Association of Self-Reported Systemic Reactions Following SARS-CoV-2 Vaccination With Immunological Response in The Danish National Cohort Study of Effectiveness and Safety of SARS-CoV-2 Vaccines (ENFORCE)

Bannister, Wendy P; Raben, Dorthe; Valentiner-Branth, Palle; Tolstrup, Martin; Larsen, Lykke; Tarp, Britta; Brouw Iversen, Mette; Schmeltz Søgaard, Ole; Rye Ostrowski, Sisse; Breinholt Stærke, Nina; Jakobsen, Marie Louise; Lindvig, Susan Olaf; Juhl, Maria Ruwald; Somuncu Johansen, Isik; Mustafa, Ahmed B; Østergaard, Lars; Dam Larsen, Fredrikke; Surland Knudsen, Lene; Klastrup, Vibeke; Wiese, Lothar; Benfield, Thomas; Toft Petersen, Kristine; Iversen, Kasper K; Nielsen, Henrik; Reekie, Joanne; Lundgren, Jens; the ENFORCE Study Group

Published in:
Open Forum Infectious Diseases

DOI:
10.1093/ofid/ofad248

Publication date:
2023

Document version:
Final published version

Open Forum Infectious Diseases

MAJOR ARTICLE

Association of Self-reported Systemic Reactions Following SARS-CoV-2 Vaccination With Immunological Response in the Danish National Cohort Study of Effectiveness and Safety of SARS-CoV-2 Vaccines (ENFORCE)

Wendy P. Bannister,[1] Dorthe Raben,[1] Palle Valentiner-Branth,[2] Martin Tolstrup,[3,4] Lykke Larsen,[5] Britta Tarp,[6] Mette Brouw Iversen,[7] Ole Schmeltz Søgaard,[3,4] Sisse Rye Ostrowski,[8,9] Nina Breinholt Stærke,[3] Marie Louise Jakobsen,[1] Susan Olaf Lindvig,[5] Maria Ruwald Juhl,[10] Isik Somuncu Johansen,[5,11] Ahmed B. Mustafa,[8,12] Lars Østergaard,[3,4] Fredrikke Dam Larsen,[3,4] Lene Surland Knudsen,[7] Vibeke Klastrup,[3] Lothar Wiese,[7] Thomas Benfield,[8,12] Kristine Toft Petersen,[10] Kasper K. Iversen,[13] Henrik Nielsen,[10,14] Joanne Reekie,[1] Jens Lundgren,[1,8] and on behalf of the ENFORCE Study Group*

[1]Centre of Excellence for Health, Immunity and Infections (CHIP), Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [2]Department of Infectious Disease Epidemiology and Prevention, Statens Serum Institut, Copenhagen, Denmark, [3]Department of Infectious Diseases, Aarhus University Hospital, Aarhus, Denmark, [4]Department of Clinical Medicine, Aarhus University, Aarhus, Denmark, [5]Department of Infectious Diseases, Odense University Hospital, Odense, Denmark, [6]Diagnostic Centre, University Research Clinic for Innovative Patient Pathways, Silkeborg Regional Hospital, Silkeborg, Denmark, [7]Department of Medicine, Zealand University Hospital, Roskilde, Denmark, [8]Department of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark, [9]Department of Clinical Immunology, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, [10]Department of Infectious Diseases, Aalborg University Hospital, Aalborg, Denmark, [11]Department of Clinical Research, University of Southern Denmark, Odense, Denmark, [12]Department of Infectious Diseases, Copenhagen University Hospital—Amager and Hvidovre, Hvidovre, Denmark, [13]Department of Cardiology and Department of Emergency Medicine, Herlev-Gentofte Hospital, Herlev, Denmark, and [14]Department of Clinical Medicine, Aalborg University, Aalborg, Denmark

Background. Side effects to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines are a key concern contributing to vaccine hesitancy, but more individuals may be encouraged if SARS-CoV-2 vaccines were known to lead to a stronger immune response.

