca d'indicació, és habitualment prescrit en les connectivopaties fora de fitxa tècnica, degut als múltiples estudis observacionals i casos clínics que recolzen la seva utilització. Es tracta d'un anticòs monoclonal quimèric/humanitzat dirigit contra els limfòcits B que presenten la proteïna transmembrana CD20+ (35). Els mecanismes d'acció condueixen a la mort cel·lular a través de quatre mecanismes principals (36):

- La unió a CD20 desencadena la cascada del complement, el que genera el complex d'atac a membrana i la posterior lisi cel·lular (36).

- Aquesta mateixa unió permet la interacció amb les cèl·lules *natural killer* a través dels receptors FC III, el que provoca una citotoxicitat cel·lular dependent d'anticossos (36).
- La porció FC del fàrmac i la presència de complement interaccionen amb els receptors corresponents dels macròfags, que s'activa i fagocita el limfòcit en qüestió (36).
- Finalment, el creuament de diverses molècules de RTX i CD20 a la capa lipídica provoca la interacció d'aquests complexes amb elements de la via de senyalització on hi participen citocines que provoquen l'apoptosi directa de la cèl·lula (36).

Els principals inhibidors de la Janus Cinasa (JAK) són el tofacitinib, el bariticinib, l'upadacitinib i el filgotinib. Duen a terme la seva acció inhibint la via JAK-STAT. Els receptors de citocines, manquen d'activitat catalítica intrínseca i depenen dels JAKs per desencadenar una resposta i una posterior modulació de l'expressió gènica. Cada receptor de citocina està aparellat amb un parell de JAK diferent (JAK1, JAK2, JAK3, TYK2), generalment com heterodímers (37). Els JAKs activats, a la seva vegada, fosforil·len la cua del receptor de citocina. El receptor fosforilat forma un lloc d'acoblament per a les STATs, que d'altra manera resideixen en el citosol. Aquestes STATs són llavors fosforilades pels JAKs abans de dissociar-se del receptor i formar heterodímers o homodímers ells mateixos. A continuació, es transloquen al nucli on actuen com factors de transcripció, regulant l'expressió gènica (38).

Tots aquests fàrmacs es consideren inmunomoduladors o immunosupressors. En l'àmbit de la Reumatologia, es consideren pacients immunossuprimits, sempre i quan estiguin rebent tractament amb:

- Prednisona o equivalent >=20mg/d >= 2 setmanes
- Metotrexate >= 0,4mg/kg/setm
- Azatioprina >= 3,0mg/kg/dia
- 6-mercaptopurina >= 1,5mg/kg/dia
- Teràpia biològica i inhibidors de Jak

Dosis menors a aquestes, es consideren immunossupressió lleu (39). Per tant, un elevat nombre (es calcula que, d'un total de 6.6% de pacients immunossuprimits a Estats Units, el 3.9% és farmacològica (40) de pacients amb malaltia sistèmica autoimmune estan en aquesta situació, pel que es consideren pacients en risc.

1.2 VACUNACIÓ

Les vacunes es defineixen com a productes biològics que realitzen la seva acció indirectament mitjançant l'estimulació del sistema immunitari, i produeixen una resposta immunitària específica i generalment amb memòria a llarg termini (41). Segons dades de l'OMS, la immunització evita, cada any, entre 3,5 i 5 milions de defuncions per malalties infeccioses com la diftèria, el tètanus, las tos ferina, la grip o el xarampió (42).

El benefici de les vacunes pot venir donat per una protecció directa, és a dir, poden evitar les malalties o disminuir-ne la gravetat en cas d'infecció en un mateix individu; o bé per una protecció indirecta en persones no vacunades, ja que, a mesura que augmenta la proporció de persones immunitzades, disminueix la probabilitat de contagi de persones en risc, el que es coneix com a immunitat col·lectiva o de grup (41).

El Sistema Immunitari desenvolupa un mecanisme de defensa contra antígens, és a dir, substàncies que poden estimular la immunitat (43). Això es coneix com a resposta immune, en la que està involucrada la producció de molècules proteiques (immunoglobulines o anticossos) per part dels limfòcits B i la de cèl·lules específiques com els limfòcits T (44). La resposta més efectiva és la desencadenada amb l'exposició a antígens en un organisme viu, tot i que no es una condició necessària (45).

Si s'analitza el mecanisme immunològic que explica la resposta vacunal amb més detall, el primer que s'ha de tenir en compte és que el sistema immune es divideix en el sistema innat i adaptatiu (46). A més, es compon de barreres anatòmiques com la pell i les membranes mucoses i barreres fisiològiques com l'augment de temperatura o l'acidesa gàstrica. Una vegada administrada la vacuna, les seves partícules són fagocitades per cèl·lules presentadores d'antígens del sistema immune innat (46). Aquestes processen les partícules i en presenten els components (subunitats proteiques, DNA viral, mRNA, partícules virals, bacteris o virus atenuats...) al sistema immune adaptatiu (47). En general, els antígens virals es presenten a través del complex major d'histocompatibilitat tipus I a cèl·lules T

CD8+, les quals activen la immunitat mediada per cèl·lules mitjançant l'alliberament de mediadors citotòxics (immunitat cel·lular) (47). Els antígens bacterians i fúngics en canvi, es presenten amb el complex major d'histocompatibilitat tipus II a les cèl·lules T CD4+ que estimulen les cèl·lules T efectores i de memòria (48). Els limfòcits T reguladors, ajuden a activar i convertir els limfòcits B a cèl·lules plasmàtiques productores d'anticossos específics d'alta afinitat (immunitat humoral) (49).

Segons la composició, les vacunes es poden classificar en vives (atenuades) i inactivades (49). Les primeres, són derivades de virus o bacteris "salvatges", els quals s'atenuen (debiliten) en un laboratori (46). Per produir una resposta, aquestes s'han de replicar en l'individu vacunat (50). Per tant, la resposta a una vacuna viva pot ser similar a la desencadenada per la infecció natural, és per això, que en el cas de les persones immunocompromeses, està contraindicada (49,50). Les vacunes inactivades, en canvi, es componen únicament de porcions de microorganismes (proteïnes o polisacàrids fonamentalment) i no poden provocar malaltia (39). La resposta fonamental a aquestes vacunes es únicament a través de la formació d'anticossos i no s'implica la resposta cel·lular, pel que la reacció immunitària es menor i, per tant, menys efectiva i duradora (51).

1.3 VACUNACIÓ I MAI

Malgrat els beneficis evidents descrits anteriorment de les vacunes, existeix evidència de Real-World data, mostrant un percentatge subòptim de pacients amb

una malaltia reumatològica vacunats. Per exemple, en un estudi realitzat amb pacients amb LES, únicament entre el 42 i el 64% havien rebut la vacuna de la grip i entre un 60 i un 67% la del pneumococ (52). Un altre estudi on s'inclouen 3920 pacients amb artritis reumatoide de la cohort multinacional COMORA, demostra que només el 17.2% dels pacients estan vacunats enfront al pneumococ i el 25.3% a la influenza (53). Altres autors han observat el mateix baix percentatge de vacunació, similar a les dades prèvies on només l'11% de 110 pacients en seguiment a Reumatologia havien rebut la vacuna contra els mateixos gèrmens (54). Aquestes dades evidencien un percentatge subòptim de persones amb malaltia autoimmune que han estat correctament vacunats seguint les guies i recomanacions de les diferents societats científiques. Els principals motius que es postulen per justificar aquesta situació interpel·len tant al col·lectiu sanitari com als pacient. Concretament serien: la manca de priorització per part del personal sanitari, la falta de temps i experiència amb la vacunació i el desconeixement per part dels pacients dels potencials riscos i beneficis tant de vacunar-se com de no fer-ho (55).

La vacunació en pacients amb una malaltia reumatològica és particular. Tot i que manca evidència en aquest camp, semblaria que aquests individus tenen menys probabilitat de respondre a les vacunes que la població sana. Tant els fàrmacs immunosupressors com l'alteració de la immunoregulació tant de la resposta innata com adaptativa causada per la pròpia malaltia podrien justificar aquesta situació, encara que no se'n coneixen exactament els mecanismes (49, 55) .

1.3.1 Resposta i seguretat a les principals vacunes

Les principals vacunes recomanades en aquests pacients perquè es considera que hi ha un augment de risc d'aquestes infeccions, per les guies oficials tant Europees com Americanes són les següents

Virus hepatitis A i B: No existeix evidència sobre l'eficàcia de la vacuna VHA ni de la del VHB en pacients amb malalties autoimmunes, però si que s'ha objectivat una forta correlació entre les concentracions d'anticossos i la seroprotecció contra la infecció (39, 56). No s'ha identificat relació entre la vacuna i l'aparició de brots (56).

Haemofilus Influenzae: S'ha demostrat que la vacuna anual contra la influenza redueix la incidència d'infecció i de les complicacions bacterianes en pacients amb malaltia autoimmune bàsicament en AR, LES i vasculitis ANCA. En LES concretament, s'ha demostrat que presenten percentatges d'immunogenicitat similars respecte els grups controls independentment del tractament, a excepció del individus que reben teràpia amb rituximab (39). No s'ha evidenciat que les vacunes interfereixin en el control de la malaltia de base ni s'han registrat efectes adversos greus (39).

Herpes varicel·la zòster: Al 2021 va aparèixer una nova vacuna recombinada inactivada contra l'HVZ, que ha permès estendre el número de pacients vacunats i està substituint l'ús de la vacuna atenuada. Ha demostrat ser més efectiva que l'anterior (entre el 91 i el 98% en persones entre 50 i 70 anys) (57,58). En pacients amb malaltia reumatològica, existeix un estudi prospectiu en pacients amb AR en

tractament amb JAKi o FAMEb. Un mes després de rebre 1 dosi, el títol d'IgG anti-VVZ va augmentar significativament independentment del tractament rebut. Un mes després de la 2 dosi, es van mantenir els nivells d'anticossos en ambdós grups i de forma comparable amb adults sans (59). En quant a la seguretat, d'entrada no s'han comunicat efectes que facin dubtar respecte el seu perfil de benefici/risc.

Streptococcus pneumòniae: No existeixen estudis recents que tinguin en compte les noves pautes terapèutiques en Reumatologia ni tampoc les noves vacunes 15 i 20 - valents. Un dels estudis més grans i més recents s'ha publicat al 2018. Es tracta d'una revisió sistemàtica i meta-anàlisi on s'avalua l'efecte de la teràpia immunosupressora amb metotrexat, anti-TNF, combinació d'ambdós i rituximab, majoritàriament en pacients amb artritis reumatoide i espondiloartritis, en la resposta a la vacuna al pneumococ pels serotips 6B i 23F. Primerament, comparen la vacuna conjugada 13-valent (PCV13) versus la de polisacàrids que inclou 23 serotips (PPSV23) (60). El que s'obté és que el percentatge de seroconversió en els controls és similar però difereix en el grup que reben tractament immunosupressor: la PPSV23 (47% vs 26%) és superior independentment del fàrmac específic que rebin (60). En el meta-anàlisi per la PCV els pacients en tractament amb metotrexat, en combinació amb metotrexat i anti-TNF i en rituximab, presenten una resposta inferior a la dels controls (60). En canvi, coherentment amb l'exposat, la vacuna PPSV23 mostra resposta disminuïda per metotrexate i també per la teràpia combinada, encara que aquesta no és significativa (60).

Virus del papil·loma humà: Aquesta vacuna està especialment recomanada en dones joves amb LES, ja que la majoria de l'evidència s'ha obtingut a partir d'aquestes pacients (61 - 63). Recentment s'ha publicat un estudi cas-control on en els resultats obtinguts s'aprecia que al setè mes, és on les pacients amb malaltia van presentar un percentatge menor de resposta de manera estadísticament significativa pels serotips 6 i 11 i als 12m pel 6 (64). També s'aprecien diferències en el mes 12 entre les pacients que prenien prednisona enfront la resposta per la variant 6 i 18 (64). En quant a seguretat, estudis poblacionals prospectius han demostrat que la vacuna del VPH no augmenta brots en persones amb malaltia pre-existent (65, 66) .

1.4 Especial menció al SARS-COV2

1.4.1 Què ha passat amb els nostres pacients durant la pandèmia?

Durant el primer any de pandèmia, es va crear un registre espanyol impulsat per la Societat Espanyola de Reumatologia anomenat COVIDSER en pacients amb patologia reumatològica concebut específicament per estudiar l'expressivitat clínica de la SARS-COV2 en aquests individus (67). Es van analitzar 7782 pacients afectats principalment d'espondiloartritis, artritis reumatoide i LES. Un 5.5% (426) es van infectar per COVID i d'aquests, el 25.2% (106) van requerir hospitalització. En l'anàlisi bivariant, dins del grup de pacients que va ingressar la majoria tenien una mitja d'edat superior, de 61.8 (54.5-73.7) anys, patien AR i duien més temps

en tractament biològic. També era més freqüent que prenguessin corticoides, i que patissin algun tipus de comorbiditat en forma de malaltia pulmonar obstructiva crònica (MPOC), diabetis, hipertensió arterial, neoplàsia, insuficiència renal i malaltia hepàtica crònica. En l'anàlisi multivariant, els factors associats amb un risc significativament augmentat d'ingrés hospitalari van ser l'edat, el sobrepès/obesitat, la malaltia hepàtica crònica, la febre i la dispnea. Ni el tipus de malaltia reumàtica ni el tractament es van associar significativament a l'hospitalització, excepte pels pacients amb anti-TNF, que van mostrar un risc menor. Contràriament, els pacients tractats amb anti-CD20 van presentar una tendència superior a ingrés, però sense arribar a establir-se una diferència estadísticament significativa.

En un altre registre multinacional amb 40 països, els pacients homes, amb comorbiditats (HTA, malaltia pulmonar, diabetis i insuficiència renal) i pacients en tractament amb prednisona a dosis iguals o superiors a 10mg/dia es van presentar un risc augmentat per ingrés hospitalari (68). Finalment, un estudi publicat al 2022, on es van incloure 200813 pacients amb diferents malalties autoimmunes i teràpia immunosupressora, els pacients en tractament amb inhibidors de JAK i RTX van presentar més hospitalització i els pacients amb RTX més mortalitat (69).

1.4.2 Resposta de les vacunes mRNA al SARS-COV2

Des de l'aparició de la pandèmia, tant la discussió sobre les recomanacions de vacunació com l'anàlisi de la possible resposta immune alterada en els nostres pacients amb malaltia sistèmica, ha agafat molta més importància, retornant aquests temes a la primera línia de debat. Per això s'han desenvolupat diversos

estudis on s'analitza la resposta immune a la vacunació. Discutirem més endavant, diferents aspectes d'aquestes vacunes ja que aquesta ha estat una de les raons que han motivat la realització del treball que es presenta. Només incidirem en que, les vacunes inicialment recomanades per les persones afectes de malalties autoimmunes eren les basades en tecnologia RNA; perquè conferien un major percentatge de seroconversió i anticossos neutralitzants en els estudis que van propiciar la seva autorització. De totes maneres, volem incidir en que aquests estudis no incloïen pacients amb malaltia autoimmunitària ni condicions d'immunosupressió ja que eren criteris d'exclusió pels assajos clínics de registre; pel que aquestes recomanacions estaven basades en aproximacions teòriques.

Poc després d'aparèixer les primeres vacunes, van generar-se estudis dirigits a valorar la resposta immunològica en els nostres pacients amb una primera dosi de vacuna mRNA. Es van obtenir resultats que, si bé potser no eren tots significatius d'entrada, s'han anat confirmant i repetint al llarg d'aquests anys. Un d'aquests primers estudis publicats va comunicar una tendència numèrica a que els pacients de més edat responien menys. No van evidenciar, però, diferències segons la malaltia, però si que van observar que els pacients en tractament amb MMF i RTX van presentar una resposta inferior, sobretot aquest últim (70).

Posteriorment, es van publicar diversos treballs avaluant la segona dosi en pacients amb malalties reumàtiques i/o amb tractaments immunosupressors, tot i que amb un temps de seguiment limitat. En un dels primers estudis avaluant l'efectivitat de la vacuna amb una o dues dosis, van objectivar que, en general, els pacients sense la segona dosi presentaven una incidència acumulada d'infecció per SARS-COV2

superior (108). A més, segons la presa de tractament immunosupressor, es va observar que entre els pacients no vacunats, van patir més infeccions els immunosuprimits (HR 1.398 (1.068 – 1.829), però sense apreciar-se diferències en quant a hospitalització; mentre que entre els pacients amb la pauta completa de la vacuna, aquells que presentaven cert grau d'immunosupressió es van infectar i van ingressar més (HR 2.173 (1.690 – 2.794). Quan s'analitzaven només els immunocompetents, aquells que van rebre la pauta completa es van infectar i es van hospitalitzar menys (HR 0.354 (0.319 – 0.392) mentre que quan es centrava l'estudi en els immunossuprimits, aquells que es van vacunar amb les dues dosis també es van infectar menys (HR 0.550 (0.387 – 0.781), però sense diferències en quant a hospitalització. Per tant, afirmar aquests resultats ens permeten constatar que la vacuna és efectiva, almenys evitant infeccions (71).

En uns altres estudis, on s'analitza la resposta en funció dels tractaments, van objectivar una seroconversió menor en aquells pacients tractats amb micofenolat, Rituximab, abatacept i prednisona (72, 73). A l'analitzar-ho més concretament, es va objectivar una resposta del 100% en controls sans tant per resposta humoral com cel·lular. En canvi, en pacients amb malaltia reumàtica sense tractament immunosupressor, aquest percentatge disminuïa fins al 80% i fins al 55% en els casos que rebien teràpia immunomoduladora. Per la resposta humoral, va objectivar-se els mateixos resultats amb el títol d'anticossos en controls sans superior als pacients amb malaltia sense tractament i la resposta d'aquests, superior als malalts amb teràpia immunosupressora. El mateixos resultats es van obtenir per la resposta cel·lular (mesurant els nivells d'interferó i IL2 in vitro) (74). S'han observat altres resultats similars, on la resposta a la segona dosi (en pacients

sense infecció prèvia) en el grup control resulta del 100% enfront a una disminució en la resposta cel·lular (segons nivells d'interferó gamma) en el grup de tractament, particularment en aquells pacients que reben teràpia amb abatacept i Rituximab, així com també hi ha una disminució de l'alliberació de diferents citocines (74).

En un altre estudi important amb una sèrie de gairebé 700 pacients comparats amb controls, es va mesurar la resposta immunològica entre dues i sis setmanes després de l'administració de la segona dosi de la vacuna, van veure que els pacients majors de 65 anys, en tractament amb anti-CD20 i en tractament amb corticoides van presentar una índex de seropositivitat menor que els controls i de manera estadísticament significativa, pel que es va concloure que l'impacte d'aquests medicaments es independent d'altres fàrmacs immunosupressors concomitants (75). Continuant amb aquesta mateixa cohort, posteriorment es va avaluar la resposta a la tercera dosi. El que s'observa es que, els que presenten pitjor resposta són el pacients en tractament amb RTX i corticoides. També es remarca que els pacients més joves que havien presenta bona resposta a la segona dosi són els que responen millor (76, 77).

Amb la tercera dosi, s'ha vist una clara manca de resposta entre 2 i 6 setmanes després de l'administració de la vacuna en pacients tractats amb Rituximab (resposta 16 cops menor) i glucocorticoides (resposta 6 cops menor) (77). Aikawa et al. han dut a terme un estudi amb grup control en que les taxes de seropositivitat d'IgG Anti-S1/S2 van augmentar significativament des del 60% fins a 93% en pacients amb malaltia reumàtica autoimmune després de 30 dies de la tercera dosi. La positivitat d'anticossos neutralitzants també va augmentar: 38% vs 81,4%

respectivament. Els anàlisis de regressió logística multivariable en el grup malaltia van revelar que l'edat avançada, el diagnòstic de vasculitis, el tractament amb prednisona ≥5 mg/dia, amb micofenolat i amb biològics estaven associats amb una menor positivitat d'IgG Anti-S1/S2 (78).

Com es podia esperar, s'observa que amb cada dosi de vacuna, la resposta immunològica contra el virus es més alta i augmenta el número de responedors. Així es demostra en un estudi publicat recentment al 2024, excepte pel grup tractat amb rituximab on el percentatge de seropositivitat es del 62%, 59% i 57% per la segona, tercera i quarta dosi respectivament, comparat amb el grup control (resposta del 100% en cadascun dels punts) (79). També es va analitzar la resposta a òmicron amb la tercera dosi per les variants sBA1 i sBA2, on no es van identificar diferències estadísticament significatives per cap tractament.

Finalment, els protocols per lluitar contra la pandèmia de la majoria de països, van incloure la quarta dosi, de la que també s'ha publicat alguna evidencia en l'àmbit de la Reumatologia. En aquesta publicació, es va seguir el protocol de pràctica clínica habitual de vacunació, i es van analitzar el resultats a 30 dies de l'administració d'aquesta. Es van incloure pacients amb pobra o nul·la resposta a la tercera vacuna i que estaven afectats d'Artritis Reumatoide (la majoria), LES i espondiloartropaties. Se'ls va indicar discontinuar el metotrexat i/o el micofenolat per rebre la quarta dosi. D'entre aquests no responedors, hi va haver un increment estadísticament significatiu en quant al percentatge de seroconversió i de nivells d'anticossos IgG anti S1/S2, però no d'activitat neutralitzant. Un treball similar, realitzat en pacients amb LES (la majoria dels quals tractats amb micofenolat) i AR van administrar la

quarta dosi al cap de 22 dies a aquells pacients poc o no responedors, aconseguint augmentar la resposta immune tant humoral com cel·lular (80).

Donada la repercussió que aparenten tenir els fàrmacs immunosupressors en la resposta vacunal, ha sorgit la qüestió sobre si es necessari canviar la dosi o el temps d'administració entre aquests i la vacuna. Sembla que ni a l'ajustar cap dels tractaments tant per dosi ni per moment d'administració canviarien la resposta (81). Del que hi ha més evidencia en aquest aspecte per la repercussió que sembla tenir però, és del RTX. Dos estudis diferents, però amb els mateixos resultats indiquen que, com més temps passa entre l'administració del fàrmac i de la vacuna, més augmenta la resposta, tant si prèviament havien fet resposta o no a les 2 dosis. I el temps òptim s'intueix que es a partir dels 6 mesos (77,82).

A banda de demostrar l'eficàcia a nivell de resposta immunitària, cal valorar també si aquestes troballes es tradueixen en un canvi en el risc de desenvolupar la infecció o en la gravetat. En aquest sentit, existeixen dades que revelen que l' hospitalització en pacients amb una sola dosi de vacuna mRNA és més elevada que amb la doble dosi, i aquesta més que amb la triple amb un 15.4%, 8% i 2.7% respectivament. En aquesta mateixa cohort el 2% dels pacients amb una única dosi van resultar èxitus, l'1.8% dels que es van administrar dues dosis i el 0.6% dels que duien tres dosis. En l'anàlisi multivariant es va confirmar que aquells individus que havien rebut menys dosi tenien més risc d'hospitalització i de mortalitat (83). Cal destacar que es va publicar prèvia a l'aparició d'òmicron, que és la variant majoritària actualment.

