Safety of baricitinib in vaccinated patients with severe and critical COVID-19 sub study of the randomised Bari-SolidAct trial

Hans-Kittil Viermyr,[a,b,c,ay] Kristian Tonby,[b,d,ay] Erica Ponzi,[e,az] Sophie Trouillet-Assant,[f,g,az] Julien Poissy,[h,i,az] José R. Arribas,[j,k] Virginie Dyon-Tafani,[f] Maude Bouscambert-Duchamp,[l,m] Lambert Assoumou,[n] Bente Halvorsen,[a,b] Nuriye Basdag Tekin,[a,b] Alpha Diallo,[ap,ax] Lucie De Gastines,[ap,ax] Ludvig A. Munthe,[o,p,q] Sarah Louise Murphy,[a,b] Thor Ueland,[a,b,ao] Annika E. Michelsen,[a,b] Fridtjof Lund-Johansen,[o,q] Pål Aukrust,[a,b] Joy Mootien,[r] Benjamin Dervieux,[s] Yoann Zerbib,[t] Jean-Christophe Richard,[u,aq] Renaud Prével,[v,ar] Denis Malvy,[w] Jean-François Timsit,[x,as,at] Nathan Peiffer-Smadja,[y,au] Damien Roux,[z] Lionel Piroth,[aa,av] Hafid Ait-Oufella,[ab] Cesar Vieira,[ac] Olav Dalgard,[d,ad] Lars Heggelund,[ae,af] Karl Erik Müller,[ae,af] Jannicke Horjen Møller,[ag] Anders Benjamin Kildal,[ah,aj] Vegard Skogen,[ai,aj] Saad Aballi,[ak] Jonas Daniel Sjøberg Øgaard,[a,b] Anne Ma Dyrhol-Riise,[b,d] Anders Tveita,[a,b,c,al] Amin Alirezaylavasani,[o] Dominique Costagliola,[n] Yazdan Yazdanpanah,[am,aw,ap,ax] Inge Christoffer Olsen,[e,ba] Tuva Børresdatter Dahl,[a,b,ba] Hassen Kared,[o,p,q,bb] Aleksander Rygh Holten,[d,an,bb] and Marius Trøseid[a,b,c,bb,*]

[a]Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway
[b]Faculty of Medicine, Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[c]Section for Clinical Immunology and Infectious Diseases, Oslo University Hospital Rikshospitalet, Oslo, Norway
[d]Department of Infectious Diseases, Oslo University Hospital Ullevål, Oslo, Norway
[e]Department of Research Support for Clinical Trials, Oslo University Hospital, Oslo, Norway
[f]Centre International de Recherche en Infectiologie (CIRI), Université Claude Bernard Lyon 1, Inserm, U1111, CNRS, UMR5308, ENS Lyon, Lyon, France
[g]Joint Research Unit Hospices Civils de Lyon-bioMerieux S.A., Hôpital Lyon Sud, Pierre-Bénite, France
[h]Université Lille, Inserm U1285, CHU Lille, Pôle de Médecine Intensive-Réanimatin, CNRS, UMR 8576, France
[i]Université Lille, Unité de Glycobiologie Structurale et Fonctionnelle (UGSF), F-59000, Lille, France
[j]Infectious Diseases Unit, Internal Medicine Department, La Paz University Hospital, IdiPAZ, Madrid, Spain
[k]Centro de Investigación Biomédica en Red de Enfermedades Infecciosas (CIBERINFEC), Madrid, Spain
[l]Hospices Civils de Lyon, Laboratoire de Virologie, Institut des Agents Infectieux de Lyon, Centre National de Référence des Virus Respiratoires France Sud, F-69317, Lyon, France
[m]Université Claude Bernard Lyon 1, Virpath, CIRI, INSERM U1111, CNRS UMR5308, ENS Lyon, F-69372, Lyon, France
[n]Sorbonne Université, INSERM, Institut Pierre Louis d'Épidémiologie et de Santé Publique (IPLESP), Paris, France
[o]Department of Immunology, Oslo University Hospital, Oslo, Norway
[p]KG Jebsen Centre for B Cell Malignancies, Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[q]Precision Immunotherapy Alliance, Institute of Clinical Medicine, University of Oslo, Oslo, Norway
[r]Intensive Care Unit, Antibiotic Stewardship Team, Groupe Hospitalier Région Mulhouse et Sud Alsace, Mulhouse, France
[s]Infectious Diseases Unit, Groupe Hospitalier Région Mulhouse et Sud Alsace, Mulhouse, France
[t]Intensive Care Department, Amiens-Picardie University Hospital, Amiens, France
[u]Medical Intensive Care Unit, Hospices Civils de Lyon, Croix-Rousse Hospital - Université Lyon 1, Lyon, France
[v]CHU Bordeaux, Medical Intensive Care Unit, Bordeaux, France
[w]Department of Infectious and Tropical Diseases, University Hospital, UMR 1219 Inserm/EMR 271 IRD, University of Bordeaux, Bordeaux, France
[x]AP-HP, Bichat Hospital, Medical and Infectious Diseases ICU (MI2), F-75018, Paris, France
[y]Université Paris Cité, Inserm, IAME, Paris, 75018, France
[z]Université Paris Cité, AP-HP, Hôpital Louis Mourier, DMU ESPRIT, Service de Médecine Intensive Réanimation, Colombes, France
[aa]Infectious Diseases Department, University Hospital, Dijon, France
[ab]Service de Médecine Intensive-Réanimation, Hôpital Saint-Antoine, AP-HP, Sorbonne Université, Paris, F-75012, France
[ac]Centro Hospitalar Universitário de Lisboa Central, Hospital Curry Cabral, Department of Intensive Care Medicine - Lisbon, Portugal
[ad]Department of Infectious Diseases, Akershus University Hospital, Norway
[ae]Department of Internal Medicine, Drammen Hospital, Vestre Viken Hospital Trust, Norway
[af]Department of Clinical Science, Bergen Integrated Diagnostic Stewardship Cluster, Faculty of Medicine, University of Bergen, Bergen, Norway

*Corresponding author. Research Institute of Internal Medicine, Oslo University Hospital Rikshospitalet, Oslo, Norway.
E-mail address: marius.troseid@medisin.uio.no (M. Trøseid).
[ay]Shared first authorship.
[az]Shared second authorship.
[ba]Shared penultimate authorship.
[bb]Shared senior authorship.

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[ag]Department of Intensive Care Medicine, Stavanger University Hospital, Stavanger, Norway
[ah]Department of Anesthesiology and Intensive Care, University Hospital of North Norway, Tromsø, Norway
[ai]Department of Infectious Diseases, University Hospital of North Norway, Tromsø, Norway
[aj]Faculty of Health Sciences, UIT The Arctic University of Norway, Norway
[ak]Department of Infectious Diseases, Østfold Hospital Kalnes, Grålum, Norway
[al]Department of Internal Medicine, Bærum Hospital, Vestre Viken Hospital Trust, 1346, Gjettum, Norway
[am]Infectious and Tropical Diseases Department, Bichat - Claude Bernard Hospital, AP-HP Nord-Université Paris Cité, Paris, France
[an]Department of Acute Medicine, Oslo University Hospital, Oslo, Norway
[ao]Thrombosis Research Center (TREC), Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway
[ap]ANRS, Maladies Infectieuses Emergentes, F-75015, Paris, France
[aq]CREATIS INSERM 1044 CNRS 5220, Villeurbanne, France
[ar]Univ Bordeaux, Centre de Recherche Cardio-Thoracique de Bordeaux, Inserm UMR 1045, Bordeaux, France
[as]Université Paris-Cité, INSERM, F-75018, Paris, France
[at]OUCTOME REA Research Network, France
[au]Service de Maladies Infectieuses et Tropicales, Hôpital Bichat Claude Bernard, AP-HP, Paris, 75018, France
[av]INSERM CIC 1432, University of Burgundy, Dijon, France
[aw]IAME INSERM UMR 1137, Université Paris Cité, Paris, France
[ax]Institut National de la Santé et de la Recherche Médicale, INSERM, F-75013, Paris, France

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Summary

Background The Bari-SolidAct randomized controlled trial compared baricitinib with placebo in patients with severe COVID-19. A post hoc analysis revealed a higher incidence of serious adverse events (SAEs) among SARS-CoV-2-vaccinated participants who had received baricitinib. This sub-study aimed to investigate whether vaccination influences the safety profile of baricitinib in patients with severe COVID-19.