Methods. Included were adult participants from the Danish National Cohort Study of Effectiveness and Safety of SARS-CoV-2 Vaccines (ENFORCE) who completed a questionnaire to assess systemic reactions following SARS-CoV-2 vaccination (BTN162b2, mRNA-1273, ChAdOx1) and had SARS-CoV-2 spike immunoglobulin G (IgG) levels measured at baseline and post–vaccine. A symptom score was developed to measure severity of systemic adverse reactions (+1 for each moderate, +2 for each severe). Post–vaccination SARS-CoV-2 spike IgG levels were compared between participants with different scores using multivariable linear regression.

Results. A total of 6528 participants were included (56.3% females; median age [interquartile range], 64 [54–75] years). After the first vaccination, no association was found between symptom score and post–vaccine dose spike IgG level ($P = .575$). Following the second vaccination, significantly higher spike IgG levels were observed according to higher symptom scores ($P < .001$); adjusted geometric mean ratios were 1.16 (95% CI, 1.04–1.30), 1.24 (95% CI, 1.09–1.41), 1.25 (95% CI, 1.06–1.46), and 1.21 (95% CI, 1.08–1.35), for scores of 2, 3, 4, and ≥5, respectively, compared with a score of 0. After adjustment for pre–vaccine dose spike IgG, this association was attenuated.

Conclusions. An association was found between more severe adverse reactions and stronger antibody response after the second vaccination but not the first, likely attributed to higher levels of preexisting immunity gained from response to first vaccination. Regardless of side effects, most people experienced an effective immune response following vaccination.

Keywords. SARS-CoV-2; COVID-19 vaccines; adverse drug reactions; antibody response.

Side effects to vaccinations are expected as an outward sign of the induced immune response. They are one of the key concerns contributing to vaccine hesitancy across the world, which varies over time, between countries, and for different vaccine types [1–4]. There are many factors that can affect the type and severity of side effects including characteristics of the recipient and those related to the vaccine itself [5]. A common assumption is that the occurrence of side effects indicates that the vaccine is working as intended, inducing protective immunity.

Although there are clear data to support that coronavirus disease 2019 (COVID-19) vaccines are effective at generating a strong immune response against the severe acute respiratory

Received 22 March 2023; editorial decision 02 May 2023; accepted 04 May 2023; published online 8 May 2023

*ENFORCE Study Group members are listed in the Acknowledgments.

Correspondence: Wendy Bannister, PhD, Centre of Excellence for Health, Immunity and Infections (CHIP), Rigshospitalet—University of Copenhagen, Section 2100, Blegdamsvej 9, DK-2100 Copenhagen, Denmark (wendy.bannister@regionh.dk); or Joanne Reekie, PhD, Centre of Excellence for Health, Immunity and Infections (CHIP), Rigshospitalet—University of Copenhagen, Section 2100, Blegdamsvej 9, DK-2100 Copenhagen, Denmark (joanne.reekie@regionh.dk).

Open Forum Infectious Diseases®

https://doi.org/10.1093/ofid/ofad248

syndrome coronavirus 2 (SARS-CoV-2) virus regardless of adverse reactions [6–9], the hypothesis put forward is that more severe reactions following vaccination are predictive of a better immunological response. Several recent studies have explored this potential relationship [10–20], but sample sizes were generally small and only a few found supporting evidence of an association.

In the Danish National Cohort Study of Effectiveness and Safety of SARS-CoV-2 vaccines (ENFORCE; www.enforce.dk), we prospectively collected self-reported adverse reactions within 2 weeks after each vaccination and performed comprehensive SARS-CoV-2 serological profiling of >6500 individuals enrolled. Our primary objective for this analysis was to investigate the association between the severity of systemic reactions after SARS-CoV-2 vaccination and immunological response.

METHODS

ENFORCE is an open-label, nonrandomized, parallel-group, phase IV study that enrolled adult (≥18 years) Danish residents before their first SARS-CoV-2 vaccination in 7 study sites across all 5 Danish regions. Participants who were scheduled to receive a vaccine as part of the Danish vaccine program were enrolled between February 2021 and August 2021. Details of the study including entry criteria and data collection have been described previously [21].

Ethics Approval and Participant Consent

This study was approved by the Ethical Committees of The Central Denmark Region. Participants provided written informed consent to participate in the study before any trial activities.

