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COVID-19 vaccine-induced antibody and T cell responses in immunosuppressed patients with inflammatory bowel disease after the third vaccine dose: a multicentre, prospective, case-control study

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Abstract:	Background COVID-19 vaccine-induced antibody responses are reduced in patients with inflammatory bowel disease (IBD) taking anti-TNF or tofacitinib after two vaccine doses. We sought to determine whether immunosuppressive treatments were associated with reduced antibody and T cell responses after a third vaccine dose. Methods 352 adults (72 healthy controls and 280 IBD) were sampled 28-49 days after a third dose of SARS-CoV-2 vaccine. IBD medications studied included thiopurines (n=65), infliximab (n=46), thiopurine/infliximab combination therapy (n=49), ustekinumab (n=44), vedolizumab (n=50) or tofacitinib (n=26). SARS-CoV-2 spike antibody binding and T cell responses were measured. Findings Geometric mean [geometric SD] anti-S1 RBD antibody concentrations increased in all groups following a third dose, but were significantly lower in patients treated with infliximab (2736.8 U/mL [4.3]; P<0.0001), infliximab and thiopurine combination (1818.3 U/mL [6.7]; P<0.0001) and tofacitinib (8071.5 U/mL [3.1]; P=0.0018) compared to controls (16774.2 U/ml [2.6]). There were no significant differences in anti-S1 RBD antibody concentrations between control subjects and thiopurine (12019.7 U/mL [2.2]; P=0.099), ustekinumab (11089.3 U/mL [2.8]; P=0.060), nor vedolizumab treated patients (13564.9 U/mL [2.4]; P=0.27). In multivariable modelling, lower anti-S1 RBD antibody concentrations were independently associated with infliximab (Geometric mean ratio 0.15, 95% CI 0.11-0.21, P<0.0001), tofacitinib (0.52, 95% CI 0.31-0.87, P=0.012) and thiopurine (0.69, 95% CI 0.51-0.95, P=0.021), but not with ustekinumab (0.64, 95% CI 0.39-1.06, P=0.083), or vedolizumab (0.84, 95% CI 0.54-1.30, P=0.43). Previous SARS-CoV-2 infection (1.58, 95% CI 1.22-2.05, P=0.00056) and older age (0.88, 95% CI 0.80-0.97, P=0.0073) were independently associated with higher and lower anti-S1 antibody concentrations respectively. Antigen specific T cell responses were similar in all groups, except for recipients of tofacitinib without evidence of previous infection, where T cell responses were significantly reduced relative to healthy controls (p=0.021). Interpretation A third dose of COVID-19 vaccine induced a boost in antibody binding in immunosuppressed patients with IBD, but these responses were reduced in patients taking infliximab, infliximab/thiopurine combination and tofacitinib. Tofacitinib was also associated with reduced T cell responses. These findings support continued prioritisation of immunosuppressed groups for further booster dosing, particularly those on anti-TNF and Janus Kinase (JAK) inhibitors. Funding Financial support was provided as a Research Grant by Pfizer Ltd.

COVID-19 vaccine-induced antibody and T cell responses in immunosuppressed patients with inflammatory bowel disease after the third vaccine dose: a multicentre, prospective, case-control study

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Abstract

Background

COVID-19 vaccine-induced antibody responses are reduced in patients with inflammatory bowel disease (IBD) taking anti-TNF or tofacitinib after two vaccine doses. We sought to determine whether immunosuppressive treatments were associated with reduced antibody and T cell responses after a third vaccine dose.

Methods

352 adults (72 healthy controls and 280 IBD) were sampled 28-49 days after a third dose of SARS-CoV-2 vaccine. IBD medications studied included thiopurines (n=65), infliximab (n=46), thiopurine/infliximab combination therapy (n=49), ustekinumab (n=44), vedolizumab (n=50) or tofacitinib (n=26). SARS-CoV-2 spike antibody binding and T cell responses were measured.

Findings

Geometric mean [geometric SD] anti-S1 RBD antibody concentrations increased in all groups following a third dose, but were significantly lower in patients treated with infliximab (2736.8 U/mL [4.3]; P<0.0001), infliximab and thiopurine combination (1818.3 U/mL [6.7]; P<0.0001) and tofacitinib (8071.5 U/mL [3.1]; P=0.0018) compared to controls (16774.2 U/ml [2.6]). There were no significant differences in anti-S1 RBD antibody concentrations between control subjects and thiopurine (12019.7 U/mL [2.2]; P=0.099), ustekinumab (11089.3 U/mL [2.8]; P=0.060), nor vedolizumab treated patients (13564.9 U/mL [2.4]; P=0.27). In multivariable modelling, lower anti-S1 RBD antibody concentrations were independently associated with infliximab (Geometric mean ratio 0.15, 95% CI 0.11-0.21, P<0.0001), tofacitinib (0.52,

95% CI 0.31-0.87, P=0.012) and thiopurine (0.69, 95% CI 0.51-0.95, P=0.021), but not with ustekinumab (0.64, 95% CI 0.39-1.06, P=0.083), or vedolizumab (0.84, 95% CI 0.54-1.30, P=0.43). Previous SARS-CoV-2 infection (1.58, 95% CI 1.22-2.05, P=0.00056) and older age (0.88, 95% CI 0.80-0.97, P=0.0073) were independently associated with higher and lower anti-S1 antibody concentrations respectively. Antigen specific T cell responses were similar in all groups, except for recipients of tofacitinib without evidence of previous infection, where T cell responses were significantly reduced relative to healthy controls (p=0.021).

Interpretation

A third dose of COVID-19 vaccine induced a boost in antibody binding in immunosuppressed patients with IBD, but these responses were reduced in patients taking infliximab, infliximab/thiopurine combination and tofacitinib. Tofacitinib was also associated with reduced T cell responses. These findings support continued prioritisation of immunosuppressed groups for further booster dosing, particularly those on anti-TNF and Janus Kinase (JAK) inhibitors.

Funding

Financial support was provided as a Research Grant by Pfizer Ltd.

Keywords

SARS-CoV-2, immune-mediated inflammatory diseases, inflammatory bowel disease, thiopurine, azathioprine, anti-TNF therapy, infliximab, vedolizumab, ustekinumab, tofacitinib, immunosuppressant, vaccine, ChAdOx1 nCoV-19, BNT162b2, mRNA1273.

Research in context

Evidence before this study

We have already demonstrated diminished COVID-19 vaccine-induced antibody responses in patients with IBD taking infliximab and tofacitinib, but not vedolizumab or thiopurine monotherapy, following two vaccine doses. Multiple studies have shown that anti-TNF treatment is associated with lower antibody responses, while CLARITY-IBD found no difference in T cell responses between infliximab and vedolizumab treated patients following a second vaccine dose. Breakthrough infection is more common in IBD patients receiving infliximab compared to vedolizumab after two vaccine doses. There are limited data on humoral and cell-mediated anti-SARS-CoV-2 immunity in patients with IBD compared to non-immunosuppressed healthy controls after three COVID-19 vaccine doses.

Added value of this study

This is the first study to evaluate humoral and cell-mediated immune responses following three doses of COVID-19 vaccine, in patients receiving different immunosuppressive treatments used in IBD. We show that, although all groups achieved a significant boost in vaccine-induced anti-SARS-CoV-2 spike antibody binding after a third dose, levels achieved were significantly reduced in those patients treated with infliximab or tofacitinib. Tofacitinib recipients also had significantly reduced T cell responses against Spike compared to healthy controls.

Implications of all the available evidence

These data show that a third dose of COVID-19 vaccine boosts S1-RBD antibody binding irrespective of immunosuppressive treatment. However, anti-TNF, anti-TNF and immunomodulator combination and tofacitinib recipients had reduced antibody responses after three doses of COVID-19 vaccine compared to healthy controls. Tofacitinib recipients also had diminished T cell responses. Future booster dosing in IBD should be considered a priority in patients receiving anti-TNF treatment or tofacitinib.

Introduction

The COVID-19 pandemic has accounted for over six million deaths as of July 2022.(1) Vaccination has been the most effective means of reducing hospitalisations and deaths.(2-4) Several vaccines have now been approved, including mRNA, adenovirus vector and protein-based platforms.(5-8) However, as patients with immune mediated inflammatory disorders such as IBD were excluded from vaccine trials, data on the efficacy of vaccines in these groups is lacking. The VIP (SARS-CoV2 Vaccination immunogenicity in Immunosuppressed inflammatory bowel disease Patients) study is a prospective multicentre study seeking to determine whether COVID-19 vaccine immunogenicity is altered in patients receiving the commonly prescribed immunosuppressive treatments. Previously, we reported that patients with IBD taking the anti-TNF treatment, infliximab or the JAK-inhibitor tofacitinib had significantly reduced anti-SARS-CoV-2 spike antibody binding compared to healthy controls after two doses of vaccine.(9) Other commonly used immunosuppressants, including thiopurines, ustekinumab and vedolizumab, were not associated with a reduction in antibody binding. Evidence is emerging that antibody levels decay more rapidly in anti-TNF treated patients with IBD and that they are at greater risk of breakthrough infection following two doses of vaccine.(10-12)

In some countries, including the United Kingdom, immunosuppressed patients have been prioritised for third primary doses and booster doses of vaccine,(13) and in the UK, uptake of third doses amongst immunosuppressed patients with IBD has been reported at 79%.(14) There are limited data about immunity following third vaccine doses in patients with IBD and interpretation is problematic due to a lack of healthy control subjects or data about cell-mediated immunity.(15) We have shown that a two-

dose schedule of mRNA vaccine is associated with higher anti-SARS-CoV-2 spike antibody binding than two doses of adenovirus vector vaccine in the immunosuppressed IBD population.(9) Whilst in North America homologous mRNA vaccine schedules have been used almost exclusively, in the United Kingdom and worldwide, heterologous vaccination schedules (for example two doses of adenovirus vaccine followed by one dose of mRNA vaccine) have been employed. Heterologous boosting is effective in healthy individuals,(16) however, further research is needed in immunosuppressed individuals. Finally, although antibody responses to SARS-CoV-2 vaccination in patients with IBD have been the subject of a growing body of research,(17-21) there is a lack of data on the impact of immunosuppressive therapies on T cell immunity post vaccination in this setting.(10, 22)

In the current study we investigated antibody and T cell-mediated immunity against SARS-CoV-2 spike following three doses of vaccine in patients with IBD that are taking commonly prescribed immunosuppressive treatments.

Methods

Study design and participants

VIP (SARS-CoV2 Vaccination immunogenicity in Immunosuppressed inflammatory bowel disease Patients) is a UK multi-centre prospective observational study (ISCRTN registration number: ISRCTN13495664) assessing the immunogenicity of SARS-CoV-2 vaccination in patients with IBD treated with six different immunosuppressive treatment regimens (thiopurine, infliximab monotherapy, infliximab and thiopurine combination therapy, ustekinumab monotherapy, vedolizumab monotherapy or tofacitinib monotherapy). Immunosuppressed IBD patients and non-immunosuppressed healthy individuals were recruited from nine UK centres.(9)

The inclusion criteria for the healthy control group were no diagnosis of IBD and no current treatment with systemic immunosuppressive therapy for any other indication. Healthy controls were not excluded if they had other medical conditions. The healthy control group was recruited from healthy volunteer databases and from staff working at medical and university centres involved in the study. Inclusion criteria for the six immunosuppressed IBD groups were an established diagnosis of Crohn's disease (CD), ulcerative colitis (UC) or inflammatory bowel disease unclassified (IBD-U) using standard definitions of IBD, and established treatment with one of six immunosuppressive regimens (thiopurine, infliximab monotherapy, infliximab and thiopurine combination therapy, ustekinumab monotherapy, vedolizumab monotherapy or tofacitinib monotherapy) for at least 12 weeks at the time of first dose of SARS-CoV-2 vaccination. Exclusion criteria were treatment with any other immunosuppressive treatments or treatment combinations including methotrexate, adalimumab and cyclosporin. Current treatment with systemic corticosteroids was not

an exclusion criterion. The full study protocol can be viewed online (https://www.vipstudy.uk/). In brief, to be eligible, participants had received three doses of an approved COVID-19 vaccine. Participants either received a homologous vaccination schedule (three doses of an mRNA vaccine) or a heterologous vaccine schedule (two doses of adenovirus vector vaccine followed by a dose of an mRNA vaccine). Anti-SARS-CoV-2 spike (S1-RBD) Ab concentrations were measured using the Elecsys anti-SARS-CoV-2 spike (S) Ab assay, 53-92 days after second vaccine dose and 28-49 days after the third vaccine dose. T cells were measured 28-49 days after the third vaccine dose.

Procedures

SARS-CoV2 Serology:

Laboratory analysis was performed at the Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust. To determine vaccine specific antibody responses the Roche Elecsys Anti-SARS-CoV-2 spike (S) electrochemiluminescence immunoassay was used.(23) This double sandwich electrochemiluminescence immunoassay uses a recombinant protein of the receptor binding domain (RBD) on the spike protein as an antigen for the determination of antibodies against SARS-CoV-2. Sample electrochemiluminescence signals are compared with internal calibration curves and quantitative values are reported as units (U)/mL. In-house validation experiments have been described previously.(17) An additional dilution step was added for samples with antibody concentrations above the analytical range of the assay following the third vaccine dose.

At entry to the VIP study (at 53-92 days after the second vaccine dose) and at 28-49 days after the third vaccine dose, all participants were tested for possible previous SARS-CoV-2 infection using the Roche Elecsys anti-SARS-CoV-2 (N) immunoassay. A concentration of greater than or equal to 0.12 U/ml was defined as a threshold below which participants were deemed to have no evidence of prior infection. Participants who reported a history of a previous positive PCR test confirming SARS-CoV-2 infection at any time were recorded as previously SARS-CoV-2 infected.

Peripheral blood mononuclear cell isolation

Whole blood was collected in lithium heparin tubes and PBMCs were isolated by density-gradient centrifugation using LymphoprepTM (Stem Cell Technologies) layered onto SepMateTM (Stem Cell Technologies) tubes. PBMC isolation was performed within 12 h of venepuncture. Purified PBMCs were cryopreserved in 10% DMSO/50% FBS and stored in liquid nitrogen pending batch analysis.

Spike-peptide specific T cell responses

IFN-γ T cell ELISpot assays were performed using pre-coated plates (Mabtech 3420-2APT) and using the protocol described previously.(10, 24, 25) Two-hundred thousand cells were seeded per well and cells were stimulated with a peptide pool, containing 18 peptides derived from SARS-CoV-2 spike protein(26) at a concentration of 10 μg/ml/peptide; the peptide pool utilises a mapped epitope pool (MEP) of 12–20mer peptides, mapped as eliciting high-prevalence CD4 responses covering diverse HLA-II haplotypes.(24, 25) Use of this spike MEP pool in otherwise healthy SARS-CoV-2 seropositive individuals elicits a T cell response in 83% of individuals at 16–18 weeks after natural SARS-CoV-2 infection and 91% of healthy individuals 2–3 weeks after

two-dose vaccination with seronegative individuals showing a level of response indistinguishable from pre-pandemic controls.(24, 25) Plates were cultured for 18–20 h before development and data were collected using an AID classic ELISpot plate reader (Autoimmun Diagnostika GMBH). In 53 cases (15%) T cell responses could not be reported, either due to insufficient blood draw, insufficient cell number harvest during PBMC extraction or technical failure of the assay. Results are expressed as differences in (delta) spot forming cells (SFC) per 10^6 PBMC between peptide stimulation and a media-only control. A response falling below 2 standard deviations above the media-only control wells was deemed to be a null response.Data were excluded if the response to the positive control anti-CD3 stimulation was <200 SFC per 10^6 PBMCs.

Outcomes

The primary outcome was anti-SARS-CoV-2 spike (S1 RBD) Ab level, measured using the Elecsys anti-SARS-CoV-2 spike (S) Ab assay, 28-49 days after third vaccine dose, adjusted by age, homologous versus heterologous vaccine schedule and history of prior infection.

Secondary outcomes were the relative increment in anti-SARS-CoV-2 spike (S1 RBD) Ab concentrations following a third vaccine dose in each study group, and spike-peptide specific T cell responses in each group following the third vaccine dose.

Variables recorded by participants were demographics (age, sex, ethnicity, comorbidities, height and weight, smoking status, and postcode), IBD disease activity (defined by patient reported outcomes [PRO2]),(27, 28) SARS-CoV-2 symptoms

aligned to the COVID-19 symptoms study (symptoms, previous testing and hospital admissions for COVID-19) and vaccine uptake (type and date of primary vaccination). Data were entered electronically into a purpose-designed REDCap database hosted at the Royal Devon and Exeter NHS Foundation Trust.(29) An additional post-third dose questionnaire was administered to capture third dose vaccination type, positive COVID-19 tests between second and third dose, and changes in IBD treatment. Participants without access to the internet or electronic device completed their questionnaires on paper case record forms that were subsequently entered by local research teams.

Statistical analysis

Sample size calculations for the VIP study have been reported previously.(9) Full details can be found in the statistical analysis plan: (https://www.vipstudy.uk/info). Statistical analyses were undertaken in R V.4.0.4 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two-tailed and values of $p<0.05$ were considered significant. We included patients with missing clinical data in analyses for which they had data and have specified the denominator for each variable. Missing clinical data affected four patients (1.1%) included in the analysis of the primary outcome, and these patients were therefore excluded from the multivariable model. No imputation of missing data was performed. Anti-S antibody concentrations are reported as geometric means and SD (Geometric SD[x] = $e^{SD[\log x]}$). Other continuous data are reported as median and IQR, and discrete data as numbers and percentages, unless otherwise stated. Figures were created in R V.4.0.4 and Graphpad Prism 9.0.0.

For the primary outcome analysis, linear regression models of log-transformed anti-SARS-CoV-2 (S) antibody concentration, adjusted for age, vaccine schedule and history of prior infection (adjustments made owing to the substantial effect of these variables on humoral responses to SARS-CoV-2 vaccination), were used to identify IBD treatment regimens associated with the concentration of anti-SARS-CoV-2 (S) antibodies. To test our primary outcome, we used multivariable linear regression models to assess the association between immunosuppressive therapies in IBD and COVID-19 vaccine-induced antibody responses, adjusted for confounders. Based on data from CLARITY-IBD, *a priori*, we included IBD medication, vaccine type (mRNA or Adenovirus), age, IBD subtype, ethnicity and smoking status.(17) Age was treated as a continuous variable in the analysis (after checking the linearity of age as a variable using simple linear regression and Runs test) and its coefficient is expressed per decade. Results are presented after exponentiation, so that the exponentiated coefficients of the model correspond to the geometric mean ratio (GMR) estimates per one unit increase associated with each binary covariate. Our analysis for the multivariable linear regression model assumed that the anti-S1 antibody data would be log normally distributed. Model diagnostics were performed to test this assumption. We subsequently performed a sensitivity analysis using a one-parameter Box-Cox transformation(30) with lambda = 0.2 (based on optimising the log-likelihood of the model) to ensure that data skew did not significantly impact our results. In addition, to account for the within patient multiple measurements of anti-SARS-CoV-2 spike (S1 RBD) Ab level (at visit 1 and visit 2), a linear mixed effects model was also performed including data from visit 1 and visit 2. The linear mixed effects model was fitted using the lmer package(31) with log(antibody concentration) as the outcome variable, the participant as a random variable for the intercept and fixed variables as specified in

the results table. The error distribution was assumed to be normal, and this assumption was checked by visual inspection of a QQ plot of the residuals. Wilcoxon matched-pairs signed-rank tests were used for comparison of post second and post third dose anti-S antibody concentrations stratified by treatment group.

