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Immune response after two doses of the BNT162b2 COVID-19 vaccine and risk of SARS-CoV-2 breakthrough infection in Tyrol, Austria: an open-label, observational phase 4 trial

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Summary

Background Correlates of protection could help to assess the extent to which a person is protected from SARS-CoV-2 infection after vaccination (so-called breakthrough infection). We aimed to clarify associations of antibody and T-cell responses after vaccination against COVID-19 with risk of a SARS-CoV-2 breakthrough infection and whether measurement of these responses enhances risk prediction.

Methods We did an open-label, phase 4 trial in two community centres in the Schwaz district of the Federal State of Tyrol, Austria, before the emergence of the omicron (B.1.1.529) variant of SARS-CoV-2. We included individuals (aged ≥16 years) a mean of 35 days (range 27–43) after they had received a second dose of the BNT162b2 (Pfizer–BioNTech) COVID-19 vaccine. We quantified associations between immunological parameters and breakthrough infection and assessed whether information on these parameters improves risk discrimination. The study is registered with the European Union Drug Regulating Authorities Clinical Trials Database, 2021-002030-16.

Findings 2760 individuals (1682 [60·9%] female, 1078 [39·1%] male, mean age 47·4 years [SD 14·5]) were enrolled into this study between May 15 and May 21, 2021, 712 (25·8%) of whom had a previous SARS-CoV-2 infection. Over a median follow-up of 5·9 months, 68 (2·5%) participants had a breakthrough infection. In models adjusted for age, sex, and previous infection, hazard ratios for breakthrough infection for having twice the immunological parameter level at baseline were 0·72 (95% CI 0·60–0·86) for anti-spike IgG, 0·80 (0·70–0·92) for neutralising antibodies in a surrogate virus neutralisation assay, 0·84 (0·58–1·21) for T-cell response after stimulation with a CD4 peptide pool, and 0·77 (0·54–1·08) for T-cell response after stimulation with a combined CD4 and CD8 peptide pool. For neutralising antibodies measured in a nested case-control sample using a pseudotyped virus neutralisation assay, the corresponding odds ratio was 0·78 (0·62–1·00). Among participants with previous infection, the corresponding hazard ratio was 0·73 (0·61–0·88) for anti-nucleocapsid Ig. Addition of anti-spike IgG information to a model containing information on age and sex improved the C-index by 0·085 (0·027–0·143).

Interpretation In contrast to T-cell response, higher levels of binding and neutralising antibodies were associated with a reduced risk of breakthrough SARS-CoV-2 infection. The assessment of anti-spike IgG enhances the prediction of incident breakthrough SARS-CoV-2 infection and could therefore be a suitable correlate of protection in practice. Our phase 4 trial measured both humoral and cellular immunity and had a 6-month follow-up period; however, the longer-term protection against emerging variants of SARS-CoV-2 remains unclear.

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Introduction

Measurable correlates of protection that help to assess to what extent a person is protected from SARS-CoV-2 infection after vaccination (so-called breakthrough infection) are useful to estimate not only the degree of protection for the individual, but also protection at the population level. Candidate biomarkers are antibodies directed against the SARS-CoV-2 spike and nucleocapsid proteins, neutralising antibodies, and markers of cellular response to vaccination. Several previous studies[1–10] have suggested inverse associations between these markers and the risk of breakthrough infection. However, the shape of associations (eg, linear, curvilinear, or presence of threshold) are unclear; few studies have concurrently measured a broad range of immunological parameters, including cellular responses; and most studies[2,6–10] have

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See Online for appendix

Research in context

Evidence before this study

We searched PubMed from database inception to April 21, 2022, with no language restrictions, for studies that investigated whether the immune response to vaccination against SARS-CoV-2 is associated with the subsequent risk of a SARS-CoV-2 breakthrough infection. We used the search terms "SARS-CoV-2" and ("antibodies" or "T-cells") and ("breakthrough infection" or "risk of incident infection"). We identified relevant publications from two clinical trials (one phase 2/3 trial of the ChAdOx1 nCoV-19 [Oxford–AstraZeneca] vaccine and one phase 3 trial of the mRNA-1273 [Moderna] vaccine) and eight observational studies (three population-based studies, three studies involving patients receiving dialysis, one study involving health-care workers, and one study involving patients with autoimmune rheumatic disease). Collectively, the studies showed inverse relationships between humoral immune responses to vaccination and subsequent risk of breakthrough infection.

Added value of this study

We took concurrent measurements of a range of immunological parameters: antibody concentrations, neutralising antibody titres, and markers of the T-cell response to vaccination. Another strength of our study is that participants received both doses of the BNT162b2 vaccine at almost the same time as each other, and therefore were exposed to the same background incidence in the population during follow-up. To our knowledge, our study is the first to analyse associations between T cells and incident SARS-CoV-2 infection using time-to-event analysis, and to quantify the added value of assessing anti-SARS-CoV-2 antibodies for predicting an individual's risk of SARS-CoV-2 infection despite vaccination.

Implications of all the available evidence

Higher concentrations of binding and neutralising antibodies were associated with greater protection from SARS-CoV-2 infection. At the population level, information on these antibody concentrations could help to refine population immunity estimates and could thereby help to enhance the prediction of the future course of the COVID-19 pandemic.

been conducted in select population subgroups, such as health-care workers or patients with renal disease. Furthermore, whether measurement of these immunological parameters can enhance the prediction of breakthrough infection risk is unclear.

We conducted an open-label, phase 4 trial among individuals who had received two doses of the BNT162b2 (Pfizer–BioNTech) COVID-19 vaccine. Our study had two aims: first, to estimate the associations between concentrations of several humoral and cellular immunological parameters and incident breakthrough SARS-CoV-2 infection; and second, to quantify the predictive value of binding and neutralising antibodies for incident breakthrough SARS-CoV-2 infection.