Study Population

We included individuals enrolled in ENFORCE who completed an assessment of adverse reactions to the first, second, or third (booster) vaccination and had pre– and post–vaccine dose assessments of SARS-CoV-2 spike immunoglobulin G (IgG) levels. Any participants who experienced a breakthrough SARS-CoV-2 infection (positive polymerase chain reaction [PCR] test after vaccination and before spike IgG assessment) were excluded from the analysis.

Data Collection

At enrollment, baseline information on age, sex, focused medical history, and vaccine type (BTN162b2, mRNA-1273, ChAdOx1) was collected. Data on any previous SARS-CoV-2 PCR tests or SARS-CoV-2 antibody measurements were extracted from the Danish national microbiology database, MiBa (Statens Serum Institut, Copenhagen, Denmark). The study protocol was approved by the Danish Medicines Agency (#2020-006003-42) and the National Committee on Health Research Ethics (#1-10-72-337-20).

Participants were asked to complete a questionnaire to report any of the following symptoms experienced within 2 weeks post–vaccination and to grade them as mild, moderate, or severe:

- systemic reactions: muscle pain, joint pain, fatigue, fever, headache, nausea, chills;
- local reactions at injection site: redness, swelling, tenderness.

Total serum levels of IgG antibodies against the SARS-CoV-2 spike protein were measured using a multiantigen serological assay (Meso Scale Diagnostics, Rockville, MD, USA) at the protocol-scheduled visits.

Definitions

For the main analysis, we developed a symptom severity score for systemic reactions experienced within the first week after vaccination, counting +1 for each moderate reaction reported and +2 for each severe reaction, so the score could go from 0 (no reaction or mild reactions only) up to a maximum of 14 (all severe reactions). We also explored alternative ways of scoring symptoms. First, we incorporated mild reactions, counting +1 for each mild reaction, +2 for each moderate, +3 for each severe, up to a maximum score of 21. Second, we additionally included local reactions using the same scoring system with a maximum possible score of 30. Finally, we included symptom assessments from the second week after the vaccination where available, taking the worst severity level for each reaction from either the first- or second-week assessment.

Immunological response was assessed by SARS-CoV-2 spike IgG levels assessed at the first protocol-scheduled visit date at least 14 days after the vaccination and before the next dose. These were planned as:

- Visit 2, after the first vaccine dose and within 5 days before the second dose.
- Visit 3, after the second vaccine dose, 3 months (±14 days) after the first dose.
  - If Visit 3 was missing (eg, for participants who received ChAdOx1 as the first dose where the timing of the second dose was close to 3 months), the next available prebooster assessment was used instead.
- Visit Xc, 28 days (±8 days) after the third (booster) dose.

Statistical Analysis

Characteristics were summarized for all participants included and were compared against those who did not meet the analysis inclusion criteria to evaluate any potential selection bias. In those with available data, we investigated the associations of moderate/severe systemic reactions after the first, second, and third vaccinations using McNemar's tests for paired nominal data.

Multivariable linear regression was used in the primary analysis to identify any significant association ($P < .05$) between symptom severity score for systemic reactions and spike IgG level after each vaccination. The analysis was adjusted for potential confounders and effect modifiers, namely time from vaccination to spike IgG assessment, age group, sex, Charlson Comorbidity Index (CCI) score (based on comorbidities in the 5 years before enrollment) [22], vaccine type, and evidence of prior SARS-CoV-2 infection, where infection before first vaccination was defined by a positive antibody result from enzyme-linked immunosorbent assay (ELISA; Wantai) testing or a positive PCR test, and where infection before next vaccination included any positive PCR tests after first vaccination. Symptom score was included as a categorical variable, and we required at least 100 participants per score category per vaccination for meaningful comparisons. We repeated the analysis adjusting for pre–vaccine dose spike IgG level to explore the effect of preexisting immunity.