Kruskal-Wallis tests, with Dunn's correction for multiple testing, were used to compare the magnitude of T cell responses (SFC/10^6 PBMCs) stratified by immunosuppressive therapy and history of prior infection. Spearman's rank correlation coefficient was calculated to determine the correlation between antibody and T cell responses.

Role of the funding source

VIP is an investigator-led, UK National Institute for Health Research COVID-19 study. Financial support was provided as a Research Grant by Pfizer Ltd.. Pfizer Ltd. had no role in study design, data collection or analysis, writing, or decision to submit for publication. Participants were included after providing informed, written consent. The sponsor was Imperial College London. The protocol is available online at https://www.vipstudy.uk. The study was registered with the ISRCTN registry.

Results

Participant characteristics

Between 18th October 2021 and 29th March 2022, 352 participants were sampled following a third dose of SARS-CoV-2 vaccine including: thiopurine (n=65), infliximab (n=46), thiopurine/infliximab combination therapy (n=49), ustekinumab (n=44), vedolizumab (n=50) or tofacitinib (n=26). There were 125 participants (35.5%) with evidence of prior SARS-CoV-2 infection. Participant characteristics are shown in Table 1.

SARS-CoV-2 (S) antibody binding following three doses of COVID-19 vaccine

We first compared post second dose and post third dose anti-SARS-CoV-2 (S) antibody concentrations in individuals stratified by immunosuppressive therapy (figure 1). Geometric mean [geometric SD] anti-S1 RBD antibody binding levels were significantly higher in healthy controls and all treatment groups following a third dose of vaccine (all $p<0.0001$).

Geometric mean [geometric SD] anti-S1 RBD antibody binding (figure 2A and B) were lower in patients treated with infliximab (2736.8 U/mL [4.3]; $P<0.0001$), infliximab and thiopurine combination (1818.3 U/mL [6.7]; $P<0.0001$) and tofacitinib (8071.5 U/mL [3.1]; $P=0.0018$) compared to controls (16774.2 U/ml [2.6]). No significant differences in anti-S1 RBD antibody binding were found between controls and thiopurine monotherapy-treated patients nor between controls and vedolizumab treated patients (13564.9 U/mL [2.4]; $P=0.27$). In ustekinumab-treated patients (11089.3 U/mL [2.8]; $P=0.060$) and thiopurine monotherapy-treated patients (12019.7 U/mL [2.2]; $P=0.099$), modest reductions in anti-S1 RBD antibody binding were observed relative to controls,

which did not reach statistical significance. One patient treated with infliximab and thiopurine combination therapy failed to mount a detectable antibody level. Anti-S1 RBD antibody binding for each vaccine schedule type (three doses mRNA (homologous) and two doses Adenovirus vector and one dose mRNA (heterologous)) stratified by study group are shown in supplementary figures 1 and 2.

In multivariable modelling (figure 3), lower anti-S1 RBD antibody concentrations were independently associated with infliximab (Geometric mean ratio 0.15, 95% CI 0.11-0.21, P<0.0001) and tofacitinib (GMR 0.52, 95% CI 0.31-0.87, P=0.012), but not with vedolizumab (GMR 0.84, 95% CI 0.54-1.30, P=0.43). The model also suggests that thiopurine (GMR 0.69, 95% CI 0.51-0.95, P=0.021) and ustekinumab (GMR 0.64, 95% CI 0.39-1.06, P=0.083) may be associated with modest reductions in anti-S1 antibody concentration, with compatible confidence intervals and p values near to 0.05. Prior infection (GMR 1.58, 95% CI 1.22-2.05, P=0.00056) and older age (GMR 0.88, 95% CI 0.80-0.97, P=0.0073) were independently associated with higher and lower anti-S1 antibody concentrations respectively. Homologous vaccination schedule, IBD subtype, ethnicity and smoking status were not associated with S1 RBD antibody binding. A linear mixed effects model, additionally adjusting for within patient multiple measurements showed no significant impact on the reported associations (supplementary table 1). After performing diagnostics to test statistical assumptions underlying the multivariable model (supplementary figure 3 and 4), a one-parameter Box-Cox transformation (supplementary figure 5) with lambda = 0.20 (based on optimising the log-likelihood of the model), demonstrated no significant impact on the treatment variables in the multivariable linear regression model (supplementary figure 6).

T cell immunity against spike following three doses of COVID-19 vaccine

In participants without evidence of prior infection, the magnitude of anti-spike T cell responses was lower in tofacitinib-treated patients compared to healthy controls (figure 4A; p=0.021). No significant differences in the magnitude of anti-spike T cell responses were observed in infection-naïve recipients of thiopurine, infliximab, thiopurine and infliximab combination therapy, ustekinumab or vedolizumab, compared to healthy controls. In individuals with laboratory confirmed evidence of previous SARS-CoV-2 infection, there were no differences observed in the magnitude of anti-spike T cell responses between the groups (figure 4A). In individuals with evidence of previous infection, T cell responses against N peptide pool were significantly reduced in ustekinumab treated patients (p=0.0018; figure 4B). There were no significant differences observed in the magnitude of T cell responses against N peptide pool between the other treatment groups and healthy controls (figure 4B). Ordering anti-spike T cell responses by the cumulative magnitude of anti-S RBD binding following three doses of COVID-19 vaccine showed discordant T cell and antibody responses in all treatment groups (figure 4C).

Discussion

This study provides important new information on the impact of different commonly used immunosuppressive drugs on T cell and antibody responses after three doses of COVID-19 vaccine. The first key finding is that patients with IBD on each of the six treatment regimens studied gain a significant boost in antibody binding levels from a third dose, supporting the decision taken in many countries to roll-out third-primary doses of vaccine to these groups. However, patients treated with infliximab or tofacitinib had reduced anti-S1 RBD antibody binding after three doses of vaccine in comparison with healthy control subjects. Patients with IBD on thiopurine monotherapy, ustekinumab or vedolizumab showed no significant reduction in antibody binding compared to control participants. These findings mirror differences seen in the previously reported VIP study following two doses of vaccine.(9)

The size of reduction in antibody binding was greatest in infliximab treated patients with an 84% reduction in antibody binding when compared to control participants. These findings are compatible with post-third dose results from CLARITY-IBD, PREVENT-COVID and HERCULES,(32-34) but contrast with a recent Canadian study in which anti-TNF therapy was not associated with a significant reduction in anti-S antibody titre following three doses of vaccine.(15) Notably, the Canadian study used 16 non-immunosuppressed patients with a diagnosis of IBD rather than healthy controls as a reference group.(15) Despite the relative reduction in antibody binding seen in anti-TNF-treated patients, our results still compare favourably with those seen in some other immunosuppressed groups such as solid organ transplant recipients, a sizeable minority of whom fail to mount any detectable response to a third dose.(35) Reassuringly for infliximab recipients, our results also show that T cell responses

following three doses of vaccine are not reduced relative to healthy controls. These data are in line with observations from CLARITY-IBD, where T cell responses were not significantly different between infliximab and vedolizumab treated patients following two doses of vaccine,(10) but we have not recapitulated the findings of the CORALE study, which showed augmentation of T cell response in anti-TNF recipients.(36) In the current study we observed that patients treated with thiopurine, infliximab, thiopurine and infliximab combination therapy, ustekinumab or vedolizumab did not differ significantly from healthy controls. However, tofacitinib treatment was associated with reduced T cell immunity against spike, indicating that this treatment impairs humoral and cell-mediated response to COVID-19 vaccination, which may mark them out as particularly vulnerable during future waves of SARS-CoV-2 infection. In the Omicron era, with post-vaccination breakthrough infection and re-infection increasingly common in immunosuppressed and non-immunsuppressed groups, translating studies of vaccine immunogenicity into practice will continue to challenge clinicians and policy makers. Studies are urgently needed to assess the relative immunogenicity of vaccines against emerging variants of concern in immunosuppressed patients with IBD, and to determine how immunogenicity corresponds to risk of severe disease and death.

Although our study has strengths including a large well-balanced cohort and both humoral and cell-mediated readouts of vaccine response, we acknowledge limitations. Firstly, the number of participants in the tofacitinib group is relatively small, and we should interpret findings in this group with caution. Modest reductions in SARS-CoV-2 antibody binding observed in the thiopurine and ustekinumab groups did not reach statistical significance. Based on these results, although we cannot be certain that

thiopurines and ustekinumab are not associated with a reduction in serological response, any differences from the healthy population are unlikely to be clinically important. In multivariable modelling we have accounted for important confounding factors associated with humoral responses to vaccination in other studies (including age, vaccine type, IBD subtype, smoking status, ethnicity, prior infection and heterologous vaccination schedules). However, confounders were not selected using a causal directed acyclic graph and we cannot exclude the possibility that our results are affected by measurement bias or residual confounding due to measurement error in the outcome variable and other measured or unmeasured confounders. IBD disease activity was assessed clinically using PRO2 and did not differ significantly between treatment groups, but we do not have information on biochemical or endoscopic activity. Previous SARS-CoV-2 infection was treated as a binary variable, but it is possible that infection with SARS-CoV-2 Variants of Concern during different waves of the pandemic differentially shape immunity.(37, 38)

In conclusion, we have shown that three doses of COVID-19 vaccine provided a significant boost in vaccine induced antibody binding in patients taking several immunosuppressive treatments commonly used in IBD, but that patients treated with infliximab or tofacitinib showed reduced antibody binding relative to healthy controls. Patients on tofacitinib additionally showed reduced vaccine induced T cell immunity against ancestral spike, raising the question of whether this group is particularly vulnerable to infection by SARS-CoV-2. Notably, vaccine induced immunity after three doses of vaccine was greater in subjects who had previously been infected with SARS-CoV2, consistent with the notion that further antigen exposure could “rescue” suboptimal responses.(25) It is possible that additional doses of vaccine recover

immunity in those patients taking immunosuppressive treatments linked to suboptimal vaccine immunogenicity, such as infliximab or tofacitinib treated patients.

Data availability statement

The study protocol including the statistical analysis plan is available at www.vipstudy.uk. Individual participant de-identified data that underlie the results reported in this article will be available immediately after publication for a period of 5 years. The data will be made available to investigators whose proposed use of the data has been approved by an independent review committee. Analyses will be restricted to the aims in the approved proposal. Proposals should be directed to nicholas.powell@ic.ac.uk. To gain access to data requestors will need to sign a data access agreement.

Ethics statements

Patient consent for publication

Not required.

Ethics approval

The Wales Research Ethics Committee 5 approved the study (REC reference: 21/WA/0105) in March 2021.

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Author Contributions

JLA, NAK, CB, JRG, CWL, RB, DA, TA and NP participated in the conception and design of this study. CB was the project manager and coordinated patient recruitment. RN coordinated serological analyses. T cell studies were performed, analysed and interpreted by DMS, CR, RB and DA. JLA, ZL, DMS, CR, NAK, HI, SA, AS, RCS, CB, ADM, GRJ, LC, FF, SS, PMI, LH, HRTW, AK, MP, KK, KP, JPT, DA, RB, AH, CWL, JRG, TA and NP were involved in the acquisition, analysis, or interpretation of data. Drafting of the manuscript was done by JLA, ZL, NAK and NP. JLA, CL, RB, TA and NP obtained funding for the study. All the authors contributed to the critical review and final approval of the manuscript. JLA, NAK, NP and TA have verified the underlying data. All authors were responsible for the decision to submit the manuscript.

Competing Interests

Dr Alexander reports sponsorship from Vifor Pharma for accommodation/travel to BSG 2019, outside the submitted work. Dr. Kennedy reports grants from AbbVie, Biogen, Celgene, Celtrion, Galapagos, MSD, Napp, Pfizer, Pharmacosmos, Roche and Takeda, consulting fees from Amgen, Bristol-Myers Squibb, Falk, Janssen, Mylan, Pharmacosmos, Galapagos, Takeda and Tillotts, personal fees from Allergan, Celltrion, Falk, Ferring, Janssen, Pharmacosmos, Takeda, Tilllotts, Galapagos, and support for attending meetings from AbbVie, Falk and Janssen outside the submitted work. Dr Saifuddin has received travel expense support from Janssen. Prof. Sebastian reports grants from Takeda, Abbvie, Tillots Pharma, Janssen, Pfizer, Biogen and personal fees from Takeda, Abbvie, Janssen, Pharmacocosmos, Biogen, Pfizer, Tillots Pharma and Falk Pharma, outside the submitted work. Dr Hart reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from AbbVie, AZ, Atlantic, Bristol-Myers Squibb, Celltrion, Falk, Galapogos, Janssen, MSD, Napp Pharmaceuticals, Pfizer, Pharmacosmos, Shire and Takeda and Global Steering Committee for Genentech, support for attending meetings from Abbvie, Takeda and Janssen, and Participation on a Data Safety Monitoring Board or Advisory Board for AbbVie, AZ, Atlantic, Bristol-Myers Squibb, Galapogos, Janssen, Pfizer and Takeda. Dr Irving reports grants and personal fees from Celltrion, grants and personal fees from Takeda, grants from MSD, grants and personal fees from Pfizer, grants and personal fees from Galapagos, personal fees from Gilead, personal fees from Abbvie, personal fees from Janssen, personal fees from BMS, personal fees from Lilly, personal fees from Arena, outside the submitted work. Dr Parkes receives unrestricted educational grants from Pfizer for genetic analyses to support the IBD BioResource and speaker fees from Janssen. Dr Jones has received

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Table 1: Characteristics of VIP study participants attending second study visit (n=352)

Variable	Level	thiopurine n = 65	combination thiopurine & infliximab n = 49	infliximab n = 46	ustekinumab n = 44	vedolizumab n = 50	tofacitinib n = 26	healthy control n = 72	p

Prior infection	Neither	66% (43/65)	59% (29/49)	65% (30/46)	70.5% (31/44)	70% (35/50)	58% (15/26)	61% (44/72)	0.59
	Swab	5% (3/65)	2% (1/49)	9% (4/46)	2% (1/44)	2% (1/50)	0% (0/26)	0% (0/72)	
	Serology	15% (10/65)	31% (15/49)	20% (9/46)	18% (8/44)	20% (10/50)	27% (7/26)	25% (18/72)	
	Both	14% (9/65)	8% (4/49)	7% (3/46)	9% (4/44)	8% (4/50)	15% (4/26)	14% (10/72)	
Age (years)		44.1 (34.6 - 54.5)	39.2 (31.1 - 52.1)	47.5 (36.1 - 56.4)	43.6 (33.1 - 56.4)	44.6 (37.0 - 59.2)	48.0 (37.9 - 54.8)	36.5 (29.0 - 50.6)	0.029
Gender	Female	55% (36/65)	49% (24/49)	48% (22/46)	52% (23/44)	33% (15/46)	31% (8/26)	65% (47/72)	0.0085
	Male	45% (29/65)	51% (25/49)	52% (24/46)	48% (21/44)	67% (31/46)	69% (18/26)	35% (25/72)	
	Other	0% (0/65)	0% (0/49)	0% (0/46)	0% (0/44)	0% (0/46)	0% (0/26)	0% (0/72)	
	Prefer not to say	0% (0/65)	0% (0/49)	0% (0/46)	0% (0/44)	0% (0/46)	0% (0/26)	0% (0/72)	
Non white		18% (12/65)	20% (10/49)	17% (8/46)	11% (5/44)	24% (11/46)	15% (4/26)	17% (12/72)	0.84
Ethnicity	White	82% (53/65)	80% (39/49)	83% (38/46)	89% (39/44)	76% (35/46)	85% (22/26)	83% (60/72)	0.91
	Asian	11% (7/65)	14% (7/49)	9% (4/46)	9% (4/44)	15% (7/46)	8% (2/26)	11% (8/72)	

	Mixed	0% (0/65)	4% (2/49)	4% (2/46)	2% (1/44)	4% (2/46)	3% (1/26)	4% (3/72)	
	Black	3% (2/65)	2% (1/49)	0% (0/46)	0% (0/44)	2% (1/46)	0% (0/26)	0% (0/72)	
	Other	5% (3/65)	0% (0/49)	4% (2/46)	0% (0/44)	2% (1/46)	4% (1/26)	1% (1/72)	
Diagnosis	Crohn's disease	43% (28/65)	61% (30/49)	67% (31/46)	98% (43/44)	44% (22/50)	8% (2/26)	(0/0)	0.00050
	Ulcerative colitis	55% (36/65)	33% (16/49)	28% (13/46)	2% (1/44)	54% (27/50)	92% (24/26)	(0/0)	
	IBD-unclassified	2% (1/65)	6% (3/49)	4% (2/46)	0% (0/44)	2% (1/50)	0% (0/26)	(0/0)	
BMI		24.2 (21.8 - 27.4)	25.1 (22.4 - 26.9)	25.2 (23.3 - 28.5)	25.7 (22.8 - 29.8)	25.0 (23.1 - 28.4)	25.3 (23.0 - 28.6)	23.4 (21.7 - 25.7)	0.067
Heart disease		2% (1/65)	0% (0/49)	2% (1/46)	0% (0/44)	7% (3/46)	0% (0/26)	0% (0/72)	0.089
Diabetes		6% (4/65)	0% (0/49)	7% (3/46)	7% (3/44)	7% (3/46)	0% (0/26)	1% (1/72)	0.22
Lung disease		11% (7/65)	14% (7/49)	15% (7/46)	9% (4/44)	7% (3/46)	12% (3/26)	8% (6/71)	0.81
Kidney disease		2% (1/65)	0% (0/49)	4% (2/46)	2% (1/44)	2% (1/46)	0% (0/26)	0% (0/72)	0.44
Cancer		2% (1/65)	0% (0/49)	2% (1/46)	0% (0/44)	2% (1/46)	0% (0/26)	0% (0/72)	0.65

Smoker	Yes	2% (1/65)	4% (2/49)	4% (2/46)	7% (3/44)	11% (5/46)	8% (2/26)	3% (2/72)	0.25
	Not currently	35% (23/65)	33% (16/49)	28% (13/46)	34% (15/44)	33% (15/46)	50% (13/26)	24% (17/72)	
	Never	63% (41/65)	63% (31/49)	67% (31/46)	59% (26/44)	57% (26/46)	42% (11/26)	74% (53/72)	
Vaccine (doses 1 & 2)	Pfizer vaccine	38% (25/65)	33% (16/49)	57% (26/46)	34% (15/44)	37% (17/46)	27% (7/26)	49% (35/72)	0.023
	Oxford - AstraZeneca vaccine	62% (40/65)	67% (33/49)	43% (20/46)	66% (29/44)	63% (29/46)	69% (18/26)	46% (33/72)	
	Moderna vaccine	0% (0/65)	0% (0/49)	0% (0/46)	0% (0/44)	0% (0/46)	4% (1/26)	6% (4/72)	
Prednisolone		3% (2/64)	6% (3/49)	9% (4/46)	5% (2/44)	9% (4/46)	15% (4/26)	(0/0)	0.41
Any prednisolone		3% (2/64)	6% (3/49)	9% (4/46)	5% (2/44)	9% (4/46)	15% (4/26)	(0/0)	0.38
Immunosuppressive therapy stopped or switched at time of third dose		2% 1/65	10% 5/49	7% 3/46	5% 2/44	4% 2/50	4% 1/26	(0/0)	0.44
Active disease (PRO2)		9% (6/65)	4% (2/47)	2% (1/46)	8% (3/40)	19% (8/43)	8% (2/25)	(0/0)	0.11
Days since third dose of vaccine		39.0 (33.0 - 44.0)	39.0 (36.0 - 44.5)	40.0 (35.0 - 46.0)	39.0 (33.5 - 44.5)	40.0 (34.7 - 43.8)	35.5 (32.0 - 40.5)	39.0 (34.0 - 44.5)	0.49

Data are median (IQR) or n/N (%), unless otherwise specified. Previous infection was defined by a concentration of anti-SARS-CoV-2 nucleocapsid antibodies of 0·12 U/mL or more or a self-reported previous PCR test confirming SARS-CoV-2

infection. P values were obtained using Fisher's exact tests for categorical variables and Kruskal Wallis tests for continuous variables.