Methods Biobanked samples from 146 participants (55 vaccinated vs. 91 unvaccinated) were analysed longitudinally for inflammation markers, humoral responses, tissue viral loads, and plasma viral antigens on days 1, 3, and 8. High-dimensional analyses, including RNA sequencing and flow cytometry, were performed on available samples. Mediation analyses were used to assess relationships between SAEs, baseline-adjusted biomarkers, and treatment-vaccination status.

Findings Vaccinated participants were older, more frequently hospitalized, had more comorbidities, and exhibited higher nasopharyngeal viral loads. Baricitinib treatment did not affect antibody responses or viral clearance, but reduced markers of T-cell and monocyte activation compared to placebo (sCD25, sCD14, sCD163, sTIM-3). Age, baseline levels of plasma viral antigen, and several inflammatory markers, as well as IL-2, IL-6, Neopterin, CXCL16, sCD14, and suPAR on day 8 were associated with the occurrence of SAEs. However, mediation analyses of markers linked to SAEs, baricitinib treatment, or vaccination status did not reveal statistically significant interactions between vaccination status and SAEs.

Interpretation This sub-study did not identify any virus- or host-related biomarkers significantly associated with the interaction between SARS-CoV-2 vaccination status and the safety of baricitinib. However, caution should be exercised due to the moderate sample size.

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Keywords: SARS-CoV-2 vaccination; COVID-19; Serious adverse events; JAK/STAT-inhibitor; Immunomodulation; Baricitinib; Inflammation

Introduction

Severe and critical SARS-CoV-2 infection is characterized by a dysregulated immune response combined with excessive systemic inflammation and immune cell activation.[1] Immunomodulatory strategies to mitigate hyperinflammation were extensively investigated during the early phases of the pandemic. One of the promising repurposed drugs was baricitinib, an anti-inflammatory Janus kinase (JAK) inhibitor, approved for the treatment of rheumatoid arthritis.[2]

JAKs and signal transducers and activators of transcription (STATs) facilitate the intracellular propagation

Research in context

Evidence before this study

The Janus kinase inhibitor baricitinib is recommended by WHO guidelines for treatment of severe and critical COVID-19 but is not approved for this indication by the European Medicines Agency. A previous study reported a potential safety signal of baricitinib in vaccinated individuals with severe and critical COVID-19, with more serious adverse events (SAEs) in this population. We searched PubMed for the terms "vaccination, baricitinib, COVID-19" up to July 16th, 2024, identifying 82 studies, but with no reports on mechanistic data or biomarkers concerning the safety of baricitinib in vaccinated patients with COVID-19.

Added value of this study

Our data show that plasma levels of viral antigen, inflammatory markers, and higher age were associated with the occurrence of SAEs, whereas vaccination status was associated with higher age and shorter duration of symptoms. There was minimal overlap between the inflammatory markers related to SAEs and those regulated by baricitinib, and our data do not provide any mechanistic link between the safety of baricitinib and vaccination status.

Implications of all the available evidence

Our data, in light of existing evidence, do not suggest that vaccination status per se is a safety concern regarding baricitinib treatment for severe and critical COVID-19.

of cytokines and growth factor signals from the cell surface to the nucleus, inducing the transcription of genes involved in inflammation and immune response.[3] Accordingly, the inhibition of JAK/STAT signalling by baricitinib reduces the transcription of genes relevant to inflammation and immunity.[4] Additionally, baricitinib has been hypothesized to exert direct anti-viral effects by restricting receptor-mediated endocytosis of the virus. However, the *in vivo* relevance of the potential anti-viral effects remains debated.[2,5–8]

Seven randomized controlled trials (RCTs) have evaluated the efficacy of baricitinib in treatment of COVID-19 showing positive, but varying results,[9–16] with a meta-analysis demonstrating an overall beneficial efficacy of baricitinib for severe COVID-19.[12] However, the studies were heterogenic in terms of study population and endpoints and were mainly conducted in the early phases of the pandemic before widespread availability of COVID-19 vaccines. The World Health Organization (WHO), The National Institute for Health and Care Excellence (NICE) in the United Kingdom, and the Infectious Diseases Society of America (IDSA), included baricitinib in treatment guidelines for severe and critical COVID-19, but the drug has not been approved by the European Medicines Agency (EMA) for this indication.[17–20]

The Bari-SolidAct trial, a randomized, double-blind, placebo-controlled trial evaluated the efficacy and safety of baricitinib for severe or critical COVID-19, but was prematurely ended in March 2022 due to external evidence from other trials indicating survival benefit of baricitinib.[13] Bari-SolidAct found no significant effect on the primary endpoint (day 60 mortality) but could not conclude due to limited sample size (n = 275). However, a significant interaction was found between vaccination status and treatment allocation with more serious adverse events (SAEs), including respiratory complications, severe infections, and deaths, in vaccinated participants receiving baricitinib. Given the high COVID-19 vaccination coverage globally,[21] it is highly relevant to explore if baricitinib causes a safety concern in vaccinated patients with severe/critical COVID-19, or whether other underlying factors could explain these findings. This sub-study of the Bari-SolidAct trial applied biobanked material to investigate temporal profiles of antibody and inflammatory responses, as well as viral clearance from baseline to day 8 in relation to vaccination status, treatment allocation, and the occurrence of SAEs. We hypothesized that baricitinib treatment could interfere with the immunological response and/or viral clearance in a non-favourable way in vaccinated participants.

Methods

Study design and participants

This is an exploratory sub-study of the Bari-SolidAct trial, a randomized, double-blind, placebo-controlled trial evaluating the efficacy and safety of baricitinib in patients with severe or critical COVID-19.[13] Details regarding sample size determination, main trial outcomes, randomization procedures and statistical methods for the main study are given in the enclosed study protocol (Supplemental Appendix) as well as at EUClinicalTrials.eu. In brief, eligible participants were adults (>18 years), with SARS-CoV-2 infection confirmed by a polymerase chain reaction (PCR) test no more than 9 days prior, who were admitted to hospital with severe or critical COVID-19.[13] The Bari-SolidAct trial was designed as an adaptive clinical trial platform enabling participation at three different levels of commitment: Level 1 with clinical data without biobank, level 2 with plasma, serum, and nasopharyngeal samples and level 3 with addition of peripheral blood mononuclear cells (PBMCs) and PAXgene tubes for mRNA analysis. Study sites in Norway, France, Spain, and Portugal participated in level 2 biobanking, whilst level 3 was only performed at Norwegian hospitals.

Intervention and sampling

Participants were recruited between 3rd June 2021 and 7th March 2022, and randomly assigned in a 1:1 ratio to receive either 4 mg baricitinib once daily or matching placebo for up to 14 days, in addition to standard of care. This sub-study analysed non-random biobanked level 2 samples (plasma, serum, and nasopharyngeal samples) at baseline (n = 141), day 3 (n = 136) and day 8 (n = 93) after inclusion, as shown in the study flowchart (Fig. 1b). Sample size was determined by availability of samples. Level 3 samples (PBMC for flow cytometry and whole blood for mRNA analysis) were available in a subset of the study-cohort at baseline (n = 23) and follow-up (n = 20). All samples were collected according to predefined time points and protocols at eligible sites as previously described.[13] Nasopharyngeal viral loads and nucleocapsid antigen were analysed at the National Centre for Viral Respiratory Infections (Hospices Civils de Lyon, France), while flow cytometry, mRNA analysis, enzyme linked immunosorbent assay (ELISA) and serology were performed at Oslo University Hospital.

Inflammatory markers

Soluble inflammatory markers were measured in plasma using either ELISA or U-plex assays. Levels of sCD163, sCD14, sCD25, soluble T cell immunoglobulin and mucin domain-containing protein [sTIM]-3, CXCL-16, monocyte chemoattractant peptide [MCP]-1, soluble urokinase plasminogen activator receptor [suPAR], and B-cell activating factor [BAFF]) were measured in duplicate by ELISA using commercially available antibodies from R&D Systems (Minneapolis, MN, USA), and PeproTech (Rocky Hill, NJ, USA) for MCP-1. The assays were performed in a 384-well format using a combination of a SELMA pipetting robot (Jena, Germany), and a BioTek dispenser/washer (Winooski, VT, USA). Absorption was read at 450 nm with wavelength correction set to 540 nm using an EIA plate reader (BioTek). Neopterin were measured by a competitive EIA (IBL International GmbH, Hamburg, Germany). Intra- and inter-assay variations were <10%. Interleukin [IL]-2, IL-6, IL10, IL-22, interferon-gamma inducible protein [IP]-10, and granulocyte-macrophage colony-stimulating factor [GM-CSF] were measured by a U-plex assay (Mesoscale Diagnostics, Rockville, MD, USA) using a QuickPlex SQ120 according to instructions from the manufacturer. Further details on catalogue numbers and reagents are provided in the Supplementary Methods section.