Due to the skewed distribution, a logarithmic transformation ($\log_{10}$) was applied to the spike IgG level. Estimates of differences of $\log_{10}$-transformed spike IgG levels from the models were then back-transformed to present adjusted geometric mean ratios, 95% CIs, and *P* values. An adjusted geometric mean ratio with a value >1 for a symptom score category indicated a higher geometric mean spike IgG level compared with that for a symptom score of 0 (ie, no symptoms), the reference category.

Sensitivity analyses were performed repeating the analysis to test if alternative ways of scoring symptoms or prior SARS-CoV-2 infection affected the results. We also tested interactions to determine whether the effect of the symptom score was significantly different ($P < .1$) according to other factors in the model.

An additional analysis was performed to explore the potential relationship between pre–vaccine dose spike IgG level and occurrence of moderate or severe systemic reactions. We used multivariable logistic regression to estimate adjusted odds ratios, 95% CIs, and *P* values and tested interactions.

SAS software, version 9.4 (SAS Institute, Cary, NC, USA), was used for all analyses.

RESULTS

Characteristics

Of 6918 participants enrolled in ENFORCE, 6531 had an assessment of adverse reactions plus baseline and post–vaccination assessments of SARS-CoV-2 spike IgG level. Among these participants, there were 12, 8, and 192 breakthrough SARS-CoV-2 infections to be excluded after the first, second, and third vaccinations, respectively. This left 6528 participants for inclusion in the analyses; 6181 had data for the first vaccination, 5932 for the second, and 4053 for the third (Supplementary Figure 1). Overall, 3676 (56.3%) were female, the median age at enrollment (interquartile range [IQR]) was 64 (54–75) years, 5162 (79.1%) had a CCI score of 0, and 335 (5.1%) had evidence of prior SARS-CoV-2 infection (Table 1). The type of vaccines received (first, second, and third, respectively) were BTN162b2 (55.8%, 60.0%, 58.4%), mRNA-1237 (38.7%, 40.0%, 41.6%), and ChAdOx1 (5.5%, 0%, 0%). The majority received the same

Table 1. Characteristics of Participants

	All[a] (n = 6528)	First Vaccination (n = 6181)	Second Vaccination (n = 5932)	Third (Booster) (n = 4053)
Age at enrollment, median (IQR), y	64 (54–75)	64 (54–75)	64 (54–75)	64 (55–74)
Sex, No. (%)				
Male	2852 (43.7)	2679 (43.3)	2559 (43.1)	1728 (42.6)
Female	3676 (56.3)	3502 (56.7)	3373 (56.9)	2325 (57.4)
CCI score categories,[b] No. (%)				
0	5162 (79.1)	4886 (79.0)	4701 (79.2)	3259 (80.4)
1–2	1156 (17.7)	1104 (17.9)	1047 (17.7)	676 (16.7)
>2	210 (3.2)	191 (3.1)	184 (3.1)	118 (2.9)
Evidence of prior SARS-CoV-2 infection,[c] No. (%)	335 (5.1)	309 (5.0)	319 (5.4)	305 (7.5)
Vaccine type, No. (%)				
BTN162b2	…	3450 (55.8)	3559 (60.0)	2367 (58.4)
mRNA-1237	…	2390 (38.7)	2373 (40.0)	1685 (41.6)
ChAdOx1	…	341 (5.5)	0 (0.0)	1 (0.0)
Time from vaccine dose to post–vaccine spike IgG assessment, median (IQR), wk	…	4 (3–5)	9 (8–10)	4 (4–5)
Pre–vaccine dose SARS-CoV-2 spike IgG level, median (IQR), BAU/mL	…	0.7 (0.4–1.6)	276.5 (86.4–819.4)	795.9 (307.4–1802.0)

Abbreviations: CCI, Charlson Comorbidity Index; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; IQR, interquartile range; PCR, polymerase chain reaction; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

[a]Participants were included if they had assessments available from at least 1 vaccination, that is, included in at least 1 of the totals for first, second, or third vaccination.

[b]Charlson Comorbidity Index score is based on comorbidities in the 5 years before enrollment.