2. HIPÒTESIS

Els pacients amb malaltia reumatològica autoimmune en tractament immunosupressor presenten una resposta immune disminuïda a les vacunes mRNA contra el SARS-COV-2 a mig termini.

2.1 Justificació de l'estudi

Es considera que els pacients amb malalties reumàtiques es troben en major risc de patir infeccions comunitàries tant per la mateixa malaltia com pels tractaments utilitzats, pel que conformen un grup particular en el calendari vacunal. Tanmateix, en la gran majoria d'estudis sobre la resposta immunitària a les vacunes, no es consideren com a tal i no s'han realitzat estudis de registre específicament dirigits a aquest col·lectiu. Per tant, no existeix evidència clara sobre la protecció real en els pacients amb malaltia reumatològica amb les pautes de vacunació habituals.

Aquest aspecte ha adquirit una importància creixent per part de diferents especialistes amb l'aparició de les vacunes contra la COVID-19. Des de l'inici de la pandèmia s'ha mostrat interès sobre els risc d'infecció dels nostres pacients, així com també sobre la gravetat un cop desenvolupen la malaltia. Posteriorment, han anat apareixent diversos estudis sobre la resposta a la vacuna també en pacients amb malalties autoimmunes.

Aquests pacients però, presenten una gran variabilitat de tractaments i d'evolució clínica. Això contribueix a que, fins al dia d'avui, manqui evidència de qualitat i treballs els dissenys dels quals s'hagin orientat a valorar específicament el tipus de vacuna, els diferents grups tant de malalties com de tractament i el manteniment de la resposta tant a mig com a llarg termini.

Per aquests motius, creiem d'interès el nostre estudi on es pretén aportar nova evidència sobre com aquests pacients responen a la vacunació i si hi ha diferències

en funció del tipus de malaltia i de tractament. Com a característica diferencial respecte altres estudis previs, ens centrarem en la resposta immune a mig termini, entre el 4-6 mesos de la vacunació. D'aquesta manera, intentarem recomanar estratègies preventives de vacunació amb l'administració de més dosis de reforç o mitjançant l'aplicació d'altres accions com podria ser la immunització passiva, en els casos disponibles.

3. OBJECTIUS

3.1 Objectiu principal

Avaluar la resposta a les vacunes mRNA contra la SARS-COV-2 a mig termini en pacients amb malalties autoimmunes reumatològiques, mitjançant la determinació dels índex de seroconversió i els anticossos neutralitzants.

3.2 Objectius secundaris

- Determinar les possibles diferències en la resposta immune a mig termini, segons els diferents tractaments immunosupressors prescrits.
- Estudiar l'efecte de les diferents malalties en aquesta resposta immune a mig termini.
- Analitzar el comportament de la resposta immunològica segons els índex de seroconversió i els anticossos neutralitzants després de rebre una tercera dosis de reforç en aquests grup de pacients.
- Avaluar l'existència de diferències en la resposta immune segons les diverses variants del virus SARS-CoV-2, reconegudes fins al moment.

4. COMPENDI DE PUBLICACIONS

4.2 Primer article

Glucocorticoids' treatment impairs the medium-term immunogenic response to SARS-CoV-2 mRNA vaccines in Systemic Lupus Erythematosus patients.

Silvia Garcia-Cirera, Joan Calvet, Antoni Berenguer-Llergo, Edwards Pradenas, Silvia Marfil, Marta Massanella, Lourdes Mateu, Benjamin Trinité, Maria Llop, Marta Arévalo, Carlos Galisteo, Cristóbal Orellana, Rafael Gómez, María Nieves Gómez-Gerique, Inma Carmona, Bonaventura Clotet, Julià Blanco, Jordi Gratacós

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Glucocorticoids' treatment impairs the medium-term immunogenic response to SARS-CoV-2 mRNA vaccines in Systemic Lupus Erythematosus patients

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Limited data exists on SARS-CoV-2 sustained-response to vaccine in patients with rheumatic diseases. This study aims to evaluate neutralizing antibodies (nAB) induced by SARS-CoV-2 vaccine after 3 to 6 months from administration in Systemic Lupus Erythematosus (SLE) patients, as a surrogate of sustained-immunological response. This cross-sectional study compared nAB titre of 39 SLE patients and 37 Healthy individuals with no previous SARS-CoV-2 infection, who had all received a complete regimen of a mRNA SARS-CoV-2 vaccine within the last 3 to 6 months. We included four lines of SLE treatment including Not-treated, Hydroxychloroquine, immunosuppressive drugs and biological therapy. Glucocorticoids were allowed in all groups. Healthy and Not-treated individuals showed the highest levels of nAB. Treated patients presented lower nAB titres compared to Healthy: a 73% decrease for First-Line patients, 56% for Second-Line treatment and 72% for Third-Line. A multivariate analysis pointed to Glucocorticoids as the most associated factor with declining nAB levels (75% decrease) in treated SLE. Furthermore, a significant reduction in nAB titres was observed for Rituximab-users compared to Healthy subjects (89% decrease). Medium-term response of SLE patients to SARS-CoV-2 mRNA vaccines is negatively impacted in Glucocorticoids and Rituximab users. These findings might help to inform recommendations in vaccination protocols for SLE patients.

The spread of coronavirus disease 2019 (COVID-19) has resulted in a severe economic and health crisis worldwide. In this adverse scenario, vaccination programs have demonstrated to play a key role in fighting the global pandemic[1]. BNT162b2 (Pfizer-BioNTech) and mRNA-1273 (Moderna), both based on the novel Messenger RNA (mRNA) technology, were the first vaccines approved by the Food and Drug Administration and the European Medicines Agency[2,3].

Patients with rheumatic diseases are considered a risk factor for community infections, although controversies exist regarding COVID-19 severity[4,5]. There still exists lack of data regarding vaccines' efficacy in vulnerable collectives with a compromised immune system, either due to a chronic pathology or to therapies targeting an autoimmune disease. Randomized controlled studies evaluating the efficacy of SARS-CoV-2 vaccines usually exclude patients of rheumatic autoimmune disease, so information about vaccines' efficacy in this group of

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		All n = 76	Control n = 37 (48.7%)	Not-treated n = 10 (13.2%)	First-Line n = 10 (13.2%)	Second-Line n = 10 (13.2%)	Third-Line n = 9 (11.8%)	p-value
Sex	Female	65 (85.5%)	30 (81.1%)	10 (100.0%)	10 (100.0%)	8 (80.0%)	7 (77.8%)	0.3315
	Male	11 (14.5%)	7 (18.9%)	0 (0.0%)	0 (0.0%)	2 (20.0%)	2 (22.2%)	
Age at sample extraction		46.0 (20.0, 82.8)	38.0 (20.0, 67.0)	56.8 (45.2, 82.8)	50.1 (34.6, 75.1)	51.5 (45.0, 81.8)	45.4 (34.8, 70.0)	< 0.0001
Time from vaccination to sample extraction (months)		3.4 (2.3, 5.5)	3.3 (2.6, 4.3)	3.3 (2.3, 4.3)	3.4 (3.3, 4.1)	3.9 (3.3, 4.4)	3.5 (3.1, 5.5)	0.0064
Vaccine type	Moderna	44 (57.9%)	17 (45.9%)	5 (50.0%)	7 (70.0%)	6 (60.0%)	9 (100.0%)	0.0329
	Pfizer	32 (42.1%)	20 (54.1%)	5 (50.0%)	3 (30.0%)	4 (40.0%)	0 (0.0%)	
Time of disease evolution (years)		14.3 (2.3, 39.4)		17.8 (7.2, 36.3)	11.7 (6.3, 36.3)	13.8 (2.3, 36.3)	16.2 (4.3, 39.4)	0.5892
Any Concomitant Treatment	No	20 (51.3%)		9 (90.0%)	7 (70.0%)	3 (30.0%)	1 (11.1%)	0.0016
	Yes	19 (48.7%)		1 (10.0%)	3 (30.0%)	7 (70.0%)	8 (88.9%)	
Concomitant Treatment with Clucocorticoids	No	30 (76.9%)		9 (90.0%)	7 (70.0%)	7 (70.0%)	7 (77.8%)	0.7575
	Yes	9 (23.1%)		1 (10.0%)	3 (30.0%)	3 (30.0%)	2 (22.2%)	
SLEDAI 2 years average	SLEDAI < 4	36 (92.3%)		9 (90.0%)	10 (100.0%)	9 (90.0%)	8 (88.9%)	
	SLEDAI [4, 6)	3 (7.7%)		1 (10.0%)	0 (0.0%)	1(10.0%)	1 (11.1%)	
SLEDAI nearest to vaccination	SLEDAI < 4	34 (87.2%)		8 (80.0%)	9 (90.0%)	9 (90.0%)	8 (88.9%)	
	SLEDAI [4, 6)	3 (7.7%)		1 (10.0%)	0 (0.0%)	1 (10.0%)	1 (11.1%)	
	SLEDAI 6 +	2 (5.1%)		1 (10.0%)	1 (10.0%)	0 (0.0%)	0 (0.0%)	

Table 1. Descriptive of subjects' characteristics in the study according to disease and treatment status. Continuous variables are described by medians, minimum and maximum values; categorical variables are summarized using absolute frequencies and percentages. p-values are derived from a Mann–Whitney (continuous) or a Fisher's test (categorical variables).

subjects is limited[6]. There currently exists recommendations for anti-viral vaccinations of individuals under immunosuppressing therapies, including specific guidelines for SARS-CoV-2 vaccination provided by different scientific societies[7].

A recently published systematic review evaluated SARS-CoV-2 infection and vaccination in rheumatic and musculoskeletal diseases. The study concluded no differences between specific diseases, a good general immunogenic response despite showing lower levels of antibodies compared to the general population and pointed to immunosuppressing therapies as the main source of impact on vaccines' response over disease condition, although this point remains controversial[5,8]. Their suggestions for future work included the separate study of specific pathologies in order confirm their findings. In this regard, Systemic Lupus Erythematosus (SLE) patients represent a highly interesting population, since they often display factors such as the deregulation of the type I interferon, the impairment of the lymphocyte functions and the use of immunosuppressive drugs. These factors might have an impact os the vaccines response, as previously described in the case of influenza A vaccination[5,9–11]. In addition, viral infections trigger both type I IFN production and B cell activation, two mechanisms frequently altered in SLE patients and, hence, likely to interfere in their immunological response to vaccines[10,12].

Immune responses to SARS-CoV-2 vaccines involve humoral and cellular arms of adaptive immunity. While the specific contribution of each arm is not yet described, T-cell responses seem to be relevant for protection against severe disease, while antibodies seem to be directly related with the protection against SARS-CoV-2 infection[13]. Although different functions of antibodies could be responsible for their protective effect, neutralizing antibodies that bind to the spike (S) glycoprotein of SARS-CoV-2 and block viral entry into target cells appear to be the most relevant ones[14]. Several experimental and epidemiological studies on SARS-CoV-2 suggest that the titre of neutralizing antibodies (nAB) could be a useful as a surrogate for protection[15], as they are for other viral infections.

The present study aims to evaluate the medium-term response to SARS-CoV-2 vaccination in SLE patients under different therapy regimes compared to healthy individuals. To do so, we quantified their levels of nAB from 3 to 6 months after their vaccination as a surrogate of protection against SARS-CoV-2 infection.

Results

Neutralizing antibody titres were assessed for a total of 76 serum samples from 39 (51%) Lupus patients and 37 (49%) healthy Controls. Lupus patients were selected to equitably cover a wide range of treatment settings, that included Non-Treated patients and subjects under First-Line (Hydroxychloroquine, 10 patients, median daily dose 300 mg), Second-Line (10 patients: Methotrexate, n = 4 median weekly dose 15 mg; Azathioprine, n = 4, median daily dose 100 mg; and Mycophenolate Mofetil, n = 2) and Third-Line therapy (9 patients, Rituximab, n = 4 and Belimumab, n = 5) (Table 1 and Supplementary Table S1). Glucocorticoids users presented a median daily dose of 3 mg of methylprednisolone.

The median age was 46 years old, although Controls were younger than Lupus patients (38 vs 53 years old median, respectively). Among Lupus patients, those treated with Third-Line therapy showed a shorter age (45 years old median) while Not-Treated patients were the oldest subjects of the series (57 years old median). Females were majority in this study (86%) with a similar proportion in Controls and patients (81 and 90%,

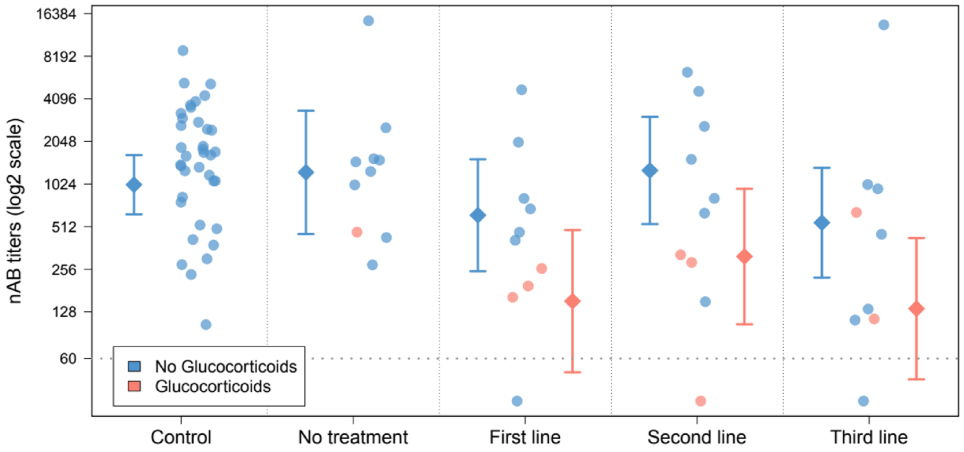

Figure 1. Neutralizing antibody (nAB) titre after SARS-CoV-2 vaccination in Healthy individuals (Controls) and Systemic Lupus Erythematosus (SLE) patients under different therapy regimens. Diamond-shaped symbols and their associated segments represent adjusted means and 95% confidence intervals of antibody titres. Estimations are derived from a linear model in which sex, age, time from vaccination, vaccine type and corticoids therapy were included as covariates for statistical control. Antibody titres were log2-transformed in order to fit the assumptions of the model and are represented in log2-scale. The horizontal dotted line indicates the detection threshold for the determinations (60). The estimation of the decrease in nAB titres in Lupus patients associated to Glucocorticoids therapy is 75% (Fold-Change = 0.25, 95% Confidence Interval = 0.10–0.63, p = 0.0037).

respectively). Moderna and Pfizer vaccines had been administered roughly equitably in the Control group (46% and 54%, respectively), while Lupus patients had been prevalently inoculated with Moderna's (69%). Subject groups were homogenous regarding time interval between vaccination and sample extraction (3.4 months median; Table 1).

Regarding SLE clinical parameters, only two patients (5%) showed an SLEDAI score above 6 in their nearest measurement to the extraction date, and none of them exceed this value as 2-years SLEDAI average. Disease evolution time was 14 years' median, ranging from 12 in First-Line to 18 years in Not-Treated patients. Patient groups were heterogeneous regarding their current regimes of concomitant therapy (from 10% in non-Treated to 89% in Third-Line treated patients), which near half of the times included therapy with Glucocorticoids (23%; Table 1).

The highest nAB titres corresponded to Healthy Control subjects (1638.0 titre median), which were similar to SLE patients not currently under treatment (1361.5 titre median). Treated patients showed considerably lower levels of nAB levels compared to Controls, a decrease estimated in 73% in patients receiving Hydroxychloroquine (First-Line therapy; p = 0.0135), 56% for patients in Second-Line (p = 0.2218) and 72% for Third-Line treated patients (p = 0.0104) (Fig. 1, Table 2 and Supplementary Table S2). Three Lupus patients (10%) showed titres of nAB below the detection threshold, while the response to vaccine was measurable for all non-Treated patients and Healthy Controls (Fig. 1).

Regarding other factors potentially associated with the vaccine response, nAB titres were inversely correlated with subject's age (Spearman Correlation = −0.284, p = 0.0128) and time from vaccination (Spearman Correlation = −0.375, p = 0.0008). A 74% decrease was also observed in patients treated with Glucocorticoids compared to non-users (p = 0.0057). No significant association was found between nAB titres and sex nor vaccine type (Fig. 1 and Table 2).

Figure 1 and Table 3 show the results from comparing nAB titres across subjects' groups after statistical control for potential confounders: sex, age, time from vaccination, vaccine type and Glucocorticoids users (see complete model in Supplementary Table S3). This analysis revealed that, although differences between Control and First- and Third-Line patients were retained with a similar magnitude effect to those observed in the univariate setting (First-Line: 70% decrease, p = 0.0179; Third-Line: 73% decrease, p = 0.0119), most of these differences were explained by the strong effect that Glucocorticoids therapy had on the nAB titres, which accounted for a 75% decrease compared to patients not prescribed with Glucocorticoids (p = 0.0037; Fig. 1, Supplementary Figure S1, Supplementary Table S3). As a result, declines in nAB titres were of a substantially higher magnitude in patients treated with Glucocorticoids than those observed in non-users (First-Line: 85%, p = 0.0022; Third-Line: 87%, p = 0.0015), where differences did not reach statistical significance (First-Line: 39%, p = 0.3136; Third-Line: 46%, p = 0.2246). Second-Line patients using Glucocorticoids also showed lower nAB levels compared to Healthy individuals although, in this case, the decrease was smaller and not statistically significant (69%, p = 0.0519) (Fig. 1, Table 3 and Supplementary Table S3; see Supplementary Table S4 for the rest of pairwise comparisons). Regarding the rest of covariates, the negative correlation observed in the univariate analysis for time from vaccination was retained after adjustment by confounders, which was estimated as a 48% decrease per month (Partial

		Median/SCC [95% CI]	p-value
Disease-treatment group	Control	1638.0 [1182.0, 2459.0]	0.0220
	Not-treated	1361.5 [429.0, 2555.0]	0.0220
	First-Line	438.5 [162.0, 2022.0]	0.0220
	Second-Line	724.0 [151.0, 4620.0]	0.0220
	Third-Line	452.0 [112.0, 1016.0]	0.0220
Sex	Female	1256.0 [809.0, 1612.0]	0.5304
	Male	762.0 [151.0, 3559.0]	0.5304
Age at sample extraction		−0.284 [−0.500, −0.058]	0.0128
Time from vaccination (months)		−0.375 [−0.575, −0.159]	0.0008
Vaccine type	Moderna	1136.0 [645.0, 1612.0]	0.6662
	Pfizer	1129.5 [467.0, 1856.0]	0.6662
Time of Disease Evolution (years)		−0.174 [−0.480, 0.133]	0.2882
Any Concomitant Treatment	No	909.5 [429.0, 1546.0]	0.1774
	Yes	452.0 [162.0, 1016.0]	0.1774
Concomitant Treatment with Glucocorticoids	No	978.5 [467.0, 1529.0]	0.0057
	Yes	259.0 [114.0, 468.0]	0.0057

Table 2. Univariate association of neutralizing antibody (nAB) levels after SARS-CoV-2 vaccination with Disease/Treatment status and demographic and clinical parameters. Association with continuous variables are estimated using Spearman Correlation Coefficients (SCC). Categorical variables, antibody levels are summarized using group medians. Range between brackets correspond to 95% confidence intervals computed with 1.000 boostrap resamples. p-values are derived from the associated SCC for continuous variables, while a Mann–Whitney (binary) or a Kruskal test (Disease/Treatment group) were used for categorical variables. SCC: Spearman Correlation Coefficient; 95% CI: 95% confidence interval.

	All		No-Glucocorticoids		Glucocorticoids	
	FC [95% CI]	p-value	FC [95% CI]	p-value	FC [95% CI]	p-value
Control (Reference)	1	–	1	–	1	–
Not-treated	0.61 [0.19, 1.94]	0.3950	1.22 [0.42, 3.52]	0.7059	0.30 [0.07, 1.25]	0.0972
First-Line	0.30 [0.11, 0.81]	0.0179	0.61 [0.23, 1.62]	0.3136	0.15 [0.05, 0.49]	0.0022
Second-Line	0.63 [0.23, 1.68]	0.3463	1.26 [0.47, 3.40]	0.6437	0.31 [0.10, 1.01]	0.0519
Third-Line	0.27 [0.10, 0.74]	0.0119	0.54 [0.20, 1.48]	0.2246	0.13 [0.04, 0.45]	0.0015

Table 3. Neutralizing antibody (nAB) levels of Systemic Lupus Erythematosus (SLE) patients after SARS-CoV-2 vaccination compared to Healthy subjects. Table cells show the Fold-Changes (FC), 95% Confidence Intervals (95% CI) and p-values derived from comparing antibody titers between each treatment line group and the Healthy subjects, which are taken as reference. Comparisons are performed for subjects receiving and not currently receiving Glucocorticoids' therapy (Corticoids and No-Corticoids columns), as well as for all patients overall (All columns). Estimations and p-values are derived from a linear model in which sex, age, time from vaccination, vaccine type and corticoids therapy were included as covariates for statistical control. FC: Fold-change; 95% CI: 95% confidence interval.

Correlation = −0.267, p = 0.0277). No statistically significant association was found for sex, age nor vaccine type (Supplementary Table S3).

A detailed analysis of specific therapy agents suggested a differential impact of drugs prescribed within Second- and Third-Lines. In the Third-Line group and in the absence of Glucocorticoids, an 89% decrease in antibody titres was observed in patients treated with Rituximab compared to Healthy Controls (p = 0.0008), while only a modest 7% was displayed by those received Belimumab (p = 0.8982). Patients under Second-Line therapy also showed different impacts regarding drug prescription without Glucocorticoids; these differences were estimated as a 39% decrease for Azathioprine and 8% for Methotrexate, although none of them reached statistical significance (p-values 0.4556 and 0.8935, respectively). Differences displayed by patients under Azathioprine were magnified by the effect of Glucocorticoids treatment (79% decrease, p = 0.0282). Although suggestive, these results at the drug level are exploratory in nature and should be interpreted with caution due to the low sample size available for the analyses (from 2 to 4 patients in the treated patients' group) (Supplementary Figure S2 and Supplementary Table S5).

Discussion

Our evaluation of the response to mRNA SARS-CoV-2 vaccination showed that, in the medium-term (3 to 6 months), the levels of nABs in patients under treatment for SLE is of a lower magnitude compared to those in controls or in non-treated patients. These lower titres in nAB are predominantly explained by the use of Glucocorticoids and Rituximab and, possibly and to a lesser extent, by the use of other immunosuppressive drugs for controlling the severity of the disease. This decrease in nAB titres are likely to be correlated with the probability of infection and, hence, might have implications in the vaccination protocols establish for SLE patients.