Figures Legends

Figure 1: Ladder plots showing anti-SARS-CoV-2 spike S1-RBD binding antibodyafter two doses (left) and three doses (right) of COVID-19 vaccine, stratified by study treatment group. Statistical analysis was performed with Wilcoxon signed-rank test (**** denotes p<0.0001).

Figure 2A: SARS-CoV-2 spike S1-RBD antibody binding 28-49 days after third dose of vaccine, stratified by study treatment group and previous infection. The wider bar represents the geometric mean, while the narrower bars are drawn one geometric SD either side of the geometric mean. 2B: Multivariable models showing coefficients of linear regression models of log(anti-SARS-CoV-2 spike antibody concentration) stratified by study treatment group.

Figure 3: Multivariable model showing exponentiated coefficients of linear regression models of log(anti-SARS-CoV-2 spike S1-RBD antibody binding). The values shown represent geometric mean ratio of S1-RBD binding associated with each variable. Age was treated as a continuous variable in the analysis and its coefficient is expressed per decade.

Figure 4. T cell immunity against SARS-CoV-2 spike and nucleocapsid in triple COVID-19 vaccinated IBD patients and healthy controls. T cell responses against SARS-CoV-2 spike mapped epitope pool (MEP) (**A**) and nucleocapsid (MEP) (**B**) in triple COVID-19 vaccinated healthy control donors (blue, n = 29 and 36) and IBD patients taking the immunomodulatory drugs thiopurine (red, n = 41 and 15), infliximab (green, n = 30 and 9), thiopurine and infliximab (purple, n = 34 and 8), ustekinumab (orange, n = 25 and 10), vedolizumab (pink, n = 31 and 12) or tofacitinib (brown, n = 12 and 7). Study donors were either SARS-CoV-2 infection naïve (closed symbols) or had been previously infected by SARS-CoV-2 (open symbols). T cell responses were measured by IFN-γ ELISpot. Previously infected donors were assayed for nucleocapsid T cell responses. The number of study participants in each group with a positive T cell response to the peptide pools is shown. Individual donor T cell responses to the spike MEP and matched data for serum S1 RBD binding antibodies (**C**) are plotted by ascending antibody binding titer for SARS-CoV-2 infection naïve healthy control donors (blue, n = 28 and 26) and SARS-CoV-2 infection naïve IBD patients taking thiopurine (red, n = 41 and 40), infliximab (green, n = 29), thiopurine and infliximab (purple, n = 33), ustekinumab (orange, n = 25), vedolizumab (pink, n = 31 and 30) or tofacitinib (brown, n = 12). (**A, B**) Statistical significance was determined using a Kruskal Wallis multiple comparison test with Dunn's correction. PBMC, peripheral blood mononuclear cells; RBD, receptor binding domain; SFC, spot forming cells.

COVID-19 vaccine-induced antibody and T cell responses in immunosuppressed patients with inflammatory bowel disease after the third vaccine dose: a multicentre, prospective, case-control study

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Abstract

Background

COVID-19 vaccine-induced antibody responses are reduced in patients with inflammatory bowel disease (IBD) taking ~~infliximab~~ anti-TNF or tofacitinib after two vaccine doses. We sought to determine whether immunosuppressive treatments were associated with reduced antibody and T cell responses after a third vaccine dose.

Methods

352 adults (72 healthy controls and 280 IBD) ~~from the prospectively recruited study cohort~~ were sampled 28-49 days after a third dose of SARS-CoV-2 vaccine. IBD medications studied included thiopurines (n=65), infliximab (n=46), thiopurine/infliximab combination therapy (n=49), ustekinumab (n=44), vedolizumab (n=50) or tofacitinib (n=26). SARS-CoV-2 spike antibody binding and T cell responses were measured.

Findings

Geometric mean [geometric SD] anti-S1 RBD antibody concentrations increased in all ~~study~~ groups following a third dose ~~of vaccine~~, but were significantly lower in patients treated with infliximab (2736.8 U/mL [4.3]; P<0.0001), infliximab and thiopurine combination (1818.3 U/mL [6.7]; P<0.0001) and tofacitinib (8071.5 U/mL [3.1]; P=0.0018) compared to controls (16774.2 U/ml [2.6]). There were no significant differences in anti-S1 RBD antibody concentrations between control subjects and thiopurine (12019.7 U/mL [2.2]; P=0.099), ustekinumab (11089.3 U/mL [2.8]; P=0.060), nor vedolizumab treated patients (13564.9 U/mL [2.4]; P=0.27). In multivariable modelling, lower anti-S1 RBD antibody concentrations were

independently associated with infliximab (Geometric mean ratio 0.15, 95% CI 0.11-0.21, P<0.0001), tofacitinib (0.52, 95% CI 0.31-0.87, P=0.012) and thiopurine (0.69, 95% CI 0.51-0.95, P=0.021), but not with ustekinumab (0.64, 95% CI 0.39-1.06, P=0.083), or vedolizumab (0.84, 95% CI 0.54-1.30, P=0.43). Previous SARS-CoV-2 infection (1.58, 95% CI 1.22-2.05, P=0.00056) and older age (0.88, 95% CI 0.80-0.97, P=0.0073) were independently associated with higher and lower anti-S1 antibody concentrations respectively. A~~However, a~~ntigen specific T cell responses were similar in ~~IBD patients in all treatment~~all groups~~ studied~~, except for recipients of tofacitinib without evidence of previous infection, where T cell responses were significantly reduced relative to healthy controls (p=0.021).

Interpretation

A third dose of COVID-19 vaccine induced a boost in antibody binding in immunosuppressed patients with IBD, but these responses were reduced in patients taking infliximab, infliximab/thiopurine combination and tofacitinib~~ therapy~~. Tofacitinib was also associated with reduced T cell responses. These findings support continued prioritisation of immunosuppressed groups for further booster dosing, particularly those on anti-TNF and Janus Kinase (JAK) inhibitors~~ who have attenuation of both serological and cell-mediated vaccine-induced immunity~~.

Funding

Financial support was provided as a Research Grant by Pfizer Ltd.

Keywords

SARS-CoV-2, immune-mediated inflammatory diseases, inflammatory bowel disease, thiopurine, azathioprine, anti-TNF therapy, infliximab, vedolizumab, ustekinumab, tofacitinib, immunosuppressant, vaccine, ChAdOx1 nCoV-19, BNT162b2, mRNA1273.

Research in context

Evidence before this study

We have already demonstrated diminished COVID-19 vaccine-induced antibody responses in patients with IBD taking infliximab and tofacitinib, but not vedolizumab or thiopurine monotherapy, following two vaccine doses. Multiple studies have shown that anti-TNF treatment is associated with lower antibody responses, while CLARITY-IBD found no difference in T cell responses between infliximab and vedolizumab treated patients following a second vaccine dose. Breakthrough infection is more common in IBD patients receiving infliximab compared to vedolizumab after two vaccine doses. There are limited data on humoral and cell-mediated anti-SARS-CoV-2 immunity in patients with IBD compared to non-immunosuppressed healthy controls after three COVID-19 vaccine doses.

Added value of this study

This is the first study to evaluate humoral and cell-mediated immune responses following three doses of COVID-19 vaccine, in patients receiving different immunosuppressive treatments used in IBD. We show that, although all groups achieved a significant boost in vaccine-induced anti-SARS-CoV-2 spike antibody binding after a third dose, levels achieved were significantly reduced in those patients

treated with infliximab or tofacitinib. Tofacitinib recipients also had significantly reduced T cell responses against Spike compared to healthy controls.

Implications of all the available evidence

These data show that a third dose of COVID-19 vaccine boosts S1-RBD antibody binding irrespective of immunosuppressive treatment. However, anti-TNF, anti-TNF and immunomodulator combination and tofacitinib recipients had reduced antibody responses after three doses of COVID-19 vaccine compared to healthy controls. Tofacitinib recipients also had diminished T cell responses. Future booster dosing in IBD should be considered a priority in patients receiving anti-TNF treatment or tofacitinib.

Introduction

The COVID-19 pandemic has accounted for over six million deaths as of July 2022.(1) Vaccination has been the most effective means of reducing hospitalisations and deaths.(2-4) Several vaccines have now been approved, including mRNA, adenovirus vector and protein-based platforms.(5-8) However, as patients with immune mediated inflammatory disorders such as IBD were excluded from vaccine trials, data on the efficacy of vaccines in these groups is lacking. The VIP (SARS-CoV2 Vaccination immunogenicity in Immunosuppressed inflammatory bowel disease Patients) study is a prospective multicentre study seeking to determine whether COVID-19 vaccine immunogenicity is altered in patients receiving the commonly prescribed immunosuppressive treatments. Previously, we reported that patients with IBD taking the anti-TNF treatment, infliximab or the JAK-inhibitor tofacitinib had significantly reduced anti-SARS-CoV-2 spike antibody binding compared to healthy controls after two doses of vaccine.(9) Other commonly used immunosuppressants, including thiopurines, ustekinumab and vedolizumab, were not associated with a reduction in antibody binding. Evidence is emerging that antibody levels decay more rapidly in anti-TNF treated patients with IBD and that they are at greater risk of breakthrough infection following two doses of vaccine.(10-12)

In some countries, including the United Kingdom, immunosuppressed patients have been prioritised for third primary doses and booster doses of vaccine,(13) and in the UK, uptake of third doses amongst immunosuppressed patients with IBD has been reported at 79%.(14) There are limited data about immunity following third vaccine doses in patients with IBD and interpretation is problematic due to a lack of healthy control subjects or data about cell-mediated immunity.(15) We have shown that a two-

dose schedule of mRNA vaccine is associated with higher anti-SARS-CoV-2 spike antibody binding than two doses of adenovirus vector vaccine in the immunosuppressed IBD population.(9) Whilst in North America homologous mRNA vaccine schedules have been used almost exclusively, in the United Kingdom and worldwide, heterologous vaccination schedules (for example two doses of adenovirus vaccine followed by one dose of mRNA vaccine) have been employed. Heterologous boosting is effective in healthy individuals,(16) however, further research is needed in immunosuppressed individuals. Finally, although antibody responses to SARS-CoV-2 vaccination in patients with IBD have been the subject of a growing body of research,(17-21) there is a lack of data on the impact of immunosuppressive therapies on T cell immunity post vaccination in this setting.(10, 22)

In the current study we investigated antibody and T cell-mediated immunity against SARS-CoV-2 spike following three doses of vaccine in patients with IBD that are taking commonly prescribed immunosuppressive treatments.

Methods

Study design and participants

VIP (SARS-CoV2 Vaccination immunogenicity in Immunosuppressed inflammatory bowel disease Patients) is a UK multi-centre prospective observational study (ISCRTN registration number: ISRCTN13495664) assessing the immunogenicity of SARS-CoV-2 vaccination in patients with IBD treated with six different immunosuppressive treatment regimens (thiopurine, infliximab monotherapy, infliximab and thiopurine combination therapy, ustekinumab monotherapy, vedolizumab monotherapy or tofacitinib monotherapy). Immunosuppressed IBD patients and non-immunosuppressed healthy individuals were recruited from nine UK centres.(9)

The inclusion criteria for the healthy control group were no diagnosis of IBD and no current treatment with systemic immunosuppressive therapy for any other indication. Healthy controls were not excluded if they had other medical conditions. The healthy control group was recruited from healthy volunteer databases and from staff working at medical and university centres involved in the study. Inclusion criteria for the six immunosuppressed IBD groups were an established diagnosis of Crohn's disease (CD), ulcerative colitis (UC) or inflammatory bowel disease unclassified (IBD-U) using standard definitions of IBD, and established treatment with one of six immunosuppressive regimens (thiopurine, infliximab monotherapy, infliximab and thiopurine combination therapy, ustekinumab monotherapy, vedolizumab monotherapy or tofacitinib monotherapy) for at least 12 weeks at the time of first dose of SARS-CoV-2 vaccination. Exclusion criteria were treatment with any other immunosuppressive treatments or treatment combinations including methotrexate, adalimumab and cyclosporin. Current treatment with systemic corticosteroids was not

an exclusion criterion. The full study protocol can be viewed online (https://www.vipstudy.uk/). In brief, to be eligible, participants had received three doses of an approved COVID-19 vaccine. Participants either received a homologous vaccination schedule (three doses of an mRNA vaccine) or a heterologous vaccine schedule (two doses of adenovirus vector vaccine followed by a dose of an mRNA vaccine). Anti-SARS-CoV-2 spike (S1-RBD) Ab concentrations were measured using the Elecsys anti-SARS-CoV-2 spike (S) Ab assay, 53-92 days after second vaccine dose and 28-49 days after the third vaccine dose. T cells were measured 28-49 days after the third vaccine dose.

Procedures

SARS-CoV2 Serology:

Laboratory analysis was performed at the Academic Department of Blood Sciences at the Royal Devon and Exeter NHS Foundation Trust. To determine vaccine specific antibody responses the Roche Elecsys Anti-SARS-CoV-2 spike (S) electrochemiluminescence immunoassay was used.(23) This double sandwich electrochemiluminescence immunoassay uses a recombinant protein of the receptor binding domain (RBD) on the spike protein as an antigen for the determination of antibodies against SARS-CoV-2. Sample electrochemiluminescence signals are compared with internal calibration curves and quantitative values are reported as units (U)/mL. In-house validation experiments have been described previously.(17) An additional dilution step was added for samples with antibody concentrations above the analytical range of the assay following the third vaccine dose.

At entry to the VIP study (at 53-92 days after the second vaccine dose) and at 28-49 days after the third vaccine dose, all participants were tested for possible previous SARS-CoV-2 infection using the Roche Elecsys anti-SARS-CoV-2 (N) immunoassay. A concentration of greater than or equal to 0.12 U/ml was defined as a threshold below which participants were deemed to have no evidence of prior infection. Participants who reported a history of a previous positive PCR test confirming SARS-CoV-2 infection at any time were recorded as previously SARS-CoV-2 infected.

Peripheral blood mononuclear cell isolation

Whole blood was collected in lithium heparin tubes and PBMCs were isolated by density-gradient centrifugation using LymphoprepTM (Stem Cell Technologies) layered onto SepMateTM (Stem Cell Technologies) tubes. PBMC isolation was performed within 12 h of venepuncture. Purified PBMCs were cryopreserved in 10% DMSO/50% FBS and stored in liquid nitrogen pending batch analysis.

Spike-peptide specific T cell responses

IFN-γ T cell ELISpot assays were performed using pre-coated plates (Mabtech 3420-2APT) and using the protocol described previously.(10, 24, 25) Two-hundred thousand cells were seeded per well and cells were stimulated with a peptide pool, containing 18 peptides derived from SARS-CoV-2 spike protein(26) at a concentration of 10 µg/ml/peptide; the peptide pool utilises a mapped epitope pool (MEP) of 12–20mer peptides, mapped as eliciting high-prevalence CD4 responses covering diverse HLA-II haplotypes.(24, 25) Use of this spike MEP pool in otherwise healthy SARS-CoV-2 seropositive individuals elicits a T cell response in 83% of individuals at 16–18 weeks after natural SARS-CoV-2 infection and 91% of healthy individuals 2–3 weeks after

two-dose vaccination with seronegative individuals showing a level of response indistinguishable from pre-pandemic controls.(24, 25) Plates were cultured for 18–20 h before development and data were collected using an AID classic ELISpot plate reader (Autoimmun Diagnostika GMBH). In 53 cases (15%) T cell responses could not be reported, either due to insufficient blood draw, insufficient cell number harvest during PBMC extraction or technical failure of the assay. Results are expressed as differences in (delta) spot forming cells (SFC) per 10^6 PBMC between peptide stimulation and a media-only control. A response falling below 2 standard deviations above the media-only control wells was deemed to be a null response. Data were excluded if the response to the positive control anti-CD3 stimulation was <200 SFC per 10^6 PBMCs.

Outcome Measures:Outcomes

The primary outcome was anti-SARS-CoV-2 spike (S1 RBD) Ab level, measured using the Elecsys anti-SARS-CoV-2 spike (S) Ab assay, 28-49 days after third vaccine dose, adjusted by age, homologous versus heterologous vaccine schedule and history of prior infection.

Secondary outcomes were the relative increment in anti-SARS-CoV-2 spike (S1 RBD) Ab concentrations following a third vaccine dose in each study group, and spike-peptide specific T cell responses in each group following the third vaccine dose.

Variables:

Variables recorded by participants were demographics (age, sex, ethnicity, comorbidities, height and weight, smoking status, and postcode), IBD disease activity

(defined by patient reported outcomes [PRO2]),(27, 28) SARS-CoV-2 symptoms aligned to the COVID-19 symptoms study (symptoms, previous testing and hospital admissions for COVID-19) and vaccine uptake (type and date of primary vaccination). Data were entered electronically into a purpose-designed REDCap database hosted at the Royal Devon and Exeter NHS Foundation Trust.(29) An additional post-third dose questionnaire was administered to capture third dose vaccination type, positive COVID-19 tests between second and third dose, and changes in IBD treatment. Participants without access to the internet or electronic device completed their questionnaires on paper case record forms that were subsequently entered by local research teams.