Methods

Study design and participants

The Shieldvacc-2 study was an open-label, phase 4 trial conducted at two community centres (in Jenbach and Zell am Ziller) in the Schwaz district of the Federal State of Tyrol, Austria. We chose this district because it conducted an ultra-rapid-rollout COVID-19 vaccination programme in March and April, 2021, providing vaccines to 66·9% of the eligible population within just 6 days per dose (dose 1, March 11–16, 2021; dose 2, April 8–13, 2021).[11]

Individuals were eligible for inclusion if they were aged at least 16 years; had received two 30 µg doses of the BNT162b2 vaccine by intramuscular injection, with the second dose having been administered 35 days (range 27–43) before enrolment; understood and agreed to comply with the study procedures; and were willing to be contacted by telephone or to complete an online diary throughout the course of the study. Exclusion criteria were previous administration of an investigational coronavirus (ie, SARS-CoV or MERS-CoV) vaccine or concurrent participation in interventional studies aimed at preventing or treating COVID-19, a contraindication to blood draws, and participation in any other interventional study within 30 days before enrolment. Eligible individuals were invited by public calls on the radio and in local newspapers to participate in the study.

At baseline (May 15–21, 2021), participants were asked to complete a questionnaire on sociodemographic characteristics, previous SARS-CoV-2 infection, and COVID-19 vaccination. Previous SARS-CoV-2 infection was based on self-report or seropositivity of Ig antibodies targeting the nucleocapsid protein (anti-N Ig) at baseline. Blood samples of up to 18 mL were drawn to enable testing of the participants' humoral and cellular immune response to vaccination at baseline and 6 months after baseline (Nov 11–18, 2021).

Written informed consent was provided by study participants or, if appropriate, by the individual's legal representative or custodian. The study was approved by the ethics committee of the Medical University of Innsbruck (1168/2021) and has been registered in the European Union Drug Regulating Authorities Clinical Trials Database (2021-002030-16). Results are reported in accordance with STROBE guidelines (appendix pp 3–4). The protocol is available online.

For the **protocol** see https://www.clinicaltrialsregister.eu/ctr-search/trial/2021-002030-16/AT

Procedures

Details on laboratory methods are provided in the appendix (p 1). In brief, to assess antibody responses,

plasma samples were collected in EDTA (edetic acid) tubes and were analysed with the Abbott SARS-CoV-2 IgG II Quant chemiluminescent microparticle immunoassay on the Alinity i instrument (Abbott Ireland, Sligo, Ireland) to measure IgG antibodies targeting the receptor-binding domain (RBD) of the spike protein (anti-S IgG); the Roche Elecsys Anti-SARS-CoV-2 electrochemiluminescent immunoassay on the Cobas e411 analyser (Roche, Mannheim, Germany) to measure anti-N Ig; and the TECO SARS-CoV-2 neutralisation antibody ELISA on the SERION Immunomat (TECOmedical, Sissach, Switzerland) to measure the inhibitory effects of neutralising antibodies blocking the interaction between angiotensin-converting enzyme 2 and the RBD of the SARS-CoV-2 spike protein. For individuals with breakthrough SARS-CoV-2 infection and control individuals who were matched by age, sex, and previous SARS-CoV-2 infection, we also measured 50% neutralising antibody titres against the ancestral (Wuhan-1) spike protein using a vesicular stomatitis virus pseudovirus assay.

To evaluate cellular immune responses, we collected additional blood samples in heparin tubes from a randomly selected subgroup of 929 participants. SARS-CoV-2-specific T-cell response was measured by a Qiagen QuantiFERON SARS-CoV-2 RUO IFNγ release assay (Qiagen, Hilden, Germany) in response to CD4 and combined CD4 and CD8 peptide pools derived from the SARS-CoV-2 spike antigen (S1 S2 RDB). The ratios of IFNγ concentrations from SARS-CoV-2-specific stimulation and the unstimulated control was defined as the stimulation index. All samples were processed centrally at the Institute of Virology of the Medical University of Innsbruck (Innsbruck, Austria).

Outcomes

The primary outcome was defined as SARS-CoV-2 infection occurring during a follow-up period of 6 months, identified by a positive PCR test, seroconversion of anti-N Ig during follow-up, or an increase to three times the anti-N Ig concentration during follow-up. To preclude underascertainment of asymptomatic or paucisymptomatic events, participants were asked to undergo SARS-CoV-2 antigen testing every 7 (range 4–10) days throughout the course of the study and to record test results and related symptoms through an online participant portal. Secondary outcomes focused on symptomatic SARS-CoV-2 infections—ie, having one or more symptoms of fever, chills, cough, breathing difficulties, muscle or limb pain, loss of sense of smell or taste, sore throat, diarrhoea, or vomiting. All recorded SARS-CoV-2 infections were rigorously validated during structured telephone interviews in terms of dates of infection, symptoms, and clinical course of infection. For infections detected through serological tests only, the date of infection was estimated using the dates of plausible contagions (eg, symptoms or close contact with infected individuals) or using the median date of all SARS-CoV-2 infections that were recorded in the study.

Statistical analysis

Baseline characteristics were summarised as n (%) for categorical variables and as mean (SD) for continuous variables, if normally distributed, or as median (IQR) otherwise. Because the distributions of immunological parameters were skewed, we $\log_2$-transformed their values for all further analyses (appendix p 6). We used transformation based on $\log_2$ because then a relative risk for a one-unit-higher $\log_2$-transformed value corresponds to a relative risk for twice the baseline value, thereby providing a more intuitive scaling than, for instance, transformation based on $\log_e$.

We used *t* tests for continuous variables and χ^2 tests for categorical variables to compare baseline characteristics of participants with and without incident breakthrough SARS-CoV-2 infection. We calculated Pearson's correlation coefficients to assess cross-sectional correlations of immunological parameters at baseline.