A higher prevalence of non-response to SARS-CoV-2 vaccination has been reported in individuals with rheumatic diseases treated with Glucocorticoids, Mycophenolate-Mofetil or Rituximab[16]. Furthermore, a hampered antibody responses to SARS-CoV-2 vaccines have been found in patients of autoimmune diseases treated with Methotrexate, Mycophenolate-Mofetil or Rituximab, and the authors suggested an extended treatment modification to improve the vaccine-induced immunogenicity[17]. Many previous studies have identified a lower short-term response to SARS-CoV-2 vaccines as measured by nAB levels in subjects with autoimmune conditions compared to healthy controls, as well as a decrease percentage of responders[16]. The immunosuppressive treatments prescription in rheumatic disease patients have also been associated to lesser levels of nAB shortly after SARS-CoV-2 vaccination, as well as to therapy with Glucocorticoids and directly to SLE condition[18,19]. Overall, these previous studies agree in reporting a weaker response to vaccination in patients with rheumatic diseases, which might be more associated to treatments with immunosuppressive drugs rather than to the specific conditions of the disease itself[20], although SLE might negatively impact to the immunogenic response[19]. Among them, Rituximab has been the more extensively studied and it has been linked to lower humoral antibody and T cell responses shortly (no more than two months) after vaccination[21,22]. Therefore, the impact of rheumatic inflammatory diseases on SARS-CoV-2 vaccine-induced response was previously evaluated at a short-term period. In our data, though, no differences in the vaccine response were found between healthy individuals and not-treated SLE patients, and this observation points to the different drug regimens and, specially, the use of Glucocorticoids or Rituximab, as the predominant cause of a lower response to the vaccine in SLE patients. Of note, therapy regimens were considered as a surrogate of SLE severity in our study by design and, therefore, it was not possible to discriminate in our data the impact of the different therapeutic options from that of the SLE conditions with a higher severity (i.e., First-, Second- and Third-Line groups).

Specifically focused on SLE studies, approximately 30% of patient experienced a low response to SARS-Cov-2 vaccines[23]. Furthermore, a quantifications of antibodies against SARS-CoV-2 in patients with Rheumatoid Arthritis (RA) and SLE after two doses of BNT162b2 resulted, in a previous work, in a 23% of patients with no detectable serological response to the vaccine. A poor humoral immune response has also been described for SLE patients treated with Glucocorticoids and immunosuppressive drugs after a single dose of mRNA vaccines[24]. SLE patients under Methotrexate or Mycophenolate-Mofetil regimes have also showed a lower antibody response against SARS-CoV-2 fifteen days after the second dose[25], although current evidence about the effect of Methotrexate on humoral response is not consistent[26,27]. None of the works mentioned above evaluated nAB as a measure of response to the vaccine. Another study on SLE patients, though, confirmed the use of Prednisone and Mycophenolate-Mofetil as independently associated with the absence of nABs six weeks after a second dose of the Sinovac-Coronovac vaccine[28]. This study suggested a positive effect of Hydroxychloroquine on the early immunogenic response, although this observation was not supported in previous works nor in our data[18].

Previous studies on SLE patients are in partial agreement with the findings reported in the present work, where Glucocorticoids and Rituximab seemed to interfere in the achievement and maintenance of an optimal nAB response to SARS-CoV-2 vaccination. As it is reported in previous studies, peripheral B cell depletion after Rituximab courses impairs the immunogenic response to SARS-CoV-2 vaccine[29]. This data supported our results where Rituximab exerted a negative impact in medium-term vaccine immunogenicity. We observed a decrease in nAB titres in SLE patients under Glucocorticoids' regimen as a concomitant therapy. It is important to highlight that, despite the median daily dose was 3 mg of Methylprednisolone, the medium-term nAB titres were significantly lower compared to non-Glucocorticoid users. Previous studies and guidelines described high doses of Glucocorticoids as a risk factor for reduced vaccine response[30]; our observation suggest that Glucocorticoid at a lower doses may also decrease this acquired immunogenic response in the medium-term[18]. Only two patients under Mycophenolate-Mofetil therapy were evaluated in our study, although it is worthy to note that one of them did not present detectable nAB titres. We did not observe Methotrexate as an obstacle for obtaining an optimal medium-term response to mRNA vaccines. Discrepancies exists in previous studies evaluating the effect of this drug in the short-term response to vaccines[26,27]. In our series, no subject under Methotrexate had received Glucocorticoids and we did not detect a decrease in its nAB titre. We did not observe either an impact of Azathioprine or Hydroxychloroquine in medium-term immunogenic response when administered alone without concomitant Glucocorticoids. To interpret the results provided, it is important to consider that our study evaluates the preservation of the immunological response in the medium-term, that is, within a period between 3 and 6 months after the second vaccination dose. In contrast, all previously published studies assessed the short-term response to vaccination (4 to 6 weeks after completion of the schedule), which might explain some of the results differences observed between these works and our own. Although it was not possible to stablish cut off points for nAB due to technical concerns, it is accepted that higher levels of nAB provides a more protection against SARS-CoV-2 infection[31]. In this regard, the use of nAB titres as a surrogate of sustained immunogenic response might help to define specific vaccination schedules for SLE patients, especially for those under Glucocorticoids and Rituximab regimens.

Strengths and limitations. The main limitation of our study arises from its cross-sectional nature, which limits the ability to establish causality. Also, SLE patients were recruited at a single clinical centre, which lim-

ited the sample size available for analyses. This is especially true for the results at the level of specific treatments within therapy lines (2 to 4 samples in the treatment groups) where results, although appealing, should be interpreted with caution. These limitations determine the exploratory nature of our findings until they are confirmed in larger and independents series of patients. Also, and although symptomatic SARS-CoV-2 infection was discarded for all participants by interview and review of clinical history, there was not availability of serology studies or history of anti-N protein antibodies for these subjects. Hence, we cannot discard the inadvertent inclusion of individuals with previous asymptomatic COVID-19 infection, a weakness that is shared by many previous studies.

On the other hand, SLE patients were selected to represent a wide range of treatment regimes, which allowed to evaluate nAB titres in different clinical scenarios. In our design, patients' therapies were considered as a surrogate of SLE severity and, although no differences in the vaccine response were found between healthy subjects and Not-treated SLE patients (a group that includes the patients with the less severe presentation), our study did not provide data to differentiate the effects of SLE conditions with higher severity from their prescribed immunosuppressive therapy.

There also exist technical concerns regarding the measurements of nAB in patients' plasma. Although nAB quantification were conducted with a set of tools and protocols previously validated[32], the procedure was not standardized to International Units per millilitre (IU/mL), as recommended by World Health Organization (WHO). These methods may be different to those used in other laboratories and, therefore it is not possible to calibrate and harmonize our results for comparison purposes, or to establish meaningful cut offs defining different levels of vaccination response that can be extrapolated to other studies.

As described above, previous work exists describing the impact of treatment in patients of autoimmune disease on SARS-CoV-2 vaccination. Most of these studies, though, jointly analysed different pathologies, focused on the short-term response (2 to 6 weeks) after inoculation or did not evaluate nAB specifically as a surrogate of risk infection. Our work is, to our knowledge, the first studying the preservation in the medium-term (3 to 6 months) of the response induced by mRNA SARS-CoV-2 vaccines, using abundance of plasma nABs as measure of this response, and focused specifically in SLE patients treated with a variety of therapy regimens.

Conclusion

Medium-term response of SLE patients to SARS-CoV-2 vaccination, as measured by the titre of nABs, may be compromised by Glucocorticoids use and other prescribed treatments aimed to control the severity of the disease. This reduced response is likely translates into a higher probability of COVID-19 infection. The confirmation of these findings in larger longitudinal series of SLE patients, might help to inform recommendations in vaccination protocols for SLE patients, especially for those under Glucocorticoids and/or Rituximab regimens.

Methods

Study design and subjects. The present work is a cross-sectional study that compares the nAB levels in the serum of 39 Systemic Lupus Erythematosus (SLE) patients and 37 healthy individuals (Controls) after vaccination against the SARS-CoV-2. None of the subjects had been previously exposed to the SARS-CoV-2, and all had received a complete two-doses schedule of BNT162b2 (Pfizer) or mRNA-1237 (Moderna) vaccines within the 3 to 6 months prior to sample extraction.

SLE patients included: 10 patients with no requirement of active SLE-specific therapy, possibly under concomitant treatment of Glucocorticoids (Not-Treated); 10 patients with mild to moderate SLE with indication of treatment with Hydroxychloroquine (First-Line); 10 patients with moderate to severe SLE with prescription of immunosuppressive drugs as Methotrexate, Azathioprine, or Mycophenolate Mofetil (Second-Line); and, finally, 9 SLE patients under biological treatment (Third-Line) with Belimumab or Rituximab. A third line treatment patient had been previously excluded from the study as it had received a complete vaccination schedule before its sample extraction (Table 1 and Supplementary Table S1). Glucocorticoids were permitted in all groups as a concomitant treatment.

After a subject was selected as potential participant, a member of the research team contacted them telephonically to expose the study and to offer their enrolment. At the inclusion data, the study was again described to the subject, who signed the informed consent and provided the blood sample. The laboratory technicians processed and conserved the sample at −80ºC until the serologic quantifications. We selected a control group of subjects from the KING study (Hospital Germans Trias i Pujol, Badalona Spain, HUGTiP, PI-20-122 and PI-20-217), without comorbidities and no previous virus exposure, who had also received a complete regimen of BNT162b2 (Pfizer) or mRNA-1237 (Moderna) vaccination within the last 3 to 6 months. This study was approved by the Local Ethical Committee at the Hospital Universitari Parc Taulí, Sabadell (2021/5093). All patients included were verbally informed and signed informed consent and all methods were performed in accordance with the relevant guidelines and regulations.

Assessments. We collected the following subjects' information: age, sex, type of vaccine (BNT162b2 (Pfizer) or mRNA-1237 (Moderna)), date of vaccination (second dose) and date of sample extraction. For SLE patients' additional clinical parameters were collected: disease evolution time, last SLE Disease Activity Index (SLEDAI) measured before sample extraction, the average of the SLEDAI measurements obtained over the last two years, and any concomitant treatment prescribed in addition to their regimen.

Neutralization assays. HEK293T cells (Integral Molecular (Cat# C-HA101), presumably of female origin) overexpressing wild-type human ACE-2 (Integral Molecular, USA) were used as target for SARS-CoV-2

spike expressing pseudovirus infection. Cells were maintained in T75 flasks with Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% foetal bovine serum and 1 µg/mL puromycin).

HIV-based pseudoviruses expressing the SARS-CoV-2 S protein and luciferase were constructed using the defective HIV plasmid, pNL4-3.Luc.R-.E-. The plasmid was obtained from the NIH AIDS Reagent Program. Expi293F cells were transfected using ExpiFectamine293 Reagent (Thermo Fisher Scientific, Waltham, MA, USA) with pNL4-3. Luc.R-.E- and SARS-CoV-2.SctΔ19 (Wuhan); at an 8:1 ratio, respectively[33]. Control pseudoviruses were generated by replacing the S protein expression plasmid with a vesicular stomatitis virus (VSV)-G protein expression plasmid as previously reported[34]. Supernatants were harvested 48 h after transfection, filtered at 0.45 µm, frozen, and titrated on HEK293T cells overexpressing wild-type human ACE-2.

Neutralization assays were performed in duplicate. Briefly, 200X the median tissue culture infectious dose (TCID50) of pseudovirus was preincubated with three-fold serial dilutions (1/60–1/14,580) of heat-inactivated plasma samples in Nunc 96-well cell culture plates (Thermo Fisher Scientific) for 1 h at 37 °C. Then 2 × 104 HEK293T/hACE2 cells treated with DEAE-Dextran (Sigma-Aldrich, St. Louis, MO, USA) were added. Results were read after 48 h using an EnSight Multimode Plate Reader and BriteLite Plus Luciferase reagent (Perkin Elmer, Waltham, MA, USA). The results were normalized and the ID50 (the reciprocal dilution inhibiting 50% of the infection) was calculated by plotting the log of plasma dilution versus response and fitting to a 4-parameter equation in Prism 8.4.3 (GraphPad Software, San Diego, CA, USA). This neutralization assay has been previously validated in a large subset of samples[32].

Statistical methods. For descriptive purposes, we used absolute and relative frequencies for categorical variables while medians, minimum and maximum values and interquartile ranges were used for continuous measurements. Univariate differences in nAB between subject groups were assessed by non-parametric techniques that included Mann–Whitney tests (binary variables), Kruskal test (categorical variables with more than one category) and Spearman Correlation (continuous variables). In these analyses, 95% confidence intervals (95% CI) for Fold-Changes between groups were computed using 1.000 bootstrap resamples stratified by condition group. Multivariate associations were assessed using linear models where sex, age at sample extraction, time from vaccination, vaccination type and glucocorticoids therapy were considered as covariates for statistical control. For doing so, antibody quantifications were log2-transformed in order to fulfil the assumptions of the model. Quantifications that did not reached the minimum detection threshold (threshold = 60) were previously assigned to a value equal to half this threshold (antibody titre = 30). Partial Correlations adjusted group means at the original scale (after undoing the log2 transformation) and FCs and their 95% CI were retrieved from the models to express the magnitude of the effects. Statistical significance was assessed using Wald tests derived from the models. Results were represented graphically using a stripchart in which the group means, and 95% CI were also included after adjustment by confounders. Statistical significance was set at the 5% threshold. All analyses were conducted using R.

Data availability

The data underlying this article is shared as Supplementary File.

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Author contributions
S.G. contributed to study conception and design, patient recruitment, data collection, data interpretation, literature search and writing the report. J.C. and A.B. contributed to study conception and design, data collection, data interpretation, literature search, data analysis and interpretation, the tables, and the figures, and writing the report. E.P., S.M., M.M., L.M. and B.T. contributed to laboratory analysis and experiments. R.G., M.N.G., and I.C. contributed to samples extraction and management, data collection and organization. M.L.L., M.A. and C.G. contributed to data collection, patients' recruitment, literature search and data interpretation. J.B., B.C., C.O. and J.G. contributed to study conception and design and data interpretation. All authors made contributions to report revision and reviewed and approved the final version of the manuscript.

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Competing interests
The authors declare no competing interests.

Additional information
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SUPPLEMENTARY INFORMATION

Table S1. Descriptive of subjects characteristics in the study according to disease and treatment status and Glucocorticoids therapy. Cells show absolute frequencies and by-row percentages.

			No Glucocorticoids	Glucocorticoids	All
Healthy	**Control**		37 (100.0%)	0 (0.0%)	37 (100%)
SLE	**Not-Treated**		9 (90.0%)	1 (10.0%)	10 (100%)
	First-Line	**Hydroxychloroquine**	7 (70.0%)	3 (30.0%)	10 (100%)
	Second-Line	**Methotrexate**	4 (100.0%)	0 (0.0%)	4 (100%)
		Azathioprine	2 (50.0%)	2 (50.0%)	4 (100%)
		Mycophenolate Mofetil	1 (50.0%)	1 (50.0%)	2 (100%)
	Third-Line	**Belimumab**	4 (80.0%)	1 (20.0%)	5 (100%)
		Rituximab	3 (75.0%)	1 (25.0%)	4 (100%)

SLE: Systemic Lupus Erythematosus

Table S2. Univariate pairwise comparisons of neutralizing antibody (nAB) levels of healthy Controls and Systemic Lupus Erythematosus (SLE) patients after SARS-CoV-2 vaccination. Cells below the table's diagonal show the Fold-Changes and corresponding 95% Confidence Intervals (between brackets), which are estimated as the ratio of medians between antibody titers between each subject group (rows) and every other group defined in the study (columns, which are taken as reference). Cells above the diagonal show the p-values associated with the corresponding comparison. For confidence intervals, a bootstrap procedure was conducted with 1.000 resamples stratified by comparison group. Statistical significance was derived from pairwise Mann-Whitney tests.

	Reference				
	Control	**Not-Treated**	**First-Line**	**Second-Line**	**Third-Line**
Control		0.38	0.01	0.22	0.01
Not-Treated	0.83 [0.27, 1.29]		0.07	0.55	0.05
First-Line	0.27 [0.12, 0.82]	0.32 [0.13, 1.12]		0.47	0.71
Second-Line	0.44 [0.17, 1.90]	0.53 [0.19, 2.73]	1.65 [0.40, 9.11]		0.39
Third-Line	0.28 [0.06, 0.70]	0.33 [0.07, 1.01]	1.03 [0.14, 3.66]	0.62 [0.06, 2.41]	

Table S3. Complete model for assessment of neutralizing antibody (nAB) levels after SARS-CoV-2 vaccination across Disease/Treatment subject groups. Results are derived from a linear model in which sex, age, time from vaccination, vaccine type and Glucocorticoids therapy were included as covariates for statistical control. Antibody titers was log2-transformed in order ot fit the assumptions of the model. Statistical significance was assessed using Wald tests (for regression coefficients) and F-tests (for overall significance of covariates). Partial Correlation for Time from Vaccination was estimated as -0.267 (95% Confidence Interval from -0.475 to -0.031).

		Beta [95%CI]	**FC [95%CI]**	**Wald test p-values**	**F-test p-value**
Disease-Treatment Group	**Control**	Ref.			0.4059
	Not-Treated	0.289 [-1.235, 1.814]	1.22 [0.42, 3.52]	0.7059	
	First-Line	-0.717 [-2.126, 0.692]	0.61 [0.23, 1.62]	0.3136	
	Second-Line	0.333 [-1.099, 1.766]	1.26 [0.47, 3.40]	0.6437	
	Third-Line	-0.892 [-2.344, 0.561]	0.54 [0.20, 1.48]	0.2246	
Sex	**Female**	Ref.			
	Male	-0.627 [-1.871, 0.617]	0.65 [0.27, 1.53]	0.3181	
Age at Sample Extraction		-0.017 [-0.052, 0.019]	0.99 [0.96, 1.01]	0.3489	
Time from Vaccination (months)		-0.948 [-1.788, -0.107]	0.52 [0.29, 0.93]	0.0277	
Vaccine Type	**Moderna**	Ref.			
	Pfizer	-0.076 [-0.933, 0.781]	0.95 [0.52, 1.72]	0.8599	
Concomitant Treatment with Corticoids	**No**	Ref.			
	Yes	-2.018 [-3.357, -0.678]	0.25 [0.10, 0.63]	0.00373	

Beta: regression coefficients; **FC:** Fold-Change; **95%CI:** 95% confidence interval; **Ref.:** Level reference for estimation of regression coefficients and Fold-Changes.

Table S4. Pairwise comparisons of neutralizing antibody (nAB) levels of Systemic Lupus Erythematosus (SLE) patients after SARS-CoV-2 vaccination. Cells below the table's diagonal show the Fold-Changes (FC) and 95% Confidence Intervals (between brackets), which are estimated as the ratio between antibody titers between each subject group (rows) and every other group defined in the study (columns, which are taken as reference). Cells above the diagonal show the p-values associated with the corresponding comparison. Results are derived from a linear model in which sex, age, time from vaccination, vaccine type and Glucocorticoids therapy were included as covariates for statistical control. Antibody titers were log2-transformed in order ot fit the assumptions of the model. Statistical significance was assessed using Wald tests.

	Reference			
	Not-Treated	**First-Line**	**Second-Line**	**Third-Line**
Not-Treated		0.22	0.96	0.2
First-Line	0.50 [0.16, 1.53]		0.18	0.83
Second-Line	1.03 [0.32, 3.32]	2.07 [0.71, 6.03]		0.13
Third-Line	0.44 [0.13, 1.54]	0.89 [0.29, 2.72]	0.43 [0.14, 1.30]	

Table S5. Neutralizing antibody (nAB) levels of Systemic Lupus Erythematosus (SLE) patients after SARS-CoV-2 vaccination compared to Healthy subjects. Table cells show the Fold-Changes (FC), 95% Confidence Intervals (95%CI) and p-values derived from comparing antibody titers between each treatment group and Healthy subjects, which are taken as reference. Comparisons are performed for subjects receiving and not currently receiving Glucocorticoids therapy (*Glucocorticoids* and *No-Glucocorticoids* columns), as well as for all patients overall (*All* columns). Estimations and p-values are derived from a linear model including treatment group and Glucocorticoids therapy as explanatory variables. Empty cells denote comparisons not evaluated due to only one sample or no samples available in the corresponding group.

			All		**No-Glucocorticoids**		**Glucocorticoids**	
			FC [95%CI]	**P-value**	**FC [95%CI]**	**P-value**	**FC [95%CI]**	**P-value**
Healthy Controls (Reference)			1.00	-	1.00	-	1.00	-
SLE	**No treatment**		0.55 [0.22, 1.37]	0.1932	1.00 [0.43, 2.31]	0.9991		
	First-Line Treatment	**Hydroxychloroquine**	0.24 [0.10, 0.57]	0.0016	0.45 [0.19, 1.08]	0.0717	0.13 [0.05, 0.39]	0.0003
	Second-Line Treatment	**Methotrexate**	0.50 [0.13, 1.88]	0.3027	0.92 [0.27, 3.15]	0.8935		
		Azathioprine	0.33 [0.10, 1.14]	0.0790	0.61 [0.16, 2.28]	0.4556	0.18 [0.05, 0.68]	0.0122
		Mycophenolate Mofetil						
	Third-Line Treatment	**Belimumab**	0.51 [0.16, 1.61]	0.2446	0.93 [0.30, 2.88]	0.8982		
		Rituximab	0.06 [0.02, 0.21]	< 0.0001	0.11 [0.03, 0.39]	0.0008		

SLE: Systemic Lupus Erythematosus; **FC:** Fold-change; **95%CI:** 95% Confidence intervals.