[c]Infection before first vaccination was defined by positive antibody result from ELISA (Wantai) testing or a positive PCR test; infection before second and third vaccinations included any infection before first vaccination, in addition to any subsequent positive PCR tests.

vaccine type at each dose (5321/5623 [94.6%] with first and second vaccination data available and 3848/3851 [99.9%] with second and third vaccination data available). The median (IQR) spike IgG levels before the first, second, and third vaccinations were 0.7 (0.4–1.6) BAU/mL, 276.5 (86.4–819.4) BAU/mL, and 796 (307.4–1802.0) BAU/mL, respectively. In 309 participants with prior SARS-CoV-2 infection at enrollment, the median (IQR) pre–vaccine dose level was 166 (55–413) BAU/mL. Median (IQR) times to post–vaccine dose assessment of spike IgG were 4 weeks (3–5), 9 weeks (8–10), and 4 weeks (4–5), respectively. For comparison, the 390 participants excluded from the analysis were slightly younger than those included (median [IQR] age, 59 [44–71] years), and a higher percentage received the ChAdOx1 vaccine (20.0% vs 6.1%). However, they had a similar distribution of males/females, CCI scores, and prior SARS-CoV-2 infections.

Self-reported Adverse Reactions

At least 1 systemic reaction was reported by 3569 (57.7%), 4048 (68.2%), and 2179 (53.8%) participants with assessments after the first, second, and third vaccinations, respectively. Mild systemic reactions were reported by 3010 (48.7%), 3418 (57.6%), and 1839 (45.4%) participants; moderate reactions were reported by 1434 (23.2%), 2110 (35.6%), and 960 (23.7%); severe reactions were reported by 509 (8.2%), 894 (15.1%), and 327 (8.1%), after the first, second, and third vaccinations, respectively.

The most commonly reported were fatigue, muscle pain, and headache, occurring more frequently and with greater severity after second vaccination (56.7%, 38.8%, and 39.2%, respectively) compared with after first (40.9%, 28.8%, and 27.1%) or third vaccination (40.4%, 26.5%, and 28.2%) (Figure 1). Of the local reactions, ~75% reported tenderness at the injection site, and <25% reported redness or swelling.

In 5623 participants with assessments available after both the first and second vaccinations, those who reported a moderate/severe systemic reaction after the first were significantly more likely to also report a moderate/severe systemic reaction after the second than those with no reactions or only mild reactions after the first (952/1451 [65.6%] vs 1310/4172 [31.4%]; $P < .001$). This was also true for second and third vaccinations (686/1530 [44.8%] vs 313/2320 [13.5%]; $P < .001$). Changes in severity of systemic adverse reactions between vaccinations are illustrated in Supplementary Figure 2, a Sankey bar chart, including 3719 participants with assessments available after all 3 vaccinations.

Association Between Severity of Systemic Reactions and Post–Vaccine Dose SARS-CoV-2 Spike IgG

Post–vaccine dose SARS-CoV-2 spike IgG levels, before the next vaccination, are displayed in Figure 2 according to symptom score. These were assessed within a detectable assay range and after the booster dose; most participants reached beyond the highest detectable limit, resulting in skewed data with