To quantify the associations between each immunological parameter and the risk of SARS-CoV-2 breakthrough infection, we estimated relative risks for having twice the concentration of immunological parameters adjusted for age (untransformed), sex (female or male), and previous SARS-CoV-2 infection (yes or no). For anti-S IgG, neutralising antibodies in a surrogate SARS-CoV-2 virus neutralisation test, T-cell response, and anti-N Ig, we analysed time-to-event data using Cox

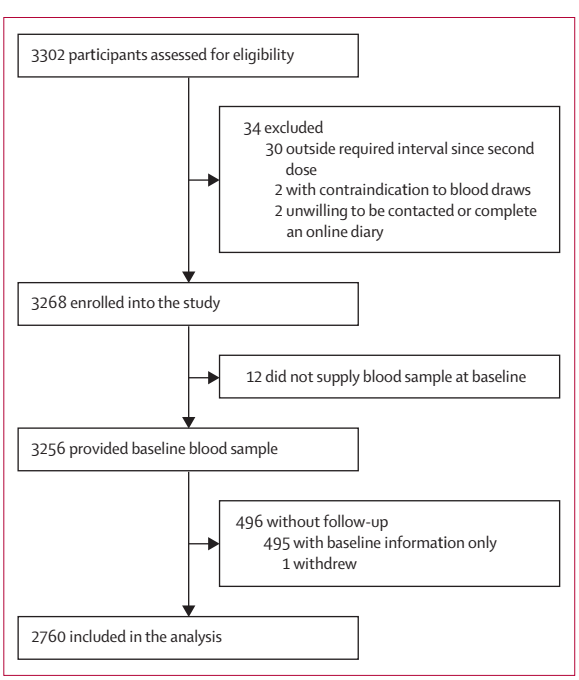

Figure 1: **Study profile**

	All participants (n=2760)	Incident SARS-CoV-2 infection		
		Yes (n=68)	No (n=2692)	p value
Age, years	47·4 (14·5)	45·2 (14·8)	47·5 (14·5)	0·20
Sex	..	..	..	0·39
Female	1682 (60·9%)	38 (55·9%)	1644 (61·1%)	..
Male	1078 (39·1%)	30 (44·1%)	1048 (38·9%)	..
Previous SARS-CoV-2 infection*	712 (25·8%)	9 (13·2%)	703 (26·1%)	0·017
Anti-S IgG				
Number measured	2760	68	2692	..
Number seropositive	2758 (99·9%)	68 (100·0%)	2690 (99·9%)	0·82
Absolute concentration, BAU/mL	1934 (1177–3120)	1384 (916–2033)	1955 (1185–3136)	0·0002
Surrogate virus neutralisation test				
Number measured	2760	68	2692	..
Number seropositive	2374 (86·0%)	53 (77·9%)	2321 (86·2%)	0·052
Absolute concentration, IU/mL	951·0 (400·3–2481·8)	518·1 (247·1–1183·5)	960·7 (409·6–2536·5)	0·0006
Pseudotyped virus neutralisation test†				
Number measured	272	68	204	..
Number seropositive	271 (99·6%)	68 (100·0%)	203 (99·5%)	0·24
Absolute reciprocal titre	355·3 (186·6–678·1)	229·3 (156·2–471·0)	396·2 (203·7–736·2)	0·019
Anti-N Ig‡				
Number measured	712	9	703	..
Number seropositive	655 (92·0%)	6 (66·7%)	649 (92·3%)	0·0039
Absolute concentration, COI	25·9 (6·2–84·6)	2·1 (0·3–4·6)	26·6 (6·7–86·2)	0·0002
CD4 peptide pool				
Number measured	929	22	907	..
Number reactive (SI ≥ 3)	494 (53·2%)	11 (50·0%)	483 (53·3%)	0·76
Number reactive or weakly reactive (SI ≥ 2)	659 (70·9%)	18 (81·8%)	641 (70·7%)	0·26
Absolute SI	3·3 (1·8–6·6)	3·1 (2·2–4·5)	3·3 (1·8–6·7)	0·29
CD4 and CD8 peptide pool				
Number measured	926	22	904	..
Number reactive (SI ≥ 3)	592 (63·9%)	13 (59·1%)	579 (64·0%)	0·63
Number reactive or weakly reactive (SI ≥ 2)	737 (79·6%)	17 (77·3%)	720 (79·6%)	0·79
Absolute SI	4·5 (2·3–9·6)	3·3 (2·6–4·9)	4·5 (2·2–9·7)	0·12

Data are mean (SD), n (%), or median (IQR), unless otherwise specified. Values are considered positive if anti-S IgG ≥7·1 BAU/mL, neutralising antibodies ≥200 IU/mL for surrogate virus neutralisation, reciprocal titre >16 for pseudotyped virus neutralisation, and COI ≥1.0 for anti-N Ig. The values of immunological parameters had skewed distributions and were $\log_2$-transformed before applying the *t* tests. Anti-N Ig=anti-nucleocapsid Ig. Anti-S IgG=anti-spike IgG. BAU=binding antibody units. COI=cutoff index. IU=international unit. SI=stimulation index. *Refers to a SARS-CoV-2 infection before study entry detected by self-report or by seropositivity of anti-N Ig at the time of enrolment. †Nested case-control sample. ‡Restricted to participants with previous SARS-CoV-2 infection.

Table: **Baseline characteristics**

regression, censoring participants at the time of SARS-CoV-2 infection, end of follow-up, or loss to follow-up, whichever came first. Participants were considered lost to follow-up if they withdrew from the study or fulfilled all of the following criteria: had more than one consecutive missing antigen test result; had no positive PCR test result; and did not provide anti-N Ig test results at the beginning and the end of the study. The proportional-hazards assumption was validated on the basis of Schoenfeld's residuals (appendix p 2). For titres of neutralising antibodies in a pseudotyped SARS-CoV-2 virus neutralisation test measured in a nested matched case-control sample, we estimated odds ratios (ORs) for breakthrough infection using conditional logistic regression, so that cases were compared with controls only in the same matched set. Secondary analyses focused on symptomatic SARS-CoV-2 infections and compared risk across categorised antibody concentrations.

To assess the incremental predictive values of measuring different immunological parameters, we quantified improvements in the C-index when adding these parameters to a model containing information on age and sex. The C-index is the preferred risk discrimination metric for time-to-event data and assesses whether the model correctly predicts the order of failure of randomly selected pairs of participants. Two-sided p values of 0·05 or lower were considered significant. Analyses were performed with Stata 15.1 and R 4.1.0.

Role of the funding source
There was no funding source for this study.

Results

Between May 15 and May 21, 2021, we screened 3302 individuals for eligibility (figure 1). We identified 3268 individuals who were eligible for inclusion, of whom 2760 participants were included in the analysis.