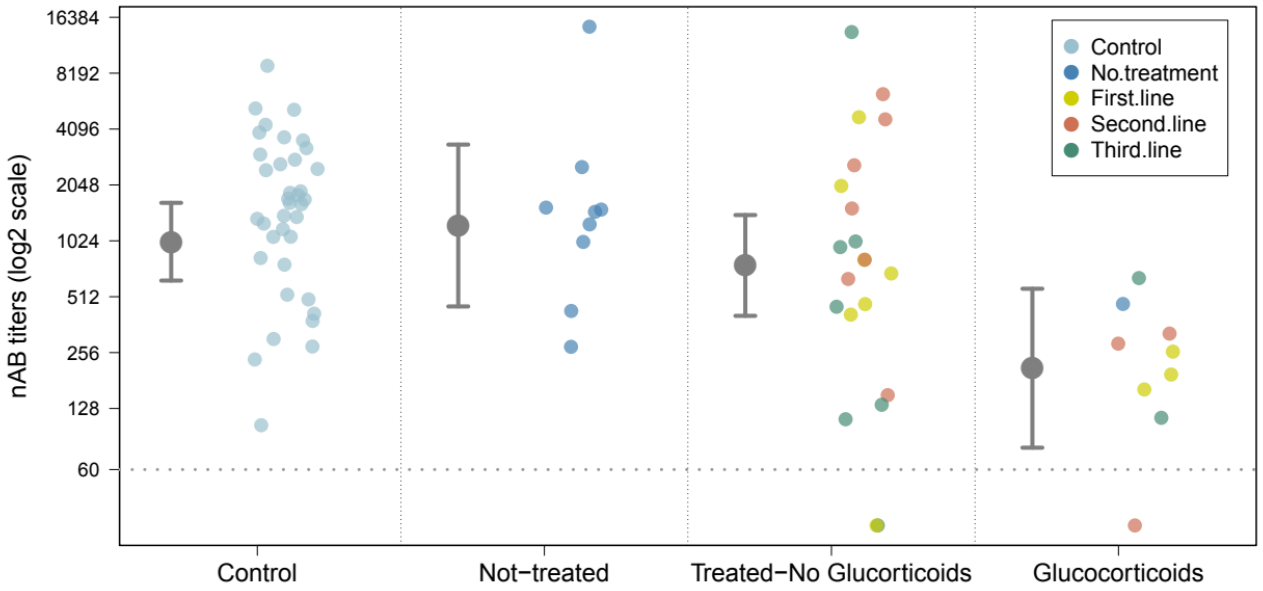

Figure S1. Neutralizing antibody (nAB) titre after SARS-CoV-2 vaccination in Healthy individuals (Controls) and Systemic Lupus Erythematosus (SLE) patients by treatment group and use of Glucocorticoids. Diamond-shaped symbols and their associated segments represent adjusted means and 95% confidence intervals of antibody titres. Estimations are derived from a linear model in which sex, age, time from vaccination, vaccine type and corticoids therapy were included as covariates for statistical control. Antibody titres were log2-transformed in order to fit the assumptions of the model and are represented in log2-scale. The horizontal dotted line indicates the detection threshold for the determinations (60). The estimation of the decrease in nAB titres in Lupus patients associated to Glucocorticoids therapy is 75% (Fold-Change = 0.25, 95% Confidence Interval = 0.10-0.63, p-value = 0.0037).

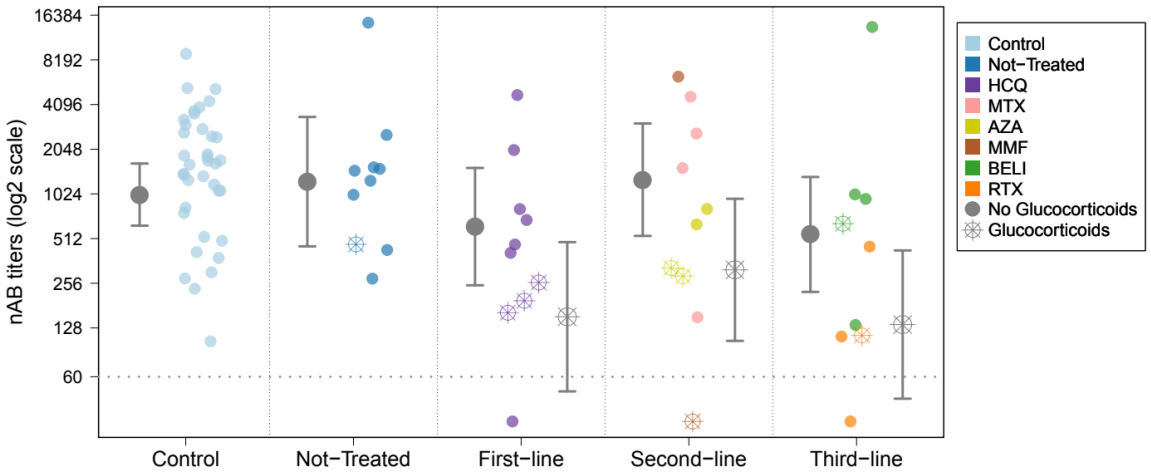

Figure S2. Neutralizing antibody (nAB) levels after SARS-CoV-2 vaccination in healthy individuals (Controls) and Systemic Lupus Erythematosus (SLE) patients under different therapy regimens. Circles represent samples of patients not treated with Glucocorticoids, while star-shaped points correspond to patients not under Glucocorticoids regimen. Data points are coloured according to their disease status (Healthy Control or SLE patient) and treatment. Gray points and their associated segments represent adjusted means and 95% confidence intervals of nAB titers. Estimations are derived from a linear model in which sex, age, time from vaccination, vaccine type and Glucocorticoids therapy were included as covariates for statistical control. nAB titers were log2-transformed in order ot fit the assumptions of the model and are represented in log2-scale. The horizontal dotted line indicates the detection threshold for the determinations (60). The estimation of the decrease in antibody titres in SLE patients associated to Glucocorticoids therapy is 75% (Fold-Change = 0.25, 95% Confidence Interval = 0.10-0.63, p-value = 0.0037). **HCQ**: Hydroxychloroquine; **MTX**: Methotrexate; **AZA**: Azathioprine; **MMF**: Mycophenolate Mofetil; **BELI**: Belimumab; **RTX**: Rituximab

4.3 Segon article

Biological and glucocorticoids treatment impair the medium-term immunogenicity to SARS-CoV-2 mRNA vaccines in autoimmune inflammatory rheumatic diseases.

Silvia Garcia-Cirera, Joan Calvet, Juan Francisco Delgado de la Poza, Antoni Berenguer-Llergo, Cristóbal Orellana, Menna Rusiñol, Maria Llop, Marta Arévalo, Alba Garcia-Pinilla, Ester Costa, Cristina Aymerich, Rafael Gómez, Anna Carreras, Jordi Gratacós

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Biological and glucocorticoids treatment impair the medium-term immunogenicity to SARS-CoV-2 mRNA vaccines in autoimmune inflammatory rheumatic diseases

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Abstract

Background This study aims to assess the sustained immunological response to the SARS-CoV-2 vaccine in patients with autoimmune inflammatory rheumatic diseases (AIRD) undergoing different treatment regimens.

Methods We conducted a prospective observational study involving 157 AIRD patients without prior COVID-19 infection. Treatment regimens included non-treatment or glucocorticoid-only (not-treated/GCs), non-biological drugs, biological therapy, and JAK inhibitors. All participants completed the two-dose vaccine schedule, and 110 of them received an additional booster dose. Serum samples were collected approximately 3–6 months after the second and third vaccine doses to measure antibodies against the Spike protein (antiS-AB) and neutralizing antibodies (nAB) targeting six SARS-CoV-2 variants.

Results Following the third dose, all patients exhibited a significant increase in antiS-AB (FC = 15, $p < 0.0001$). Patients under biological therapy had lower titres compared to the non-biological (66% decrease, $p = 0.038$) and the not-treated/GCs group (62% decrease, $p = 0.0132$), with the latter persisting after the booster dose (86% decrease, $p = 0.0027$). GC use was associated with lower antiS-AB levels in the biological group (87% decrease, $p = 0.0124$), although not statistically significant after confounders adjustment. nABs showed the highest positivity rates for the wild-type strain before (50%) and after the booster dose (93%), while the Omicron variant exhibited the lowest rates (11% and 55%, respectively). All variants demonstrated similar positivity patterns and good concordance with antiS-AB (AUCs from 0.896 to 0.997).

Conclusions The SARS-CoV-2 vaccine booster strategy effectively elicited a sustained antibody immune response in AIRD patients. However, patients under biological therapies exhibited a reduced response to the booster dose, particularly when combined with GCs.

Keywords Autoimmune disease, Immune response, COVID19, Neutralizing antibodies

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Introduction.

Patients with autoimmune inflammatory rheumatic diseases (AIRD) are at a higher risk of infections, including COVID19, compared to the general population [1–3]. This increased susceptibility can be attributed to the inflammatory burden and comorbidities associated with their condition, as well as to treatments used to manage AIRD, including glucocorticoids, immunosuppressive agents, and immunomodulatory therapies [4, 5]. However, the specific contributions of the disease and these treatments to this imbalance in infection susceptibility remains unclear [6].

The introduction of SARS-CoV-2 vaccination dramatically decreased the severity of the infection. Previous research has documented the safety and effectiveness of SARS-CoV-2 vaccines in patients with AIRD [7–9]. Nevertheless, concerns persist regarding the durability of vaccine-induced protection in this population. Several studies, including a systematic review, have investigated the seroconversion and vaccine response among AIRD patients [10, 11], and have observed a rate improvement of this response following vaccination. These studies have also indicated that the medications prescribed to AIRD patients, particularly glucocorticoids, mycophenolate–mofetil, and rituximab, may have a greater impact on the diminished vaccine response than the underlying autoimmune disorder itself [12–14]. Moreover, it is important to note that, patients suffering from AIRD presented an impairment in both, the humoral and cellular immune induction response compared to healthy controls [15]. In line with previous research, our group has recently published a study highlighting the detrimental effect of glucocorticoids on the immune response of individuals with systemic lupus erythematosus (SLE), regardless of their treatment regimen, in comparison to healthy individuals [16]. Other works have reported a significant decline in the humoral immune response among AIRD patients 6 months after receiving the second dose of the vaccine [17].

The administration of a third dose (booster) of the SARS-CoV-2 vaccine has generally shown to enhance immunity in most individuals. However, a subset of patients with compromised immune systems may not attain an optimal immune response even after receiving a booster, highlighting the need for further research to identify the most effective approach for this specific population [18–20]. A recent study on AIRD patients observed that older age, vasculitis, and the use of medications such as prednisone, mycophenolate–mofetil, and biologic therapies (belimumab, rituximab, and abatacept) were associated with lower levels of IgG and neutralizing antibodies in the short term (1 month) after vaccination [18]. Another study found that rituximab and glucocorticoids were linked to a diminished humoral response, with no impact in the cellular immunity, to the third dose of the SARS-CoV-2 vaccine, consistent with previous research highlighting the negative impact of rituximab on immune protection in AIRD patients [19, 20]. Nevertheless, individuals with AIRD who received three vaccine doses still experienced less severe SARS-CoV-2 infections and a reduced risk of hospital admission compared to those who received two doses or remained unvaccinated [21].

Despite previous studies, there is still limited understanding of the medium-term response (3 and 6 months) to the SARS-CoV-2 vaccine in patients with AIRD, particularly in relation to the third dose and its correlation with the level of protection achieved after the second dose. The lack of comprehensive data hampers the development of strategies to ensure sustained protection against COVID-19 in this vulnerable population, and it remains unclear whether additional doses or alternative strategies are necessary to achieve long-term immunity.

Our study aims to address these limitations by evaluating the sustained response to the SARS-CoV-2 vaccine in patients with various AIRD who receive different therapy regimens. For this purpose, we assessed the levels of Spike (S) protein antibodies (antiS-AB) and neutralizing antibodies (nAB) against multiple SARS-CoV-2 variants between 3 and 6 months after the second and third vaccine doses. These antibody levels serve as a measure of protection against SARS-CoV-2 infection, allowing us to assess the medium-term effectiveness of the vaccine, identify individuals with impaired antibody immune response, and explore the potential benefits of the booster strategy in AIRD patients.

Methods

Study design and subjects

The present work is a prospective observational study aimed at comparing the immune response to the SARS-CoV-2 vaccine following the initial two-dose regimen and the booster (third dose) of BNT162b2 (Pfizer) or mRNA-1237 (Moderna) vaccines. We included 157 patients from the outpatient Rheumatology department diagnosed with Rheumatoid Arthritis (RA), Systemic Lupus Erythematosus (SLE), Giant Cell Arteritis (GCA), Psoriatic Arthritis (PsA), and Axial Spondylitis B27 + (SpA). These patients were on different treatment regimens, including no treatment or treatment with glucocorticoids only (not-treated/GCs), non-biological drugs (non-biological), biological therapy (biological), and JAK inhibitors (JAKi). Among the enrolled patients, 43 were using glucocorticoids (GCs) at the time of recruitment. Of them, 25 were on prednisone 5 mg/day or less, corresponding to patients with SLE [7], RA [11], and PsA [7]. The

remaining 18 subjects were patients with GCA, of which only 6 patients were prescribed with more than 5 mg/day. None of the subjects had been previously exposed to SARS-CoV-2 infection before the two sample extractions. All participants had received a complete two-dose schedule of SARS-CoV-2 vaccines approximately 3–6 months prior to the first sample extraction and, out of them, 110 also received a booster dose within the same interval before the second sample extraction. Blood samples were collected at two time points, approximately between 3 and 6 months after the second dose and after the booster, to assess their sustained immunological response. The collected samples were processed by laboratory technicians and stored at -80ºC for subsequent serologic determinations. The samples were evaluated for titers of anti-S protein antibody (antiS-AB), as well as neutralizing antibodies (nAB) targeting wild-type SARS-CoV-2 and variants B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), B.1.1.529 (Omicron), and P.1 (Gamma).

Assessments

We collected various demographic and clinical information: from the subjects, including age, sex, type of vaccine received (BNT162b2 or mRNA-1237), dates of vaccination (second and third doses), dates of sample extraction, specific rheumatic disease, treatment regimen for the disease, and information on current glucocorticoid use as a binary variable.

Antibodies against S and N SARS-CoV-2 protein

Antibody response to SARS-CoV-2 S protein was measured using the Elecsys® Anti-SARS-CoV-2 S test (Roche Diagnostics International Ltd, Rotkreuz, Switzerland, quantitative) according to manufacture instructions. In these experiments, the standards and international units proposed by the WHO for the determination of antibodies against SARS-CoV-2 (Binding Antibody Units, BAU/ml) [22] were used. Based on previous studies [23], a value of 260 has been established as the minimum to consider the presence of a protective level against SARS-CoV-2 and, therefore, the minimum value to consider a patient as a responder to the vaccine.

Neutralization assays

We employed the SARS-CoV-2 Variants Neutralizing Antibody 6-Plex ProcartaPlex™ Panel kit (Thermofisher Scientific, Waltham, MA, USA) in accordance with the manufacturer's instructions to determine nAB against several SARS-CoV-2 variants. The method involves a competitive assay between the ACE2 protein and the antibodies produced by the patient after vaccination and binding to the S protein of the several variants of SARS-CoV-2: wild type, B.1.1.529 (Omicron), B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta) and P.1 (Gamma). The Luminex® 200™ system (LuminexCorp, Austin, TX, USA) was used to evaluate the assay, which makes use of the Luminex xMAP technology. For result interpretation, we followed the manufacturer's recommended cutoff points, established after screening 160 healthy samples, to determine the vaccine response for each SARS-CoV-2 variant.

Statistical methods

For descriptive purposes, categorical variables were presented as absolute and relative frequencies, while continuous measurements were described using medians, minimum and maximum values, and interquartile ranges. Univariate differences between subject groups were assessed by non-parametric techniques that included, Mann–Whitney tests (binary variables), Kruskal test (categorical variables with more than one category) and Fisher's tests for contingency tables. nAB were analysed as binary variables according to their positivity for immune response (see previous section).

For the analysis of antiS-AB, 95% confidence intervals (95%CI) for Fold-Changes (FC) between groups were computed using 1,000 bootstrap resamples stratified by the condition group. Multivariate associations were evaluated using a mixed-effects linear model. The fixed effects included sex, age, rheumatic condition, treatment type, use of glucocorticoids, type of vaccine, sample type (pre- or post-third dose), and time from the previous SARS-CoV-2 vaccine dose. Interactions between various factors were also included in the model, such as sex and sample type, treatment and sample type, rheumatic condition and sample type, use of glucocorticoids and sample type, time from second and third vaccine dose and sample type, glucocorticoids use and treatment regimen, and glucocorticoids use and rheumatic condition. Individual effects were considered as random effects in the model. When comparing treatment groups, only rheumatic conditions represented in the treatment groups involved in the comparisons were included in the analysis. Similarly, when analysing specific rheumatic conditions, only the treatment groups that included those conditions were considered. To fulfil the assumptions of the model, antibody quantifications were log2-transformed. Quantifications that did not reach the minimum detection threshold (threshold = 0.4) were assigned a value equal to half this threshold (antibody titre = 0.2). The adjusted group means at the original scale were retrieved from the models after undoing the log2 transformation. FCs and their 95%CI were obtained to express the magnitude of the effects. Statistical significance was determined using Wald tests derived from the models. The results were visually represented using a stripchart, which included the

group means and 95%CI after adjustment for confounders. A significance level of 5% was used.

The predictive value of anti-S-AB titres and nAB positivity was evaluated using Receiver Operating Characteristic (ROC) analysis and the corresponding Area Under the Curve (AUC). Total accuracy, sensitivity, and specificity were determined for the optimal threshold, which was defined as the point on the ROC curve that is closest to the top-left corner (representing perfect sensitivity and specificity). Confidence intervals at a 95% confidence level were computed using bootstrap resampling. All the data analyses were performed using R [24].

Results

Patients description

We enrolled a cohort of 157 patients with various rheumatic conditions who had received a full two-dose regimen of mRNA-based SARS-CoV-2 vaccination and had no previous COVID-19 infection (Table 1). The rheumatic conditions represented in the study included Systemic Lupus Erythematosus (SLE, 22.3%), Rheumatoid Arthritis (RA, 22.9%), HLA-B27 positive Ankylosing Spondylitis (B27-AS, 14%), Psoriatic Arthritis (PSA, 25.5%), and Giant Cell Arteritis (GCA, 15.3%). Due to the specificities of each disease, there was heterogeneity in age and sex distributions across the groups. The proportion of women ranged from 32.5% in PSA to 89% in SLE, and patient age ranged from 52 years in B27-AS to 76 years in GCA (Table 1).

All patients underwent baseline sample collection prior to receiving the third dose of the SARS-CoV-2 vaccine. The median time interval between this baseline sample and the administration of the previous (second) dose was 5.3 months, although it was slightly shorter for SLE patients (3.5 months). In addition, a follow-up sample was obtained from 110 patients (70%) after the administration of the third dose. Loss of follow-up occurred due to patient mortality (3, 1.9%), COVID-19 infection (17, 10.8%), or patients not attending their sample extraction appointments (27, 17.2%). These patients showed half of the anti-S ABs average levels, lower presence of nABs for all variants analyzed, lower prevalence of SLE, higher frequency of RA, and similar distributions for the rest of parameters analyzed compared to subjects with both baseline and follow-up samples available (Additional file 2: Table S1).. The median time interval between the third dose and the follow-up sample was 3.8 months, with a range of 2.2 to 7.0 months across the entire series. The majority of patients in all disease groups (64% overall) were administered the mRNA-1273 vaccine, except for GCA patients who primarily received the BNT162b2 vaccine (92%) (Table 1).

The treatment regimens varied among the disease groups, reflecting differences in the current therapeutic

Table 1 Study patients' characteristics

		All $n=157$	Systemic lupus erythematosus 35 (22.3%)	Rheumatoid arthritis 36 (22.9%)	B27-ankylosing spondylitis 22 (14.0%)	Psoriatic arthritis 40 (25.5%)	Giant cell arteritis 24 (15.3%)	*p* value
Sex—Female		100 (63.7%)	31 (88.6%)	30 (83.3%)	10 (45.5%)	13 (32.5%)	16 (66.7%)	< 0.0001
Age at third dose of SARS-CoV-2 vaccine		58.8 (33.5, 88.2)	53.3 (34.9, 83.0)	64.5 (47.4, 74.1)	51.9 (33.5, 75.5)	58.3 (39.4, 88.2)	75.6 (55.9, 83.9)	< 0.0001
Sample post-third SARS-CoV-2 provided		110 (70.1%)	20 (57.1%)	32 (88.9%)	17 (77.3%)	23 (57.5%)	18 (75.0%)	0.0105
Treatment type	Not treated or Glucorticoids only	25 (15.9%)	10 (28.6%)	0 (0.0%)	7 (31.8%)	0 (0.0%)	8 (33.3%)	< 0.0001
	Non-biological	48 (30.6%)	17 (48.6%)	8 (22.2%)	0 (0.0%)	16 (40.0%)	7 (29.2%)	
	Biological	69 (43.9%)	8 (22.9%)	18 (50.0%)	15 (68.2%)	19 (47.5%)	9 (37.5%)	
	JAK-inhibitors	15 (9.6%)	0 (0.0%)	10 (27.8%)	0 (0.0%)	5 (12.5%)	0 (0.0%)	
Glucocorticoids use		43 (27.4%)	7 (20.0%)	11 (30.6%)	0 (0.0%)	7 (17.5%)	18 (75.0%)	< 0.0001
mRNA-based vaccine type	mRNA-1273	100 (63.7%)	25 (71.4%)	30 (83.3%)	18 (81.8%)	25 (62.5%)	2 (8.3%)	< 0.0001
	BNT162b2	57 (36.3%)	10 (28.6%)	6 (16.7%)	4 (18.2%)	15 (37.5%)	22 (91.7%)	
2nd SARS-CoV-2 vaccine dose—baseline time interval (months)		5.3 (2.3, 7.6)	3.5 (2.3, 5.5)	5.3 (4.4, 7.0)	5.5 (3.4, 7.3)	5.4 (4.2, 7.0)	5.7 (4.9, 7.6)	< 0.0001
3rd SARS-CoV-2 vaccine dose—follow-up time interval (months)		3.8 (2.2, 7.0)	4.0 (2.8, 7.0)	3.6 (2.5, 7.0)	4.1 (2.7, 6.9)	3.5 (2.2, 5.4)	4.5 (2.9, 6.5)	0.1488
2nd to 3rd dose of the SARS-CoV-2 vaccine time interval (months)		6.7 (3.6, 11.1)	6.8 (3.6, 11.1)	6.7 (5.0, 8.5)	6.8 (5.3, 8.7)	6.8 (5.6, 10.0)	6.9 (5.9, 8.6)	0.2615

approaches for these conditions. Approximately half of the patients with SLE (49%) and PSA (40%) were prescribed non-biological drugs, while none of the B27-AS patients received this type of treatment. In the B27-AS group, the predominant treatment prescription was biological agents (68%), which was also the therapy of choice for 50% of RA patients and 48% of PSA patients. JAK inhibitors (JAKi) were prescribed exclusively for patients with RA (28%) and PSA (13%). The use of glucocorticoids (GCs) also varied across disease groups, ranging from no B27-AS patients to 75% of GCA patients (Table 1).

Anti-S protein antibodies levels prior to the third dose of the SARS-CoV-2 vaccine

Baseline titers of antiS-AB were significantly lower in patients receiving biological therapy compared to those who were not treated or were treated with GCs only (not-treated/GCs; 62% decrease, p value = 0.0132) and patients under non-biological drugs (66% decrease, p value = 0.038) (Fig. 1, Table 2 and Additional file 2: Table S2). These differences remained significant even after statistical control for potential confounders, (90% decrease and p value = 0.00007 compared to not-treated/GCs group; 71% decrease and p value = 0.0072 compared to the non-biological group; Table 3). There was also substantial variation among patients with different rheumatic conditions prior to the third SARS-CoV-2 vaccine dose, ranging from 239 (GCA) to 796 (PSA) BAU/ml (Additional file 2: Table S3). After adjusting for potential confounders, SLE patients displayed the lowest baseline antibody titers compared to other rheumatic conditions, with fold changes ranging from 3.21 (B27-AS, p value = 0.0850) to 8.27 (PSA, p value = 0.0027; Additional file 2: Table S4).