~~*Ethical consideration and role of funders*~~

~~VIP is an investigator-led, UK National Institute for Health Research COVID-19 study. Financial support was provided as a Research Grant by Pfizer Ltd.. Pfizer Ltd. had no role in study design, data collection or analysis, writing, or decision to submit for publication. Participants were included after providing informed, written consent. The sponsor was Imperial College London. The protocol is available online at https://www.vipstudy.uk. The study was registered with the ISRCTN registry.~~

Statistical analysis~~s~~:

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Sample size calculations for the VIP study have been reported previously.(9) Full details can be found in the statistical analysis plan: (https://www.vipstudy.uk/info). Statistical analyses were undertaken in R V.4.0.4 (R Foundation for Statistical Computing, Vienna, Austria). All tests were two-tailed and values of $p<0.05$ were considered significant. We included patients with missing clinical data in analyses for

which they had data and have specified the denominator for each variable. Missing clinical data affected four patients (1.1%) included in the analysis of the primary outcome, and these patients were therefore excluded from the multivariable model. No imputation of missing data was performed. Anti-S antibody concentrations are reported as geometric means and SD (Geometric SD[x] = $e^{SD[\log x]}$). Other continuous data are reported as median and IQR, and discrete data as numbers and percentages, unless otherwise stated. Figures were created in R V.4.0.4 and Graphpad Prism 9.0.0.

For the primary outcome analysis, linear regression models of log-transformed anti-SARS-CoV-2 (S) antibody concentration, adjusted for age, vaccine schedule and history of prior infection (adjustments made owing to the substantial effect of these variables on humoral responses to SARS-CoV-2 vaccination), were used to identify IBD treatment regimens associated with the concentration of anti-SARS-CoV-2 (S) antibodies. To test our primary outcome, we used multivariable linear regression models to assess the association between immunosuppressive therapies in IBD and COVID-19 vaccine-induced antibody responses, adjusted for confounders. Based on data from CLARITY-IBD, *a priori*, we included IBD medication, vaccine type (mRNA or Adenovirus), age, IBD subtype, ethnicity and smoking status.(17) Age was treated as a continuous variable in the analysis (after checking the linearity of age as a variable using simple linear regression and Runs test) and its coefficient is expressed per decade. Results are presented after exponentiation, so that the exponentiated coefficients of the model correspond to the geometric mean ratio (GMR) estimates per one unit increase associated with each binary covariate. Our analysis for the multivariable linear regression model assumed that the anti-S1 antibody data would be log normally distributed. Model diagnostics were performed to test this assumption.

We subsequently performed a sensitivity analysis using a one-parameter Box-Cox transformation(30) with lambda = 0.2 (based on optimising the log-likelihood of the model) to ensure that data skew did not significantly impact our results. In addition, t~~T~~o account for the within patient multiple measurements of anti-SARS-CoV-2 spike (S1 RBD) Ab level (at visit 1 and visit 2), a linear mixed effects model was also performed including data from visit 1 and visit 2. The linear mixed effects model was fitted using the lmer package(31) with log(antibody concentration) as the outcome variable, the participant as a random variable for the intercept and fixed variables as specified in the results table. The error distribution was assumed to be normal, and this assumption was checked by visual inspection of a QQ plot of the residuals. Wilcoxon matched-~~-~~pairs signed-~~-~~rank tests were used for comparison of post second and post third dose anti-S antibody concentrations stratified by treatment group.

Kruskal-Wallis tests, with Dunn's correction for multiple testing, were used to compare the magnitude of T cell responses (SFC/10^6 PBMCs) stratified by immunosuppressive therapy and history of prior infection. Spearman's rank correlation coefficient was calculated to determine the correlation between antibody and T cell responses.

~~Ethical consideration and role~~Role of the funding source~~ers~~

VIP is an investigator-led, UK National Institute for Health Research COVID-19 study. Financial support was provided as a Research Grant by Pfizer Ltd.. Pfizer Ltd. had no role in study design, data collection or analysis, writing, or decision to submit for publication. Participants were included after providing informed, written consent. The sponsor was Imperial College London. The protocol is available online at https://www.vipstudy.uk. The study was registered with the ISRCTN registry.

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Results

Participant characteristics

Between 18th October 2021 and 29th March 2022, 352 participants were sampled following a third dose of SARS-CoV-2 vaccine including: thiopurine (n=65), infliximab (n=46), thiopurine/infliximab combination therapy (n=49), ustekinumab (n=44), vedolizumab (n=50) or tofacitinib (n=26). There were 125 participants (35.5%) with evidence of prior SARS-CoV-2 infection. Participant characteristics are shown in Table 1.

SARS-CoV-2 (S) antibody binding following three doses of COVID-19 vaccine

We first compared post second dose and post third dose anti-SARS-CoV-2 (S) antibody concentrations in individuals stratified by immunosuppressive therapy (figure 1). Geometric mean [geometric SD] anti-S1 RBD antibody binding levels were significantly higher in healthy controls and all treatment groups following a third dose of vaccine (all p<0.0001).

Geometric mean [geometric SD] anti-S1 RBD antibody binding (figure 2A and B) were lower in patients treated with infliximab (2736.8 U/mL [4.3]; P<0.0001), infliximab and thiopurine combination (1818.3 U/mL [6.7]; P<0.0001) and tofacitinib (8071.5 U/mL [3.1]; P=0.0018) compared to controls (16774.2 U/ml [2.6]). No significant differences in anti-S1 RBD antibody binding were found between controls and thiopurine monotherapy-treated patients ~~(12019.7 U/mL [2.2]; P=0.099),~~ nor between controls and vedolizumab treated patients (13564.9 U/mL [2.4]; P=0.27). In ustekinumab-treated patients (11089.3 U/mL [2.8]; P=0.060) and thiopurine monotherapy-treated patients (12019.7 U/mL [2.2]; P=0.099), modest reductions in anti-S1 RBD antibody

binding were observed relative to controls, which did not reach statistical significance~~ustekinumab treated patients (11089.3 U/mL [2.8]; P=0.060), nor between controls and vedolizumab treated patients (13564.9 U/mL [2.4]; P=0.27)~~. One patient treated with infliximab and thiopurine combination therapy failed to mount a detectable antibody level. Anti-S1 RBD antibody binding for each vaccine schedule type (three doses mRNA (homologous) and two doses Adenovirus vector and one dose mRNA (heterologous)) stratified by study group are shown in supplementary figures 1 and 2.

In multivariable modelling (figure 3), lower anti-S1 RBD antibody concentrations were independently associated with infliximab (Geometric mean ratio 0.15, 95% CI 0.11-0.21, P<0.0001) and~~,~~ tofacitinib (GMR 0.52, 95% CI 0.31-0.87, P=0.012), but not with vedolizumab (GMR 0.84, 95% CI 0.54-1.30, P=0.43). ~~and~~ The model also suggests that t~~t~~hiopurine (GMR 0.69, 95% CI 0.51-0.95, P=0.021) and~~, but not with~~ ustekinumab (GMR 0.64, 95% CI 0.39-1.06, P=0.083) may be associated with modest reductions in anti-S1 antibody concentration, with compatible confidence intervals and p values near to 0.05. ~~, or vedolizumab (GMR 0.84, 95% CI 0.54-1.30, P=0.43).~~ Prior infection (GMR 1.58, 95% CI 1.22-2.05, P=0.00056) and older age (GMR 0.88, 95% CI 0.80-0.97, P=0.0073) were independently associated with higher and lower anti-S1 antibody concentrations respectively. Homologous vaccination schedule, IBD subtype, ethnicity and smoking status were not associated with S1 RBD antibody binding. A linear mixed effects model, additionally adjusting for within patient multiple measurements showed no significant impact on the reported associations (supplementary table 1). After performing diagnostics to test statistical assumptions underlying the multivariable model (supplementary figure 3 and 4), ~~we further ensured that data skew did not impact our results by performing a sensitivity analysis using~~ a

one-parameter Box-Cox transformation (supplementary figure 5~~4~~) with lambda = 0.20 (based on optimising the log-likelihood of the model), ~~which showed~~ demonstrated no significant impact on the treatment variables in the multivariable linear regression model (supplementary figure 6~~5~~).

T cell immunity against spike following three doses of COVID-19 vaccine

In participants without evidence of prior infection, the magnitude of anti-spike T cell responses was lower in tofacitinib-treated patients compared to healthy controls (figure 4A; p=0.021). No significant differences in the magnitude of anti-spike T cell responses were observed in infection-naïve recipients of thiopurine, infliximab, thiopurine and infliximab combination therapy, ustekinumab or vedolizumab, compared to healthy controls. In individuals with laboratory confirmed evidence of previous SARS-CoV-2 infection, there were no differences observed in the magnitude of anti-spike T cell responses between the groups (figure 4A). In individuals with evidence of previous infection, T cell responses against N peptide pool were significantly reduced in ustekinumab treated patients (p=0.0018; figure 4B). There were no significant differences observed in the magnitude of T cell responses against N peptide pool between the other treatment groups and healthy controls (figure 4B). Ordering anti-spike T cell responses by the cumulative magnitude of anti-S RBD binding following three doses of COVID-19 vaccine showed discordant T cell and antibody responses in all treatment groups (figure 4C).

Discussion

This study provides important new information on the impact of different commonly used immunosuppressive drugs on T cell and antibody responses after three doses of COVID-19 vaccine. The first key finding is that patients with IBD on each of the six treatment regimens studied gain a significant boost in antibody binding levels from a third dose, supporting the decision taken in many countries to roll-out third-primary doses of vaccine to these groups. However, patients treated with infliximab or tofacitinib had reduced anti-S1 RBD antibody binding after three doses of vaccine in comparison with healthy control subjects. Patients with IBD on thiopurine monotherapy, ustekinumab or vedolizumab showed no significant reduction in antibody binding compared to control participants. These findings mirror differences seen in the previously reported VIP study following two doses of vaccine.(9)

The size of reduction in antibody binding was greatest in infliximab treated patients with a n 84% reduction in antibody binding when compared to control participants. These findings are compatible with post-third dose results from CLARITY-IBD, PREVENT-COVID and HERCULES,(32-34) but contrast with a recent Canadian study in which anti-TNF therapy was not associated with a significant reduction in anti-S antibody titre following three doses of vaccine.(15) Notably, the Canadian study used 16 non-immunosuppressed patients with a diagnosis of IBD rather than healthy controls as a reference group.(15) Despite the relative reduction in antibody binding seen in anti-TNF-treated patients, our results still compare favourably with those seen in some other immunosuppressed groups such as solid organ transplant recipients, a sizeable minority of whom fail to mount any detectable response to a third dose.(35) Reassuringly for infliximab recipients, our results also

show that T cell responses following three doses of vaccine are not reduced relative to healthy controls. These data are in line with observations from CLARITY-IBD, where T cell responses were not significantly different between infliximab and vedolizumab treated patients following two doses of vaccine,(10) but we have not recapitulated the findings of the CORALE study, which showed augmentation of T cell response in anti-TNF recipients.(36) In the current study we observed that patients treated with thiopurine, infliximab, thiopurine and infliximab combination therapy, ustekinumab or vedolizumab did not differ significantly from healthy controls. However, tofacitinib treatment was associated with reduced T cell immunity against spike, indicating that this treatment impairs humoral and cell-mediated response to COVID-19 vaccination, which may mark them out as particularly vulnerable during future waves of SARS-CoV-2 infection. In the Omicron era, with post-vaccination breakthrough infection and re-infection increasingly common in immunosuppressed and non-immunsuppressed groups, translating studies of vaccine immunogenicity into practice will continue to challenge clinicians and policy makers. Studies are urgently needed to assess the relative immunogenicity of vaccines against emerging variants of concern in immunosuppressed patients with IBD, and to determine how immunogenicity corresponds to risk of severe disease and death.

Although our study has strengths including a large well-balanced cohort and both humoral and cell-mediated readouts of vaccine response, we acknowledge limitations. Firstly, the number of participants in the tofacitinib group is relatively small, and we should interpret findings in this group with caution. Modest reductions in SARS-CoV-2 antibody binding observed in the thiopurine and ustekinumab groups did not reach statistical significance. Based on these results, although we cannot be certain that

thiopurines and ustekinumab are not associated with a reduction in serological response, any differences from the healthy population are unlikely to be clinically important. In multivariable modelling we have accounted for important confounding factors associated with humoral responses to vaccination in other studies (including age, vaccine type, IBD subtype, smoking status, ethnicity, prior infection and heterologous vaccination schedules). However, ~~but~~ confounders were not selected using a causal directed acyclic graph and we cannot exclude the possibility that our results are affected by measurement bias or residual confounding due to measurement error in the outcome variable and other measured or unmeasured confounders. ~~– other unmeasured confounding factors.~~ IBD disease activity was assessed clinically using PRO2 and did not differ significantly between treatment groups, but we do not have information on biochemical or endoscopic activity. Previous SARS-CoV-2 infection was treated as a binary variable, but it is possible that infection with SARS-CoV-2 Variants of Concern during different waves of the pandemic differentially shape immunity.(37, 38)

In conclusion, we have shown that three doses of COVID-19 vaccine provided a significant boost in vaccine induced antibody binding in patients taking several immunosuppressive treatments commonly used in IBD, but that patients treated with infliximab or tofacitinib showed reduced antibody binding relative to healthy controls. Patients on tofacitinib additionally showed reduced vaccine induced T cell immunity against ancestral spike, raising the question of whether this group is particularly vulnerable to infection by SARS-CoV-2. Notably, vaccine induced immunity after three doses of vaccine was greater in subjects who had previously been infected with SARS-CoV2, consistent with the notion that further antigen exposure could “rescue”

suboptimal responses.(25) It is possible that additional doses of vaccine recover immunity in those patients taking immunosuppressive treatments linked to suboptimal vaccine immunogenicity, such as infliximab or tofacitinib treated patients.

Data availability statement

The study protocol including the statistical analysis plan is available at www.vipstudy.uk. Individual participant de-identified data that underlie the results reported in this article will be available immediately after publication for a period of 5 years. The data will be made available to investigators whose proposed use of the data has been approved by an independent review committee. Analyses will be restricted to the aims in the approved proposal. Proposals should be directed to nicholas.powell@ic.ac.uk. To gain access to data requestors will need to sign a data access agreement.

Ethics statements

Patient consent for publication

Not required.

Ethics approval

The Wales Research Ethics Committee 5 approved the study (REC reference: 21/WA/0105) in March 2021.

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Author Contributions

JLA, NAK, CB, JRG, CWL, RB, DA, TA and NP participated in the conception and design of this study. CB was the project manager and coordinated patient recruitment. RN coordinated serological analyses. T cell studies were performed, analysed and interpreted by DMS, CR, RB and DA. JLA, ZL, DMS, CR, NAK, HI, SA, AS, RCS, CB, ADM, GRJ, LC, FF, SS, PMI, LH, HRTW, AK, MP, KK, KP, JPT, DA, RB, AH, CWL, JRG, TA and NP were involved in the acquisition, analysis, or interpretation of data. Drafting of the manuscript was done by JLA, ZL, NAK and NP. JLA, CL, RB, TA and NP obtained funding for the study. All the authors contributed to the critical review and final approval of the manuscript. JLA, NAK, NP and TA have verified the underlying data. All authors were responsible for the decision to submit the manuscript.

Competing Interests

Dr Alexander reports sponsorship from Vifor Pharma for accommodation/travel to BSG 2019, outside the submitted work. Dr. Kennedy reports grants from AbbVie, Biogen, Celgene, Celtrion, Galapagos, MSD, Napp, Pfizer, Pharmacosmos, Roche and Takeda, consulting fees from Amgen, Bristol-Myers Squibb, Falk, Janssen, Mylan, Pharmacosmos, Galapagos, Takeda and Tillotts, personal fees from Allergan, Celltrion, Falk, Ferring, Janssen, Pharmacosmos, Takeda, Tilllotts, Galapagos, and support for attending meetings from AbbVie, Falk and Janssen outside the submitted work. Dr Saifuddin has received travel expense support from Janssen. Prof. Sebastian reports grants from Takeda, Abbvie, Tillots Pharma, Janssen, Pfizer, Biogen and personal fees from Takeda, Abbvie, Janssen, Pharmacocosmos, Biogen, Pfizer, Tillots Pharma and Falk Pharma, outside the submitted work. Dr Hart reports payment or honoraria for lectures, presentations, speakers bureaus, manuscript writing or educational events from AbbVie, AZ, Atlantic, Bristol-Myers Squibb, Celltrion, Falk, Galapogos, Janssen, MSD, Napp Pharmaceuticals, Pfizer, Pharmacosmos, Shire and Takeda and Global Steering Committee for Genentech, support for attending meetings from Abbvie, Takeda and Janssen, and Participation on a Data Safety Monitoring Board or Advisory Board for AbbVie, AZ, Atlantic, Bristol-Myers Squibb, Galapogos, Janssen, Pfizer and Takeda. Dr Irving reports grants and personal fees from Celltrion, grants and personal fees from Takeda, grants from MSD, grants and personal fees from Pfizer, grants and personal fees from Galapagos, personal fees from Gilead, personal fees from Abbvie, personal fees from Janssen, personal fees from BMS, personal fees from Lilly, personal fees from Arena, outside the submitted work. Dr Parkes receives unrestricted educational grants from Pfizer for genetic analyses to support the IBD BioResource and speaker fees from Janssen. Dr Jones has received

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Table 1: Characteristics of VIP study participants attending second study visit (n=352)

Variable	Level	thiopurine n = 65	combination thiopurine & infliximab n = 49	infliximab n = 46	ustekinumab n = 44	vedolizumab n = 50	tofacitinib n = 26	healthy control n = 72	p

Prior infection	Neither	66.2% (43/65)	59.2% (29/49)	65.2% (30/46)	70.5% (31/44)	70.0% (35/50)	587.7% (15/26)	61.1% (44/72)	0.59
	Swab	54.6% (3/65)	2.0% (1/49)	98.7% (4/46)	2.3% (1/44)	2.0% (1/50)	0.0% (0/26)	0.0% (0/72)	
	Serology	15.4% (10/65)	310.6% (15/49)	2019.6% (9/46)	18.2% (8/44)	20.0% (10/50)	276.9% (7/26)	25.0% (18/72)	
	Both	143.8% (9/65)	8.2% (4/49)	76.5% (3/46)	9.1% (4/44)	8.0% (4/50)	15.4% (4/26)	143.9% (10/72)	
Age (years)		44.1 (34.6 - 54.5)	39.2 (31.1 - 52.1)	47.5 (36.1 - 56.4)	43.6 (33.1 - 56.4)	44.6 (37.0 - 59.2)	48.0 (37.9 - 54.8)	36.5 (29.0 - 50.6)	0.029
Gender	Female	55.4% (36/65)	49.0% (24/49)	47.8% (22/46)	52.3% (23/44)	332.6% (15/46)	310.8% (8/26)	65.3% (47/72)	0.0085
	Male	454.6% (29/65)	511.0% (25/49)	52.2% (24/46)	487.7% (21/44)	67.4% (31/46)	69.2% (18/26)	354.7% (25/72)	
	Other	0.0% (0/65)	0.0% (0/49)	0.0% (0/46)	0.0% (0/44)	0.0% (0/46)	0.0% (0/26)	0.0% (0/72)	
	Prefer not to say	0.0% (0/65)	0.0% (0/49)	0.0% (0/46)	0.0% (0/44)	0.0% (0/46)	0.0% (0/26)	0.0% (0/72)	
Non white		188.5% (12/65)	20.4% (10/49)	17.4% (8/46)	11.4% (5/44)	243.9% (11/46)	15.4% (4/26)	16.7% (12/72)	0.84
Ethnicity	White	821.5% (53/65)	8079.6% (39/49)	832.6% (38/46)	898.6% (39/44)	76.1% (35/46)	854.6% (22/26)	83.3% (60/72)	0.91
	Asian	110.8% (7/65)	14.3% (7/49)	98.7% (4/46)	9.1% (4/44)	15.2% (7/46)	87.7% (2/26)	11.1% (8/72)	