Baseline characteristics are shown in the table. Overall, the mean age of participants was 47·4 years (SD 14·5); 1682 (60·9%) were female and 1078 (39·1%) were male. 712 (25·8%) of 2760 participants had a previous SARS-CoV-2 infection, which had occurred a median of 6·1 months (IQR 5·0–6·6) before enrolment. Median time since vaccination was 67 days (65–68) for dose 1 and 39 days (37–40) for dose 2. Vaccinations were generally well tolerated (appendix p 7). The most common complaints within 1 week after vaccination were pain at the injection site (1867 [67·6%] participants after dose 1 and 1469 [53·2%] after dose 2), fatigue (602 [21·8%] participants after dose 1 and 1108 [40·1%] after dose 2), and headache (329 [11·9%] participants after dose 1 and 649 [23·5%] after dose 2; appendix p 7).

Except for two participants who were immunosuppressed, all individuals were seropositive for anti-S IgG; 2374 (86·0%) of 2760 participants were seropositive for neutralising antibodies in the surrogate virus neutralisation test, and 271 (99·6%) of 272 participants were seropositive for neutralising antibodies in the pseudotyped virus neutralisation test (table 1). Among the 712 participants with previous SARS-CoV-2 infection, 655 (92·0%) were seropositive for anti-N Ig. Significantly lower titres of anti-S IgG (p=0·0002), neutralising antibodies in the surrogate (p=0·0006) and the pseudotyped (p=0·019) virus

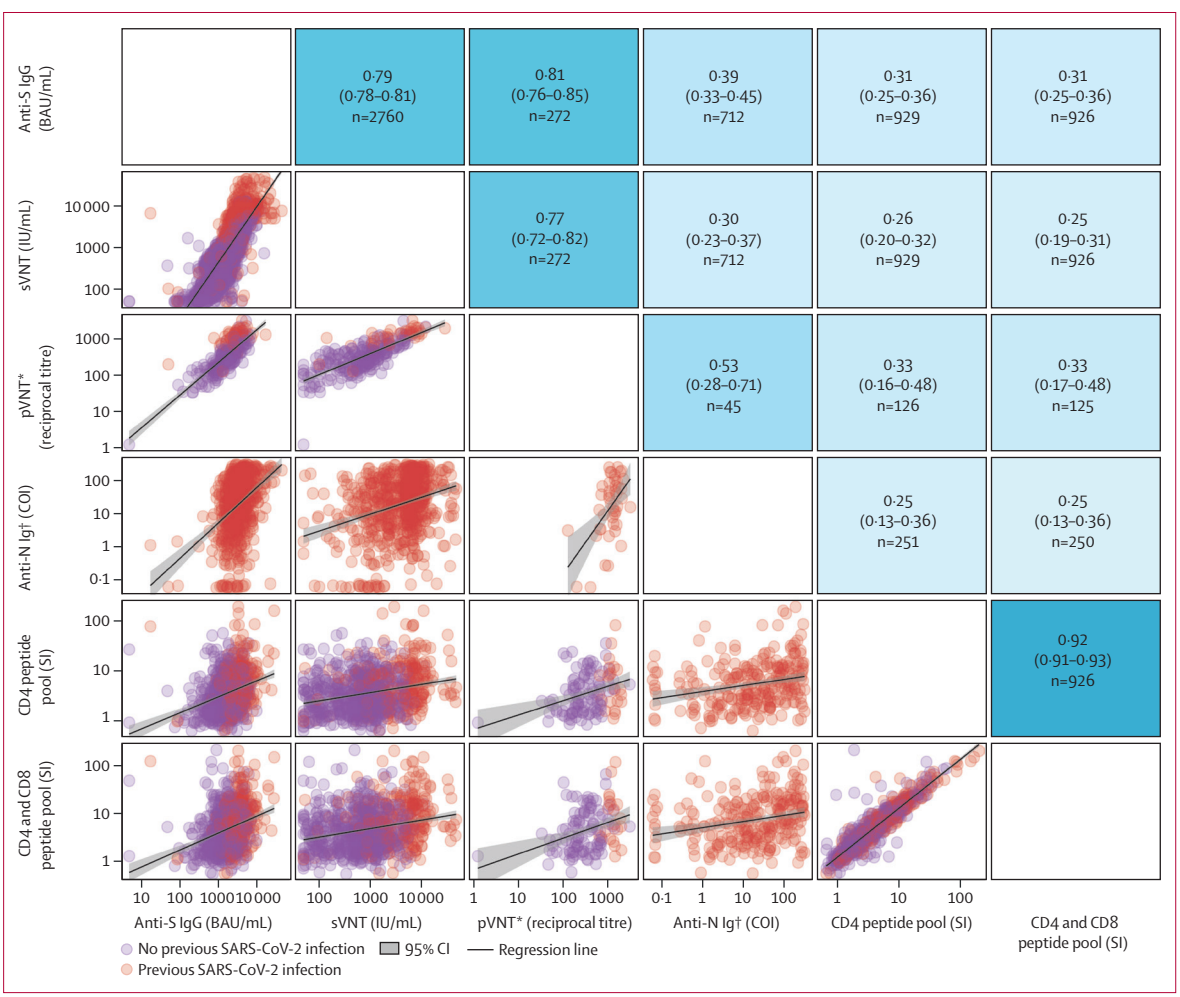

Figure 2: **Correlation coefficients and scatter plots of baseline levels of immunological parameters**
For all analyses we used $\log_2$-transformed values of immunological parameters. The upper part of the matrix shows unadjusted Pearson's correlation coefficients (95% CIs) and numbers of participants. Areas are shaded according to the magnitude of the point estimates of Pearson's correlation coefficients, with darker shading indicating values closer to 1. The lower part of the matrix depicts scatter plots of different immunological parameters, with both axes presented on a log scale. Anti-N Ig=anti-nucleocapsid Ig. Anti-S IgG=anti-spike IgG. BAU=binding antibody units. COI=cutoff index. IU=international unit. pVNT=pseudotyped SARS-CoV-2 virus neutralisation test. SI=stimulation index. sVNT=surrogate SARS-CoV-2 virus neutralisation test. *The analyses of pVNT values were restricted to the nested case-control sample. †The analyses of anti-N Ig values were restricted to participants with previous SARS-CoV-2 infection.

neutralisation tests, and anti-N Ig (p=0·0002) were seen in participants with a SARS-CoV-2 infection during follow-up than in those without. Among the participants in whom cellular response was analysed, 659 (70·9%) of 929 showed some reactivity against the CD4 peptide pool and 737 (79·3%) of 926 to combined CD4 and CD8 peptides. Median stimulation indices of CD4 peptides were 3·3 (IQR 1·8–6·6) and of combined CD4 and CD8 peptides were 4·5 (2·3–9·6).