Anti-S protein antibody levels after the third dose of the SARS-CoV-2 vaccine

After the administration of the third dose of the SARS-CoV-2 vaccine, patients' samples demonstrated elevated titers of antiS-AB compared to the pre-third dose samples (FC = 15.29, p value < 0.0001; Fig. 1). This increase was observed in roughly all patients in the cohort, regardless of their specific treatment regimen (Fold-Changes, FCs from 10.51 in the JAKi group to 22.35 for patients under biological therapy, p value < 0.03 in all cases; Fig. 1, Table 2, and Additional file 2: Table S5), as well as their rheumatic condition (FCs ranging from 18.07 in GCA patients to 23.30 in the SLE group, p value < 0.04 in all cases; Additional file 1: Figure S1 and Additional file 2: Tables S3 and S6). After control for potential confounders, the magnitude of this titer's raise was not statistically significant across groups defined by the specific

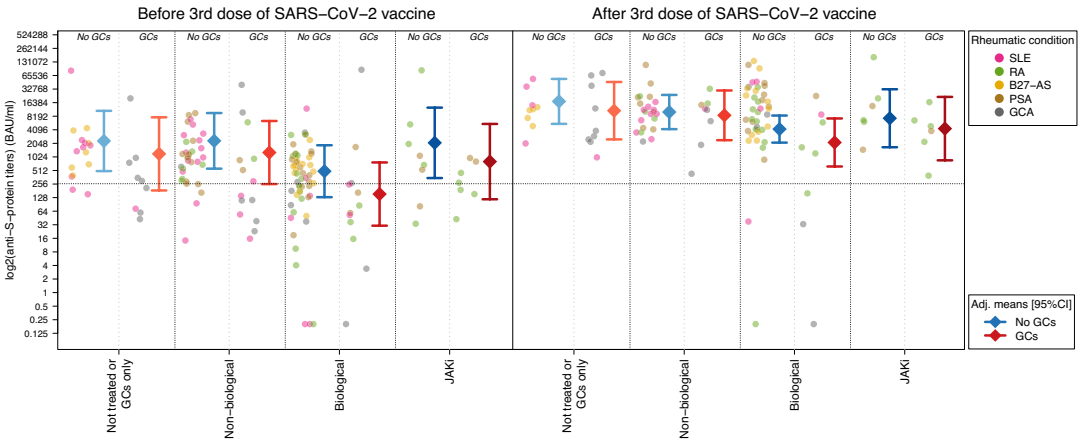

Fig. 1 Anti-S protein antibodies titers by treatment groups before and after a third dose of mRNA-based SARS-CoV-2 vaccine. The circle-shaped dots show the levels of anti-S protein antibodies of the patients' baseline and follow-up samples. The diamond-shaped points and the adjacent segments display the adjusted group means of the titer values and their corresponding 95% confidence intervals, respectively. Dot colors indicate the patients' disease condition. Adjusted means were derived from a mixed-effects linear model where sex, age, rheumatic condition, treatment type, use of glucocorticoids, type of vaccine, sample type (pre- or post-third dose) and time from previous dose where included as fixed effects, as well as the interaction between: sex and sample type; treatment and sample type; rheumatic condition and sample type; use of glucocorticoids and sample type; time from second and third vaccine dose and sample type; glucocorticoids use and treatment regimen; and glucorticoids use and rheumatic condition. Individual effects were included as random effects in this model. The *y*-axis is in log2-scale, while the value labels are displayed in the original scale of the titer values (BAU/ml). The horizontal dotted line indicates a value of 260 BAU/ml, which is typically used as a threshold to define a positive response to the vaccine. *SLE* Systemic Lupus Erythematosus, *RA* Rheumatoid Arthritis, *B27-AS* HLA-B27 positive Ankylosing Spondylitis, *PSA* Psoriatic Arthritis, *GCA* Giant Cell Arteritis, *GCs* Glucocorticoids; *JAKi* JAK inhibitors

Table 2 Anti-S protein antibody levels and neutralizing antibodies positivity (nABs) by treatment group before (pre-third dose) and after (post-third dose) a third dose of the SARS-CoV-2 mRNA-based vaccine

	Pre-third dose					Post-third dose				
	Not-treated or GCs only *n* = 25	**Non-biological *n* = 48**	**Biological *n* = 68**	**JAKi *n* = 15**	***p* value**	**Not-treated or GCs only *n* = 18**	**Non-biological *n* = 30**	**Biological *n* = 50**	**JAKi *n* = 12**	***p* value**
Anti-S-protein antibody (BAU/ml)	761 [43, 83890]	857 [14, 40700]	288 [0, 87880]	534 [34, 85340]	0.0111	11445 [1002, 74400]	10850 [433, 112590]	7966 [0, 137780]	6374 [393, 166760]	0.8029
Wild type—Positive	19 (76.0%)	37 (77.1%)	37 (54.4%)	10 (66.7%)	0.0523	18 (100%)	30 (100%)	45 (90.0%)	12 (100%)	0.1561
B.1.1.529—Positive	4 (16.0%)	8 (16.7%)	3 (4.4%)	2 (13.3%)	0.09088	10 (55.6%)	18 (60.0%)	25 (50.0%)	7 (58.3%)	0.8489
B.1.1.7—Positive	11 (44.0%)	23 (47.9%)	19 (27.9%)	5 (33.3%)	0.1380	17 (94.4%)	29 (96.7%)	44 (88.0%)	10 (83.3%)	0.3733
B.1.351—Positive	10 (41.7%)	19 (39.6%)	12 (17.9%)	3 (20.0%)	0.0265	14 (87.5%)	29 (96.7%)	41 (83.7%)	10 (83.3%)	0.3002
B.1.617.2—Positive	11 (44.0%)	21 (43.8%)	16 (23.5%)	4 (26.7%)	0.0730	17 (94.4%)	29 (96.7%)	43 (86.0%)	10 (83.3%)	0.3146
P.1—Positive	10 (40.0%)	18 (37.5%)	15 (22.1%)	4 (26.7%)	0.1959	14 (77.8%)	28 (93.3%)	42 (84.0%)	10 (83.3%)	0.45344

Cells show the medians and ranges of anti-S protein antibody titers, and the frequencies and percentages of positivity for nABs of diferents SARS-CoV-2 variants including: Wild type, B.1.1.529 (Omicron), B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta) and P.1 (Gamma). The last row display the rheumatic conditions of patients represented in each treatment group and sample draw. *p* values are derived from a Kruskal–Wallis tests comparing treatment groups within each sample time point. JAKi: JAK inhibitors; GCs: Glucocorticoids

Table 3 Comparison of anti-S protein antibody titers between treatment groups within each time point of sample extraction

	Pre-third dose		Post-third dose	
	FC [95%CI]	***p* value**	**FC [95%CI]**	***p* value**
Non-biological—not treated or GCs only	0.62 [0.21, 1.90]	0.4059	0.42 [0.12, 1.50]	0.1845
Biological—not treated or GCs only	0.10 [0.03, 0.31]	0.00007	0.14 [0.04, 0.53]	0.0027
Biological—non-biological	0.29 [0.12, 0.72]	0.0072	0.49 [0.17, 1.43]	0.1904
JAKi—non-biological	0.50 [0.13, 1.90]	0.3066	0.37 [0.08, 1.66]	0.1935
JAKi—biological	3.21 [0.89, 11.55]	0.0742	1.15 [0.29, 4.59]	0.8483

Cells show Fold-changes (FC), 95% confidence intervals (CI) and I values of the corresponding comparison. FCs and *p* values were derived from a mixed-effects linear model where sex, age, rheumatic condition, treatment type, use of glucocorticoids, type of vaccine, sample type (pre- or post-3rd dose) and time from previous dose where included as fixed effects, as well as the interaction between: sex and sample type; treatment and sample type; rheumatic condition and sample type; use of glucocorticoids and sample type; time from second and third vaccine dose and sample type; glucocorticoids use and treatment regimen; and glucorticoids use and rheumatic condition. Individual effects were included as random effects in this model. *SLE* Systemic Lupus Erythematosus, *RA* Rheumatoid Arthritis, *B27-AS* HLA-B27 positive Ankylosing Spondylitis, *PSA* Psoriatic Arthritis, *GCA* Giant Cell Arteritis, *GCs* Glucocorticoids; *JAKi* JAK inhibitors, *GCs* Glucocorticoids, *FC* Fold-Change, *95%CI* 95% confidence interval

rheumatic disease or by their prescribed therapy (interaction *p* values = 0.5300 and 0.3059, respectively). However, it is noteworthy that this increase was notably higher in men (Fold Change, FC = 27.13) compared to women (FC = 8.62, interaction *p* value = 0.0095).

Similarly, to the baseline samples, post-third dose levels in the Biological group were significantly lower compared to the not-treated/GCs group, even after control for confounders (86% decrease, *p* value = 0.0027; Table 3). Only five patients (5%) reached a value under 260 BAU/ml after the third dose, a threshold typically used to define a positive response to the vaccine and, notably, all of them were under biological therapy (Fig. 1). After the third dose, the differences observed in baseline for SLE compared to the rest of rheumatic conditions became less pronounced (FCs ranging from 1.46 to 4.30) and lost their statistical significance (Additional file 2: Table S4).

Glucocorticoids and anti-S protein antibody levels

Pre-third dose antibody levels were consistently lower in patients receiving glucocorticoids across all treatment groups, with decreases ranging from 83% (Biological) to 69% (JAKi) (Additional file 2: Table S7). These decreases were statistically significant for untreated patients (80% decrease, *p* value = 0.0272) and those receiving biological therapy (83% decrease, *p* value = 0.0402). The differences in post-third dose samples became less pronounced except for patients treated with biological drugs, where GCs users still exhibited a statistically significant 87% decrease in antiS-AB titers compared to non-users (*p* value = 0.0124; Additional file 2: Table S7). None of these differences remained statistical significant after controlling for potential confounders, although they still showed considerable magnitude in some cases (Table 4). Overall, the use of GCs was associated with a 58% decrease in baseline antiS-AB levels and a 36% decrease after the administration of the third SARS-CoV-2 vaccine dose, although these differences did not reach statistical significance (*p* values 0.0577 and 0.2931, respectively; Table 4).

Table 4 Comparison of anti-S protein antibody titers between users and non-users of glucocroticoids (GCs) within each treatment group and time point of sample extraction

	Pre-third dose		Post-third dose	
	FC [95%CI]	***p* value**	**FC [95%CI]**	***p* value**
Overall	0.42 [0.22, 1.21]	0.0577	0.64 [0.22, 1.84]	0.4079
Non-biological	0.56 [0.17, 1.03]	0.3903	0.80 [0.19, 3.38]	0.7636
Biological	0.33 [0.10, 1.13]	0.0775	0.47 [0.12, 1.80]	0.2706
JAKi	0.38 [0.06, 2.56]	0.3180	0.54 [0.07, 3.91]	0.5418

Cells show Fold-changes (FC), 95% confidence intervals (CI) and *p* values of the corresponding comparison. FCs and *p* values were derived from a mixed-effects linear model where sex, age, rheumatic condition, treatment type, use of glucocorticoids, type of vaccine, sample type (pre- or post-third dose) and time from previous dose where included as fixed effects, as well as the interaction between: sex and sample type; treatment and sample type; rheumatic condition and sample type; use of glucocorticoids and sample type; time from second and third vaccine dose and sample type; glucocorticoids use and treatment regimen; and glucorticoids use and rheumatic condition. Individual effects were included as random effects in this model. *SLE* Systemic Lupus Erythematosus, *RA* Rheumatoid Arthritis, *B27-AS* HLA-B27 positive Ankylosing Spondylitis, *PSA* Psoriatic Arthritis, *GCA* Giant Cell Arteritis, *GCs* Glucocorticoids; *JAKi* JAK inhibitors, *GCs* Glucocorticoids, *FC* Fold-Change, *95%CI* 95% confidence interval

Regarding rheumatic conditions, up to 75% decrease in baseline antiS-AB levels was observed in GCs users after adjusting for confounders (25% decrease in SLE and GCA patients; Additional file 2: Table S8). However, these differences were not statistically significant (*p* values 0.0922 and 0.1515, respectively) and their magnitude substantially diminished after the administration of the third dose of the SARS-CoV-2 vaccine (Additional file 2: Table S8).

Neutralizing antibodies for SARS-CoV-2 variants

As expected, neutralizing antibodies (nAB) against the wild-type strain exhibited the highest rates of positivity before and after the administration of the third dose (50% and 93%, respectively; p value < 0.002 for all variants). In contrast, the B.1.1.529 variant (Omicron) showed the lowest positivity across all patient groups (11% and 55%, respectively; all p values < 0.002). Notably, among the 27 patients who experienced a COVID-19 infection between the second and third vaccine administration, none of them demonstrated evidence of neutralizing antibodies against the B.1.1.529 variant, which was epidemiologically predominant during the study period. The variants B.1.1.7 (Alpha), B.1.351 (Beta), B.1.617.2 (Delta), and P.1 (Gamma) displayed similar levels of positivity, approximately 30% before the third dose and 90% after the third dose.

Despite overall differences in positivity, all variants demonstrated a consistent pattern across treatment groups, similar to that observed for antiS-AB titers (Table 2, Fig. 2, and Additional file 1: Figures S2 to S4). This pattern included a lower proportion of positivity at baseline among patients receiving biological therapy (54% for wild type, 4% for B.1.1.529, and 18–28% for other variants) or JAK inhibitors (67%, 13% and 20–33%, respectively) compared to those receiving non-biological drugs (77%, 17% and 38–48%, respectively) or no treatment other than glucocorticoids (76%, 16% and 40–44%, respectively).

Following the administration of the third dose of the SARS-CoV-2 vaccine, the positivity for variants B.1.1.7, B.1.351, B.1.617.2, and P.1 increased to near 90% in untreated patients (79–94%) and the non-biological group (93–97%). However, post-third dose positivity was slightly lower in patients under biological drugs (84–86%) or JAK inhibitors (83.3%) for these variants. This lower positivity in the biological group after the third dose was also observed for the wild-type strain (90% vs. 100% in the other treatment groups) and the B.1.1.529 variant (50% vs. 56–60%). However, differences in the nAB response across treatment groups were only statistically significant for variant B.1.351 in the pre-third dose sample (p value = 0.0265) (Table 2). No statistically significant differences in the positivity of any variant were found across groups of patients defined by their rheumatic condition after the administration of the third vaccine dose (Additional file 2: Table S3).

The presence of neutralizing antibodies (nABs) also showed a similar trend to that observed for antiS-AB in relation to glucocorticoid (GCs) prescription. GCs users had a lower frequency of nAB positivity, particularly after receiving the third dose of the SARS-CoV-2 vaccine (Additional file 2: Table S9). Statistically significant decreases in nAB positivity were observed for variants B.1.1.7 (19%, p value = 0.0050), B.1.351 (19%, p value = 0.00097), and B.1617.2 (18%, p value = 0.0109) in the post-third dose sample (Additional file 2: Table S9). These decreases were even more pronounced in patients treated with biological drugs, where GCs users experienced a decrease ranging from 44.2% to 55.4% (p values < 0.05 in all cases; Additional file 2: Table S10).

Finally, there was good concordance between the levels of nABs and antiS-AB, as evidenced by the AUC values measuring the ability of antiS-AB titres to predict variant positivity. These AUC values ranged from 0.896 (wild-type strain in pre-third dose samples) to 0.997 (variant B.1.1.7 in the post-third dose samples), with sensitivities ranging from 78% (wild type in pre-third dose) to 99% (B.1.617.2 in post-third dose), and specificities from 75% (P.1 in post-third dose) to 100% (wild type, B.1.1.7 and B.1.351 in post-third dose) (Additional file 1: Figures S5 and S6).

Discussion

The present study aimed to investigate the medium-term effects of SARS-CoV-2 vaccination (3–6 months) after the second and third vaccine doses in patients with AIRD. For doing so, we employed a cohort of 157 AIRD patients who had received a full two-dose regimen of mRNA-based SARS-CoV-2 vaccination and had no previous COVID-19 infection. Our primary objectives were to assess the immune response following vaccination, examine the impact of different treatment regimens on this response, and analyze the presence of nAB against various variants of the SARS-CoV-2. Our data revealed three significant findings. First, we observed a notable enhancement in the immune response through the implementation of a booster strategy in the majority of AIRD patients, regardless of their specific rheumatic condition or therapeutic option. Secondly, we identified a negative impact on the vaccine response among patients undergoing biological treatment compared to those receiving other therapeutic options. Finally, we found that patients prescribed with GCs exhibited a diminished response to the vaccine in comparison to non-GCs users, especially in patients under biological therapy.

We observed a notable improvement in immune induction after administering a booster dose to patients with AIRD, including those who had a limited response following the second dose. This improvement was evident in the overall increase in levels of both antiS-ABs (FC = 15.29) and nABs against different variants of the SARS-CoV-2 (from 43% to 59%) and, of note, it was higher in men compared to women. Previous works have consistently shown that this enhanced immunological response is associated with a lower incidence of

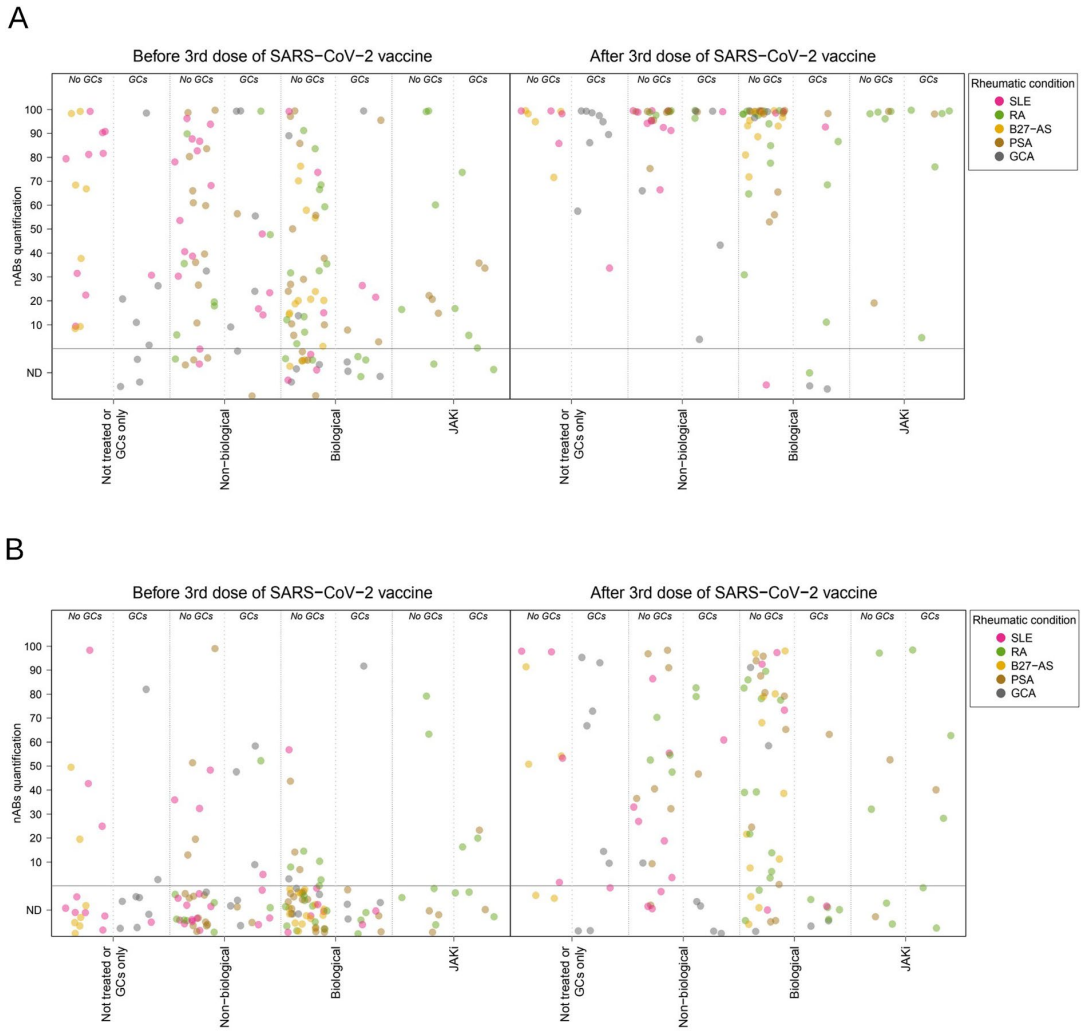

Fig. 2 Quantification of neutralizing antibodies by treatment regimen before and after a third dose of mRNA-based SARS-CoV-2 vaccine for variants Wild type (**A**) and B.1.1.529 (Omicron) (**B**). The dots show the quantitative estimations of neutralizing antibodies abundance of the patients' baseline and follow-up samples. Dot colors indicate the patients' rheumatic condition. Horizontal dotted lines display the threshold for no detection of antibody (ND) and positivity. *SLE* Systemic Lupus Erythematosus, *RA* Rheumatoid Arthritis, *B27-AS* HLA-B27 positive Ankylosing Spondylitis, *PSA* Psoriatic Arthritis, *GCA* Giant Cell Arteritis, *GCs* Glucocorticoids; *JAKi* JAK inhibitors, *nABs* neutralizing antibodies

severe SARS-CoV-2 infections and hospital admissions in AIRD patients who received a triple-vaccination regimen, compared to those who received only two doses or remained unvaccinated [21]. Other studies focusing on patients with AIRD, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), and psoriatic arthritis (PsA), have demonstrated an improved immunological response four weeks after administering a booster vaccine scheme [25, 26]. It is worth noting that, in these works, patients initially exhibited lower levels of nABs compared to healthy individuals [27]. However, following the booster dose, AIRD patients experienced a more significant increase in their nAB levels compared to the control group. Our data align with these previous observations and demonstrate a sustained immunological response in AIRD patients following the booster vaccination, as indicated by the increase in antiS-ABs and nABs before and after the third vaccination dose. These findings emphasize the effectiveness of booster doses in enhancing the immune response and

generating a sustained immunological defense against SARS-CoV-2 in patients with AIRD.