SARS-CoV-2 serology

Antibodies to SARS-CoV-2 antigens were quantified using a multiplexed bead-based assay.[22] Briefly, serum diluted 1:1000 was incubated for 1 h with bead-based arrays containing full length spike protein (spike-FL) and the receptor-binding domain (RBD). The arrays were labelled with fluorescent anti-human IgG Fc and analysed by flow cytometry. Signal intensity was defined as the median fluorescence intensity (MFI) of beads coupled with viral proteins divided by the MFI of beads with no viral protein (relative MFI, rMFI). Neutralizing antibodies were measured by incubation of arrays with serum diluted 1:100, labelling with digoxigenin-conjugated ACE2 and fluorescent anti-digoxigenin. Anti-spike antibody levels were converted to binding antibody units (BAU)/ml as described in detail previously, while anti-nucleocapsid antibody levels are reported as rMFI.[22] Further details on catalogue numbers and reagents are provided in the Supplementary Methods section.

Virological markers

Plasma SARS-CoV-2 nucleocapsid antigen levels (viral Ag) were quantitatively detected using Single Molecule Array (Simoa) as described.[23] All samples were analysed using a Quanterix assay and HD- X Analyzer (Quanterix, Billerica, MA). Lower level of detection (LoQ) was 0.12 pg/ml, with a positivity threshold set at 2.69 pg/ml. Normalized nasopharyngeal viral loads were measured as previously described.[13]

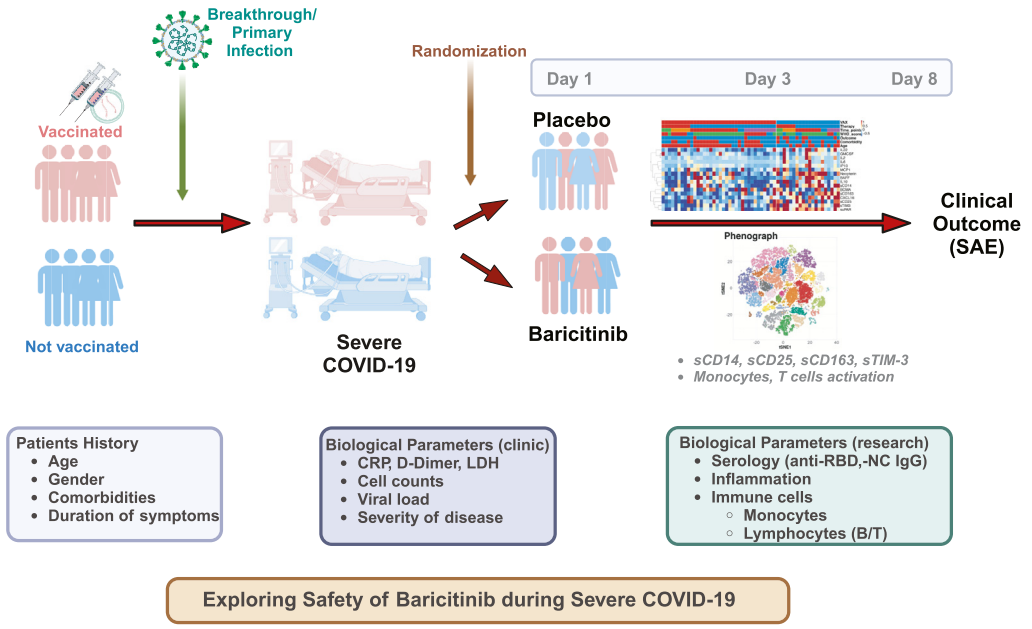
Isolation and sequencing of RNA

Total RNA was isolated from BD PAXgene™ Blood RNA tubes using MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Invitrogen™, Waltham, MA) following the manufacturer's instructions. To remove contaminated adapters and low-quality reads with phred score below 30 in the pair-end mode, the fastp (v0.23.0) was used.[24] Filtered reads were mapped to the human transcriptome (Gencode Human Release H37), and transcripts were quantified with 200 bootstrap iterations by Salmon (v1.5.2).[25,26] The Salmon outputs were summarized to gene-level and imported into DESeq2 (v1.34.0) via tximeta (v1.12.3).[27,28] DESeq2 was applied to assess differential gene expression. Haemoglobin mRNAs were removed both during library preparation, and bioinformatically for better accuracy before the analysis of differentially expressed genes.[29] Differentially expressed genes with more than 50 counts, a 2-fold change of more than ± 1 and a p-value <0.05 were uploaded to Metascape for gene annotation analyses.[30] The annotated gene ontology pathways were further analysed with Gene Set Enrichment Analysis (GSEA, fgsea 1.30.0).[31,32]

Flow cytometry

Thawed cryo-preserved PBMC samples (2×10^6) were transferred to a 96-well U-bottom plate. Cells were first stained with Fc block (BD Biosciences) for 5 min at room temperature, washed twice, and stained with Blue Live Dead (Thermo Fischer) for 10 min at room temperature. Cells were washed again and stained with pre-titrated antibodies according to manufacturer protocols: BUV805-Mouse Anti-Human CD14 (612902, clone

a



b

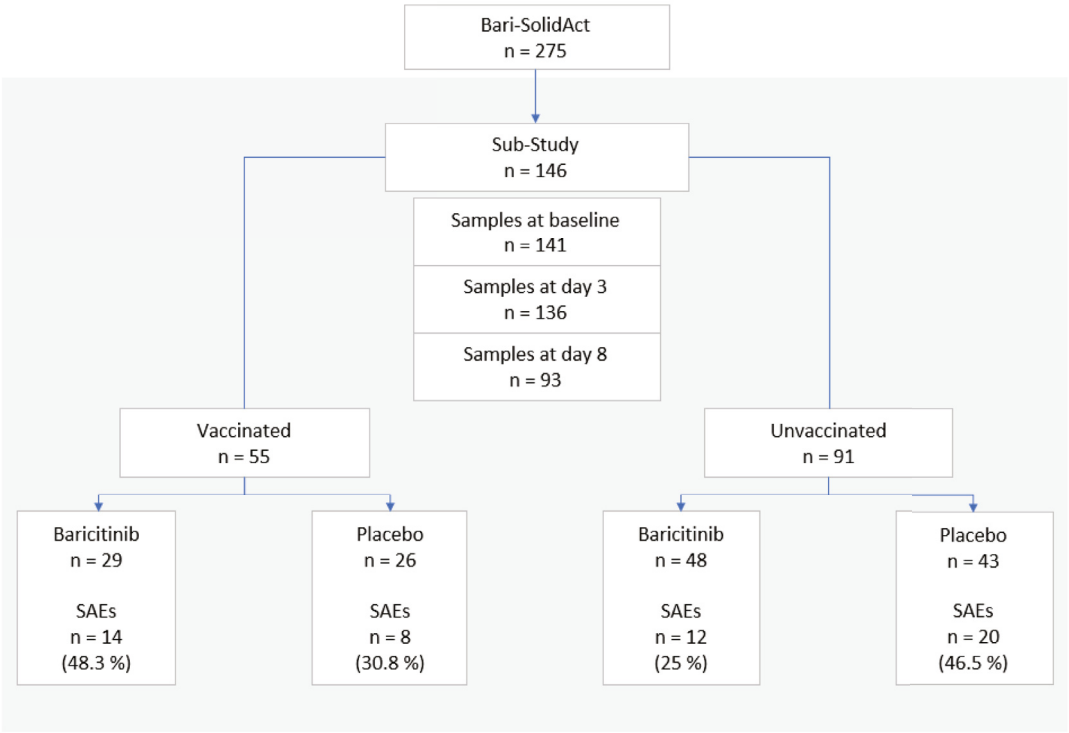


Fig. 1: **Study cohort and end points**. a. Graphical abstract of study cohort, workflow, and readouts. (NC, nucleocapsid; RBD, receptor binding domain; SAE, serious adverse events). b. Flow chart of the Bari-Solid Act's sub-study including availability of biobanked samples at baseline, day 3 and day 8.