Scatter plots and correlations of baseline immunological parameters are shown in figure 2. A strong positive correlation between anti-S IgG concentrations and titres of neutralising antibodies was observed in the surrogate (r=0·79 [95% CI 0·78–0·81]) and the pseudotyped (0·81 [0·76–0·85]) virus neutralisation tests. By comparison, anti-S IgG concentrations and titres of neutralising antibodies correlated more weakly with anti-N Ig. Also, correlations between humoral and cellular immune parameters were weak, ranging from 0·25 to 0·33, whereas responses to CD4 and combined CD4 and CD8 peptide pools were very strongly correlated with each other (0·92 [0·91–0·93]).

Cumulative SARS-CoV-2 incidence among both study participants and the overall population in the district of Schwaz sharply increased in the last third of follow-up (ie, mid-September to mid-November, 2021; appendix p 8). Over a median follow-up of 5·9 months (IQR 5·8–5·9), corresponding to 14 995 person-days at risk, we recorded 68 SARS-CoV-2 incident breakthrough infections. Infections occurred between Aug 1 and Nov 15, 2021; as such, the majority were likely to have been caused by the delta variant (B.1.617.2) as this was the dominant variant in the region during this time. 53 (77·9%) of the 68 infections were symptomatic. The most common symptoms were cough (39 [57·3%] participants), loss of taste or smell (31 [45·6%]), muscle or limb pain (30 [44·1%]), and fever or chills (25 [36·8%]; appendix p 9). One participant who was infected with SARS-CoV-2 required admission to hospital; no participants died as a result of SARS-CoV-2 infection or from any other causes during the study.

The hazard ratio (HR) for breakthrough infection adjusted for age, sex, and previous infection was 0·72 (95% CI 0·60–0·86; p=0·0003) for having twice the anti-S IgG concentration and 0·80 (0·70–0·92; p=0·0022) for having twice the concentration of neutralising antibodies in the surrogate virus neutralisation test (figure 3). The corresponding OR for neutralising antibodies in the pseudotyped virus neutralisation test was 0·78 (95% CI 0·62–1·00; p=0·047). By contrast, we observed no significant associations for cellular immunity in the response of either the CD4 peptide pool (HR 0·84 [95% CI 0·58–1·21]; p=0·34) or the combination of CD4 and CD8 peptide pools (0·77 [0·54–1·08]; p=0·13) with incident breakthrough SARS-CoV-2 infection.

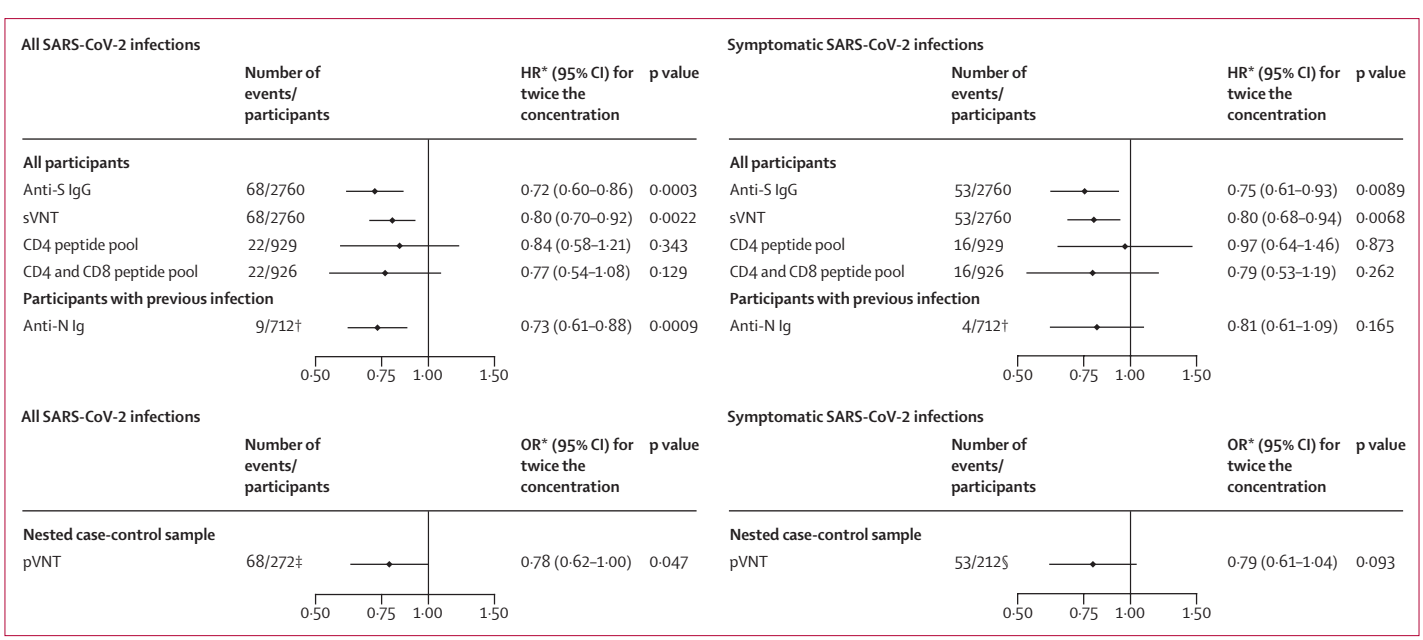

Figure 3: **Associations of baseline levels of immunological parameters with incident breakthrough SARS-CoV-2 infection**