Second, it was observed that patients undergoing biological treatment exhibited a lower level of immunological protection to SARS-CoV-2, and a higher proportion of these patients remained below the protective threshold both for antiS-ABs and nABs, even following the administration of a booster. Previous studies have reported that, in general, most patients with AIRD regained a humoral response six weeks after receiving the booster dose, except for those undergoing rituximab treatment [19]. However, there is ongoing controversy surrounding the specific biological and immunosuppressive agents that have the most negative impact on the immune response. Among the different treatment regimens, rituximab appears to exert the most negative effect on this response [20]. Previous studies have also shown that biological drugs, in general, are associated with higher risks of lower immunological response rates [27, 28], with notable exceptions such as IL17 inhibitors, which do not seem to produce this undesirable effect [29]. Among non-biological immunosuppressive drugs, salazopyrin has showed no impact in the immune response against the SARS-CoV-2, while mycophenolate clearly impacts the level of the immunological protection. There are uncertainties regarding the impact of other treatments, which may be influenced by factors such as age and concomitant conditions [29, 30]. A recent large cohort study involving patients with various AIRD, predominantly rheumatoid arthritis (RA), and receiving multiple immunomodulatory treatment regimens, found that compared to antimalarials, anti-CD20 monoclonal antibodies, CTLA-4 Ig, mycophenolate, IL6 inhibitors, JAK inhibitors, and TNF inhibitors showed adjusted hazard ratios ranging from 5.20 to 1.70, indicating a compromised immune response to the SARS-CoV-2 vaccine [31]. While CD20 inhibitors may potentially benefit from new passive immunity or vaccines, other biologics and certain immunomodulators or immunosuppressive drugs like mycophenolate require careful monitoring of the immune response to make informed decisions regarding the best strategies for these patients, taking into account concerns about long-term efficacy [32]. In addition, a recent study reported an accelerated decline in the immunological response among AIRD patients after receiving the third dose of the SARS-CoV-2 vaccine, highlighting the importance of diligent follow-up to enhance the protective strategy [33]. Unfortunately, our study's sample size did not allow for specific comparisons between drugs within each treatment group, thus unable to contribute to this controversy.

The findings of our study also provide relevant insights into the impact of GC use on the immunological response to SARS-CoV-2 vaccination in AIRD patients. Consistent with a previous work of our group [16], we observed a significant decrease in baseline antiS-AB levels in patients receiving GCs, indicating a compromised immune response in the mid-term after the second dose of the vaccine. This decrease ranged from 69% (JAKi) to up to 83% in patients receiving biological therapy and, although it became less pronounced after the administration of the third vaccine dose, it remained statistically significant for patients treated with biological drugs. In the overall series, we observed a statistically significant 64% decrease in antiS-AB levels associated with GCs before the administration of the third vaccine dose, even after controlling for potential confounders. Regarding specific rheumatic conditions, it is worth noting that we observed a 75% decrease in antiS-AB titers in SLE patients which, although not statistically significant in our present data, is consistent with our previous work [16]. The impact of GCs on nABs was also evident, as the frequency of positive responses to the vaccines was significantly lower in GCs users even in post-third dose samples (16–19%), where this decrease was statistically significant for three out of the five variants assessed. Notably, patients treated with biological drugs exhibited more pronounced declines, with reductions in nAB positivity ranging from 44% to 55%. These findings underscore the importance of further research in larger cohorts to better understand the complex interplay between GC use and the immunological response to SARS-CoV-2 vaccination in AIRD patients.

Compared to other SARS-CoV-2 variants, the proportion of vaccine responders analyzed for nABs against the B.1.1.529 (Omicron) was comparatively lower, indicating a higher number of patients below the protection threshold. In addition, the improvement in antibody levels against the Omicron variant after the booster administration was less pronounced compared to other variants. These findings align with previous studies that have reported the potential immune evasion of the Omicron variant in AIRD patients, highlighting the need for novel strategies in their immunization [34]. Subsequent studies in AIRD patients have confirmed these findings, demonstrating a diminished immunological response specifically to the Omicron variant [27]. Moreover, a previous study conducted among vaccinated AIRD patients reported a higher incidence of breakthrough infections during the Omicron wave compared to previous rates [35]. It is noteworthy that the risk of contracting the Omicron variant was found to be lower in patients with hybrid immunization (those who had a previous active COVID-19 infection) compared to fully vaccinated patients. Among the fully vaccinated patients, a 17% rate of breakthrough infections was observed during

the Omicron era [36]. Importantly, in our dataset, all 17 patients who experienced breakthrough infections between the two sample collections exhibited Omicron nAB levels below the predetermined protection threshold, indicating a lack of immunological response in these individuals. In light of these emerging SARS-CoV-2 variants, there is a pressing need for ongoing research to enhance vaccine effectiveness and explore strategies such as monoclonal antiviral treatments [37], to ensure adequate protection against SARS-CoV-2 for AIRD patients, who may be particularly vulnerable to the evolving landscape of viral variants.

The present study has several limitations inherent to its observational nature conducted in a clinical practice setting, which involved patients with diverse autoimmune-mediated rheumatic diseases and various treatment regimens. Some of the treatments considered were specific to rheumatic conditions to some extent, which made it challenging to statistically control for potential confounders and discern between disease and therapy effects. This complexity impaired the effective sample size in certain comparisons and may influence the interpretation of the results. In addition, the diversity in treatment regimens and the relatively small number of patients enrolled restricted our ability to conduct a comprehensive comparison between specific drugs to identify differences in immune responses among different biological agents (Additional file 2: Table S11).

Roughly 30% of patients did not undergo a post-third dose sample due to death (2%), SARS-CoV-2 infection (11%) or not attending the extraction appointment (17%). Overall, these patients showed lower levels of anti-S ABs and a lower presence of nABs for all variants analyzed, which might indicate a poorer overall health status. However, they also showed largely similar characteristics to those contributing with two samples to the study. In addition, the magnitude of the anti-S antibody titer raises after the third vaccine dose did not differ significantly across groups defined by the specific rheumatic disease or by their prescribed therapy. These observations suggest a minimal impact of follow-up losses on the conclusions of our study. Another limitation of our study is that, while our analysis focused on the specific therapies administered for rheumatic conditions, we did not account for other medications that patients might be taking, and which could potentially influence vaccine response.

Despite these limitations, our findings suggest that treatment regimens have a greater influence on the decline of the immunological response rather than the underlying disease itself. To further confirm these findings, future studies should aim to address these limitations by including a larger sample size, accounting for a wider range of AIRD conditions, and standardizing treatment regimens. On the other hand, a notable strength of our study was the evaluation conducted at approximately 3–6 months following the administration of the second and third vaccine doses. This extended timeframe allowed us to assess the sustainability of the immunological response in patients with AIRD and, hence, the durability of the immune protection conferred by the SARS-CoV-2 vaccination in this population.

In conclusion, our study demonstrates the effectiveness of booster doses in enhancing the immune response and generating a sustained immunological defense against SARS-CoV-2 in patients AIRD, as indicated by increased serum levels of antiS-ABs and nABs. However, our findings suggest that patients under biological therapy or GC treatment may experience an impaired immune response to the vaccine, potentially increasing their risk of COVID-19 infection and disease severity. The emergence of divergent virus variants emphasizes the need for ongoing research to enhance vaccine effectiveness and explore alternative strategies to ensure adequate protection for AIRD patients. Future studies should focus on optimizing vaccine response in these vulnerable populations and identifying strategies to address the challenges posed by evolving virus variants.

Abbreviations

AIRD	Autoimmune Inflammatory Rheumatic Diseases
antiS-AB	Antibodies against the Spike protein
AUC	Area Under the Curve
SpA	Axial Spondylitis B27 +
BAU/ml	Binding Antibody Units
CI	Confidence intervals
FC	Fold-Changes
GCs	Glucocorticoids
GCA	Giant Cell Arteritis
nAB	Neutralizing antibodies
PsA	Psoriatic Arthritis
SLE	Systemic Lupus Erythematosus
RA	Rheumatoid Arthritis
ROC	Receiver Operating Characteristic

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40001-023-01620-7.

Additional file 1: Figure S1. Anti-S protein antibodies titers by rheumatic disease groups before and after a third dose of mRNA-based SARSCoV-2 vaccine. **Figure S2**. Quantification of neutralizing antibodies for B.1.1.7 (Alpha) variant of SARS-CoV-2 by treatment regimen before and after a third dose of mRNA-based SARS-CoV-2 vaccine. **Figure S3**. Quantification of neutralizing antibodies for B.1.351 (Beta) variant of SARS-CoV-2 by treatment regimen before and after a third dose of mRNA-based SARS-CoV-2 vaccine. **Figure S4**. Quantification of neutralizing antibodies for B.1.617.2 (Delta) variant of SARS-CoV-2 by treatment regimen before and after a third dose of mRNA-based SARS-CoV-2 vaccine. **Figure S5**. Concordance of anti-S protein and neutralizing antibodies in pre-third dose samples. **Figure S6**. Concordance of anti-S protein and neutralizing antibodies in post-third dose samples.

Additional file 2: Table S1. Patients' characteristics by sample availability for analysis. **Table S2**. Fold-changes (FC), boostrap 95% confidence intervals (CI) and p-values for comparison of treatment groups within each time point of sample extraction. P-values are derived from a Mann-Whitney's test. JAKi: JAK inhibitors; GCs: Glucocorticoids. **Table S3**. Anti-S protein antibody levels and neutralizing antibodies positivity (nABs) by rhuematic condition group before (Pre-3rd dose) and after (Post-3rd dose) of a third dose of the SARS-CoV-2 mRNA-based vaccine. **Table S4**. Comparison of anti-S protein antibody titers between rheumatic condition groups within each time point of sample extraction. **Table S5**. Increase of anti-S protein antibody levels after the third dose of the SARS-CoV-2 vaccine by treatment group. **Table S6**. Increase of anti-S protein antibody levels after the third dose of the SARS-CoV-2 vaccine by rheumatic condition. **Table S7**. Anti-S protein antibody levels by treatment group before (Pre-3rd dose) and after (Post-3rd dose) a third dose of the SARS-CoV-2 mRNA-based vaccine. **Table S8** Comparison of anti-S protein antibody titers between users and non-users of Glucocroticoids (GCs) within each rheumatic condition and time point of sample extraction. Cells show Fold-changes (FC), 95% confidence intervals (CI) and p-values of the corresponding comparison. **Table S9**. Positivity of neutralizing antibodies by glucorcorticoids use in the overall series. **Table S10**. Positivity of neutralizing antibodies by glucorcorticoids use in patients under biological treatment. **Table S11**. Distribution of biological treatments by use of Glucocorticoids (GCs).

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Author contributions
SG contributed to study conception and design, patient recruitment, data collection, data interpretation, literature search and writing the report. JC and AB contributed to study conception and design, data collection, data interpretation, literature search, data analysis and interpretation, the tables, and the figures, and writing the report. JFD and AS contributed to laboratory analysis and experiments. CA and RG, and AC contributed to samples extraction and management, data collection and organization. MLL, MA, AGP, EC and CO contributed to data collection, patients' recruitment, literature search and data interpretation. CO and JG contributed to study conception and design and data interpretation. All authors made contributions to report revision and reviewed and approved the final version of the manuscript.

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Availability of data and materials
The data sets used and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
This study was approved by the Local Ethical Committee at the Hospital Universitari Parc Taulí, Sabadell (2021/5093). All patients included were verbally informed and signed informed consent and all methods were performed in accordance with the relevant guidelines and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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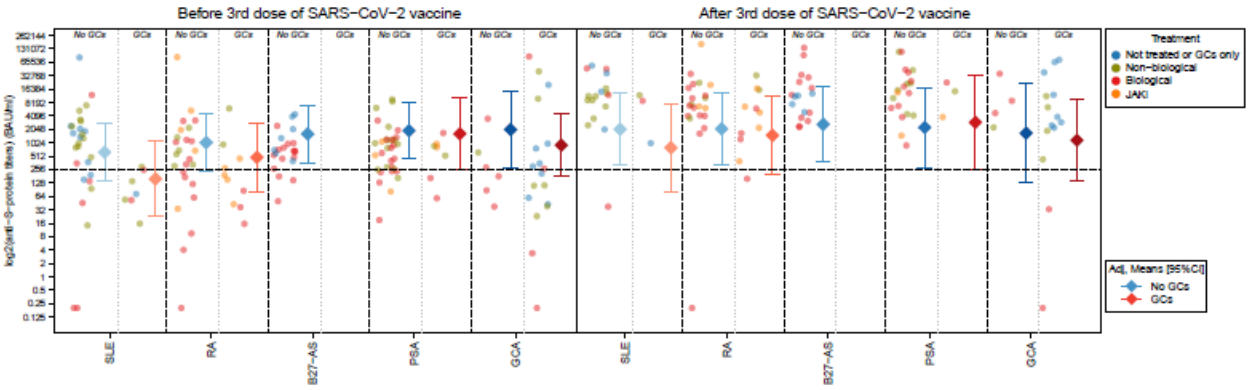
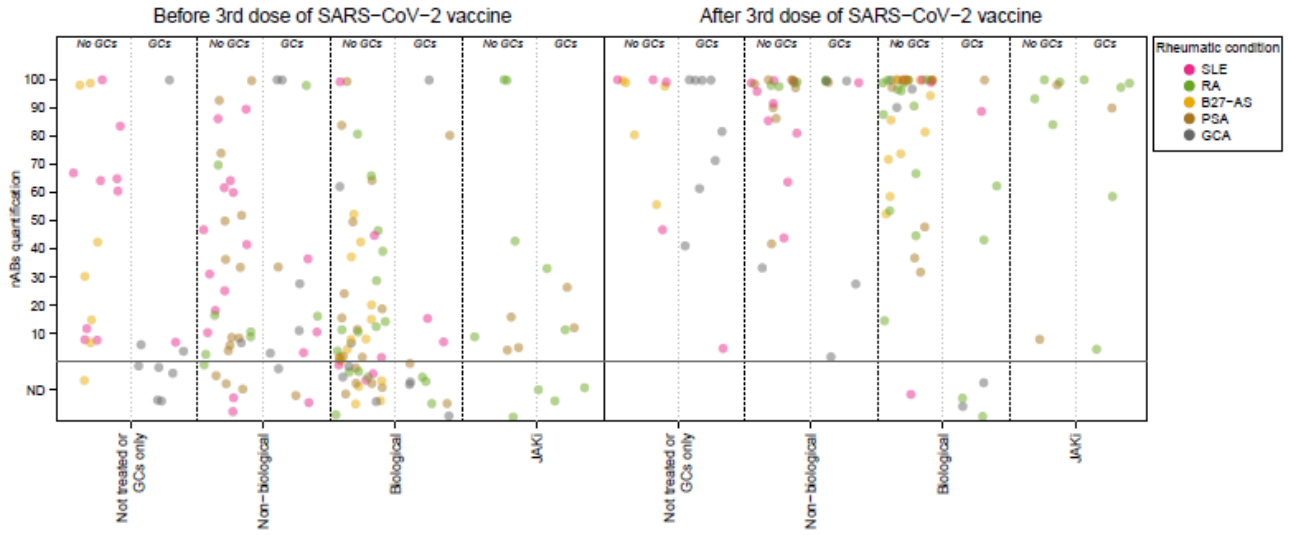
Publisher's Note

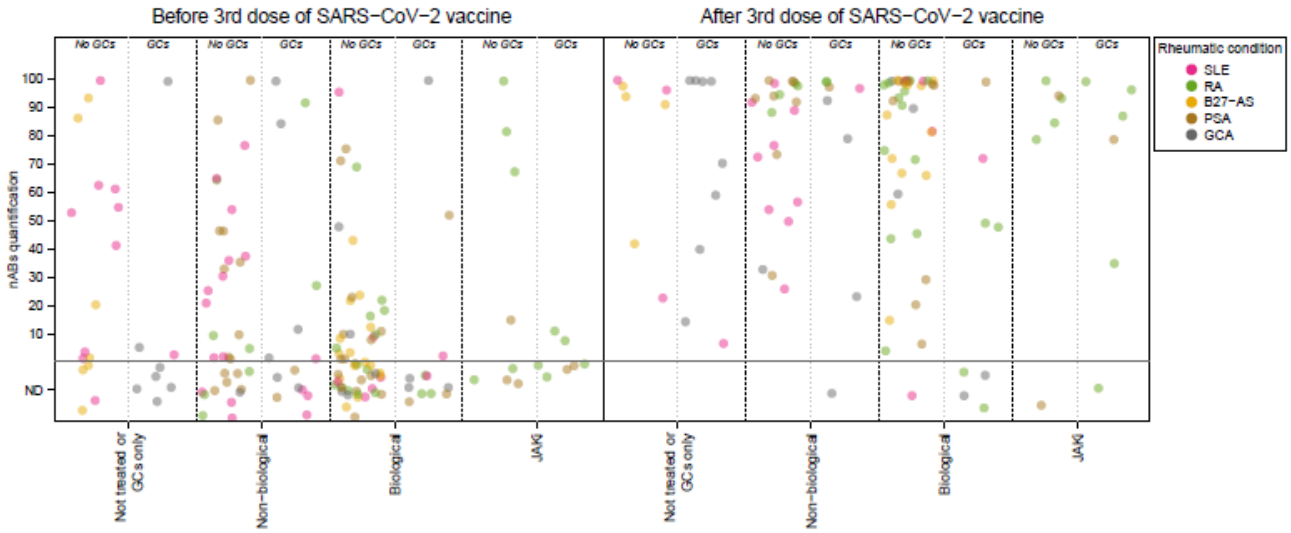
SUPPLEMENTARY INFORMATION



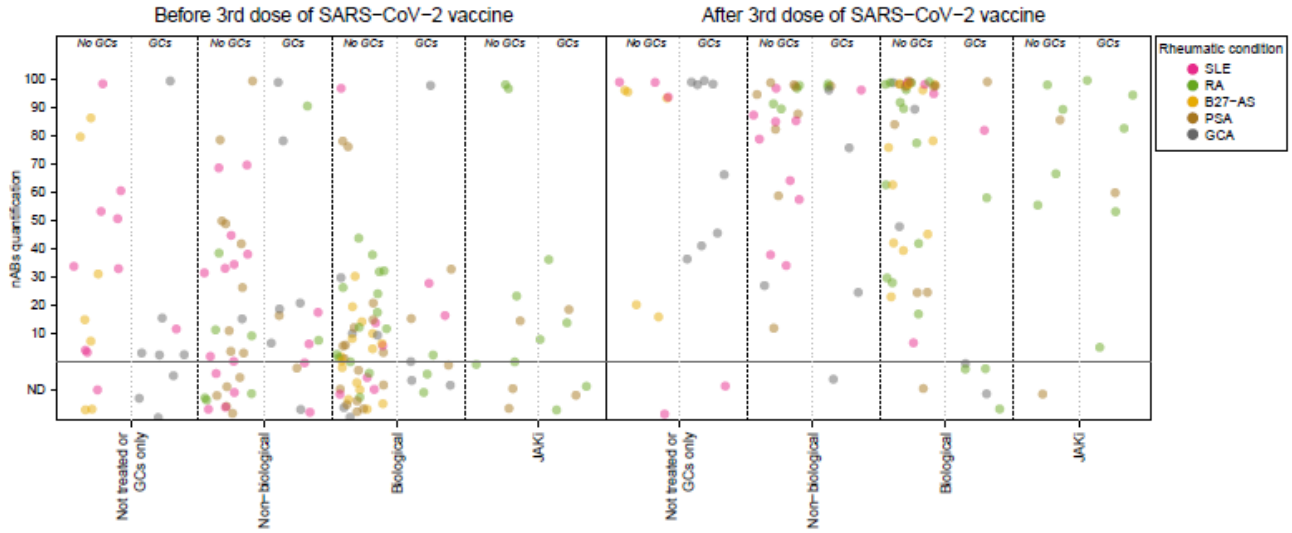
Suplementary Figure S1. Anti-S protein antibodies titers by rheumatic disease groups before and after a third dose of mRNA-based SARS-CoV-2 vaccine. The circle-shaped dots show the levels of anti-S protein antibodies of the patients' baseline and follow-up samples. The diamond-shaped points and the adjacent segments display the adjusted group means of the titer valuues and their corresponding 95% confidence intervals, respectively. Dot colors indicate the patients' treatment regimen. Adjusted means were derived from a mixed-effects linear model where sex, age, rheumatic condition, treatment type, use of glucocorticoids, type of vaccine, sample type (pre- or post-3rd dose) and time from previous dose were included as fixed effects, as well as the interaction between: sex and sample type; treatment and sample type; rheumatic condition and sample type; use of glucocorticoids and sample type; time from 2nd and third vaccine dose and sample type; glucocorticoids use and treatment regimen; and glucorticoids use and rheumatic condition. Individual effects were included as random effects in this model. The y-axis is in log2-scale, while the value labels are displayed in the original scale of the titer values (BAU/ml), The horizontal dotted line indicates a value of 260 BAU/ml, which is typically used as a threshold to define a positive response to the vaccine. **SLE**: Systemic Lupus Erythematosus; **RA:** Rheumatoid Arthritis; **B27-AS:** HLA-B27 positive Ankylosing Spondylitis; **PSA:** Psoriatic Arthritis; **GCA:** Giant Cell Arteritis; **GCs:** Glucocorticoids; **JAKi:** JAK inhibitors.



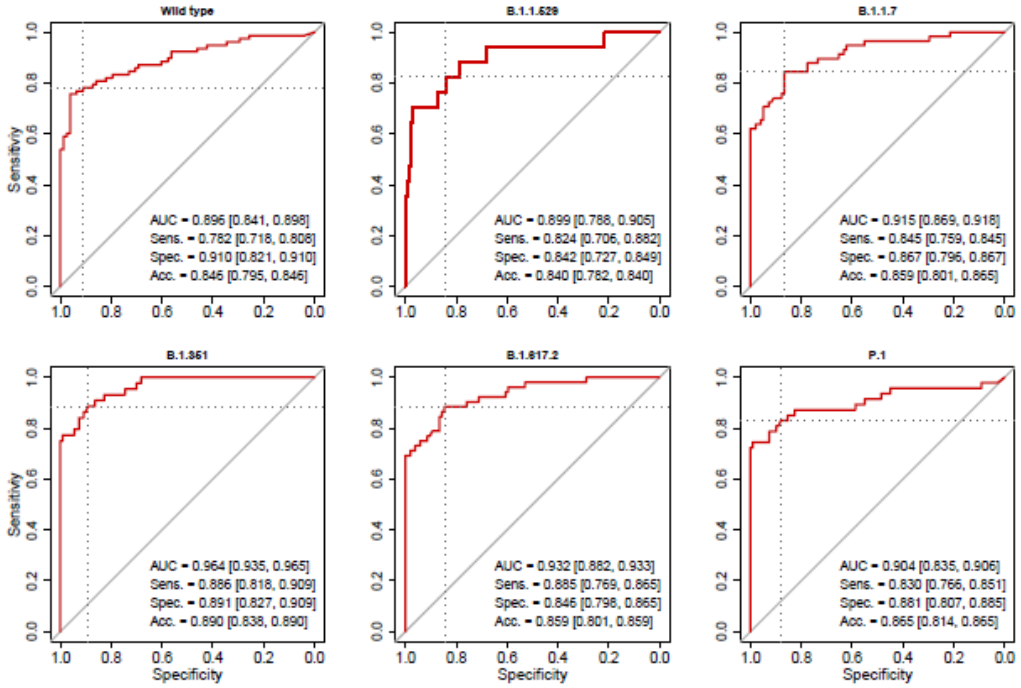
Suplementary Figure S2. Quantification of neutralizing antibodies for **B.1.1.7** (Alpha) variant of SARS-CoV-2 by treatment regimen before and after a third dose of mRNA-based SARS-CoV-2 vaccine. The dots show the quantitative estimations of neutralizing antibodies abundance of the patients' baseline and follow-up samples. Dot colors indicate the patients' rheumatic condition. Horizontal dotted lines display the threshold for no detection of antibody (**ND**) and positivity. **SLE**: Systemic Lupus Erythematosus; **RA:** Rheumatoid Arthritis; **B27-AS:** HLA-B27 positive Ankylosing Spondylitis; **PSA:** Psoriatic Arthritis; **GCA:** Giant Cell Arteritis; **GCs:** Glucocorticoids; **JAKi:** JAK inhibitors; **nABs:** neutralizing antibodies.