	Mixed	0.0% (0/65)	4.1% (2/49)	4.3% (2/46)	2.3% (1/44)	4.3% (2/46)	3.8% (1/26)	4.2% (3/72)	
	Black	3.1% (2/65)	2.0% (1/49)	0.0% (0/46)	0.0% (0/44)	2.2% (1/46)	0.0% (0/26)	0.0% (0/72)	
	Other	54.6% (3/65)	0.0% (0/49)	4.3% (2/46)	0.0% (0/44)	2.2% (1/46)	43.8% (1/26)	1.4% (1/72)	
Diagnosis	Crohn's disease	43.1% (28/65)	61.2% (30/49)	67.4% (31/46)	987.7% (43/44)	44.0% (22/50)	87.7% (2/26)	NaN% (0/0)	0.00050
	Ulcerative colitis	55.4% (36/65)	332.7% (16/49)	28.3% (13/46)	2.3% (1/44)	54.0% (27/50)	92.3% (24/26)	NaN% (0/0)	
	IBD-unclassified	21.5% (1/65)	6.1% (3/49)	4.3% (2/46)	0.0% (0/44)	2.0% (1/50)	0.0% (0/26)	NaN% (0/0)	
BMI		24.2 (21.8 - 27.4)	25.1 (22.4 - 26.9)	25.2 (23.3 - 28.5)	25.7 (22.8 - 29.8)	25.0 (23.1 - 28.4)	25.3 (23.0 - 28.6)	23.4 (21.7 - 25.7)	0.067
Heart disease		21.5% (1/65)	0.0% (0/49)	2.2% (1/46)	0.0% (0/44)	76.5% (3/46)	0.0% (0/26)	0.0% (0/72)	0.089
Diabetes		6.2% (4/65)	0.0% (0/49)	76.5% (3/46)	76.8% (3/44)	76.5% (3/46)	0.0% (0/26)	1.4% (1/72)	0.22
Lung disease		110.8% (7/65)	14.3% (7/49)	15.2% (7/46)	9.1% (4/44)	76.5% (3/46)	121.5% (3/26)	88.5% (6/71)	0.81
Kidney disease		21.5% (1/65)	0.0% (0/49)	4.3% (2/46)	2.3% (1/44)	2.2% (1/46)	0.0% (0/26)	0.0% (0/72)	0.44
Cancer		21.5% (1/65)	0.0% (0/49)	2.2% (1/46)	0.0% (0/44)	2.2% (1/46)	0.0% (0/26)	0.0% (0/72)	0.65

Smoker	Yes	21.5% (1/65)	4.1% (2/49)	4.3% (2/46)	76.8% (3/44)	110.9% (5/46)	87.7% (2/26)	32.8% (2/72)	0.25
	Not currently	35.4% (23/65)	32.7% (16/49)	28.3% (13/46)	34.1% (15/44)	32.6% (15/46)	50.0% (13/26)	243.6% (17/72)	
	Never	63.1% (41/65)	63.3% (31/49)	67.4% (31/46)	59.1% (26/44)	576.5% (26/46)	42.3% (11/26)	743.6% (53/72)	
Vaccine (doses 1 & 2)	Pfizer vaccine	388.5% (25/65)	32.7% (16/49)	576.5% (26/46)	34.1% (15/44)	377.0% (17/46)	276.9% (7/26)	498.6% (35/72)	0.023
	Oxford - AstraZeneca vaccine	621.5% (40/65)	67.3% (33/49)	43.5% (20/46)	665.9% (29/44)	63.0% (29/46)	69.2% (18/26)	465.8% (33/72)	
	Moderna vaccine	0.0% (0/65)	0.0% (0/49)	0.0% (0/46)	0.0% (0/44)	0.0% (0/46)	43.8% (1/26)	5.6% (4/72)	
Prednisolone		3.1% (2/64)	6.1% (3/49)	98.7% (4/46)	4.5% (2/44)	98.7% (4/46)	15.4% (4/26)	NaN% (0/0)	0.41
Any prednisolone		3.1% (2/64)	6.1% (3/49)	98.7% (4/46)	4.5% (2/44)	98.7% (4/46)	15.4% (4/26)	NaN% (0/0)	0.38
Immunosuppressive therapy stopped or switched at time of third dose		21.5% 1/65	10.2% 5/49	76.5% 3/46	4.5% 2/44	4.0% 2/50	43.8% 1/26	NaN% (0/0)	0.44
Active disease (PRO2)		9.2% (6/65)	4.3% (2/47)	2.2% (1/46)	87.5% (3/40)	198.6% (8/43)	8.0% (2/25)	NaN% (0/0)	0.11
Days since third dose of vaccine		39.0 (33.0 - 44.0)	39.0 (36.0 - 44.5)	40.0 (35.0 - 46.0)	39.0 (33.5 - 44.5)	40.0 (34.7 - 43.8)	35.5 (32.0 - 40.5)	39.0 (34.0 - 44.5)	0.49

Data are median (IQR) or n/N (%), unless otherwise specified. Previous infection was defined by a concentration of anti-SARS-CoV-2 nucleocapsid antibodies of 0·12 U/mL or more or a self-reported previous PCR test confirming SARS-CoV-2

infection. P values were obtained using Fisher's exact tests for categorical variables and Kruskal Wallis tests for continuous variables.

Figures Legends

Figure 1: Ladder plots showing anti-SARS-CoV-2 spike S1-RBD binding antibodyafter two doses (left) and three doses (right) of COVID-19 vaccine, stratified by study treatment group. Statistical analysis was performed with Wilcoxon signed-rank test (**** denotes p<0.0001).

Figure 2A: SARS-CoV-2 spike S1-RBD antibody binding 28-49 days after third dose of vaccine, stratified by study treatment group and previous infection. The wider bar represents the geometric mean, while the narrower bars are drawn one geometric SD either side of the geometric mean. 2B: Multivariable models showing coefficients of linear regression models of log(anti-SARS-CoV-2 spike antibody concentration) stratified by study treatment group.

Figure 3: Multivariable model showing exponentiated coefficients of linear regression models of log(anti-SARS-CoV-2 spike S1-RBD antibody binding). The values shown represent geometric mean ratio of S1-RBD binding associated with each variable. Age was treated as a continuous variable in the analysis and its coefficient is expressed per decade.

Figure 4. T cell immunity against SARS-CoV-2 spike and nucleocapsid in triple COVID-19 vaccinated IBD patients and healthy controls. T cell responses against SARS-CoV-2 spike mapped epitope pool (MEP) (**A**) and nucleocapsid (MEP) (**B**) in triple COVID-19 vaccinated healthy control donors (blue, n = 29 and 36) and IBD patients taking the immunomodulatory drugs thiopurine (red, n = 41 and 15), infliximab (green, n = 30 and 9), thiopurine and infliximab (purple, n = 34 and 8), ustekinumab (orange, n = 25 and 10), vedolizumab (pink, n = 31 and 12) or tofacitinib (brown, n = 12 and 7). Study donors were either SARS-CoV-2 infection naïve (closed symbols) or had been previously infected by SARS-CoV-2 (open symbols). T cell responses were measured by IFN-γ ELISpot. Previously infected donors were assayed for nucleocapsid T cell responses. The number of study participants in each group with a positive T cell response to the peptide pools is shown. Individual donor T cell responses to the spike MEP and matched data for serum S1 RBD binding antibodies (**C**) are plotted by ascending antibody binding titer for SARS-CoV-2 infection naïve healthy control donors (blue, n = 28 and 26) and SARS-CoV-2 infection naïve IBD patients taking thiopurine (red, n = 41 and 40), infliximab (green, n = 29), thiopurine and infliximab (purple, n = 33), ustekinumab (orange, n = 25), vedolizumab (pink, n = 31 and 30) or tofacitinib (brown, n = 12). (**A, B**) Statistical significance was determined using a Kruskal Wallis multiple comparison test with Dunn's correction. PBMC, peripheral blood mononuclear cells; RBD, receptor binding domain; SFC, spot forming cells.

Manuscript reference number: thelancetgastrohep-D-21-01206R1

Title: COVID-19 vaccine-induced antibody and T cell responses in immunosuppressed patients with inflammatory bowel disease after the third vaccine dose

The authors would like to thank the Reviewers for their comments on our manuscript. We have now addressed all the points raised and include our response to each of the specific comments below:

REVIEWERS' COMMENTS:

Reviewer #1: This study assesses COVID-19 vaccine-induced antibody and T cell responses in immunosuppressed patients with inflammatory bowel disease after the third vaccine dose. There are several major and minor concerns about the statistics and methodology of the paper.

Major
Methods
1) Lines 328-329: Please mention the range for missing data proportions.

Four participants (1.1%) had missing clinical data relevant to the primary outcome analysis, such that they could not be included in the multivariable model. This information has been added to the Methods.

2) Line 334: The assumptions underlying linear regression model including Normality and homogeneity of variance of residuals as well as linearity for quantitative predictors should be assessed.

Response: We thank the reviewer for requesting these assessments. Our analysis for the multivariable linear regression model assumed that the anti-S1 antibody data would be log normally distributed. In fact, model diagnostics (supplementary figure 4) demonstrate that the data do not quite fit a log normal distribution. We have therefore performed a sensitivity analysis using a one-parameter Box Cox transformation with lambda = 0.20 (based on optimising the log-likelihood of the model). We have included the subsequent diagnostics plot (supplementary figure 5) and the results of this model as supplementary figure 6. The impact on the outcomes of the multivariable linear model was minimal compared to the original model results. Variables that were associated with significant GMR change in anti-S antibody concentration in the original analysis (thiopurine, infliximab, tofacitinib, prior infection, age) remain significant, and variables which were non-significant remain non-significant, with the exception of non-white ethnicity, which changes from marginally non-significant (p=0.099) to marginally significant (p=0.016).

We include the non-transformed model to allow easier interpretation of the coefficients.

3) Lines 334-343: A minimally sufficient set of confounders should have been selected using a causal directed acyclic graph.

Response: The identification of confounders was based on data from the CLARITY-IBD study (DOI: 10.1136/gutjnl-2021-324789), which showed that IBD medication, vaccine type, age, IBD subtype, ethnicity and smoking status were all associated with anti-SARS-CoV-2 spike antibody concentration following a single dose of vaccine. These confounders were pre-defined in the Statistical Analysis Plan (SAP). For the post-third dose analysis, we additionally included prior infection as a covariate, owing to the substantial impact this variable has on anti-SARS-CoV-2 spike antibody concentration. Given the relatively small sample size of the VIP study, we were wary of including too many covariates in the multivariable model. We did not construct a causal directed acyclic graph (DAG) and we agree this might have been included, had it been done at the time of designing the SAP. We have added the absence of a causal DAG as a limitation in the discussion section.

4) Lines 343-344: Inclusion of the variable age as a continuous variable in the model imposes a linearity assumption which should be assessed.

We thank the reviewer for raising this point. To confirm that the variable age satisfied our assumption of linearity, we ran a simple linear regression analysis of age versus the outcome variable (i.e. log-anti-S concentration). The linear regression plot is included as supplementary figure 3. Runs test for deviation from linearity (p=0.67) indicated non-deviation from linearity.

5) Lines 345-347: It is important to note that the exponentiated coefficient for a quantitative predictor would be geometric mean ratio per one unit increase in the variable.

This point has now been noted in the text.

6) Line 348: Please provide more details for the linear mixed-effects model including the outcome variable, the error distribution, the predictors, and the random effect term and its assumed distribution.

We thank the reviewer for asking for these details, which we have now added to the Statistics section of the methods.

Results

7) Line 382: This is an extreme example of overreliance on significance testing which should be avoided, noting that a P-value of 0.06 is not much different from P-value of 0.04. The clinical importance of the results should be considered based on appropriate point and interval estimates e.g., see the following paper:
Greenland S, Mansournia MA, Joffe M. To curb research misreporting, replace significance and confidence by compatibility: A Preventive Medicine golden jubilee article. Preventive Medicine. 2022 Jul 3:107127.

We thank the reviewer for raising this point and we agree that a p-value of 0.06 does not necessarily signify that there is no difference between the groups. The point and interval estimates here suggest that there may be a modest difference between ustekinumab-treated patients and controls. However, this difference is unlikely to be of clinical importance. As suggested in the Greenland paper, we have tempered the description of these results accordingly.

8) Lines 390-392: Based on the geometric mean ratio estimate with 95% confidence interval (CI), the results for ustekinumab and thiopurine are not much different. The 95% CIs should be considered as compatibility intervals; Please see the reference mentioned in the previous comment.

We thank the reviewer for this comment and agree that the results for ustekinumab and thiopurine are similar. We also accept the notion that their confidence intervals are compatible with each other. We have therefore changed how we report these results, noting that both treatments may be associated with a modest reduction in antibody concentration and putting more emphasis on their compatibility, whilst also following journal guidance on unbiased reporting of results.

9) Table 1: In the footnote, please mention the statistical tests you used for obtaining P-values.

These tests have been added.

10) Supplementary Table 1: Please omit redundant information such as SE, df, and t statistic value.

These redundant values have now been omitted.

11) Supplementary Table 1: Please report 95% CIs for the model coefficients instead of SEs.

We now report 95% CIs for the model coefficients.

12) Supplementary Table 1: The intercept estimate cannot be interpreted as the variable age was not centered. Regardless, it seems to be redundant and should be omitted.

The intercept estimate has now been omitted.

13) Supplementary Table 1: The presentation of P-values is poor. To be consistent with the text, report P-values with two meaningful digits; any P-value less than 0.0001 should be reported "<0.0001".

P-values have been changed to be consistent with the text.

Discussion
14) The measurement bias/residual confounding due to measurement error in the outcome variable and confounders such as smoking status should be highlighted as an important limitation of the study in the Discussion.

We thank the reviewer for raising this point and we agree that it should be highlighted. The Discussion text has been amended accordingly.

Minor
15) Line 345: The term "coefficients" should be replaced with "exponentiated coefficients"

This has been changed.

16) Lines 349-350: Please change the term "Wilcoxon matched pairs signed rank tests" to "Wilcoxon matched-pairs signed-rank tests".

This has been changed.

17) Table 1, etc: Please avoid spurious precision in the presentation of numbers e.g., report percentages without any decimal given the small sample sizes (in the denominator).

Percentages in table 1 are now presented without any decimals.

18) Line 402: Please change the term "Box Cox transformation" to "Box-Cox transformation".

This has been changed.

19) Line 402: In the Statistics section, please explain your Box-Cox transformation analysis.

An explanation of the Box-Cox transformation analysis has been added to the Statistics section with the appropriate reference.

Reviewer #2: The authors sought to determine whether immunosuppressive

treatments were associated with reduced antibody and T cell responses after a third vaccine dose. SARS-CoV-2 spike antibody binding and T cell responses were measured. I have some comments:

1-Why azathioprine decrease the antibodies levels in multivariate analysis and not in univariate analysis

We thank the reviewer for raising this question. As the reviewer notes, the p-values for the comparison between thiopurines and controls were 0.099 in the primary analysis (figure 2B) and 0.021 in the broader multivariable model (figure 3). The key difference between these two analyses is that only thiopurine monotherapy-treated patients are included in the primary analysis, whereas patients treated with thiopurine monotherapy and thiopurine in combination with infliximab are included in the broader multivariable model. In addition, the broader model includes covariates such as smoking, ethnicity and IBD subtype, which may modulate the association between thiopurine treatment and SARS-CoV-2 spike antibody binding.

We also agree with the points highlighted by reviewer 1 that p-values which sit just above and below 0.05 should be interpreted with caution. We have therefore tempered the description of our findings in relation to thiopurines, recognising that our results may indicate a modest reduction in SARS-CoV-2 spike antibody binding. We have also made reference to this issue in the limitations section.

2-Looking the figure 2 the decrease levels of antibodies was only showed in total but not with homologous vaccination for all groups, if it is true, the conclusions and abstract showed be reviewed

We thank the reviewer for raising this question. To clarify, the data shown in figure 2A are for all participants, including both heterologous and homologous vaccination recipients. Conscious of the relatively small numbers of patients in certain groups when stratifying by vaccination schedule, but recognising the potential importance of this variable to SARS-CoV-2 spike antibody binding, we have accounted for vaccination schedule (homologous versus heterologous) as a covariate in the primary analysis (figure 2B). We also show the raw data, stratified by vaccination schedule, in supplementary figures 1 and 2.

3-The results with tofacitinib in T-cells could be related with sample size and low statistical power

We agree with the reviewer that the findings relating to T cell responses in the tofacitinib could be a consequence of the sample size and limited statistical power. We focus on this point as the first study limitation in the Discussion.

Reviewer #3: This study seeks to evaluate the impact of immunosuppression of the immunologic response to a 3 dose series of COVID vaccination among persons with IBD, in terms of antibody and T-cell responses. Work from this group (and others) has

consistently shown that persons with IBD who are using anti-TNF blockers tend to have lower antibody titres against spike protein and faster decay of antibody levels. T cell responses tend to be equivalent. Previouss work by this group show a correlation between antibody levels to spike protein and breakthrough infections, though this has not been consistently reported. T cell mediated immunity has also been postulated to protect against breakthrough infection, and may play a role in preventing re-infection and serious disease in persons who do not have a serologic response to vaccination. though previous work by this group approx 20% of persons with IBD who received 2 doses of COVD vaccination have a low TCR response to COVID-associated peptides.

This study is novel in that it reports on the immunologic and T-cell response in the 4-7 weeks following a 3rd dose of COVID vaccine (all with mRNA) in a population where many received the less effective adenovirus vector vaccine for the first 2 doses. The results mirror previous work in the serological aspect, with IFX (and especially anti-TNFs in combination with thiopurines) having lower levels of serologic response. Additional novel findings include low T-cell response to Nucleocapsid peptides in UST users (though numbers are small) and poor T-cell immunity in tofacitinib users.

These data are an important addition to the body of knowledge on the immunologic response in IBD patients using immunosuppressive medications. However, while there are several issues that I believe the authorship groups should consider addressing prior to publication

We thank the reviewer for these positive comments.