Symptomatic SARS-CoV-2 infection was defined as having one or more symptoms of fever or chills, cough, breathing difficulties, muscle or limb pain, loss of sense of smell or taste, sore throat, diarrhoea, or vomiting. Previous SARS-CoV-2 infection was based on self-report or seropositivity of anti-N Ig at the time of enrolment. For additional information on participants with previous SARS-CoV-2 infection and with incident breakthrough SARS-CoV-2 infection, see the appendix (p 5). Cox regression was applied for anti-S IgG, sVNT, CD4 peptide pool, CD4 and CD8 peptide pool, and anti-N Ig and conditional logistic regression for pVNT. Immunological parameters were entered as $\log_2$-transformed continuous terms. Anti-N Ig=anti-nucleocapsid Ig. Anti-S IgG=anti-spike IgG. HR=hazard ratio. OR=odds ratio. pVNT=pseudotyped SARS-CoV-2 virus neutralisation test. sVNT=surrogate SARS-CoV-2 virus neutralisation test. *HRs and ORs were adjusted for age, sex, and previous SARS-CoV-2 infection. †The analysis of anti-N Ig was restricted to participants with previous SARS-CoV-2 infection. ‡pVNT was measured in a subset of 68 participants infected with SARS-CoV-2 and 204 individual-matched controls. §pVNT was measured in a subset of 53 participants with symptomatic SARS-CoV-2 infection and 159 individual-matched controls.

Among 712 participants with previous SARS-CoV-2 infection, 9 (1·3%) had a breakthrough infection during follow-up (appendix p 5). The HR of breakthrough infection for anti-N Ig in the group with previous infection was 0·73 (0·61–0·88; p=0·0009).

Secondary analyses restricted to symptomatic SARS-CoV-2 infections yielded broadly similar results (figure 3). In secondary analyses quantifying associations across categorised antibody concentrations (figure 4), p values for trend were 0·001 or lower and associations were log-linear, with no evidence of any thresholds that would clearly delineate population groups at high versus low risk. Cumulative incidence plots according to category of immunological parameter are shown in the appendix (p 10).

Finally, to assess the incremental value of immunological parameters for predicting SARS-CoV-2 breakthrough infection, we quantified improvements in the C-index when adding these parameters to a base model including information on age and sex (figure 5). The base model had a C-index of 0·562 (95% CI 0·494 to 0·631). Separate addition of immunological parameters provided significant improvements in the C-index of 0·085 (0·027 to 0·143; p=0·0043) for anti-S IgG, 0·079 (0·011 to 0·147; p=0·023) for neutralising antibodies in the surrogate virus neutralisation test, 0·054 (0·002 to 0·106; p=0·040) for anti-N Ig, and 0·088 (0·026 to 0·151; p=0·0056) for the combination of anti-S IgG and anti-N Ig. The combination of anti-S IgG and anti-N Ig did not yield a significantly higher C-index than measurement of anti-S IgG alone (0·004 [–0·030 to 0·038]; p=0·82). As a benchmark, the addition of information on self-reported previous infection led to a change in C-index of 0·015 (–0·037 to 0·067; p=0·58) compared with the base model.

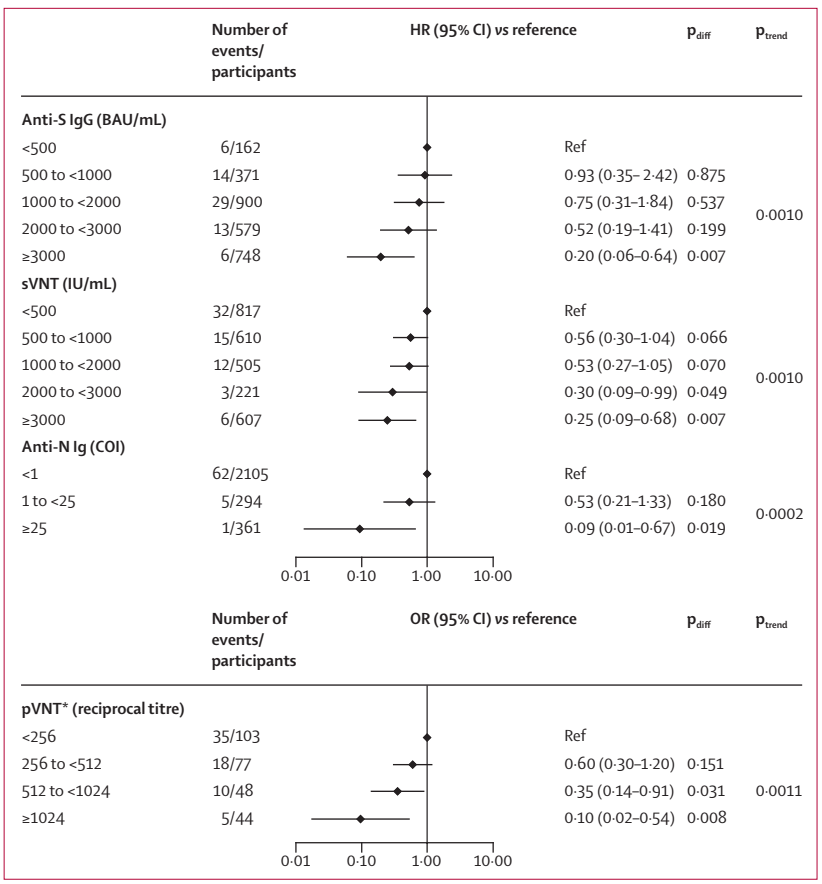

Figure 4: **Associations of categorised baseline levels of binding and neutralising antibody levels with incident breakthrough SARS-CoV-2 infection**

Cox regression was applied for anti-S IgG, sVNT, and anti-N Ig, and conditional logistic regression was applied for pVNT. For anti-N Ig, the regression model was adjusted for age and sex (and not for previous SARS-CoV-2 infection due to collinearity) and for anti-S IgG, sVNT, and pVNT the model was additionally adjusted for previous SARS-CoV-2 infection as established by the seropositivity of anti-N Ig at the time of enrolment or self-report. p_{trend} indicates the p value of the likelihood ratio test comparing regression models including categories of antibody concentrations as a continuous variable and without antibody information. For additional information on participants with previous SARS-CoV-2 infection and with incident breakthrough SARS-CoV-2 infection, see the appendix (p 5). Anti-N Ig=anti-nucleocapsid Ig. Anti-S IgG=anti-spike IgG. BAU=binding antibody units. COI=cutoff index. HR=hazard ratio. IU=international unit. OR=odds ratio. pVNT=pseudotyped SARS-CoV-2 virus neutralisation test. sVNT=surrogate SARS-CoV-2 virus neutralisation test. *pVNT was measured in a nested case-control sample of 68 participants and 204 individual-matched controls.