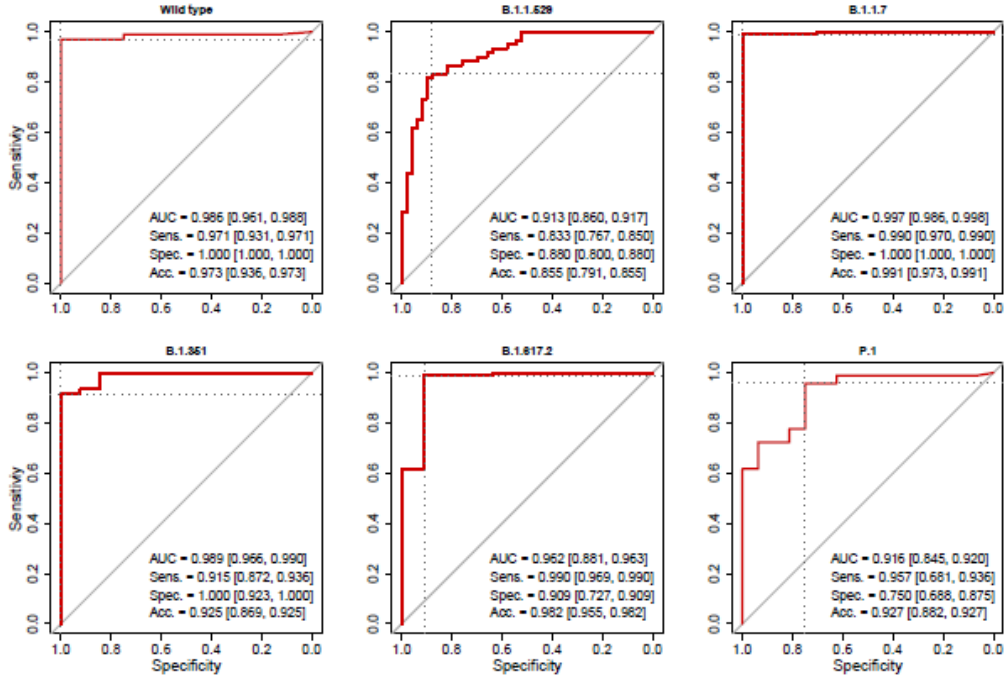
Suplementary Figure S3. Quantification of neutralizing antibodies for **B.1.351** (Beta) variant of SARS-CoV-2 by treatment regimen before and after a third dose of mRNA-based SARS-CoV-2 vaccine. The dots show the quantitative estimations of neutralizing antibodies abundance of the patients' baseline and follow-up samples. Dot colors indicate the patients' rheumatic condition. Horizontal dotted lines display the threshold for no detection of antibody (**ND**) and positivity. **SLE**: Systemic Lupus Erythematosus; **RA:** Rheumatoid Arthritis; **B27-AS:** HLA-B27 positive Ankylosing Spondylitis; **PSA:** Psoriatic Arthritis; **GCA:** Giant Cell Arteritis; **GCs:** Glucocorticoids; **JAKi:** JAK inhibitors; **nABs:** neutralizing antibodies.



Suplementary Figure S4. Quantification of neutralizing antibodies for **P.1** (Gamma) variant of SARS-CoV-2 by treatment regimen before and after a third dose of mRNA-based SARS-CoV-2 vaccine. The dots show the quantitative estimations of neutralizing antibodies abundance of the patients' baseline and follow-up samples. Dot colors indicate the patients' rheumatic condition. Horizontal dotted lines display the threshold for no detection of antibody (**ND**) and positivity. **SLE**: Systemic Lupus Erythematosus; **RA:** Rheumatoid Arthritis; **B27-AS:** HLA-B27 positive Ankylosing Spondylitis; **PSA:** Psoriatic Arthritis; **GCA:** Giant Cell Arteritis; **GCs:** Glucocorticoids; **JAKi:** JAK inhibitors; **nABs:** neutralizing antibodies.



Suplementary Figure S5. Concordance of anti-S protein and neutralizing antibodies in pre-third dose samples. Receiver Operating Characteristics (ROC) and their corresponding Area Under the Curve (**AUC**) assessing the ability of anti-S protein antibody titers to predict neutralizing antibody positivity. The legends show the values of AUC, sensitivity (**Sens.**), Specificity (**Spec.**) and overall acuraccy in predicting the variant positivity as well as their corresponding bootstrap 95% confidence intervals. Total accuracy, sensitivity, and specificity are displayed for the optimal threshold, defined as the ROC point closest to the top-left part of the plot (perfect sensitivity and specificity). **AUC:** Area Under the Curve; **Sens.:** sensitivity; **Spec.:** specificity; **Acc.:** accuracy.



Suplementary Figure S6. Concordance of anti-S protein and neutralizing antibodies in post-third dose samples. Receiver Operating Characteristics (ROC) and their corresponding Area Under the Curve (**AUC**) assessing the ability of anti-S protein antibody titers to predict neutralizing antibody positivity. The legends show the values of AUC, sensitivity (**Sens.**), Specificity (**Spec.**) and overall acuraccy in predicting the variant positivity as well as their corresponding bootstrap 95% confidence intervals. Total accuracy, sensitivity, and specificity are displayed for the optimal threshold, defined as the ROC point closest to the top-left part of the plot (perfect sensitivity and specificity). **AUC:** Area Under the Curve; **Sens.:** sensitivity; **Spec.:** specificity; **Acc.:** accuracy.

Table S1. Patients' characteristics by sample availability for analysis. The table presents the distribution of key parameters analyzed for all patients in our series (All, n = 157), those with pre- and post-third dose samples (Two samples available, n = 110), and those contributing to the study with an only sample collected before the third vaccine dose (One sample only, n = 47). For the later group, the table further provides descriptives for patients who failed to complete follow-up due to death (Exitus, n = 3), SARS-CoV-2 infection after the pre-third dose sample (COVID-19 infection, n = 17), and other reasons related to the study logistics (Logistics, n = 27). Cells display the medians and ranges for continuous variables (anti-S protein antibody titers and age), and the frequencies/percentages for categorical variables, including positivity for neutralizing antibodies (nABs) against diferents SARS-CoV-2 variants, such as: **Wild type, B.1.1.529** (Omicron), **B.1.1.7** (Alpha), **B.1.351** (Beta), **B.1.617.2** (Delta) and **P.1** (Gamma). The last column presents p-values from Mann-Whitney tests (continuous variables) of a Fisher's tests (categorical variables) comparing patients with and without second sample available for analyses. **SLE:** Systemic Lupus Erythematosus; **RA:** Rheumatoid Arthritis; **B27-AS:** HLA-B27 positive Ankylosing Spondylitis; **PSA:** Psoriatic Arthritis; **GCA:** Giant Cell Arteritis; **JAKi:** JAK inhibitors.

		All n=157	Two samples available n=110 (70.1%)	One sample only (pre-third dose)				P-value (Two vs One samples)
				Exitus n=3 (1.9%)	COVID-19 n=17 (10.8%)	Logistics n=27 (17.2%)	All n=47 (29.9%)	
Anti-S-protein antibody (BAU/ml)		537.0 (0.2, 87880.0)	618.0 (0.0, 85340.0)	233.0 (23.0, 1693.0)	295.0 (0.2, 8672.0)	332.0 (9.4, 87880.0)	295.0 (0.2, 87880.0)	0.135461
Wild type - Positive		78 (50.0%)	61 (56.0%)	1 (33.3%)	8 (47.1%)	8 (29.6%)	17 (36.2%)	0.035666
B.1.1.529 - Positive		17 (10.9%)	15 (13.8%)	0 (0.0%)	0 (0.0%)	2 (7.4%)	2 (4.3%)	0.097276
B.1.1.7 - Positive		58 (37.2%)	42 (38.5%)	1 (33.3%)	8 (47.1%)	7 (25.9%)	16 (34.0%)	0.718381
B.1.351 - Positive		44 (28.6%)	34 (31.8%)	1 (33.3%)	4 (23.5%)	5 (18.5%)	10 (21.3%)	0.245299
B.1.617.2 - Positive		52 (33.3%)	40 (36.7%)	1 (33.3%)	6 (35.3%)	5 (18.5%)	12 (25.5%)	0.198702
P.1 - Positive		47 (30.1%)	36 (33.0%)	2 (66.7%)	5 (29.4%)	4 (14.8%)	11 (23.4%)	0.258635
Sex - Female		100 (63.7%)	70 (63.6%)	0 (0.0%)	15 (88.2%)	15 (55.6%)	30 (63.8%)	>0.9999
Age at third dose of SARS-CoV-2 vaccine		58.8 (33.5, 88.2)	59.2 (33.5, 88.2)	68.7 (61.0, 83.8)	56.8 (41.7, 85.0)	58.2 (43.4, 79.6)	58.3 (41.7, 85.0)	0.557594
Rheumatic condition	LES	35 (22.3%)	20 (18.2%)	0 (0.0%)	9 (52.9%)	6 (22.2%)	15 (31.9%)	0.010199
	AR	36 (22.9%)	32 (29.1%)	0 (0.0%)	0 (0.0%)	4 (14.8%)	4 (8.5%)	
	EAB27	22 (14.0%)	17 (15.5%)	0 (0.0%)	2 (11.8%)	3 (11.1%)	5 (10.6%)	
	APSO	40 (25.5%)	23 (20.9%)	2 (66.7%)	5 (29.4%)	10 (37.0%)	17 (36.2%)	
	VGV	24 (15.3%)	18 (16.4%)	1 (33.3%)	1 (5.9%)	4 (14.8%)	6 (12.8%)	
Treatmen type	Not treated or Glucorticoids only	25 (15.9%)	18 (16.4%)	0 (0.0%)	3 (17.6%)	4 (14.8%)	7 (14.9%)	0.558644
	Immunosupressive	48 (30.6%)	30 (27.3%)	1 (33.3%)	9 (52.9%)	8 (29.6%)	18 (38.3%)	
	Biological	69 (43.9%)	50 (45.5%)	2 (66.7%)	4 (23.5%)	13 (48.1%)	19 (40.4%)	
	JAKi	15 (9.6%)	12 (10.9%)	0 (0.0%)	1 (5.9%)	2 (7.4%)	3 (6.4%)	
Glucocorticoids use		43 (27.4%)	31 (28.2%)	2 (66.7%)	5 (29.4%)	5 (18.5%)	12 (25.5%)	0.845809
mRNA-based vaccine type	mRNA-1273	100 (63.7%)	71 (64.5%)	2 (66.7%)	11 (64.7%)	16 (59.3%)	29 (61.7%)	0.856361
	BNT162b2	57 (36.3%)	39 (35.5%)	1 (33.3%)	6 (35.3%)	11 (40.7%)	18 (38.3%)	

Supplementary Table S2. Fold-changes (FC), boostrap 95% confidence intervals (CI) and p-values for comparison of treatment groups within each time point of sample extraction. P-values are derived from a Mann-Whitney's test. **JAKi:** JAK inhibitors; **GCs:** Glucocorticoids.

	Pre-3rd dose		Post-3rd dose	
	FC [95%CI]	**P-value**	**FC [95%CI]**	**P-value**
Non-biological - Not treated or GCs only	1.13 [0.43, 2.52]	0.8799	0.95 [0.39, 3.18]	0.9830
Biological - Not treated or GCs only	0.38 [0.14, 1.06]	0.0132	0.70 [0.31, 2.46]	0.5973
JAKi - Not treated or GCs only	0.70 [0.15, 2.14]	0.3565	0.56 [0.24, 2.26]	0.4718
Biological - Non-biological	0.34 [0.21, 0.72]	0.0038	0.73 [0.39, 1.49]	0.6837
JAKi - Non-biological	0.62 [0.18, 1.41]	0.3329	0.59 [0.30, 1.47]	0.2776
JAKi - Biological	1.85 [0.44, 3.85]	0.3032	0.80 [0.33, 2.21]	0.6304

Supplementary Table S4. Comparison of anti-S protein antibody titers between rheumatic condition groups within each time point of sample extraction. Cells show Fold-changes (FC), 95% confidence intervals (CI) and p-values of the corresponding comparison. FCs and p-values were derived from a mixed-effects linear model where sex, age, rheumatic condition, treatment type, use of glucocorticoids, type of vaccine, sample type (pre- or post-3rd dose) and time from previous dose where included as fixed effects, as well as the interaction between: sex and sample type; treatment and sample type; rheumatic condition and sample type; use of glucocorticoids and sample type; time from 2nd and third vaccine dose and sample type; glucocorticoids use and treatment regimen; and glucorticoids use and rheumatic condition. Individual effects were included as random effects in this model. **SLE:** Systemic Lupus Erythematosus; **RA:** Rheumatoid Arthritis; **B27-AS:** HLA-B27 positive Ankylosing Spondylitis; **PSA:** Psoriatic Arthritis; **GCA:** Giant Cell Arteritis; **JAKi:** JAK inhibitors; **GCs:** Glucocorticoids; **FC:** Fold-Change; **95%CI:** 95% confidence interval.

	Pre-3rd dose		Post-3rd dose	
	FC [95%CI]	**P-value**	**FC [95%CI]**	**P-value**
RA - SLE	3.31 [0.87, 12.60]	0.0788	2.58 [0.64, 10.43]	0.1844
B27-AS - SLE	3.21 [0.85, 12.13]	0.0850	1.34 [0.33, 5.51]	0.6860
PSA - SLE	8.27 [2.08, 32.80]	0.0027	3.74 [0.85, 16.59]	0.0821
GCA - SLE	5.14 [1.15, 22.94]	0.0317	1.31 [0.27, 6.50]	0.7373
B27-AS - RA	0.97 [0.20, 4.60]	0.9695	0.52 [0.10, 2.81]	0.4469
PSA - RA	2.50 [0.79, 7.93]	0.1208	1.45 [0.38, 5.52]	0.5830
GCA - RA	1.55 [0.38, 6.36]	0.5406	0.51 [0.11, 2.39]	0.3933
PSA - B27-AS	2.57 [0.57, 11.60]	0.2186	2.80 [0.53, 14.64]	0.22353
GCA - B27-AS	1.60 [0.31, 8.28]	0.57438	0.98 [0.16, 6.05]	0.9840
GCA - PSA	0.62 [0.15, 2.53]	0.5074	0.35 [0.07, 1.77]	0.2054

Supplementary Table S5. Increase of anti-S protein antibody levels after the third dose of the SARS-CoV-2 vaccine by treatment group. Cells show the Fold-changes (FC), bootstrap 95% confidence intervals (CI) and p-values comparing the the patients' titer levels after and before the administration of the third vaccine's dose. P-values are derived from a Wilcoxon test using only patients with both samples available. **JAKi:** JAK inhibitors; **GCs:** Glucocorticoids.

	FC [95%CI]	**P-value**
Not treated or GCs only	16.89 [3.97, 50.67]	0.0066
Non-biological	11.26 [5.63, 19.19]	< 0.0001
Biological	22.35 [8.78, 49.27]	< 0.0001
JAKi	10.51 [3.22, 32.23]	0.02686

Supplementary Table S6. Increase of anti-S protein antibody levels after the third dose of the SARS-CoV-2 vaccine by rheumatic condition. Cells show the Fold-changes (FC), bootstrap 95% confidence intervals (CI) and p-values comparing the the patients' titer levels after and before the administration of the third vaccine's dose. P-values are derived from a Wilcoxon test using only patients with both samples available.

	FC [95%CI]	P-value
Systemic Lupus Erythematosus	23.30 [7.21, 62.25]	0.0010
Rheumatoid Arthritis	19.20 [6.71, 38.39]	< 0.0001
HLA-B27 positive Ankylosing Spondylitis	17.45 [6.99, 35.33]	< 0.0001
Psoriatic Arthritis	18.28 [7.89, 41.83]	< 0.0001
Giant Cell Arteritis	18.07 [6.28, 80.06]	0.0373

Supplementary Table S7. Anti-S protein antibody levels by treatment group before (Pre-3rd dose) and after (Post-3rd dose) a third dose of the SARS-CoV-2 mRNA-based vaccine. Cells show the medians an ranges of anti-S protein antibody titers. P-values are derived from a Mann-Whiteny test comparing treatment groups within each sample time point. **JAKi:** JAK inhibitors; **GCs:** Glucocorticoids.

	Pre-3rd dose			**Post-3rd dose**		
	No GCs	**Gcs**	**P-value**	**No GCs**	**Gcs**	**P-value**
Not treated or GCs only	1522 [152, 83890]	300 [43, 20363]	0.0272	11712 [2037, 55080]	3881 [1002, 74400]	0.6272
Non-biological	996 [14, 9567]	218 [16, 40700]	0.0538	10338 [2246, 112590]	11643 [433, 33200]	0.8512
Biological	419 [0, 12069]	72 [0, 87880]	0.0402	10890 [0, 137780]	1447 [0, 23022]	0.0124
JAKi	882 [34, 85340]	274 [43, 967]	0.2030	10229 [1479, 166760]	4378 [393, 16823]	0.1495

Supplementary Table S8 Comparison of anti-S protein antibody titers between users and non-users of Glucocroticoids (GCs) within each rheumatic condition and time point of sample extraction. Cells show Fold-changes (FC), 95% confidence intervals (CI) and p-values of the corresponding comparison. FCs and p-values were derived from a mixed-effects linear model where sex, age, rheumatic condition, treatment type, use of glucocorticoids, type of vaccine, sample type (pre- or post-3rd dose) and time from previous dose where included as fixed effects, as well as the interaction between: sex and sample type; treatment and sample type; rheumatic condition and sample type; use of glucocorticoids and sample type; time from 2nd and third vaccine dose and sample type; glucocorticoids use and treatment regimen; and glucorticoids use and rheumatic condition. Individual effects were included as random effects in this model. **SLE:** Systemic Lupus Erythematosus; **RA:** Rheumatoid Arthritis; **B27-AS:** HLA-B27 positive Ankylosing Spondylitis; **PSA:** Psoriatic Arthritis; **GCA:** Giant Cell Arteritis; **JAKi:** JAK inhibitors; **GCs:** Glucocorticoids; **FC:** Fold-Change; **95%CI:** 95% confidence interval.

	Pre-3rd dose		**Post-3rd dose**	
	FC [95%CI]	**P-value**	**FC [95%CI]**	**P-value**
SLE	0.25 [0.05, 1.25]	0.0911	0.39 [0.07, 2.20]	0.2832
RA	0.46 [0.11, 1.89]	0.2837	0.71 [0.17, 2.91]	0.6384
PSA	0.85 [0.17, 4.24]	0.8435	1.31 [0.23, 7.47]	0.7639
CGA	0.25 [0.04, 1.69]	0.1563	0.39 [0.05, 2.82]	0.3494

Supplementary Table S9. Positivity of neutralizing antibodies by glucorcorticoids use in the overall series. Cells show, for different variants of the SARS-CoV-2, the absolute frequency and the percentage of positive immune response, and the p-value for the comparison of this frequencies derived from a exact Fisher's test. Variants of SARS-Cov-2 are: **B.1.1.529** (Omicron), **B.1.1.7** (Alpha), **B.1.351** (Beta), **B.1.617.2** (Delta) and **P.1** (Gamma). **GCs:** Glucocorticoids.

	Pre-3rd dose			Post-3rd dose		
	No GCs N(%)	**GCs N(%)**	**P-value**	**No GCs N(%)**	**GCs N(%)**	**P-value**
Wild type	84 (73.7%)	19 (45.2%)	0.0012	77 (97.5%)	28 (90.3%)	0.1349
B.1.1.529	12 (10.5%)	5 (11.9%)	0.7780	47 (59.5%)	13 (41.9%)	0.1359
B.1.1.7	47 (41.2%)	11 (26.2%)	0.0955	76 (96.2%)	24 (77.4%)	0.0050
B.1.351	37 (32.7%)	7 (17.1%)	0.0698	71 (93.4%)	23 (74.2%)	0.0097
B.1.617.2	43 (37.7%)	9 (21.4%)	0.0588	75 (94.9%)	24 (77.4%)	0.0109
P.1	38 (33.3%)	9 (21.4%)	0.1726	71 (89.9%)	23 (74.2%)	0.0670

Supplementary Table S10. Positivity of neutralizing antibodies by glucorcorticoids use in patients under biological treatment. Cells show, for different variants of the SARS-CoV-2, the absolute frequency and the percentage of positive immune response, and the p-value for the comparison of this frequencies derived from a exact Fisher's test. Variants of SARS-Cov-2 are: **B.1.1.529** (Omicron), **B.1.1.7** (Alpha), **B.1.351** (Beta), **B.1.617.2** (Delta) and **P.1** (Gamma). **GCs:** Glucocorticoids.

	Pre-3rd dose			Post-3rd dose		
	No GCs N(%)	**GCs N(%)**	**P-value**	**No GCs N(%)**	**GCs N(%)**	**P-value**
Wild type	34 (60.7%)	3 (25.0%)	0.0299	40 (95.2%)	5 (62.5%)	0.0242
B.1.1.529	2 (3.6%)	1 (8.3%)	0.4469	24 (57.1%)	1 (12.5%)	0.0488
B.1.1.7	17 (30.4%)	2 (16.7%)	0.4866	40 (95.2%)	4 (50.0%)	0.0039
B.1.351	10 (18.2%)	2 (16.7%)	0.9999	37 (90.2%)	4 (50.0%)	0.0171
B.1.617.2	14 (25.0%)	2 (16.7%)	0.7171	39 (92.9%)	4 (50.0%)	0.0085
P.1	12 (21.4%)	3 (25.0%)	0.7188	39 (92.9%)	3 (37.5%)	0.0012

Table S11. Distribution of biological treatments by use of Glucocorticoids (GCs).