1. One of the major limitations in this study is the lack of information on biochemical/endoscopic disease activity at the time of receipt of 3rd dose. As tofacitinib is most commonly used as rescue therapy for persons with relapse or treatment resistant disease, how much do they belive that residua disease activity could be a contributor to a lack of T-cell response in infection naive tofacitinib users

We thank the reviewer for raising this issue. In the context of the United Kingdom's rapid COVID vaccination programme, mandating endoscopic assessment of patients in the VIP study was not deemed practical. However, we agree with the reviewer that the lack of information on biochemical and endoscopic disease activity is a limitation of the study, and we have added this to the limitations section of the Discussion.

Despite this limitation, several factors reassure us that residual disease activity is unlikely to be a significant contributor to the lack of T-cell response in infection naive tofacitinib users. Firstly, according to PRO2 assessment (data in table 1 of manuscript) only 8% of patients in the tofacitinib group had clinically active disease and there was no significant difference in PRO2 scores between treatment groups. In univariate analysis in the CLARITY-IBD study (http://dx.doi.org/10.1136/gutjnl-2021-324789) active disease was not associated with serological response to two doses of either BNT162b2 (p=0.32) or ChAdOx1 nCoV-19 (p=0.51) vaccination. Secondly, an inclusion criterion for the study was that patients needed to be established on their current immunosuppressive therapy for at least 12 weeks prior to the first vaccine dose. We would expect that patients with ongoing active disease after 12 weeks of tofacitinib therapy would have had their treatment

discontinued. Thirdly, at the time of the third vaccine dose participants were asked if they had remained on, or stopped/switched immunosuppressive therapy. Of the 26 tofactinib-treated patients, only one patient had stopped tofacitinib at this point (>6 months after entering the study).

2. What is the significance of the differences in T-cell response to S- and N- peptides. Both are reported; what do we know about their relative and/or combined importance to prevention of infection? How can we better contextualize the ustekinumab findings? Could this be a multiple comparisons phenomenon?

We thank the reviewer for raising this interesting point. T cell responses are posited to play an important role in protection from SARS-CoV-2 infection, although, in contrast to neutralising antibody responses, definitive evidence linking T cell responses to clinical correlates remains limited. The relative importance of T cell responses against S- and N-peptides is not known. With regard to ustekinumab, our data suggest that whilst T cell responses against S-peptide post third vaccine dose are preserved in ustekinumab-treated patients relative to healthy controls, there may be a deficit in N-peptide responses in those ustekinumab-treated patients with prior infection. Although the statistical significance of this finding stands up to correction for multiple comparisons, we share the reviewer's caution that this finding may represent an artefact of small sample size. Notably, data from SECURE-IBD (https://doi.org/10.1053/j.gastro.2021.09.011) suggest that ustekinumab-treated patients with IBD were at lower risk of hospitalisation or death than those not on ustekinumab (RR 0.44, 95% CI 0.36 to 0.54). Consequently, we have chosen not to draw any conclusions about the clinical implication of this result.

3. We are now in an era where vaccines no longer appear to significantly protect against re-infection against the most prevalent circulating strains of SARS-COV-2 (BA5 and related variants). What is the level of evidence supporting the relevance of T-cell and serologic response to severity of re-infections (as opposed to re-infection itself).

We are grateful to the reviewer for raising this important point. Although the effectiveness of currently available vaccines is undoubtedly lower against Omicron variants, the relative attenuation of severe disease in vaccinated groups is likely attributable to the partial protection conferred by the residual neutralizing antibodies and the activation of primed B cell and T cell memory (doi: 10.1038/s41586-022-04460-3; doi: 10.1016/j.cell.2022.04.009). In the IBD context, it is notable that time to re-infection in the Omicron era has been shorter in infliximab-treated than vedolizumab-treated patients in the CLARITY study (doi: 10.1136/gutjnl-2022-327570). There is currently limited direct evidence on the contribution of T cell and serologic responses to the severity of re-infection, but studies in both non-immunocompromised (doi:10.1016/j.cell.2020.08.017 & doi:10.1016/j.cell.2020.09.038) and immunocompromised groups (doi:10.1038/s41591-021-01386-7) have shown robust T cell responses correlate with better outcomes to primary SARS-CoV-2 infection.

In recognition of this point, we have revised the Discussion to include additional context on re-infection and variants of concern, signposting the existing uncertainties about the

relevance of immune response to vaccination, and thus the urgent need for further studies.

4. In the discussion, he authorship refers to a "6-fold decline" in antibody levels. Perhaps this is pedantic, but "-fold" should only be used to refer to increases better to say an 83% reduction in circulating antibody concentrations

We thank the reviewer for this correction, and we have amended the manuscript accordingly.

5. As a broad point, the speed which the contours of the COVID pandemic change outpace our ability to perform research that is actionable and not merely forensic. How should (or can) the practicing clinician use this information, in the context of a clinical landscape that is vastly different than the one this study was performed in

We agree with the reviewer that the dynamic nature of the COVID pandemic, and particularly the emergence of SARS-CoV-2 variants with vaccine-escape capacity, makes translating findings from vaccine immunogenicity studies into clinical practice challenging. An acceptance of this broad point has been added to the Discussion.

Thankfully, despite the high rates of infection and re-infection in vaccinated individuals with Omicron variants, currently available vaccines have remained relatively effective in preventing severe disease and death (discussed further in response to point 3). However, a minority of immunosuppressed patients with IBD have chosen not to be vaccinated (https://doi.org/10.1016/S2468-1253(21)00347-2) and others have not completed a full three dose primary schedule. Moreover, we and others have demonstrated that patients with IBD treated with infliximab or tofacitinib have diminished responses to vaccination, which exposes them to a potential higher risk of infection. In the event of future COVID-19 waves of infection and new more virulent variants, prioritisation of such higher risk groups for booster doses of vaccination (especially in resource-limited settings) will be critical. Policy makers may also choose to select such patients for newly available pre-exposure prophylaxis treatments (https://appg-vulnerablegroups.org/fileadmin/user_upload/Prophylactic_clinical_consensus_statement.pdf).

Reviewer #4: The investigators of VIP present their data on post third dose of COVID-19 vaccine evaluating antibody and T cell responses after a third dose. They found that antibody concentrations were lower in patients on anti-TNF therapy, thiopurine, and tofacitinib and no impact by other biologics.
All patients had T cells responses.

A couple of questions and concerns.

A) Please clarify in the inclusion criteria if patients continued on the same treatment regimen for the third blood draw. E.g. if someone was on infliximab and switched to vedolizumab for third dose, was this person excluded if kept where they kept in infliximab group?

We thank the reviewer for raising this important question. A small proportion of patients in each treatment group were not on the same treatment regimen at the time of their third vaccine dose (range 2-10%). In accordance with our pre-defined Statistical Analysis Plan, we did not exclude these participants from the analysis of post-third dose responses and these participants were analysed in the treatment groups they belonged to at the time of their first and second vaccine doses. We have added a row to the demographics (table 1) showing the number of participants in each group who underwent a change in their treatment.

No patients on infliximab and methotrexate were included?

Patients on methotrexate were excluded from the VIP study but the impact of methotrexate and infliximab combination therapy has been reported on in our sister study CLARITY-IBD (https://doi.org/10.1038/s41467-022-28517-z and http://dx.doi.org/10.1136/gutjnl-2022-327570).

b) please clarify if heterologous boosting was only mRNA to viral vector, or mRNA to different mRNA counted as heterologous boosting

We thank the reviewer for raising this question. Heterologous boosting constituted only participants who received two doses of adenovirus vector vaccine followed by a dose of mRNA vaccine. Homologous boosting included participants who received three doses of any mRNA vaccine. A small number of the homologous booster group (n=22; 15%) received either two doses of BNT162b2 (Pfizer/BioNTech) followed by a single dose of mRNA1273 (Moderna) or two doses of mRNA1273 (Moderna) followed by a single dose of BNT162b2 (Pfizer/BioNTech). We have clarified this point in the Methods section.

c) it appears T cell responses were not done in all patients. Please clarify how it was determined in whom T cells response were evaluated.
All clarify a T cell response was seen in all patient with IBD?

T cell responses were reported in 299 participants (85% of cohort). There were several reasons why T cell responses could not be reported in all participants, none of which are anticipated to have introduced bias:

1. *Insufficient blood draw*
2. *Insufficient cell number harvested during PBMC extraction*
3. *Technical failure of the elispot assay – defined as response to the positive control anti-CD3 stimulation of <200 SFC per 106 PBMCs.*

These points have been added to the Methods section. A null T cell response was seen in two healthy controls, two thiopurine-treated patients, zero infliximab monotherapy-treated patients, five thiopurine and infliximab combination-treated patients, one ustekinumab-treated patient, one vedolizumab-treated patient and two tofacitinib-treated patients (figure 4A).

d) Table 1 is hard to read please revise.

The table has been reformatted according to the journal requirements.

[ED: please see the formatting used in the first VIP paper]

e) was every patient with IBD seropositive after the third dose? And all HC?

We thank the reviewer for raising this question. In the first VIP paper (https://doi.org/10.1016/S2468-1253(22)00005-X), using the same Roche assay as used in the current study, we reported on rates of seroconversion, defined as an antibody concentration of 15 U/mL. This threshold correlated with 20% neutralisation in a viral pseudo-neutralisation assay described previously (http://dx.doi.org/10.1136/gutjnl-2021-324789). One patient treated with infliximab and thiopurine combination therapy failed to reach the 15U/ml threshold after three doses of vaccine. In fact, this patient had no detectable antibody response after three doses, and we have added this information to the Results section. We have elected not to refer to seroconversion or seropositivity. This decision was taken in the context of new variants of concern, including Omicron with its immune-escape capabilities, which have made measures of seroconversion less viable.

f) the CORRALE IBD has showing boosting augmentation of T cell response by ANTI-tnf therapy. Did the authors evaluate for this possibility?

We thank the reviewer for highlighting the T cell response data from the CORALE study showing an augmented T cell response in anti-TNF recipients. In our study we saw no significant difference in T cell response between anti-TNF-treated patients and controls after three doses of COVID-19 vaccine. Correspondingly, CLARITY-IBD showed no significant difference in T cell responses between infliximab-treated patients and vedolizumab-treated patients, although there was a trend seen towards higher responses to two doses of BNT162b2 vaccine (but not adenovirus vector vaccine) in the infliximab group.

Given that our current study looked at T cell responses after a third dose of vaccine (as opposed to two doses in CORALE), included patients receiving a heterologous vaccination schedule, and used a different assay (Elispot in VIP as opposed to T-cell receptor β sequencing of blood genomic DNA in CORALE), it is difficult to draw firm conclusions regarding the contrasting findings in the two studies. Nonetheless, we have revised our Discussion to include reference to the CORALE data.

g) the authors should expand to discussion to discuss other studies evaluating immune response after third dose from PREVENT COVID-19 and HERCULES. These studies should be included and discussed.

We thank the reviewer for highlighting these important studies. PREVENT COVID-19 and HERCULES were not discussed in the first submission of the manuscript, but we agree these are important additions to the literature. We are over the limit for the number of references (30 references) for a research article in this journal, but we would like to include them at the discretion of the Editor.

h) it would be important to discuss how patients with IBD compared to solid organ transplant.

We thank the reviewer for raising this suggestion. We agree that solid organ transplant makes for an interesting comparator group, given the high burden of immunosuppressive therapy that these patients receive and the well-reported sub-optimal immune response to COVID vaccination. Accepting that comparisons between studies are challenging due to the use of differing immunosuppressive regimens, sampling protocols, experimental assays and analytical approaches, we have added a note to the Discussion reflecting that the results in our study suggest IBD patient responses to third doses of COVID vaccination compare favourably to those of solid organ transplant recipients.

EDITORS' GENERAL POINTS:

- Your revised paper should have fewer than 3500 words (4500 for randomised trials; not including references, COI statements, abstract etc) and a maximum of 30 references (unless it is a systematic review or meta-analysis). The abstract should be structured (background, methods, findings, interpretation, funding) and should be less than 300 words long.

We are within the overall word count. We are slightly over the maximum number of references and the abstract word count (although we have reduced the abstract since the first submission), as we were for the first VIP paper. We would be happy to reduce the references and length of abstract further at the editor's discretion.

- The main text should be structured as follows: Introduction, Methods, Results, Discussion. Note that subheadings should only be used within the Methods section. Our preferences is to structure the Methods section as: Study design and participants, Procedures, Outcomes, Statistical analysis, Role of the funding source (see below)
- Generally, please ensure that all results are presented in a balanced, unbiased way, including clearly stating where findings are non-significant. Note that we often edit manuscripts post-acceptance to ensure the unbiased presentation of results, but it is preferable for authors to ensure this is the case at revision stage.
- The study title should include a study descriptor—eg, case-control study. Titles should be non-declamatory (ie, not state the findings of the paper).
- Please check with your co-authors, and confirm that all names are spelt correctly, and affiliation details for each author are listed correctly (including department, institution, city, state [if applicable], and country). We cannot guarantee that we will be able to correct names and affiliations after publication of your article.
- Please ensure that you include full first names for all authors and please supply (after author names on the title page) one preferred degree per author and indicate in the authorship if any authors are full professors. Note that we can only have one corresponding author, whose full mailing address (including postal code, if applicable) and email address should be included.

- If your author line includes a study group (eg, 'on behalf of the XXXX trial study group'), collaborators' names and affiliations may be listed at the end of the paper or in the appendix. Additionally, if you wish the names of collaborators within a study group to appear on PubMed, please upload with your revision a separate Word document with a list of names of the study group members presented as a two-column table. First and middle names or initials should be placed in the first column, and surnames in the second column. Names should be ordered as you wish them to appear on PubMed. The table will not be included in the paper itself - it's simply used to make sure that PubMed adds the names correctly.
- References
    - References should be in Vancouver style. Many references are missing details, please ensure they are corrected.
- All research articles must contain a data sharing statement, to be included at the end of the manuscript. For more information on these required statements see the Data sharing section of the Information for Authors (https://thelancet.com/pb-assets/Lancet/authors/tlgastro-info-for-authors.pdf) and (https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(17)31282-5/fulltext)

REQUIRED CHECKLISTS:
Please confirm that your study conforms to the relevant guidelines by completing and returning the checklist:

STROBE - Observational studies
— http://www.thelancet.com/journals/lancet/article/PIIS0140-6736(07)61602-X/fulltext
For more info: http://www.equator-network.org/

FORMS:

- We require completed, signed, author contribution forms from all authors listed (that they agree with the submission and content and to being listed), declaring their contribution to the article, and stating the role of the funding source. The form can be downloaded at:

http://download.thelancet.com/pb/assets/raw/Lancet/authors/tlgas-author-signatures.pdf

- We require completed ICMJE declaration forms from all authors, listing any potential conflicts of interest. Forms must be returned for each author, even if no declaration is being made. The form can be found at:

http://www.icmje.org/conflicts-of-interest (scroll down and click on the blue download link)

Figure 1

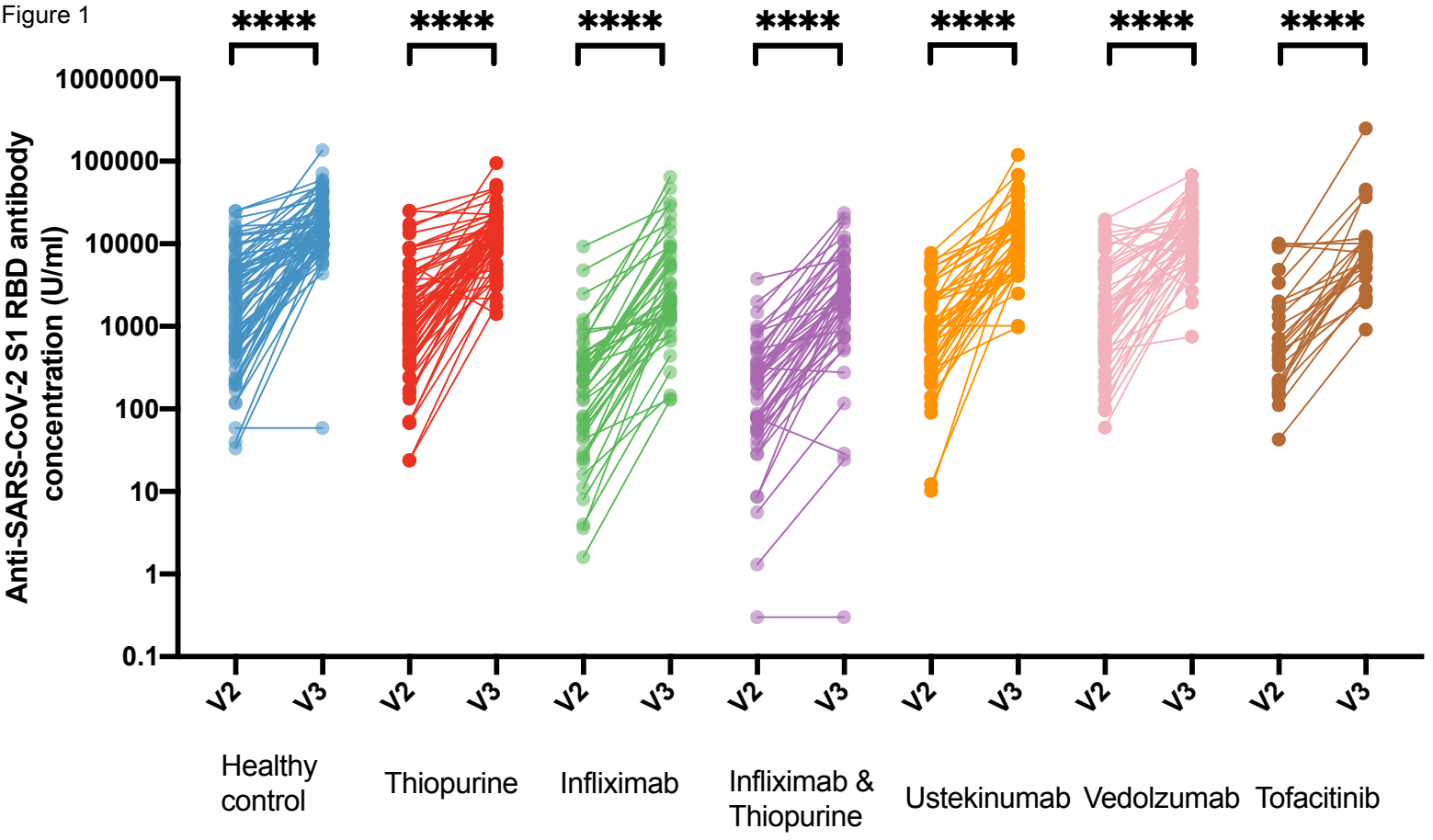
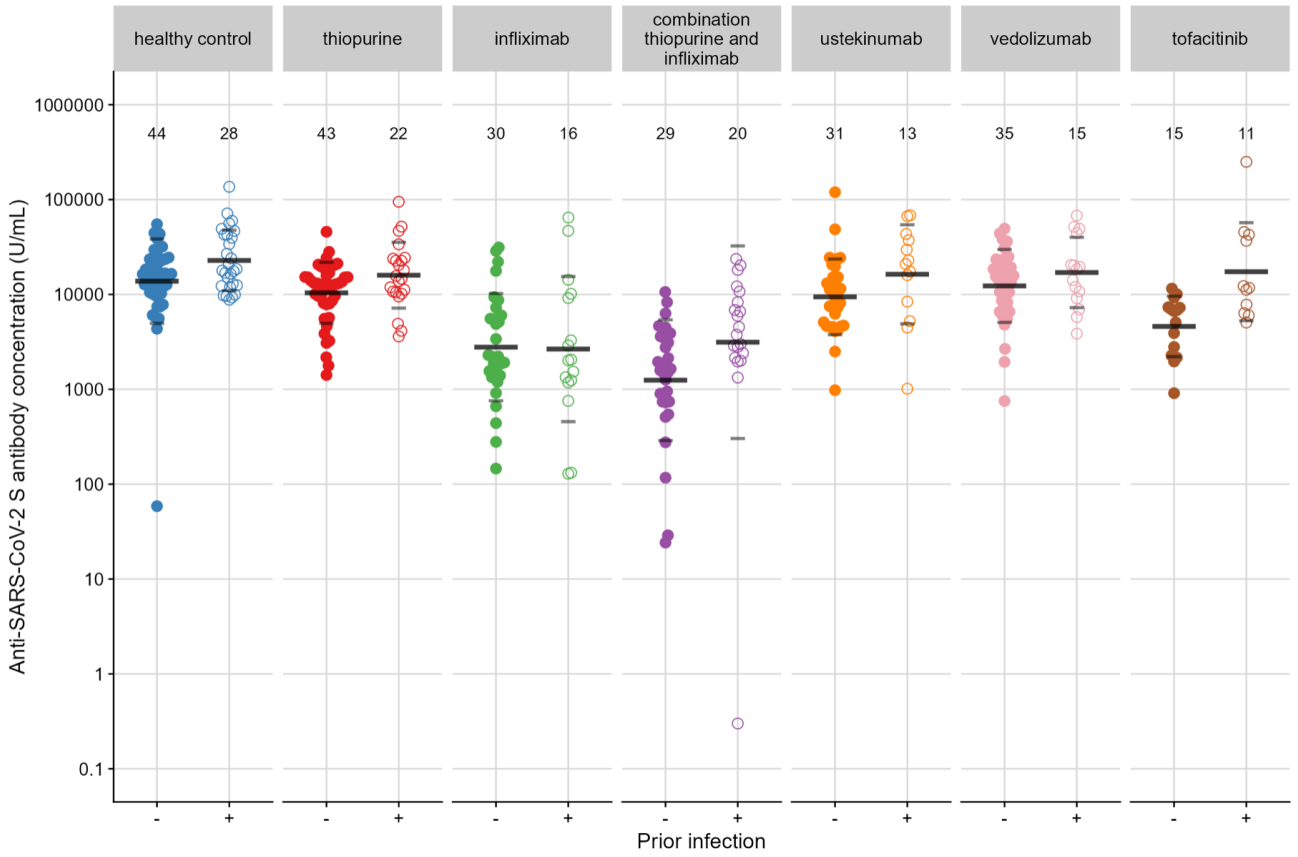