M5E2), BD Biosciences, BUV737-Mouse Anti-Human CD38 (612824, clone HB7), BD Biosciences, BUV661-Mouse Anti-Human CD4 (612962, clone SK3), BUV615-Mouse Anti-Human CD56 (613002, clone NCAM16.2), BD Biosciences, BUV563-Mouse Anti-Human CD45RO (748369, clone UCHL1), BD Biosciences, BUV395-Mouse Anti-Human CD163 (745572, clone GHI/61), BD Biosciences, BV711-Mouse Anti-Human TIM-3 (565566, clone 7D3), BD Biosciences, BV750-Mouse Anti-Human CD8 (747097, clone SK1), BD Biosciences, BV786-Mouse Anti-Human CD57 (393329, clone QA17A04), BD Biosciences, BV650-Mouse Anti-Human CD161 (563864, clone DX12), BD Biosciences, BV605-Mouse Anti-Human CD127 (562662, clone HIL-7R-M21), BD Biosciences, BV570-Mouse Anti-Human CD3 (300436, clone UCHT1), BD Biosciences, BV480-Rat Anti-Human CXCR5 (566142, clone RF8B2), BD Biosciences, BV421-Mouse Anti-Human PD-1 (329920, clone EH12.2H7), BD Biosciences, A700-Mouse Anti-Human CD27 (565116, clone M-T271), BD Biosciences, APC-H7-Mouse Anti-Human HLA-DR (307618, clone L243), Biolegend, APC-Mouse Anti-Human CXCR3 (353712, clone G025H7), BD Biosciences, PE -Mouse Anti-Human pan-KIR (312708, clone DX9), PE-Cy-5-Mouse Anti-Human CD123 (551065, clone 9F5), PE-CF594-Mouse Anti-Human CD39 (567668, clone A1), BD Biosciences, PE-Cy-7-Mouse Anti-Human CCR6 (353418, clone GO34E3), BD Biosciences, PerCP-Cy5.5 Mouse Anti-Human CD16 (302027, clone 3G8), Biolegend, and BB515-Mouse Anti-Human CD25 (567318, clone BC96), BD Biosciences for 30 min on ice. Cells were fixed overnight in 1% PFA, and samples were acquired on BD FACSymphony (BD Biosciences).

Statistics

The distribution of continuous variables was assessed with histograms and the Shapiro–Wilk test. Demographics and baseline characteristics are presented with median and interquartile ranges (IQR) for continuous variables, and number and percentages, for categorical variables. Categorical data was compared using chi-square test while the Mann–Whitney U test was used for continuous data. Comparative analyses of anti-SARS-CoV-2 IgG antibody titers and inflammatory marker expression are presented by GraphPad Prism version 10.1. Differences between the control and test groups were initially tested using the Mann–Whitney U test for unpaired data and the Wilcoxon test for paired samples in the longitudinal follow-up. Linear models adjusted for age and sex were used to analyse the association between inflammatory markers, serology, viral antigen, and viral loads at baseline with vaccination status. For longitudinal measurements a linear mixed model was used with baseline, age, sex, time, vaccination status or treatment, and the interaction between time and vaccine and treatment as fixed effects, and with a random intercept by participant resulting in a compound symmetry covariance structure.

Correlations between continuous variables were assessed using the Spearman method. A correlation matrix was calculated comparing clinical and serological marker variables in a pairwise fashion, using the corr.test function from the psych CRAN package; the corrplot package was subsequently used to graphically display the correlation matrix, with p-values adjusted for multiple testing using the Bonferroni method. Spearman's correlation coefficients were indicated by a heat scale whereby the red colour indicates a positive correlation, and the blue colour indicates a negative correlation. The volcano plots and the correlation matrix were integrated as a package in CYTOGRAPHER®.

For the prediction of SAEs, a logistic model adjusted for age and sex was used. Mediation analyses were performed with any SAE as the outcome (adjusted for sex and age), nasopharyngeal viral load, viral Ag, and soluble markers of inflammation as mediators (adjusted for baseline level) and treatment and/or vaccination status as exposure. For the mediation analyses a probit model was used for the SAE outcome. Any two-sided p-value below 0.05 was considered statistically significant. Statistical analyses were performed using R version 4.3.1 and STATA version 18 for the mediation analyses.

Ethical considerations

The trial was conducted in accordance with ICH E6 (R2) Good Clinical Practice and the ethical principles of the Declaration of Helsinki. Informed consent by the study participant or legally authorised representative was given prior to inclusion in the study. The trial is accepted under the Clinical Trial Regulation (CTR), euclinicaltrials.eu (EU CT number 2022-500385-99-00).

Role of funders

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

Results

Clinical characteristics according to vaccination status

Baseline characteristics for the study participants (n = 146) are provided in Table 1, and the workflow of analyses is illustrated in Fig. 1a. Vaccinated participants (n = 55) were older (68 [IQR: 59.5–76.0] years vs. 55 [IQR: 45.0–64.5] years, $p < 0.0001$) and had more underlying comorbidities ($p = 0.028$), particularly pulmonary ($p = 0.014$) and cardiovascular ($p < 0.0001$) diseases. They also had lower levels of ferritin ($p = 0.0063$) and LDH ($p = 0.0018$). Furthermore, a significantly higher fraction of vaccinated participants (40%, 22/55) had less than 7 days of symptoms before treatment randomization compared to non-vaccinated

participants (16.5%, 15/91) (p = 0.0015). Consistent with results from the main study,[13] vaccinated participants treated with baricitinib had a higher proportion of SAEs (48%, 14/29) compared to unvaccinated participants (25%, 12/48) treated with baricitinib (Fig. 1b). A detailed description of SAEs according to treatment and vaccination status is provided in Supplementary Table S1. Among the vaccinated participants, six had received one dose, 42 had received two doses, and three had received three doses. Of these, 36 had received an mRNA vaccine, 15 had received a viral vector vaccine, and for the remaining four participants, the number and type of vaccine was unknown.

Serology, virology and inflammatory markers according to vaccination status

As shown in Fig. 2a, most vaccinated participants had detectable baseline anti-RBD SARS-CoV-2 IgG antibody levels within the expected range of responders (green, >2000 BAU/ml), and the few non-responders (white, <200 BAU/ml), at baseline had levels within or close to the responder range by day 8. In contrast, anti-nucleocapsid IgG antibody titers were lower in vaccinated compared to non-vaccinated participants at baseline but increased to almost similar levels by day 8 (Fig. 2b). In accordance with this, and as shown in a volcano plot representing clinical laboratory data from the entire cohort, the main difference between vaccinated and non-vaccinated participants was related to serological responses, with an increase of anti-spike and -RBD IgG antibodies in vaccinated participants and of anti-nucleocapsid IgG antibodies in non-vaccinated participants at all time points (Fig. 2c).

To further explore immunological differences between vaccinated and unvaccinated participants, we extracted RNA from whole blood and performed RNA sequencing from a subset of participants with available samples (n = 23). At baseline, there was a total of 55 differentially expressed genes (DEGs) between vaccinated and unvaccinated participants. Of these DEGs, 8 were upregulated, and 47 were downregulated in vaccinated compared to unvaccinated participants. Pathway analysis revealed that the genes that were most differentially expressed between vaccinated and unvaccinated participants were related to adaptive immune responses, in particular immunoglobulin production (Fig. 2d and e).

In line with the results from the main study,[13] vaccinated participants had significantly higher baseline nasopharyngeal viral loads (p = 0.014). However, vaccination status did not affect the concentration of SARS-CoV-2 viral antigen levels in plasma. Both nasopharyngeal viral loads and plasma viral antigen decreased from baseline to almost non-detectable levels at day 8 regardless of the vaccination status (Fig. 2f and g).

We further assessed whether vaccination status influenced the overall inflammatory response at baseline. No significant differences were detected in the concentration of circulating inflammatory or immunoregulatory cytokines, chemokines, soluble markers of innate immunity, or indirect soluble markers of T cell and monocyte activation (Supplementary Fig. S1). However, network analyses of soluble inflammatory, and immune markers combined with the identification of immune cells (detected by flow cytometry), revealed specific correlation between inflammatory markers and subsets of immune cells in vaccinated participants at baseline (Supplementary Fig. S2). The potential cellular sources of the released surface markers, and the correlation between cells and secreted proteins are described in Supplementary Fig. S3.