	No GCs	GCs
anti-TFN	21 (87.5%)	3 (12.5%)
Baricitinib	0 (0.0%)	1 (100.0%)
Belimumab	3 (75.0%)	1 (25.0%)
IL-17	17 (94.4%)	1 (5.6%)
Mycophenolate mofetil	2 (100.0%)	0 (0.0%)
Rituximab	4 (100.0%)	0 (0.0%)
Sulfasalazine	0 (0.0%)	1 (100.0%)
Tocilizumab	10 (62.5%)	6 (37.5%)
Ustekinumab	1 (50.0%)	1 (50.0%)

5. RESUM GLOBAL DELS RESULTATS

Degut a les particularitats intrínseques de cada malaltia, els grups resultants presenten diferents característiques basals tant demogràfiques com de tractament. En LES, AR, i VGV la majoria de la població són dones, al contrari que en les espondiloartropaties (EA B27 i APSO). També observem diferències respecte l'edat dels pacients essent els que presenten VGV els de més edat. La utilització de glucocorticoides també difereix entre els grups, possiblement per un biaix en la prescripció segons cada entitat, i com és esperable, els pacients afectes de VGV presenten un percentatge major d'ús de glucocorticoides. Mantenint les mateixes proporcions observables en pràctica clínica, els pacients amb major percentatge de tractament biològic són el grup d'AR i APSO. Els dos treballs presenten homogeneïtat en el moment d'avaluació de la resposta immune entre la vacunació i l'extracció de les mostres tant per la segona com per la tercera dosi de vacuna: 5.3 (2.3, 7.6) i 3.8 (2.2, 7.0) mesos, respectivament.

En ambdós treballs publicats, els resultats obtinguts suggereixen que, independentment de la malaltia, els pacients que estan tractats amb fàrmacs biològics presenten una resposta vacunal menor que la resta d'individus. I aquesta encara és menor si associen tractament amb corticoides, inclús quedant un número significatiu de pacients per sota del límit d'anticossos que es considera protector cara a desenvolupar infecció. Aquests resultats s'observen tant pel número d'anticossos anti-proteïna S com d'anticossos neutralitzants i es calcula que, tant els pacients en tractament biològic com els que associen corticoides, presenten una resposta de més del 80% menor respecte els altres. Es cert però, que amb la 3a dosi de reforç, tots els grups augmenten la resposta i la majoria assoleixen nivells òptims. Tanmateix, aquests grups esmentats segueixen sent els que presenten una

pitjor resposta immune a la vacunació, mantenint una disminució de la resposta en el grup de tractament biològic associat a corticoides d'un 87%. Per altra banda, hem pogut observar que els pacients que únicament prenen corticoides, també presenten una resposta significativament disminuïda amb la segona dosi de la vacuna, tot i que aquesta diferència respecte als que no en prenen desapareix amb la dosi de reforç.

També vam analitzar la resposta vacunal davant de diferents variants - *Wild-tipe, alpha (B1.1.7), beta (B 1.351), delta (B 1.1.529), Omicron (B1.1.529), gamma (P.1)* – de les quals la resposta més alta de totes les variants va ser la wild-tipe, amb (50% pre-3ª dosis i 93% post 3a dosis amb diferències estadísticament significatives), amb un patró relativament similar en la resta de variant analitzades exceptuant en omicron. Per tant, la resposta més baixa va ser per la variant omicron (11% pre-3ª dosis i 55% post 3ª dosis també amb diferències significatives) que, com s'ha comentat, seria la que mostraria uns nivells de resposta immune diferencials respecte a la resta de variants.

6. RESUM GLOBAL DE LA DISCUSSIÓ

El nostre treball es focalitza en l'avaluació de la resposta immune a mig termini a la vacuna contra el SARS-CoV-2 en pacients amb malaltia reumàtica sistèmica autoimmune. El resultat principal del treball es basa en la detecció d'uns nivells menors de resposta immune en aquells pacients en règim de teràpia biològica i principalment, quan concomitantment reben glucocorticoides. Dels nostres resultats, podem també observar que aquest efecte de la teràpia biològica i dels glucocorticoides és independent de la malaltia i que la resposta a la vacuna inicial és menor en les noves variants. Igualment, s'objectiva una efectivitat adequada de l'estratègia de revacunació pel que fa a l'augment de pacients responedors a la vacunació, mesurada per anticossos neutralitzants i percentatge de seroconversió. Finalment, es posa de manifest que, l'efectivitat de la vacuna baixa dràsticament amb l'aparició de les noves variants, com passa amb Omicron.

Des de l'inici de la pandèmia per COVID-19, el risc d'infecció en pacients amb malalties autoimmunes ha estat motiu d'investigació. Existeixen diversos estudis prospectius que demostren que els pacients amb malalties autoimmunitàries presenten un risc augmentat d'infeccions, ja sigui per alteracions immunològiques derivades de la pròpia malaltia com pels fàrmacs immunosupressors utilitzats per tractar-les. Per tant, l'avaluació de la resposta immune a la vacuna SARS-CoV-2 en aquest col·lectiu de pacients és d'interès cara a plantejar la possibilitat d'estratègies diferencials de vacunació o protecció.

Una de les mesures principals per combatre infeccions és la prevenció mitjançant vacunes tant contra virus com bacteris. Les que actualment es troben disponibles

en pràctica clínica, han hagut de sotmetre's a múltiples estudis d'eficàcia i efectivitat. Tanmateix, com succeeix amb altres malalties, els pacients amb una alteració immunològica, com serien els individus afectes d'una malaltia inflamatòria autoimmune reumàtica, habitualment queden exclosos d'aquests estudis de registre per vacunes i, per tant, s'han de valorar específicament en estudis de pràctica clínica real, o tanmateix, fer-ne un seguiment més exhaustiu per avaluar la seva aplicabilitat i efectivitat en vida real. Actualment no hi ha suficient evidència o pràcticament no n'existeix sobre l'efecte que poden tenir aquestes malalties o els tractaments utilitzats en la resposta vacunal davant de les vacunes disponibles; i les guies o recomanacions es basen en consensos d'experts.

Com sabem, les malalties autoimmunes es caracteritzen per l'existència d'autoanticossos i reaccions inflamatòries perpetuades degut a la pèrdua de tolerància immune i un sistema immune desregulat, el que provoca dany i mal funcionament dels òrgans objectiu (83). Aquestes lesions mediades pel sistema immune també existeixen en la COVID-19 (84). La infecció per SARS-CoV-2 indueix reaccions immunes, que poden tenir implicacions importants en el desenvolupament d'estratègies de vacunació contra aquest virus. I és que l'acció de les cèl·lules T té un paper central en el control de la infecció per SARS-CoV-2. Les cèl·lules T CD4+ i CD8+ específiques d'antigen i les respostes d'anticossos neutralitzants tenen un rol protector contra el SARS-CoV-2, mentre que les respostes immunes adaptatives deteriorades com les que s'observen en les malalties autoimmunes, com la disminució de cèl·lules T naïves, poden conduir a resultats desfavorables de la malaltia (85, 86). Per tant, la resposta immune és una arma de doble tall en la COVID-19, amb resultats afectats pel grau de desequilibri

de citocines i activació de cèl·lules immunes. La producció i alliberament excessiu de citocines i quimiocines pro inflamatòries poden causar danys greus als òrgans en casos crítics, cosa que també s'observa en malalties autoimmunes (84).

Des de l'inici de la pandèmia de COVID-19 i l'aparició de les vacunes contra el virus SARS-COV2 per combatre-la, sembla que hi ha hagut un canvi de consciència sobre com podem protegir millor els nostres pacients davant de les infeccions, que representen una de les principals causes de morbi-mortalitat en aquesta població (87, 88); tot i que segueix sense haver-hi recomanacions consistents en aquest aspecte.

Una de les poblacions més àmpliament estudiades foren els pacients amb LES. Concretament, existeixen treballs previs amb mostres de fins a 226 pacients que confirmen pitjors resultats per la infecció per COVID-19 en individus amb aquesta malaltia. Especialment pel que fa a la necessitat l'hospitalització per infecció més greu (68,89,90). També n'hi ha d'altres que demostren que els pacients vacunats presenten menys incidència d'infecció només amb una dosi, encara que a curt termini (màxim 3 mesos) (88).

En base a aquesta informació prèvia de la revisió de la literatura, es dissenya el primer estudi centrat en avaluar la resposta immunològica mantinguda a mig termini, als 3-6 mesos de la segona dosi de vacuna mRNA contra la SARS-COV2, exclusivament en pacients amb LES. El disseny incloïa pacients en diferents línies

de tractament comparat amb controls sans, per determinar específicament la influència de la teràpia sobre aquesta resposta immune.

Els resultats mostren, com a primera dada interesant, uns nivells d'anticossos neutralitzants inferiors en els pacients amb LES respecte el grup control. Aquesta observació concorda amb altres publicacions prèvies, on s'objectiva una resposta disminuïda aproximadament en el 30% dels casos i inclús, en un 23%, presenten una resposta serològica indetectable (91). En el nostre cas, els pacients amb LES, van mostrar una reducció del 75% d'anticossos anti-S després de la segona dosi de vacuna respecte els controls. Amb l'administració de la tercera dosis, tot i que no tenim comparativa amb un grup control, els pacients amb LES augmenten de forma estadísticament significativa els nivells d'anticossos neutralitzants. Aquest patró de resposta augmentada després del booster és consistent amb estudis previs (92) i esperable amb l'estratègia de vacunació proposada. Així , pel que es pot considerar una estratègia acceptable en els pacients amb LES, que augmenta el nombre de pacients responedors.

Analitzant els pacients amb una resposta immune inferior, els individus que rebien glucocorticoides i fàrmacs biològics són els que van presentar una resposta a la vacuna més deficitària. En un estudi semblant al nostre, van obtenir resultats similars als presentats pel nostre grup. Els pacients amb LES evidenciaven uns nivells d'anticossos tant pre com post vacunació significativament menors que els controls. A més, es va associar una resposta encara pitjor en aquells pacients que rebien prednisona, MMF, combinació d'un FAME i prednisona i combinació de 2 FAMEs, mentre que els que van presentar l'augment més elevat d'anticossos van

ser aquells que prenien únicament antipalúdics o no prenien cap medicació (91). D'aquesta forma s'evidencia un impacte negatiu d'algunes medicacions en la resposta a la vacuna. En base a aquestes dades, una possible alternativa seria monitoritzar de forma més estreta a aquest grup de pacients en teràpia biològica i sobretot si prenen glucocorticoides de forma concomitant, per avaluar periòdicament la seva resposta a la vacuna i poder emetre recomanacions específiques, ja siguin en forma de calendari de vacunació diferencial, tenir-los en compte com a candidats per rebre tractaments en casos de contacte o infecció o per instaurar mesures preventives amb vacunació passiva .

Basant-nos en els resultats obtinguts en el primer treball, vam considerar d'interès seguir estudiant i analitzant de forma concreta, la resposta vacunal, però en aquest cas, incloent els pacients amb malaltia reumàtica. Per aquets motiu vam desenvolupar un segon estudi on s'analitza la resposta immune, també a mig termini, als 3-6 mesos de la vacunació, en pacients amb diferents malalties reumàtiques i diverses línies de tractament, cara a estudiar possibles especificitats per malaltia o tractament, i analitzar el possible impacte tant de la teràpia biològica com del tractament concomitant amb corticoides. De forma global, els resultats evidencien que els pacients en tractament biològic (especialment si s'associen a corticoides) presenten una resposta immune mesurada a través, tant de nivells d'anticossos neutralitzants com d'anticossos anti-proteïna S a mig termini, inferiors a la resta de pacients analitzats. De forma rellevant, aquesta tendència observada és independent de la malaltia avaluada. Aquesta observació va en concordança amb altres publicacions prèvies (93), que remarquen la importància d'alguns

tractaments antireumàtics més que de les pròpies malalties, en la resposta immune a la vacunació SARS-CoV-2. Un estudi previ on s'inclouen diverses malalties autoimmunes de diferents especialitats com serien des de connectivopaties, artritis inflamatòries, malalties inflamatòries intestinals o malalties neurològiques autoimmunes, va objectivar una bona resposta a 2 dosis de la vacuna mRNA de manera global. Tanmateix, aquells que es trobaven en tractament amb bloquejadors de cèl·lules B i glucocorticoides van presentar absència o un nivell disminuït de títols d'anticossos neutralitzants (94). Un altre estudi previ presenta resultats similars plantejant un potencial efecte negatiu en la resposta immune a la vacuna en els pacients en teràpia biològica (excepte el Rituximab que no ha estat inclòs en aquest anàlisi) i sobretot en els casos en combinació amb FAMEs sintètics. No van poder analitzar l'efecte dels corticoides de forma individualitzada degut al baix número de pacients que rebien aquesta teràpia (93). Com s'ha mencionat anteriorment, els glucocorticoides tenen un efecte immunosupressor tan quantitatiu com qualitatiu que pot augmentar el risc d'infeccions, especialment fúngiques i parasitàries (95). S'ha vist que el risc d'infecció augmenta amb la dosi i la duració del tractament i es manté baix en aquells pacients exposats a dosis baixes. Es postula que la presa dels glucocorticoides a la mínima dosi possible i a la nit, durant la fase de "turn-of" del TNF, podria disminuir el risc d'infeccions en aquests pacients (96).

Centrant-nos en el grup de fàrmacs biològics, la revisió de la literatura mostra que els més àmpliament estudiats són els anti-TNF. El TNF és una citocina essencial en la defensa de l'hoste, ja que en cas d'infecció, facilita la comunicació cel·lular (97). S'ha demostrat àmpliament el risc d'infeccions i sobretot el risc de reactivar

infeccions latents com el VHB i la tuberculosi, motiu pel qual es realitzen protocols de cribratge abans d'iniciar-los. De totes maneres aquesta troballa que implicaria una disminució de la resposta immune en pacients tractats amb antiTNF és inconsistent respecte a la infecció per SARS-CoV-2; tot i que la informació actual indicaria un risc augmentat en els pacients exposats.

Referent al rituximab, nosaltres no hem pogut comparar els tipus de biològic entre ells per qüestions de mida de la mostra, pel que hem considerat els fàrmacs biològics com a grup. La potència estadística analitzant els biològics per famílies no ens permetia extreure conclusions fermes; tot i que el rituximab apareixia com a clarament influent de forma negativa en la resposta vacunal. Basant-nos en la la literatura publicada, posiciona el el rituximab com el fàrmac biològic que té un efecte més deleteri en la resposta immunitària vacunal contra la SARS-COV-2 (98). En quant a la infecció per COVID, estudis que valoren la severitat de les infeccions en funció de les hospitalitzacions i les defuncions presenten resultats inconsistents tot i que la majoria coincideixen en que, dins dels biològics, els pacients amb Rituximab presenten pitjors resultats que la resta (99-101). De totes maneres, encara no s'han identificat de forma consistent quins serien els biològics més influents en la disminució de la resposta immune. En aquest sentit, la revisió de la literatura posiciona a tots els biològics com d'alt risc per evitar una resposta immune satisfactòria amb excepció dels inhibidors de la IL-17 (102, 103) . Tot i que en els nostres resultats s'evidencia clarament un efecte negatiu en la resposta immune a la vacuna SARS-CoV-2 en el grup dels fàrmacs biològics i no en els grups dels fàrmacs immunosupressors sintètics, per qüestions de mida de mostra i potència estadística no hem pogut analitzar individualment l'efecte d'aquests tractaments,

pel que no podem descartar algun pugui tenir un cert impacte negatiu, sobretot quan es prescriuen en combinació amb glucocorticoides. La revisió de la literatura posiciona el micofenolat mofetil (MMF) com a fàrmac amb un impacte negatiu respecte a la resposta vacunal (104), pel que caldria avaluar amb major deteniment els pacients sota aquesta teràpia.

Una de les troballes més rellevants del nostre estudi seria que, malgrat que altres estudis previs orienten a que són les dosis altes de corticoides les que es consideren un factor de risc per l'escassa resposta immune (101), els pacients de la nostra mostra prenien una mitjana de 3mg de metilprednisolona, i tot i així van resultar perjudicials en el desenvolupament d'una resposta immune òptima. En un altre estudi on es valoren específicament els possibles factors implicats en pacients que presenten una mala resposta a la vacunació, un 60.3% dels pacients que van presentar negativitat dels anticossos neutralitzants contra el SARS-COV-2, estaven reben glucocorticoides. De forma destacada, només una minoria (el 6.3%) de pacients estaven rebent dosis superiors a 5mg, el que suggereix, i concorda amb la nostra observació, que els glucocorticoides són un factor de risc de pobra resposta immunològica vacunal a mig termini independentment de la dosis pautada (104).

Si bé es cert que, la resposta augmenta amb la tercera dosi de reforç, inclús en aquells pacients en que presentaven nivells per sota del llindar de protecció després de la primera pauta de vacunació (primera dosis + record), els pacients amb els tractaments que acabem de mencionar (teràpia biològica i glucocorticoides)

segueixen mostrant una resposta inferior respecte la resta. Aquest augment global en la resposta immune a la vacuna, resulta ser del 43% al 59% en els nostres resultats. Es més, existeix evidència ferma publicada prèviament que demostra que, aquest augment de la resposta vacunal a conseqüència de la tercera dosi, s'associa a menor incidència i gravetat d'infecció per SARS-COV2, així com també menor hospitalització (105). Aquest fet confirmaria la utilitat i viabilitat de la pauta vacunal utilitzada a nivell global, però caldria mantenir amb especial vigilància a alguns pacients en concret, com serien sobretot els que reben teràpia biològica i/o glucocorticoides.

Com a característica diferencial del nostre treball, la majoria d'estudis publicats fins als moment de la notificació dels nostres resultats, valoren la resposta vacunal tant per la segona com per la tercera dosis en el moment immediat o a curt termini, dins de les primeres 4-6 setmanes post administració de la dosi de vacuna. En canvi, en els nostres estudis s'han extret les mostres al cap de 3-6 mesos de cada dosi de vacuna per poder estudiar la resposta mantinguda a mig termini, pel que alguns dels resultats es poden veure discordants per aquest motiu; però pensem que les nostres dades transmeten una idea millor del que pot succeir a llarg termini. De totes maneres, caldrà seguir investigant si aquesta és la millor estratègia per afrontar el risc d'infecció i quines serien les millors recomanacions pels pacients amb malalties reumàtiques autoimmunes.

6.1 Limitacions i fortaleses

La principal limitació del nostre estudi prové de la seva naturalesa observacional, que limita la capacitat d'establir causalitat. A més, els pacients van ser reclutats en un únic centre clínic, el que limita els tractaments dins de les línies de teràpia on els resultats, tot i que atractius, s'han d'interpretar amb precaució. Aquestes limitacions determinen la naturalesa exploratòria dels nostres descobriments fins que siguin confirmats en sèries més grans i independents de pacients. A més, i tot i que la infecció simptomàtica per SARS-CoV-2 va ser descartada per a tots els participants mitjançant entrevista i revisió de la història clínica, no es disposava d'estudis de serologia ni d'història d'anticossos anti-proteïna N per a aquests subjectes. Per tant, no podem descartar la inclusió inadvertida d'individus amb infecció prèvia asimptomàtica de COVID-19, una debilitat que comparteixen molts estudis anteriorment publicats.

Alguns dels tractaments considerats eren específics per a condicions reumàtiques en certa mesura, la qual cosa va dificultar el control estadístic dels possibles factors de confusió i discernir entre els efectes de la malaltia i la teràpia. Aquesta complexitat va reduir la mida efectiva de la mostra en certes comparacions i pot influir en la interpretació dels resultats, motiu pel que no hem pogut informar sobre especificitats de certs tractaments.

Malgrat aquestes limitacions, els nostres descobriments suggereixen que els règims de tractament tenen una major influència en la disminució de la resposta immunològica més que la malaltia subjacent en si. Per confirmar aquests resultats, els futurs estudis haurien d'adreçar aquestes limitacions incloent una mostra més

gran, tenint en compte una gamma més àmplia de condicions de les malalties reumatològiques i estandarditzant els règims de tractament.

D'altra banda, un notable punt fort del nostre estudi va ser l'avaluació realitzada aproximadament entre 3 i 6 mesos després de l'administració de la segona i tercera dosi de la vacuna. Aquest període prolongat ens va permetre avaluar la sostenibilitat de la resposta immunològica en pacients amb malalties autoimmunes i, per tant, la durabilitat de la protecció immune conferida per la vacunació contra el SARS-CoV-2 en aquesta població resultant també un fet original del nostre treball.

7. CONCLUSIONS

- L’augment de forma mantinguda dels nivells d’anticossos anti-proteïna S i anti- neutralitzants contra SARS-CoV-2 evidencia que l’administració d’una dosis de reforç (tercera dosi) és una estratègia globalment efectiva en pacients amb malaltia reumàtica autoimmune. .

- Els pacients en tractament biològic i, especialment associats a glucocorticoides són els que presenten una pitjor resposta immune a la vacuna SARS-CoV-2.

- Aquesta resposta disminuïda pot traduir un potencial augment del risc d’infecció en els pacients amb malaltia reumàtica autoimmune.

- La identificació de pacients amb una cobertura vacunal deficitària és d’interès per seleccionar candidats per recomanar calendaris de vacunació específics, per rebre de forma preferent tractaments antivirals en casos d’infecció confirmada i dissenyar estratègies de vacunació passiva en casos de disponibilitat.

- L’escassa cobertura de la variant Òmicron amb les vacunes actuals, genera la necessitat de continuar investigant per poder optimitzar l’estratègia de vacunació o trobar alternatives per englobar totes les variants.

8. LÍNEES DE FUTUR

En aquesta tesi doctoral s'ha treballat en la resposta vacunal davant el SARS-CoV-2 en un tipologia concreta de pacients, els que pateixen una malaltia autoimmune reumatològica. Inicialment, vam estudiar i analitzar els factors de pobra resposta en LES i seguidament en un grup més ampli de malalties. A dia d'avui, segueix mancant evidència sobre quina és la millor estratègia pel maneig d'aquests individus que considerem, segueixen essent de risc i mereixen una menció especial per part de les recomanacions establertes per les autoritats sanitàries. Per això, considerem que les investigacions futures haurien de centrar-se en augmentar l'evidència i definir sobre quins individus actuar. Per això, creiem que és necessari seguir investigant en la línia de:

- Valorar augmentar la mostra per poder realitzar comparacions entre tractaments i no solament entre grups de fàrmacs.

- Realitzar un anàlisi més precís sobre els potencials factors de risc de mala resposta, incloent el tractament concomitant, l'activitat de la malaltia, etc.

- Cercar biomarcadors implicats en la resposta immune per poder predir la resposta vacunal.

- En cas de confirmar-se les nostres hipòtesis futures, podríem establir pautes específiques segons malaltia i tractament (monitorització, espaiar dosi de fàrmac i vacuna, immunització passiva, etc.).

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