****
****
****
****
****
****
****
Anti-SARS-CoV-2 S1 RBD antibody concentration (U/ml)
1000000
100000
10000
1000
100
10
1
0.1
V2
V3
Healthy control
Thiopurine
Infliximab
Infliximab & Thiopurine
Ustekinumab
Vedolzumab
Tofacitinib

Figure 2

A



B

Variable	N	Geometric mean ratio (GMR)	GMR (95% CI)	p
thiopurine	65/137		0·78 (0·58, 1·05)	0·099
Homologous vaccination	64/137		1·17 (0·87, 1·58)	0·30
Age (per decade)	137/137		0·92 (0·82, 1·03)	0·17
Prior infection	50/137		1·52 (1·13, 2·05)	0·0064

Variable	N	Geometric mean ratio (GMR)	GMR (95% CI)	p
combination thiopurine and infliximab	47/119		0·15 (0·10, 0·22)	<0·0001
Homologous vaccination	54/119		1·11 (0·73, 1·68)	0·62
Age (per decade)	119/119		0·90 (0·77, 1·05)	0·17
Prior infection	47/119		2·20 (1·50, 3·22)	<0·0001

Variable	N	Geometric mean ratio (GMR)	GMR (95% CI)	p
infliximab	46/118		0·17 (0·11, 0·26)	<0·0001
Homologous vaccination	65/118		2·00 (1·30, 3·10)	0·0020
Age (per decade)	118/118		0·93 (0·78, 1·11)	0·41
Prior infection	44/118		1·25 (0·82, 1·91)	0·30

Variable	N	Geometric mean ratio (GMR)	GMR (95% CI)	p
ustekinumab	44/116		0·70 (0·48, 1·02)	0·060
Homologous vaccination	54/116		0·98 (0·64, 1·48)	0·90
Age (per decade)	116/116		0·97 (0·83, 1·14)	0·74
Prior infection	41/116		1·67 (1·15, 2·43)	0·0071

Variable	N	Geometric mean ratio (GMR)	GMR (95% CI)	p
vedolizumab	46/118		0·82 (0·58, 1·17)	0·27
Homologous vaccination	56/118		1·57 (1·09, 2·26)	0·015
Age (per decade)	118/118		1·07 (0·93, 1·22)	0·35
Prior infection	39/118		1·47 (1·04, 2·08)	0·031

Variable	N	Geometric mean ratio (GMR)	GMR (95% CI)	p
tofacitinib	26/98		0·49 (0·31, 0·76)	0·0018
Homologous vaccination	47/98		1·44 (0·88, 2·36)	0·14
Age (per decade)	98/98		1·08 (0·89, 1·30)	0·42
Prior infection	39/98		1·95 (1·30, 2·92)	0·0014

Figure 3

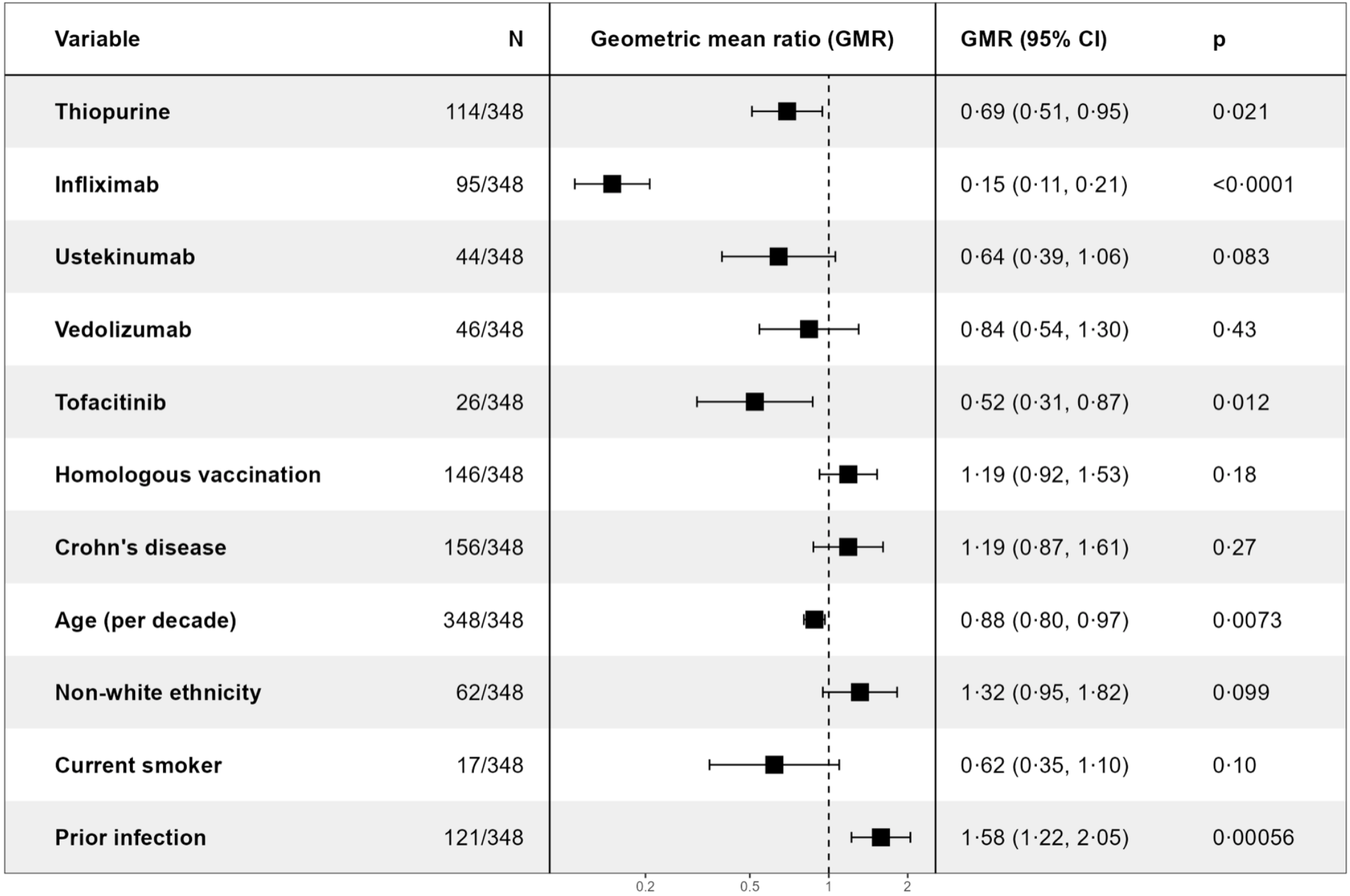
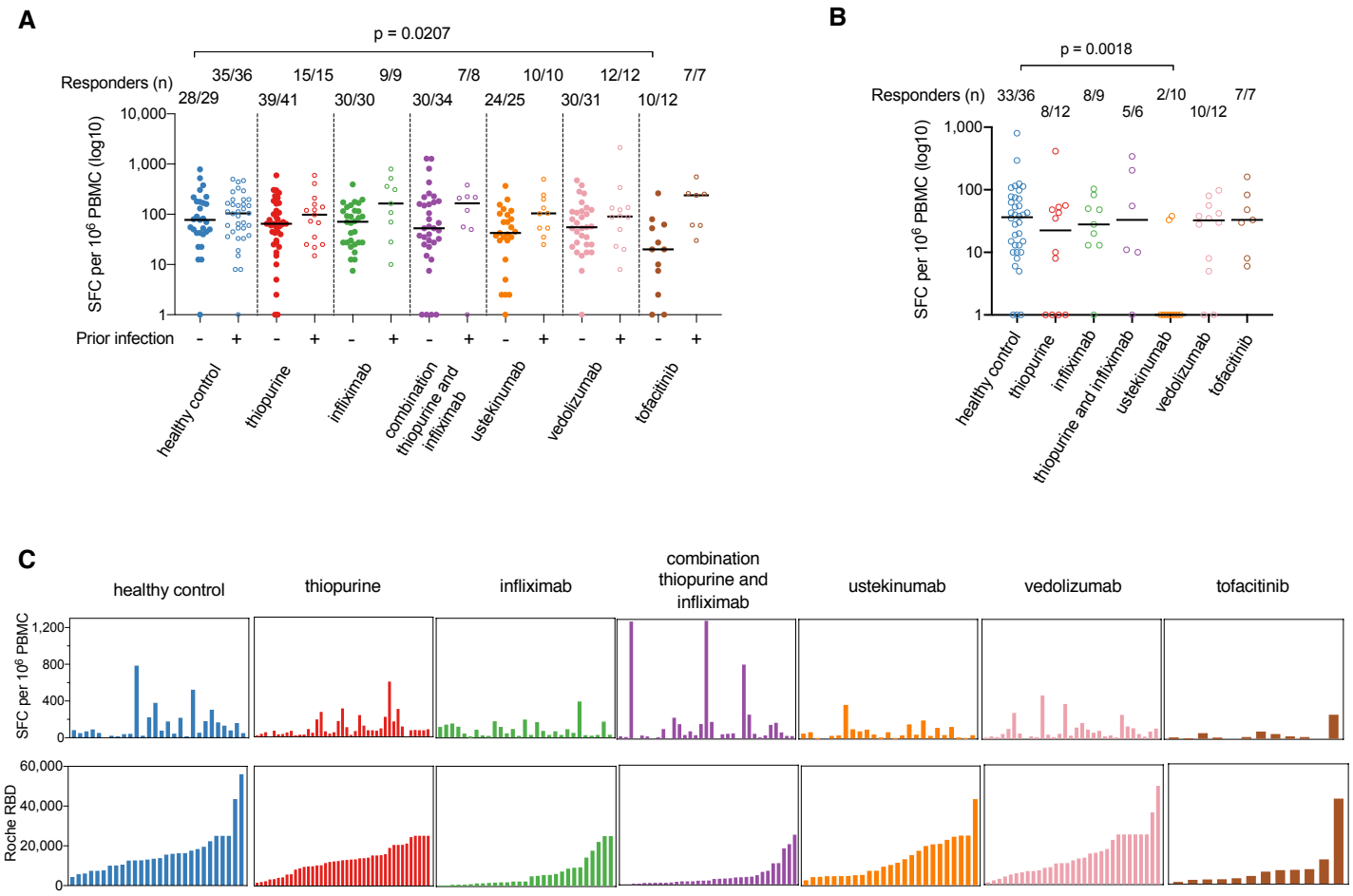

Variable
N
Geometric mean ratio (GMR)
GMR (95% CI)
p
Thiopurine
114/348
0·69 (0·51, 0·95)
0·021
Infliximab
95/348
0·15 (0·11, 0·21)
<0·0001
Ustekinumab
44/348
0·64 (0·39, 1·06)
0·083
Vedolizumab
46/348
0·84 (0·54, 1·30)
0·43
Tofacitinib
26/348
0·52 (0·31, 0·87)
0·012
Homologous vaccination
146/348
1·19 (0·92, 1·53)
0·18
Crohn's disease
156/348
1·19 (0·87, 1·61)
0·27
Age (per decade)
348/348
0·88 (0·80, 0·97)
0·0073
Non-white ethnicity
62/348
1·32 (0·95, 1·82)
0·099
Current smoker
17/348
0·62 (0·35, 1·10)
0·10
Prior infection
121/348
1·58 (1·22, 2·05)
0·00056
0.2
0.5
1
2

Figure 4

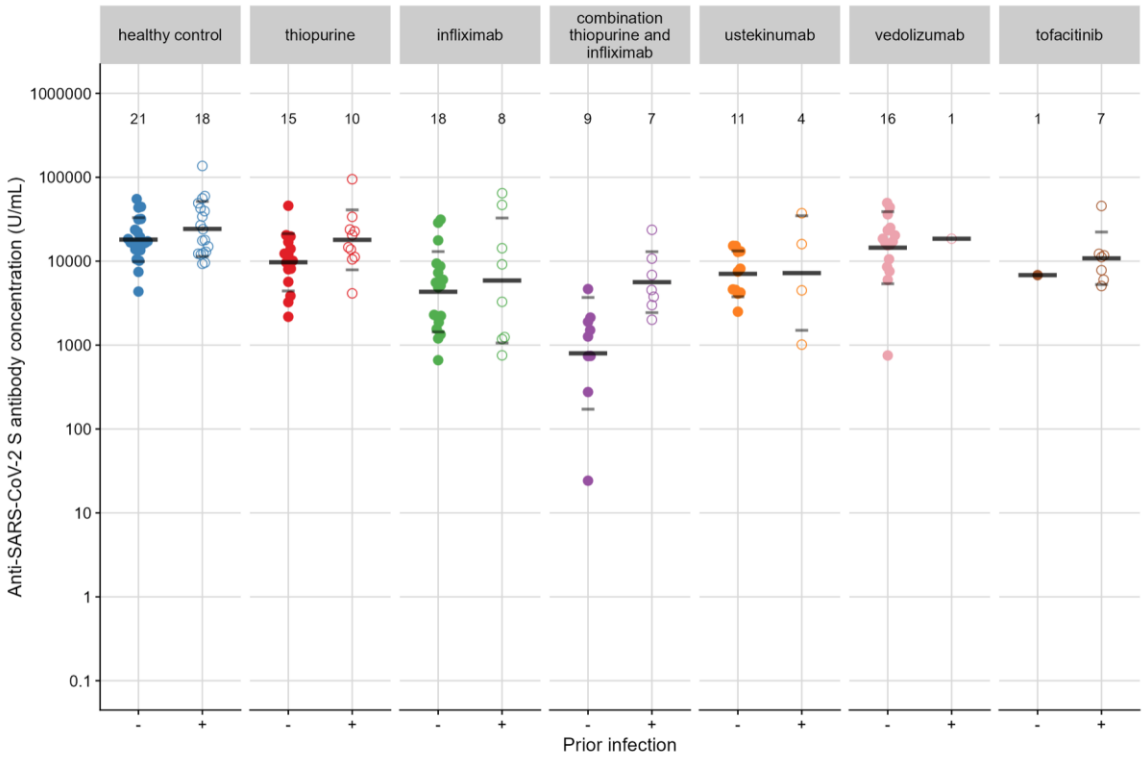

A
p = 0.0207
Responders (n)
35/36
15/15
9/9
7/8
10/10
12/12
7/7
28/29
39/41
30/30
30/34
24/25
30/31
10/12
SFC per 10^6 PBMC (log10)
10,000
1,000
100
10
1
Prior infection
- + - + - + - + - + - + - +
healthy control
thiopurine
infliximab
combination thiopurine and infliximab
ustekinumab
vedolizumab
tofacitinib
B
p = 0.0018
Responders (n)
33/36
8/12
8/9
5/6
2/10
10/12
7/7
SFC per 10^6 PBMC (log10)
1,000
100
10
1
healthy control
thiopurine
infliximab
thiopurine and infliximab
ustekinumab
vedolizumab
tofacitinib
C
healthy control
thiopurine
infliximab
combination thiopurine and infliximab
ustekinumab
vedolizumab
tofacitinib
SFC per 10^6 PBMC
1,200
800
400
0
Roche RBD
60,000
40,000
20,000
0

COVID-19 vaccine-induced antibody and T cell responses in immunosuppressed patients with inflammatory bowel disease after the third vaccine dose (VIP): a multicentre, prospective, case-control study

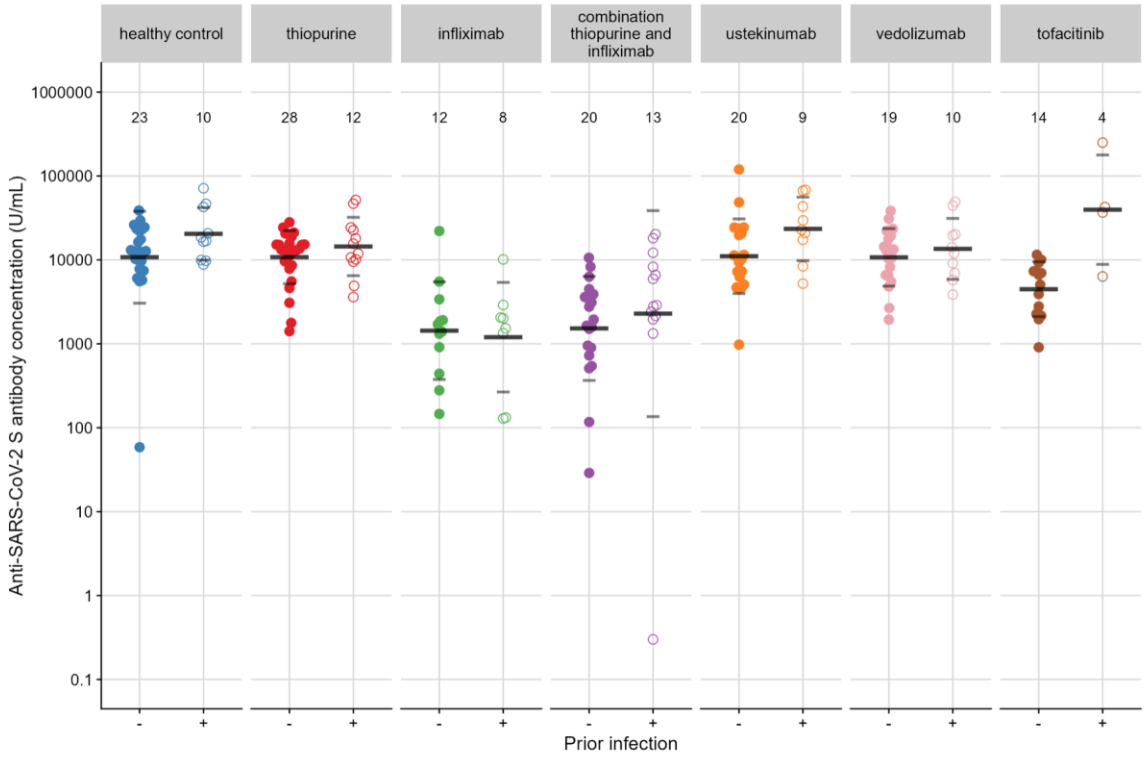
Supplementary Material

Supplementary table 1: Linear mixed effects model including visit 1 (post second vaccine dose) and visit 2 (post third vaccine dose) anti-S1 RBD antibody concentrations. Study visit was analysed as a fixed effect.