	Vaccinated (n = 55)	Unvaccinated (n = 91)	p-value
Age (years), median (IQR)	68.0 (59.5–76.0)	55.0 (45.0–64.5)	<0.0001
Country, n/N (%)			0.034
France	23/55 (41.8)	49/91 (53.9)	
Norway	24/55 (43.6)	39/91 (42.9)	
Others	8/55 (14.6)	3/91 (3.3)	
Male gender, n/N (%)	41/55 (74.6)	69/91 (75.8)	0.86
Comorbidities, n/N (%)			
Any comorbidity	47/55 (85.5)	63/91 (69.2)	0.028
Obesity	17/55 (30.9)	34/91 (37.4)	0.67
Hypertension	30/55 (54.6)	17/91 (18.7)	<0.0001
Diabetes	16/55 (29.1)	15/91 (16.5)	0.071
Pulmonary disease	18/55 (32.7)	14/91 (15.4)	0.014
Cardiovascular disease	21/55 (38.2)	9/91 (9.9)	<0.0001
WHO score, n/N (%)			0.12
6	49/55 (89.1)	72/91 (79.1)	
7–9	6/55 (10.9)	19/91 (20.9)	
Days from symptom onset before randomization, n/N (%)			0.0015
≤7	22/55 (40)	15/91 (16.5)	
≥8	33/55 (60)	76/91 (83.5)	
Remdesivir	1/55 (1.8)	0/91 (0.0)	0.20
Systemic steroids	49/55 (89.1)	79/91 (86.8)	0.95
Biochemistry, median (IQR)			
Lymphocyte count (10^9 cells/L)	0.8 (0.5–1.1)	0.8 (0.5–1.0)	0.87
Neutrophil count (10^9 cells/L)	6.1 (4.1–9.8)	6.2 (4.3–8.1)	0.76
CRP (mg/L)	103.5 (57.4–188.5)	90 (45.9–130.0)	0.14
Ferritin (ng/ml)	868.5 (440.3–1473.3)	1380 (876.0–2010.0)	0.0063
LDH (U/L)	376.5 (265.3–471.0)	440 (351.0–563.5)	0.0018

Demographic, clinical, and biochemical characteristics of 146 patients hospitalized for COVID-19 according to SARS-CoV-2 vaccination status. LDH, Lactate dehydrogenase; CRP, C-reactive protein.

Table 1: **Baseline demographics and clinical characteristics.**

Baricitinib reduces markers of T-cell and monocyte activation but does not interfere with antibody response or viral clearance

Given the lower levels of anti-nucleocapsid IgG antibodies in vaccinated participants at baseline, we specifically assessed whether baricitinib could interfere with the development of the anti-SARS-CoV-2 serological

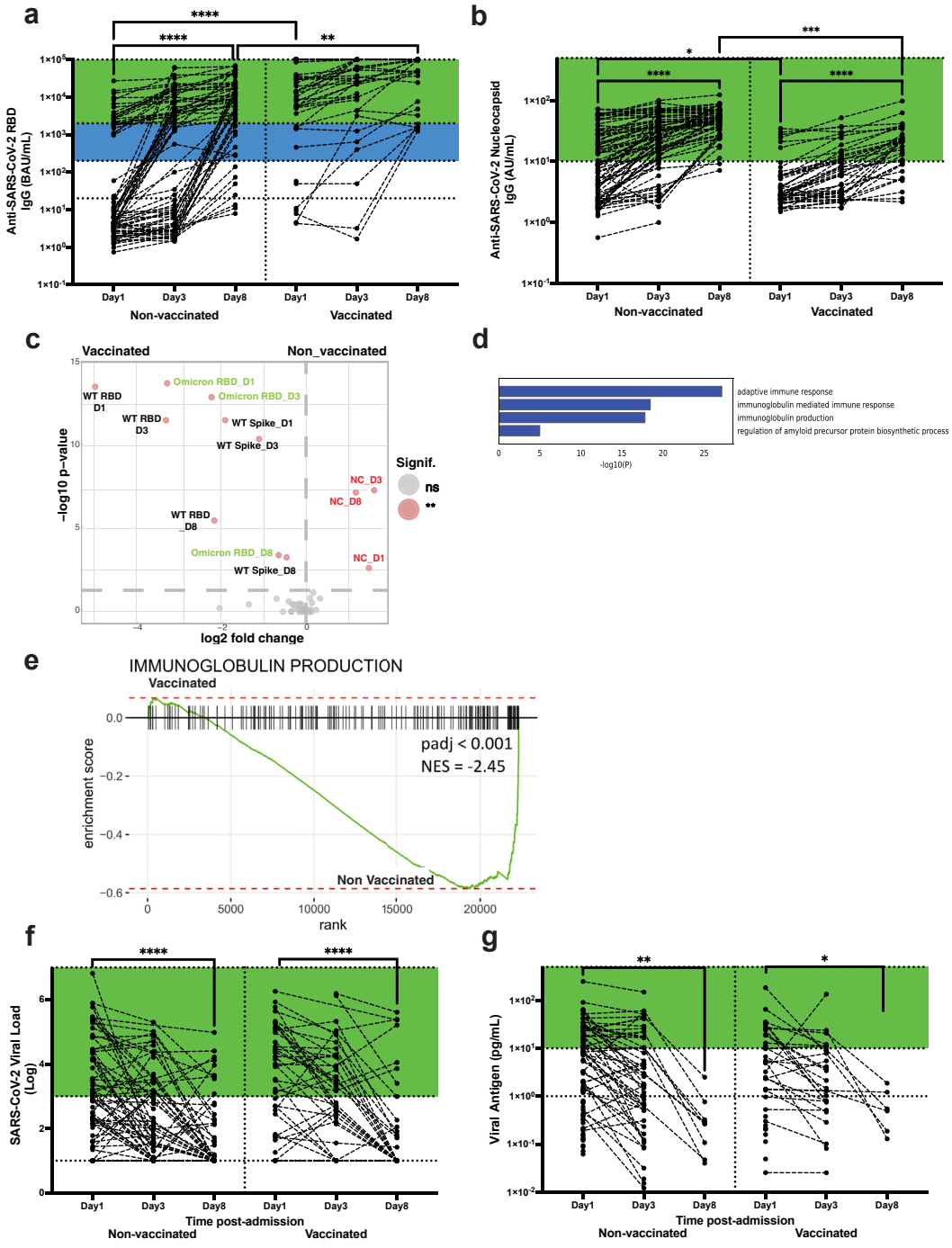


***Fig. 2:* Serology response and viral clearance according to vaccination status**. a. Longitudinal follow-up of anti-SARS-CoV-2 RBD IgG and (b) of anti-SARS-CoV-2 nucleocapsid IgG titers during COVID-19 hospitalization. Green area delimits high serological response against SARS-CoV-2 with a cut-off of 2000 BAU/mL for anti-SARS-CoV-2 RBD IgG and 10 AU/mL for anti-NC IgG. Blue area delimits intermediate anti-RBD IgG response (200–2000 BAU/mL). c. Specific humoral signature of vaccinated patients with COVID-19. Visualization by volcano plots of the significant clinical and serological parameters detected longitudinally in vaccinated and non-vaccinated COVID-19 hospitalized patients. Significance levels from the Mann–Whitney tests are indicated: ** for $p < 0.01$. (WT, wild type; RBD, receptor binding domain; NC, nucleocapsid, D1-D3-D8 represent baseline, day 3 and day 8 respectively. d. Functional analysis of whole blood transcriptome comparing differentially expressed genes (DEGs) between vaccinated and unvaccinated patients with COVID-19 at admission using Metascape, revealing Gene Ontology

responses. The relative increase of both anti-spike- and anti-nucleocapsid IgG antibodies was however comparable between the two treatment groups from baseline to day 8 (Fig. 3a and b), irrespective of vaccination status (Supplementary Fig. S4). Previous studies have suggested that baricitinib could have a direct anti-viral effect,[2,5–8] we therefore investigated viral clearance through the measurement of both plasma SARS-CoV-2 viral antigen and nasopharyngeal viral load. The kinetics of these viral biomarkers were similar throughout the first 8 days regardless of treatment allocation and vaccination status (Supplementary Fig. S4).