Discussion

In this study, involving 2760 participants aged at least 16 years, we evaluated humoral and cellular immunological parameters after two doses of the BNT162b2 vaccine as potential correlates of protection against SARS-CoV-2 infection over a 6-month follow-up period. We observed strong inverse log-linear associations between the risk of incident SARS-CoV-2 breakthrough infections (independent of age, sex, and previous infection) and anti-S IgG, titres of neutralising antibodies, and—in people who were infected with SARS-CoV-2 before inclusion in the study—concentrations of anti-N Ig. By contrast, no significant association was found between levels of cellular immune response to vaccination and breakthrough infection risk. Finally, we provide data on the usefulness of anti-S IgG concentrations in predicting breakthrough infection, showing that including information on anti-S IgG provided a substantial improvement in risk discrimination over and beyond a model containing information on age and sex.

Our findings corroborate previous data from clinical trials and observational studies showing inverse relationships between humoral immune responses to vaccination and subsequent risk of breakthrough infection. The COV002 trial—a phase 2/3 trial of the ChAdOx1 nCoV-19 (Oxford–AstraZeneca) vaccine—measured anti-S IgG, anti-RBD IgG, and titres of neutralising antibodies in a pseudotyped and a live-virus neutralisation assay 28 days after receipt of the second dose, and found that higher concentrations were linked to a significantly reduced risk of symptomatic infection over an approximately 3-month follow-up.[1] Similarly, over a 2·7-month follow-up, the COVE trial—a phase 3 trial of mRNA-1273 (Moderna)—found HRs for breakthrough infection associated with ten times the concentrations of

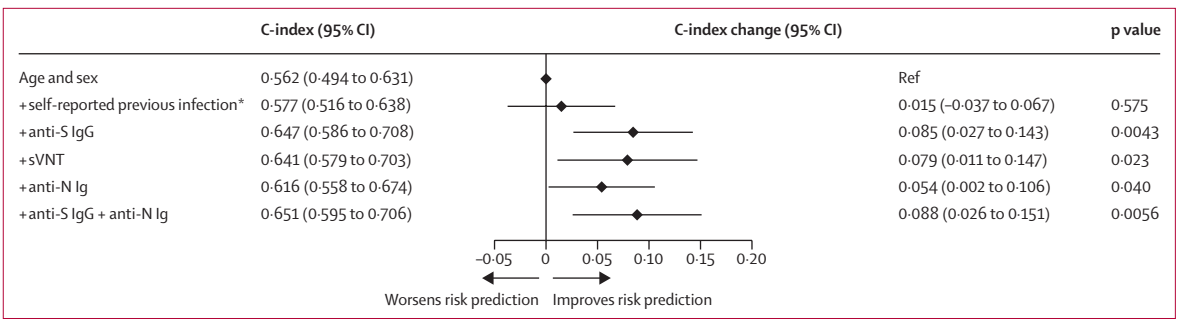

Figure 5: **Improvement in prediction of incident breakthrough SARS-CoV-2 infection when including information on anti-SARS-CoV-2 antibodies and previous SARS-CoV-2 infection**

Participants with complete data for all variables are included in analyses (2760 participants; 68 incident SARS-CoV-2 breakthrough infections). A C-index of 1·0 indicates perfect prediction of the order of failure; a C-index of 0·5 is achieved purely by chance. Immunological parameters were entered as $\log_2$-transformed continuous terms. sVNT=surrogate SARS-CoV-2 virus neutralisation test. *Refers to a SARS-CoV-2 infection before study entry established by self-report.

antibodies of 0·66 (0·50–0·88) for anti-S IgG, 0·57 (0·40–0·82) for anti-RBD IgG, and 0·42 (0·27–0·65) for neutralising antibodies in a pseudotyped virus neutralisation assay measured 28 days after the second dose.[2] Compared with these trials, effect sizes for anti-S IgG in our study were stronger and robust for both the outcome of any infection and symptomatic infection.

Our results are also in agreement with previous observational studies that were conducted in samples of vaccinated individuals in the community,[3–5] health-care workers,[6] patients with autoimmune rheumatic diseases,[7] and patients undergoing dialysis.[8–10] However, whereas the majority of earlier studies compared risk across categories (eg, dichotomising the study population at arbitrary cutoffs for anti-S IgG concentrations), our study revealed associations that corresponded to a log-linear fit, thereby suggesting that the stronger the immune response the lower the risk of breakthrough infection without evidence for a threshold or saturation effect.

In terms of T-cell responses, the proportion of participants classified as reactive in our study was lower than in previous reports,[12–14] which might be related to differing cutoffs for reactivity, assay performances, vaccination regimes, or timings of measurement. However, consistent with earlier studies of individuals who received the BNT162b2 vaccine,[15,16] we did not detect measurable differences in post-vaccination T-cell response between people with or without breakthrough infection. This lack of difference might be explained by the main function of T cells—ie, facilitating early viral clearance[17] and hence circumventing severe clinical course rather than preventing primary infection. The shorter incubation time (2–3 days) of the delta variant of SARS-CoV-2 compared with previously circulating variants could also limit the potential of T cells to avert symptomatic disease, whereas T cells have more time to respond to protect from severe disease. The fact that the majority of breakthrough infections were mild could support this idea, making it impossible to detect any appreciable difference in T-cell response between the groups. That the major mechanism of protection from acquiring the infection comes from neutralising antibodies is further supported by data from previous studies showing non-significant effects of vaccine-induced antibodies in preventing infection with variants—for example the omicron variant—that are highly mutated at the binding sites of neutralising antibodies.[18] Our study ended before the emergence of the omicron variant in the region, making it impossible to comment on any such change in the breakthrough infection pattern. Furthermore, other parts of the adaptive immune system (eg, mucosal antibodies and tissue resident T cells) might contribute to protection, but were beyond the scope of the present study.