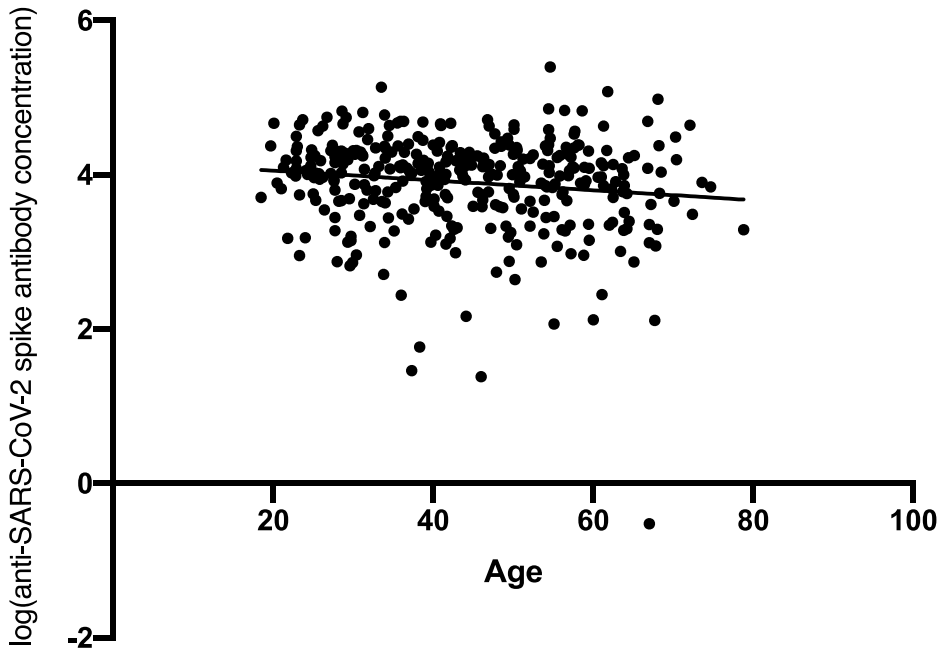
Variable	Estimate	95% CIs	P-value
Thiopurine	0.85	0.64 - 1.14	0.29
Infliximab	0.13	0.09 - 0.18	<0.0001
Ustekinumab	0.72	0.45 – 1.15	0.17
Vedolizumab	1.04	0.69 - 1.56	0.86
Tofacitinib	0.57	0.35 – 0.92	0.021
Visit: visit 2	16.75	13.94 – 20.13	<0.0001
mRNA vaccine effect on visit 1	3.30	2.50 – 4.34	<0.0001
Baseline mRNA vaccine effect on visit 2 (i.e. homologous vaccination)	1.19	0.90 – 1.56	0.22
Crohn's disease	1.08	0.81 – 1.44	0.60
Age (per decade)	0.84	0.77 – 0.91	<0.0001
Non-white ethnicity	1.05	0.78 – 1.43	0.74
Current smoker	0.67	0.39 – 1.15	0.15
Prior infection (assessed separately for each visit)	2.27	1.80 – 2.87	<0.0001



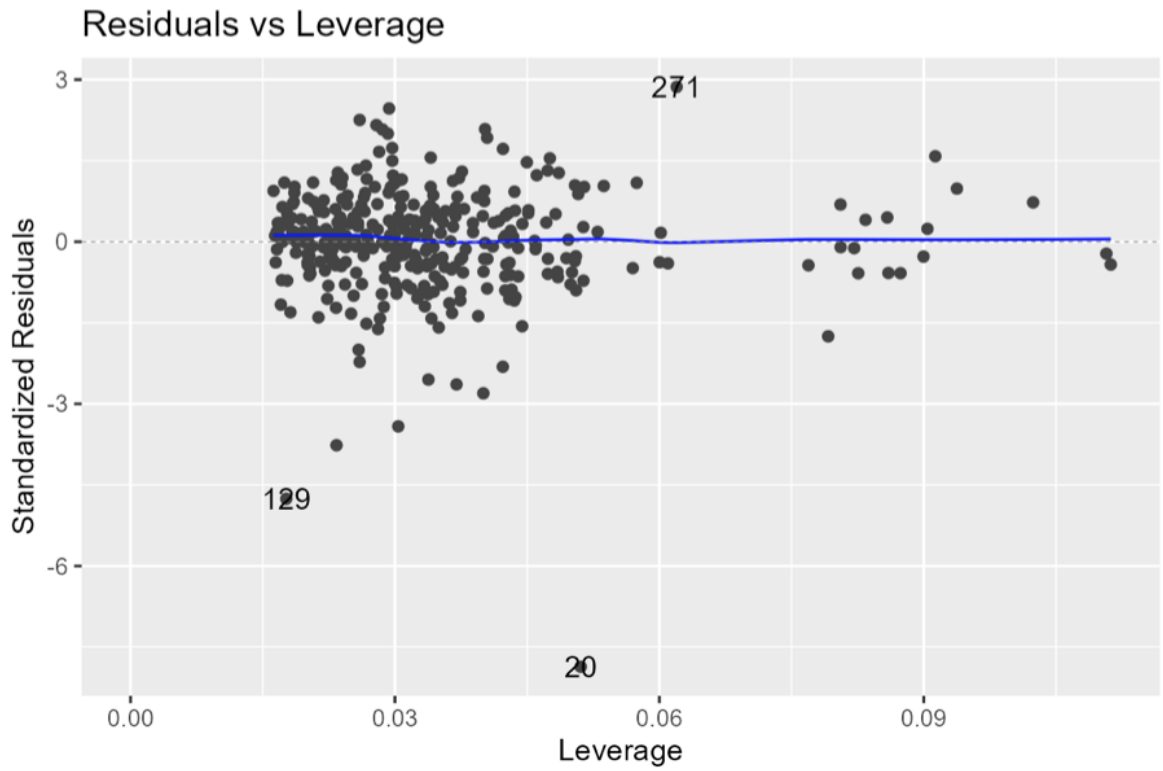
Supplementary figure 1: Anti-SARS-CoV-2 spike antibody concentration in participants receiving a homologous vaccine schedule (three doses of mRNA vaccine) stratified by study group and previous infection status. The wider bar represents the geometric mean, while the narrower bars are one geometric SD either side of the geometric mean.



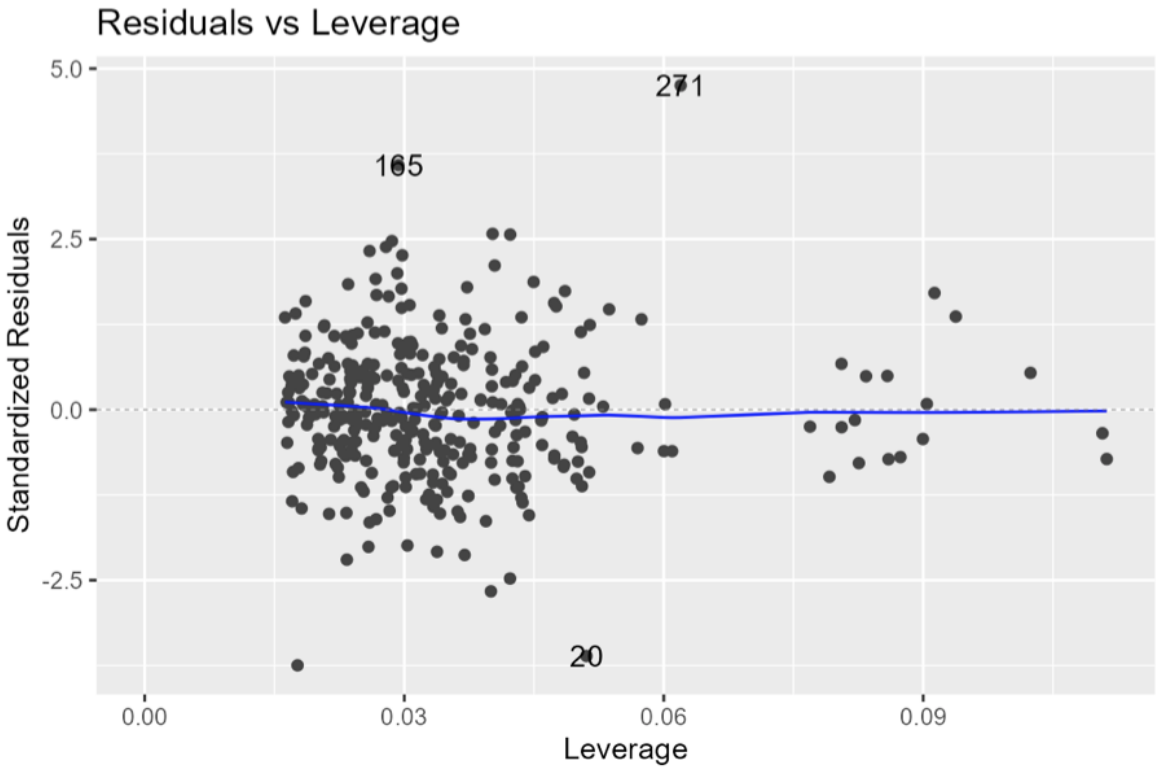
Supplementary figure 2: Anti-SARS-CoV-2 spike antibody concentration in participants receiving a heterologous vaccine schedule (two doses of adenovirus vector vaccine and one dose of mRNA vaccine) stratified by study group and previous infection status. The wider bar represents the geometric mean, while the narrower bars are one geometric SD either side of the geometric mean.



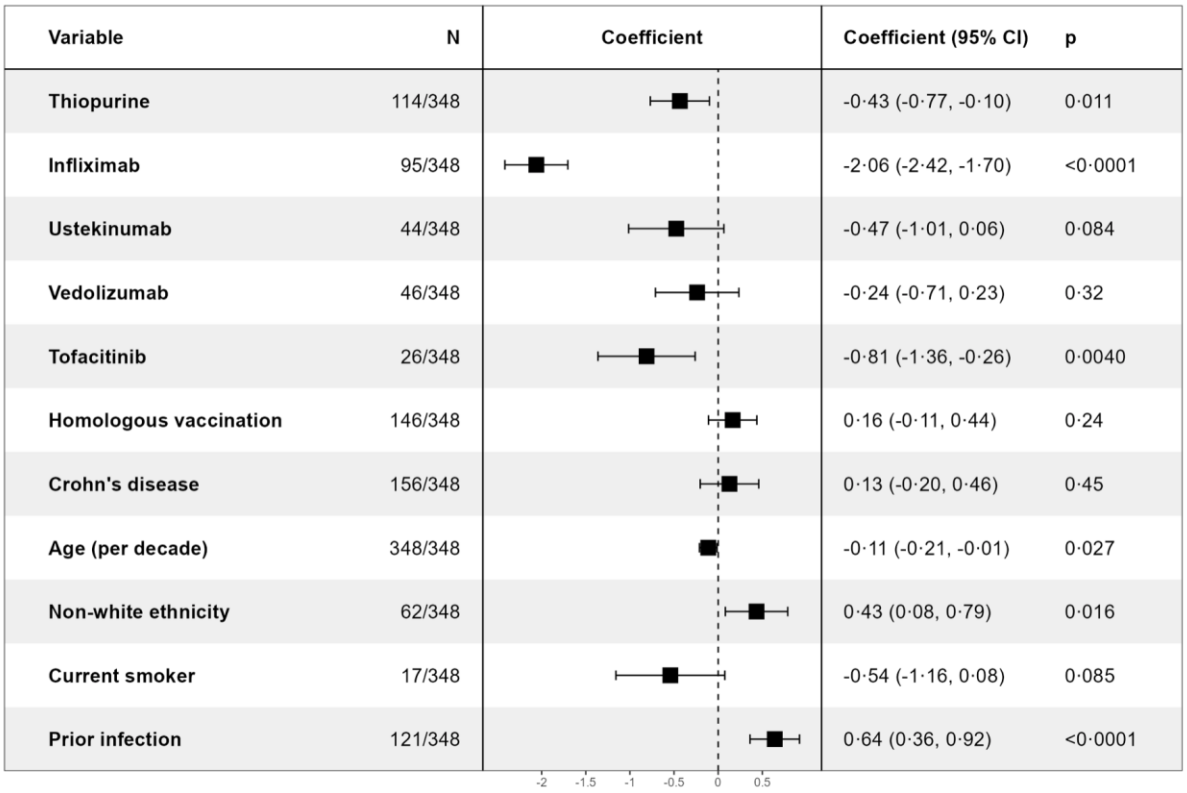
Supplementary figure 3: Simple linear regression model of age versus log[anti-SARS-CoV-2 spike antibody concentration] (R^2=0.02; p=0.0091). Runs test for deviation from linearity (p=0.67) indicated non-deviation from linearity.



Supplementary figure 4: Diagnostics plot showing distribution of residuals in the multivariable linear regression model (figure 3) following log transformation.



Supplementary figure 5: Diagnostics plot showing distribution of residuals in the multivariable linear regression model (figure 3) following Box Cox transformation with lambda = 0.20 (based on optimising the log-likelihood of the model).



Supplementary figure 6: Sensitivity analysis using a one-parameter Box Cox transformation with lambda = 0.20 (based on optimising the log-likelihood of the model). Multivariable model showing exponentiated coefficients of linear regression models of log(anti-SARS-CoV-2 spike antibody concentration). Results are for individuals without evidence of previous SARS-CoV-2 infection. The values shown represent geometric mean ratio estimates of S1 level associated with each variable. Age was treated as a continuous variable in the analysis and its coefficient is expressed per decade.

First Name	Surname
Ijeoma	Chukwurah
Sulaimaan	Haq
Parita	Shah
Stephanie	Wilken-Smith
Anitha	Ramanathan
Mikin	Patel
Lidia	Romanczuk
Rebecca	King
Jason	Domingo
Djamila	Shamtally
Vivien	Mendoza
Joanne	Sanchez
Hannah	Stark
Bridget	Knight
Louise	Bee
Charmaine	Estember
Anna	Barnes
Darcy	Watkins
Sam	Stone
John	Kirkwood
Marian	Parkinson
Helen	Gardner-Thorpe
Kate	Covil
Lauranne	Derikx
Beatriz	Gros Alcalde
Irish	Lee
Bessie	Cipriano
Giuseppe	Ruocco
Manisha	Baden
Graham	Cooke
Katrina	Pollock
Evgenia	Kourampa
Ciro	Pasquale
Elena	Robisco-Diaz
Suhaylah	Bhatti

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No.	Recommendation	Page No.	Relevant text from manuscript
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	4-5	
Introduction				
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	8-9	
Objectives	3	State specific objectives, including any prespecified hypotheses	9	
Methods				
Study design	4	Present key elements of study design early in the paper	10	
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	10-11	
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	10-11	
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	13-14	
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	13-14	
Bias	9	Describe any efforts to address potential sources of bias	14-15	
Study size	10	Explain how the study size was arrived at	14	

Continued on next page

Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	14
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	14-16
		(*b*) Describe any methods used to examine subgroups and interactions	14-16
		(*c*) Explain how missing data were addressed	14
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	N/A
		(*e*) Describe any sensitivity analyses	15
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	17
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Table 1
		(b) Indicate number of participants with missing data for each variable of interest	14
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	*N/A*
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	17
		Cross-sectional study—Report numbers of outcome events or summary measures	*N/A*
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	17-19
		(*b*) Report category boundaries when continuous variables were categorized	Table 1
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A

Continued on next page

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	18
Discussion			
Key results	18	Summarise key results with reference to study objectives	20-21
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	21-22
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22
Generalisability	21	Discuss the generalisability (external validity) of the study results	22
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	16

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

First Name	Surname
Ijeoma	Chukwurah
Sulaimaan	Haq
Parita	Shah
Stephanie	Wilken-Smith
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Mikin	Patel
Lidia	Romanczuk
Rebecca	King
Jason	Domingo
Djamila	Shamtally
Vivien	Mendoza
Joanne	Sanchez
Hannah	Stark
Bridget	Knight
Louise	Bee
Charmaine	Estember
Anna	Barnes
Darcy	Watkins
Sam	Stone
John	Kirkwood
Marian	Parkinson
Helen	Gardner-Thorpe
Kate	Covil
Lauranne	Derikx
Beatriz	Gros Alcalde
Irish	Lee
Bessie	Cipriano
Giuseppe	Ruocco
Manisha	Baden
Graham	Cooke
Katrina	Pollock
Evgenia	Kourampa
Ciro	Pasquale
Elena	Robisco-Diaz
Suhaylah	Bhatti