The main effect of baricitinib was the regulation of pro-inflammatory molecules including the T-cell activation marker soluble (s) CD25,[33] as visualized in the volcano plots in Fig. 3c. We identified the cellular source of sCD25 in participants with available cellular samples by flow cytometry staining of PBMCs. This revealed the regulation of T cell activation by baricitinib, as shown by the reduced quantity of CD4 T cells expressing CD25. We also found reduced expression of CD38 on both CD4 and CD8 T cells, as well as B cells (Fig. 3d).

Baricitinib also affected the myeloid cell population. The soluble markers of monocyte activation (sCD14, sCD163, and sTIM3) were lower in the baricitinib group at day 8 compared with the placebo arm (Fig. 3c and Supplementary Fig. S5A). The concentration of suPAR, which has been identified as an early predictor of severe COVID-19,[34] was also lower in the baricitinib arm compared with placebo at day 3 and 8 (Fig. 3c and Supplementary Fig. S5A). In baseline-adjusted mixed model analyses, the effect of baricitinib on all the measured biomarkers of inflammation revealed no significant differences between vaccinated and unvaccinated participants (Fig. 3e and Supplemental Fig. S5B). Cellular network analyses also revealed immunomodulating activity of baricitinib, including stronger correlations between various immune cell subsets in participants treated with baricitinib compared with placebo (Supplementary Fig. S6).

Inflammatory markers and plasma viral antigen associate with SAEs

Several demographic, clinical, and biochemical factors differed significantly between vaccinated and unvaccinated participants at baseline (Table 1). In a multivariate logistic regression analysis including these variables, age showed a significant effect on SAEs ($p = 0.014$), while no effect was found for treatment arm, vaccination status, comorbidity, duration of symptoms before randomization or country. Given this finding, we adjust all models for age and sex. Participants who experienced at least one SAE were on average 6.2 years older than those who did not experience any SAEs (median 64 [IQR 55–72] years vs. 57 [IQR 47–66] years, $p = 0.0003$). Although the presence of a comorbidity was not identified as an independent predictor of SAEs ($p = 0.099$), participants with comorbid conditions had significantly higher levels of several inflammatory markers compared to those without comorbidities (Supplementary Fig. S7). Several inflammatory markers clustered according to occurrence of SAEs (sTIM-3, suPAR, Neopterin, sCD14), whereas no obvious clustering was observed according to vaccination status, treatment allocation or detectable viral loads at baseline (Fig. 4a). In a model adjusted for age and sex, the baseline values of plasma viral antigen, and the concentration of sCD25, sTIM-3, suPAR, Neopterin, IP-10, sCD14, IL-22, D-dimer, and LDH were associated with occurrence of SAEs (Supplementary Table S2 and Supplementary Fig. S8). In contrast, there was no association between baseline levels of anti-SARS-CoV-2 IgG antibodies and the occurrence of SAEs.

We next investigated whether the temporal profiles of viral and inflammatory markers could be related to occurrence of SAEs. As shown in Fig. 4b, several inflammatory markers, in particular IL-6, CXCL16, and suPAR, had markedly increased levels at day 8 in participants experiencing SAEs. Potential interaction between the inflammatory molecules and the resulting networks are depicted in Fig. 4c to highlight correlations associated with SAEs at day 8.

Targeted mediation analysis of biomarkers does not explain the interaction between vaccination status and baricitinib on the occurrence of SAEs

Finally, to investigate if the occurrence of SAEs could be mediated through changes in the biomarkers associated with SAEs, baricitinib treatment or vaccination status, we performed targeted mediation analyses on baseline-adjusted changes in sCD25, sTIM-3, and suPAR (associated with SAEs and baricitinib treatment), IL-6, CXCL-16 (increase in levels associated with SAEs), plasma viral antigen (associated with SAEs), and nasopharyngeal viral load (associated with vaccination status). The outcome was the occurrence of any SAE adjusted for sex and age. However, we could not demonstrate that any statistically significant interactions between vaccination status and the occurrence of SAEs were mediated by changes in any of these markers (Supplementary Table S3).

pathways related to adaptive immunity to be the most regulated between the two groups (GO:0002250, GO:0016064, and GO:0002377). e. Gene Set Enrichment Analysis demonstrating that transcripts related to Immunoglobulin production was significantly enriched in the non-vaccinated patients with COVID-19 compared to the vaccinated, f. Nasopharyngeal and (g) Plasma SARS-CoV-2 viral loads in patients with COVID-19 during hospitalization according to vaccination status. Green area represents values of detected viral loads and antigenemia above the median value. Significance levels from the Wilcoxon tests are indicated: *, **, **** for $p < 0.05$, $p < 0.01$, and $p < 0.0001$ respectively.