We also evaluated the added value of measuring anti-SARS-CoV-2 antibodies for predicting an individual's risk of breakthrough infection. On the basis of our findings in the risk discrimination analysis and considering complexity and cost, anti-S IgG appears to be the most suitable measurable correlate of protection in practice, yielding a large improvement in the C-index by 0·085 (95% CI 0·027–0·143, p=0·0043) when added to prediction models. Information on anti-N Ig showed no incremental predictive value when included alongside information on anti-S IgG. To our knowledge, no previous study has investigated the usefulness of these immunological parameters in SARS-CoV-2 risk prediction.

In another set of analyses, we examined cross-sectional correlations of different immunological parameters elicited by vaccination. Together with evidence from other studies,[2,19–21] the strong correlation we observed between anti-S IgG and neutralising antibodies indicates a high potential of anti-S IgG to quantitatively reflect neutralising capacities for SARS-CoV-2, at least before the emergence of the omicron variant. In participants with previous SARS-CoV-2 infections, the only moderate correlation of anti-N Ig with other parameters can be explained by the stronger waning of anti-N Ig than anti-S IgG over time after SARS-CoV-2 infection.[22] Furthermore, the anti-S IgG response is invoked by both previous infection and vaccination, whereas anti-N Ig response is elicited only

after infection, potentially distorting the correlation. This notion is supported by previous data from before COVID-19 vaccines were available, which showed a considerable degree of correlation between anti-N and anti-S IgG antibodies generated after infection.[23] Our finding of poor correlations between antibodies and T-cell responses is consistent with some other studies showing no correlation to only moderate correlation between humoral and cellular immune parameters in vaccinated[24–26] and convalescent[27–29] individuals.

Our study has several strengths. It has a prospective design, is adequately sized, covers a 6-month follow-up after the second vaccine dose, and compared immunological parameters for humoral and cellular immunity measured simultaneously with validated assays. In addition, participants received their two doses of the BNT162b2 vaccine at almost the same time as each other, and were therefore exposed to the same background incidence in the population during follow-up. To our knowledge, our study is the first to analyse, in vaccinated individuals, the associations between T cells and incident breakthrough SARS-CoV-2 infection using time-to-event analysis. Furthermore, all incident breakthrough SARS-CoV-2 infections and related symptoms were validated rigorously in structured telephone interviews.

Our study also has limitations. First, cellular immune parameters were available for only a subgroup of participants, thereby limiting statistical power. Second, the QuantiFERON SARS-CoV-2 RUO IFNγ release assay was limited to the measurement of IFNγ production after stimulation with CD4 and combined CD4 and CD8 peptide pools; as such, detailed characterisation of the T-cell response in terms of the source of IFNγ (CD4 or CD8 T cells), phenotypical analysis, and further functional analysis of T cells was not possible. Third, owing to assay limitations, there is some imprecision in quantifying very high titres of neutralising antibodies (>5000 international units [IU]/mL for surrogate and >1024 reciprocal titre for pseudotyped virus neutralisation tests). Fourth, the proportion of participants with previous SARS-CoV-2 infection was relatively high (25·8%), which could be related to a higher motivation of this group to participate in the study. However, another study in the same district estimated 24·0% (95% CI 22·5–25·6) of the population to have had a previous infection by March, 2021,[30] thereby endorsing the generalisability of our findings. Ascertainment of previous infection was of high quality as it was conducted by trained staff and confirmed by a positive anti-N Ig measurement in 92% of cases. Fifth, compared with the REDUCE study[11] that was directly integrated into the district's vaccination campaign, participants in our study were on average 2·8 years older and more commonly female (both p<0·0001), suggesting some over-representation of these population subgroups (appendix p 11). Sixth, we conducted our study during a period in which delta was the predominant SARS-CoV-2 variant, and associations with the omicron variant might be weaker. Finally, these analyses were conducted on samples taken after participants had received two doses of the BNT162b2 vaccine, and might not apply to participants who received other SARS-CoV-2 vaccines or those who received BNT162b2 booster doses.

In conclusion, in contrast to the T-cell response, higher levels of binding and neutralising antibodies after two doses of the BNT162b2 vaccine were associated with reduced risk of incident breakthrough SARS-CoV-2 infection. Assessment of anti-S IgG concentrations enhances prediction of incident breakthrough SARS-CoV-2 infection and might therefore be a suitable measurable correlate of protection in practice.

Contributors

PW, DvL, FK, WB, ZB, and JK conceived the study. WB, DvL, SS, and LF designed the questionnaire and the online data collection. ZB, AR, JK, and WB were responsible for laboratory tests. LS, LT, and PW accessed and verified the data. LS and LT cleaned the data. LS and PW did the statistical data analysis. HS, BF, TH, DB, MS, CO, DvL, and WB were responsible for on-site data acquisition. DvL, PW, FK, HU, and WB supervised the study. LS, WB, and PW drafted the manuscript. All authors critically reviewed and approved the final manuscript for publication. WB and PW had full access to all data in the study and accept responsibility to submit for publication.

Declaration of interests

DB holds stocks of Pfizer. The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and Newcastle disease virus-based COVID-19 vaccines that list FK as a co-inventor. FK has received support from the National Institutes of Health (NIH) National Institute of Allergy and Infectious Diseases Collaborative Influenza Vaccine Innovation Centers (contract 75N93019C00051), NIH Centers of Excellence for Influenza Research and Response (75N93021C00014), the JPB Foundation and the Open Philanthropy Project (research grant 2020-215611, 5384), and the NIH National Cancer Institute (contract 75N91019D00024, task order 75N91020F00003). FK's laboratory is also collaborating with Pfizer on animal models of SARS-CoV-2. FK has received royalties or licences from Avimex and Kantaro. The Icahn School of Medicine at Mount Sinai has spun out a company, Kantaro, to market serological tests for SARS-CoV-2. FK has consulted for Pfizer (before 2020), and is currently consulting for Pfizer, Seqirus, Third Rock Ventures, and Avimex. FK has received payment or honoraria for academic lectures over the past 2 years. All other authors declare no competing interests.

Data sharing

Data on COVID-19 cases in the district of Schwaz, Austria, are publicly available from Open Data Österreich (https://www.data.gv.at/). Tabular data on the Shieldvacc-2 cohort can be requested from the corresponding authors by researchers who submit a methodologically sound proposal (including a statistical analysis plan); participant-level data on the Shieldvacc-2 cohort cannot be shared owing to regulatory restrictions.

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