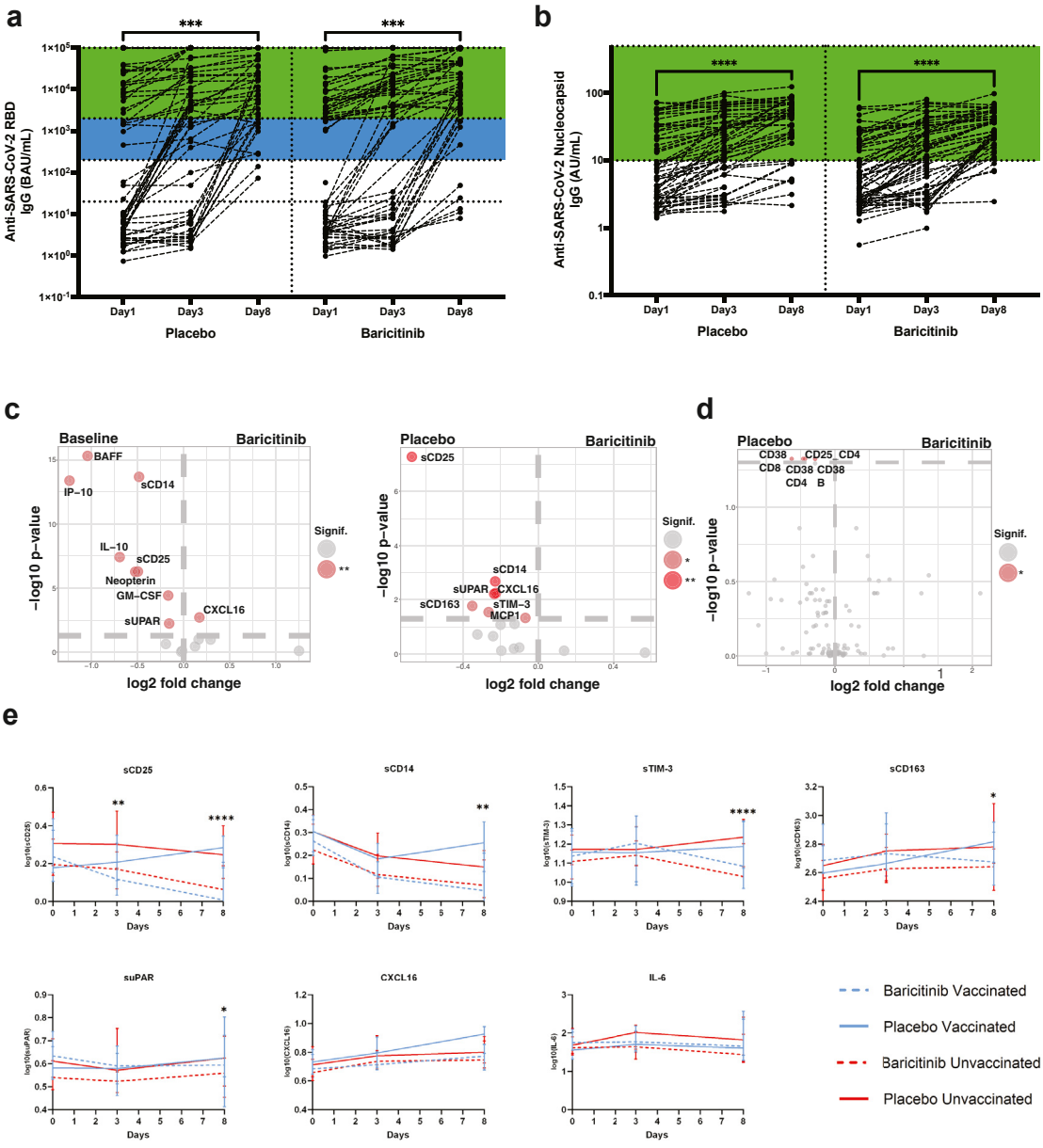


***Fig. 3*: The effect of baricitinib on serology response and inflammation**. a. Longitudinal follow-up of anti-SARS-CoV-2 RBD IgG and (b) of anti-SARS-CoV-2 nucleocapsid IgG titers during COVID-19 hospitalization. Green and blue areas delimit serological responses as in Fig. 2a and b. c. Specific inflammatory signature of baricitinib-treated patients with COVID-19. Visualization by volcano plots of the significant inflammatory molecules detected longitudinally in placebo and baricitinib-treated COVID-19 hospitalized patients. Significance levels from the Mann–Whitney tests are indicated: *, ** for $p < 0.05$ and $p < 0.01$. d. Specific cellular signature of baricitinib-treated patients with COVID-19. Visualization by volcano plots of the significant cellular subset's enrichment in placebo vs. baricitinib-treated COVID-19 hospitalized patients. The frequencies of immune cells were assessed by flow cytometry on longitudinally cryo-preserved PBMCs. Significance levels from the Mann–Whitney tests are indicated: * for $p < 0.05$. e. Longitudinal follow-up of pro-inflammatory molecules in study participants stratified according to the treatment allocation and vaccination status. Red and blue lines represent unvaccinated and vaccinated patients respectively, with stipulated lines for baricitinib-treated patients, and solid lines for placebo-treated patients. Data are represented as medians and interquartile ranges (IQRs). Statistical analysis was performed by comparing baseline adjusted serum concentration in mixed models in participants receiving baricitinib or placebo on day 3 and day 8, and represented with * $p < 0.05$, **$p < 0.01$, ****$p < 0.0001$.

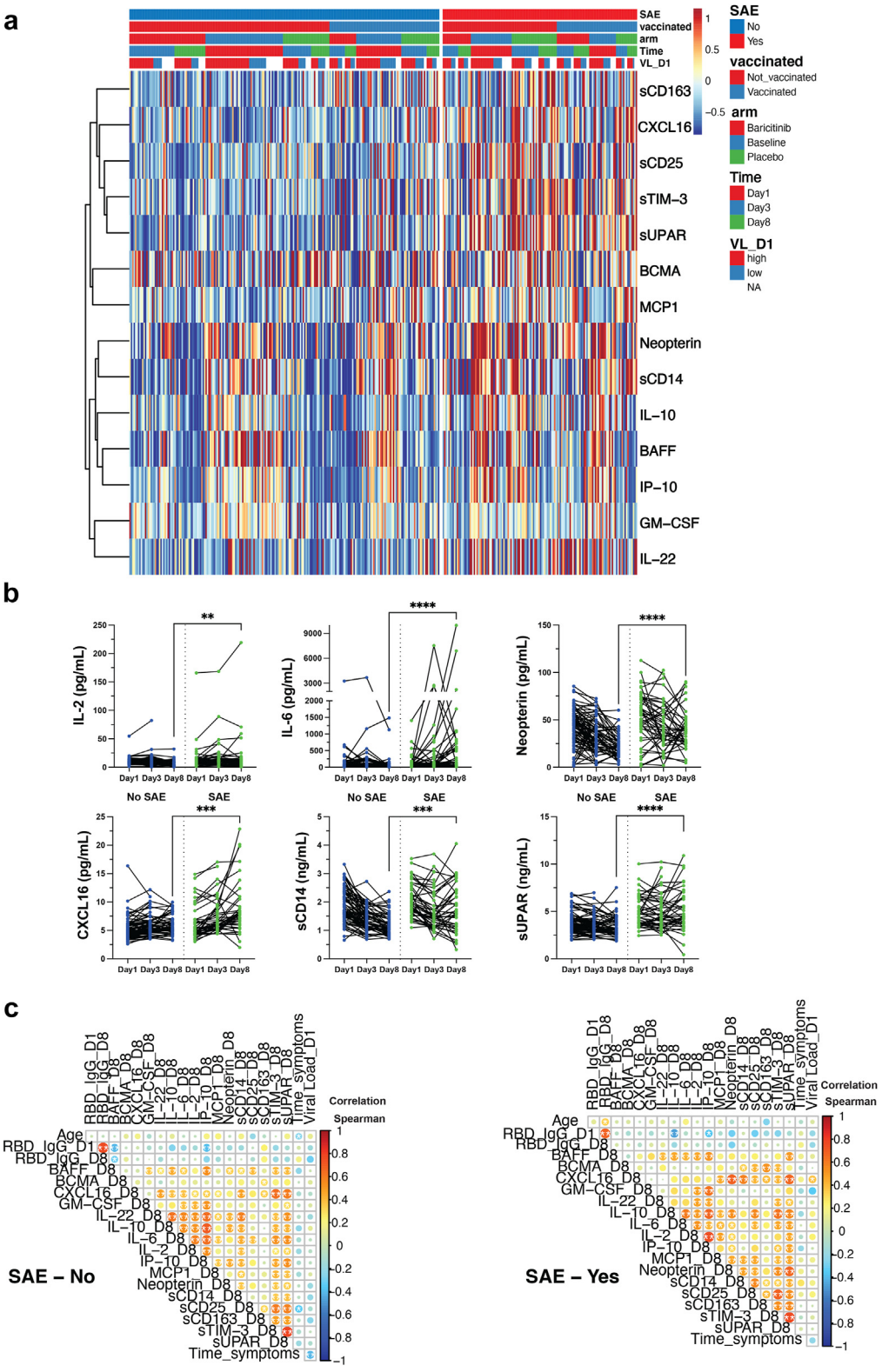


Fig. 4: **Inflammatory markers and SAE**. a. Proteomic signature of SAE after severe COVID-19. Visualization by a heat map of normalized inflammatory markers according to the occurrence of SAE, vaccination status, treatment arm, time-point, and nasopharyngeal SARS-CoV-2 viral

Discussion

In this sub-study of the randomized clinical trial Bari-SolidAct, we investigated a potential safety signal in vaccinated participants treated with baricitinib,[13] by exploring host and viral factors in relation to vaccination status, treatment allocation and occurrence the of SAEs. We report the following key findings:

I) Vaccinated participants were older, had more comorbidities, and exhibited a stronger humoral response at baseline (higher titers of anti-RBD IgG but lower levels of anti-nucleocapsid IgG antibodies), with no significant differences in pro-inflammatory cytokine concentrations. II) Baricitinib treatment did not influence anti-SARS-CoV-2 antibody responses or viral clearance but significantly reduced systemic inflammation, as reflected by decreased peripheral T-cell and monocyte activation markers, and suPAR concentration, a general marker of inflammation. III) Age, plasma viral antigen levels, and several inflammatory markers were associated with the occurrence of SAEs. IV) There was minimal overlap between inflammatory markers associated with SAEs and those regulated by baricitinib, and none of the investigated biomarkers in our mediation analysis could link baricitinib or vaccination status to the occurrence of SAEs.

Previous studies investigating baricitinib in COVID-19 did not include detailed data on cytokine concentrations.[9–12,14–16] Our study demonstrates a significant decrease in markers of T-cell and monocyte activation (sCD25, sCD14, sTIM-3, sCD163) and suPAR levels[33] in participants receiving baricitinib. This aligns with previous *in vitro* studies from healthy donors and patients with rheumatoid arthritis, showing dose dependent JAK-STAT inhibition and T cell modulation by baricitinib.[35] Notably, significantly higher baseline levels of sCD25, suPAR, and sTIM-3 were observed in participants who later developed SAEs. Inflammatory markers not regulated by baricitinib, including IL-6 and CXCL-16, were also associated with the occurrence of SAEs. Mediation analysis did not show that the occurrence of SAEs was mediated through changes in any of these markers. Nonetheless, our findings demonstrate broad anti-inflammatory effects of baricitinib in patients hospitalized due to COVID-19.

Age and comorbidities differed significantly between vaccinated and unvaccinated participants. This may partly reflect the early prioritization of elderly individuals with underlying conditions in vaccination programs to reduce deaths and disease burden caused by the pandemic.[36] Notably, more than 90% of participants in this sub-study were enrolled from September 2021 onward, by which time most should have been prioritized for vaccination with at least one dose.[21,37] Despite this, the majority of participants in the study were not vaccinated. As expected, and consistent with previous studies, vaccinated participants displayed higher titers of anti-RBD IgG antibodies at baseline and throughout the follow-up period.[38,39] However, vaccinated participants had lower levels of anti-nucleocapsid IgG antibodies, and differentially regulated expression of genes in PBMC related to immunoglobulin production at baseline. This finding may reflect previous SARS-CoV-2 infection in some unvaccinated individuals.[40] Alternatively, it could be attributed to immune imprinting which may favour anti-RBD over anti-nucleocapsid IgG production in certain vaccinated participants,[41,42] or to immunosenecence in older participants, which could affect overall production of antibodies.[43] Nonetheless, the increase in anti-nucleocapsid IgG antibodies from baseline to day 8 was similar in vaccinated and non-vaccinated participants. Additionally, 40% of the vaccinated participants entered the trial after ≤7 days of symptoms, allowing less time for de-novo antibody production. Short symptom duration was associated with higher nasopharyngeal viral load, suggesting that these individuals entered the trial in an earlier phase of the disease, with possible ongoing viral replication.

The association between nasopharyngeal viral loads and clinical outcomes has been widely investigated with conflicting results.[44–48] Recently, a large prospective cohort study (n = 2043) showed that elderly patients had significantly elevated peak viral loads, and together with patients with underlying health conditions, they had a protracted course of viral clearance compared to younger patients.[39] Additionally, findings from the ACTIVE/TICO trial showed that elevated plasma levels of viral nucleocapsid antigen were associated with mortality in patients with severe COVID-19, especially those with elevated inflammatory markers and high need of respiratory support.[49] In line with this, participants in the Bari-SolidAct trial who experienced SAEs (including deaths) during the trial, had significantly higher baseline levels of viral nucleocapsid antigen.

Differences in antiviral therapy may have influenced the risk-benefit-ratio of baricitinib in previous trials, as only 3% of participants in the Bari-SolidAct trial[13] received remdesivir at study inclusion compared to

load at baseline (VL_D1). Serological inflammatory markers were automatically clustered according to their co-expression. (SAE, serious adverse events; VL, viral load; NA, not available) b. Longitudinal inflammatory profiles of serum from patients with/without SAE during COVID-19 hospitalization. Significance levels from the Mann–Whitney tests are indicated: **, ***, **** for $p < 0.01$, $p < 0.001$ and $p < 0.0001$ respectively. c. Clinical and inflammatory networks of SAE during severe COVID-19. The correlogram described the significant correlation between clinical (age, anti-RBD IgG, duration symptoms, and viral load) and inflammatory markers detected in patients with COVID-19, with or without SAE. Pearson correlations with FDR are indicated with * for $p < 0.05$ and ** for $p < 0.01$.

19% in the COV-BARRIER trial,[10] 20% in the RECOVERY trial,[12] and 100% in the ACTT-2 trial.[9] This may be particularly important for patients with a short duration of symptoms and a high viral load. Although baricitinib has been suggested to exert antiviral effects,[5,6] it showed no effect on viral clearance measured by nasopharyngeal viral RNA levels or plasma viral antigen levels in the present study.

While our study utilizes valuable biobanked material from the Bari-SolidAct trial, it has several limitations. This sub-study investigates a safety signal that was identified post-hoc in the Bari-SolidAct trial, which was terminated before reaching its estimated sample size. The seriousness of the finding (increased incidence of SAEs among vaccinated participants treated with baricitinib) necessitated further investigation, despite the inherent risk of Type I errors when conducting multiple comparisons and post-hoc subgroup analyses. While our investigation was comprehensive, the initial sample size limitation is further enhanced by our reliance on non-random inclusion of samples from a subgroup of the original participants, resulting in a moderate sample size. Furthermore, the limited availability of samples for mRNA analysis and flow cytometry, the absence of lower respiratory tract samples, and our selection of candidate biomarkers, may have left some relevant mechanisms unexplored, limiting the robustness of our conclusion. Moreover, the groups of vaccinated and unvaccinated participants differ in key risk factors, such as age and comorbidities, likely to be important explanatory elements for the main outcome. Although adjustments were made for age differences, the imbalance may still affect the generalizability of our findings to all vaccinated individuals treated with baricitinib, necessitating cautious interpretation of our results. Furthermore, both outcomes and measured variables may have been influenced by the effects of systemic corticosteroids, which were given to most participants in accordance with current treatment guidelines, as well as rescue therapy with tocilizumab or increased dose of corticosteroids administered to 27% of participants.

In conclusion, the increased incidence of SAEs among vaccinated participants treated with baricitinib in the Bari-SolidAct could potentially have caused a serious safety concern underlining the importance of this study. However, our data did not provide a mechanistic explanation for the potential interaction between SARS-CoV-2 vaccination status and baricitinib treatment on the incidence of SAEs in the Bari-SolidAct trial, which could have occurred by chance. Although caution should be taken due to moderate sample size, our findings are in line with a recently submitted individual participant data meta-analysis of JAK inhibitors for COVID-19 (preprint), finding no safety signal in vaccinated participants.[50]

Contributors

HKV, KT, HK, ARH, and MT wrote the first draft of the article. MT, ICO, JAR, LA, and DC wrote the protocol with contribution from other members of the writing committee of Bari-SolidAct. EP, HKV, HK, and ICO performed statistical analyses, with input from DC and LA. AD and LDG were responsible for pharmacovigilance and recording of serious adverse events. Figures included in the article were made by HK, HKV, TBD, and SLM. YY, MT, ICO, JP, DC, LAM, and HK obtained funding for the study. STA, VDT, and MB performed virological analyses. BH and NBT organized the biobanking. SLM, JDSØ, HKV, and TBD performed and interpreted mRNA analyses. TU, PA, BH, and AEM planned and performed ELISA and other analyses of soluble inflammatory markers. FL-J was responsible for serological analysis. JM, BD, YZ, J-CR, RP, DM, J-FT, NP-F, DR, LP, HA-O, CV, OD, LH, KEM, JHM, ABK, VS, SA, AMD, and AT included study participants and biobanked material. LAM, AA, and HK planned and performed flow cytometry analyses. HKV, EP, ICO, and HK have accessed and verified the underlying data. All authors edited the manuscript for important intellectual content. All authors gave final approval of the version to be published and had final responsibility for the decision to submit for publication.

Data sharing statement

Deidentified, individual participant data, along with a data dictionary describing variables in the dataset, will be made available to researchers whose proposed purpose of use is approved by the EU-SolidAct Trial Steering Committee. To request the dataset, please address directly to the corresponding author (marius.troseid@medisin.uio.no) or to Inge Christoffer Olsen (i.c.olsen@medisin.uio.no) to obtain a data access form. All requests will be evaluated by the Trial Management Team and the EU-SolidAct Trial Steering Committee. For accepted requests, data will be shared after signing a data transfer agreement with the study sponsor. Data will be shared via a secure online procedure. Related documents, such as the study protocol, statistical analysis plan, and informed consent form, will be made available (with publication) on request to the corresponding author. The data will be open access for the informed consent form, protocol, and statistical analysis plan.

Consent for publication

Not applicable.

Declaration of interests

MT has been a pro bono member of the scientific advisory board for Lilly. JP reports lecture fees from Gilead, Shionogi, and Mundipharma, as well as payment for expert testimony from Gilead, Shionogi, Eumedica, and Pfizer, and support for attending meetings from Gilead, and Shionogi. ARH reports personal fee from Pfizer (2021) for lectures outside the submitted work. RP reports personal fees from MSD (2024) for one lecture and from Gilead (2023) and Pfizer (2023) for congress attendance. LAM reports Helse Sør-Øst UiO and Research Council of Norway grant for developing cellular analyses of COVID-19 (2020–2022), grant from KG Jebsen Stiftelsen, and grant from The Coalition for Epidemic Preparedness Innovation to monitor immune responses in patients (2021–2023). DC reports personal fees from Pfizer (2022) for a lecture outside the submitted work. BD reports support from Amgen for congress attendance. J-FT report honoraria from Shionogi, Merck, Pfizer, and Advanz as well as participation in advisory board for Gilead, Merck, Menarini, and Biomerieux. AEM reports stocks in Pfizer. LP reports honoraria from Gilead, GSK, Moderna, Pfizer, and ViiV Healthcare, as well as support for attending meetings from MSD and Pfizer. YZ reports payments for lectures from Gilead, Shionogi, and Mundipharma, payment for expert testimony from Gilead, Shionogi, Eumedica, and Pfizer, as well as support for attending meetings from Gilead and Shionogi. MB reports support for attending meetings from Gilead and Shionogi. JM reports support for attending meetings from Pfizer and Menarini as well as participation in advisory board for MSD.

All other authors have nothing to declare.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105511.

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