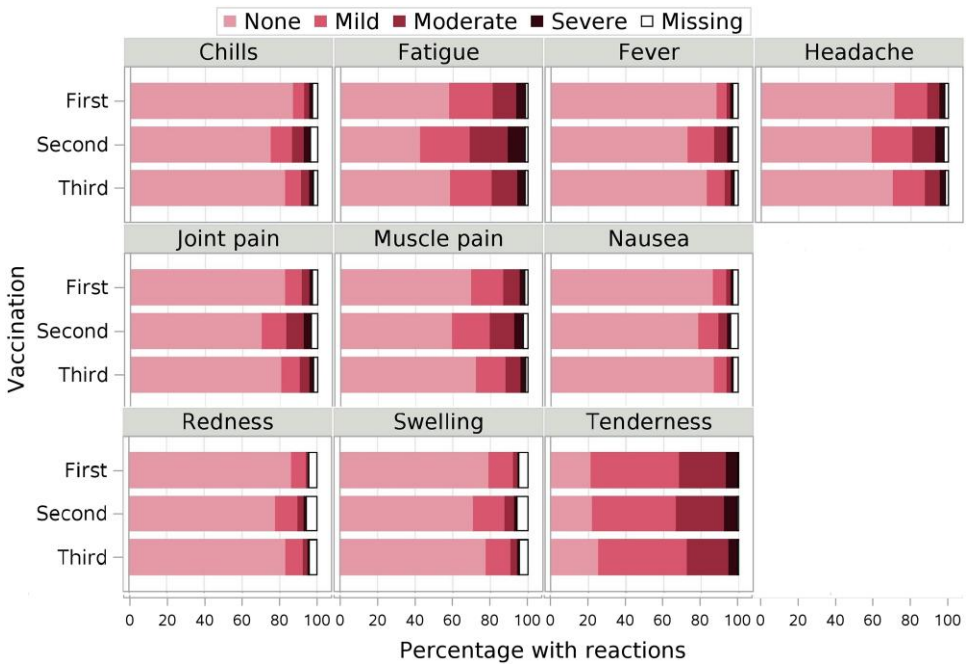

Figure 1. Severity of self-reported systemic and local reactions following vaccination. Systemic reactions were chills, fatigue, fever, headache, joint pain, muscle pain, and nausea. Local reactions at the injection site were redness, swelling, and tenderness. Percentages are out of total participants who completed a questionnaire to assess adverse reactions within 1 week following each vaccination.

decreased sensitivity for our analysis. For this reason, we present data following the first and second vaccinations here. Data for the booster vaccination are presented in a Supplementary Appendix (Supplementary Figure 4), recognizing this limitation.

The median spike IgG levels after the first vaccination varied between approximately 250 and 450 BAU/mL across participants with different symptom scores. After the second vaccination, most participants had a high antibody response, and there was a steady increase in the median (IQR) level according to higher symptom scores from 1686 (728–3679) BAU/mL to 3952 (2315–4396) BAU/mL for scores of 0 compared with ≥5.

For the primary adjusted linear regression analysis, estimates of geometric mean post–vaccine dose spike IgG levels were compared between participants with different symptom severity scores with the reference group with a score of 0 (Figure 3). After the first vaccination, we found no significant association between symptom score and spike IgG level (global $P = .575$). However, following the second vaccination, the association in the primary analysis was highly significant (global $P < .001$), with participants who scored 2 or more (at least 2 moderate or 1 severe reaction) having significantly higher post–vaccination spike IgG levels than those who had a score of 0; adjusted geometric mean ratios were 1.16 (95% CI, 1.04–1.30), 1.24 (95% CI, 1.09–1.41), 1.25 (95% CI, 1.06–1.46), and 1.21 (95% CI, 1.08–1.35), for scores of 2, 3, 4, and ≥5, respectively.

To test the effect of preexisting immunity, we adjusted for pre–vaccine dose spike IgG. Estimated geometric mean ratios remained similar for the first vaccination ($P = .741$), but for the second vaccination, the significant association with post-vaccine spike IgG disappeared, and all geometric mean ratios were between 0.93 and 1.02 ($P = .537$).

Different methods of scoring severity of adverse reactions were explored in sensitivity analyses, and results were broadly consistent, presented in Supplementary Figure 3. Results were also consistent when we excluded participants with known prior SARS-CoV-2 infection and when outliers (values outside of mean ± 2 SDs) were removed.

We tested interactions between symptom score and sex, age, baseline CCI, vaccine type, prior SARS-CoV-2 infection, and time from vaccination to spike IgG assessment and identified a significant interaction with baseline CCI after the second vaccination. Therefore, we stratified the analysis and found that in 184 participants with a CCI score of >2, there was no association between severity of reactions and post–vaccine dose spike IgG level ($P = .906$). Although the interaction between symptom score and vaccine type was not significant, there were slightly higher post–vaccine dose spike IgG levels for higher symptom scores in those who received the mRNA-1273 vaccine compared with the main analysis, with estimated spike IgG up to 29% higher for symptom scores ≥1 vs 0 after the second vaccination ($P < .001$). For BTN162b2, although the highest scores of 4 or ≥5 had higher post–vaccine dose spike IgG levels, the association was not significant ($P = .118$).

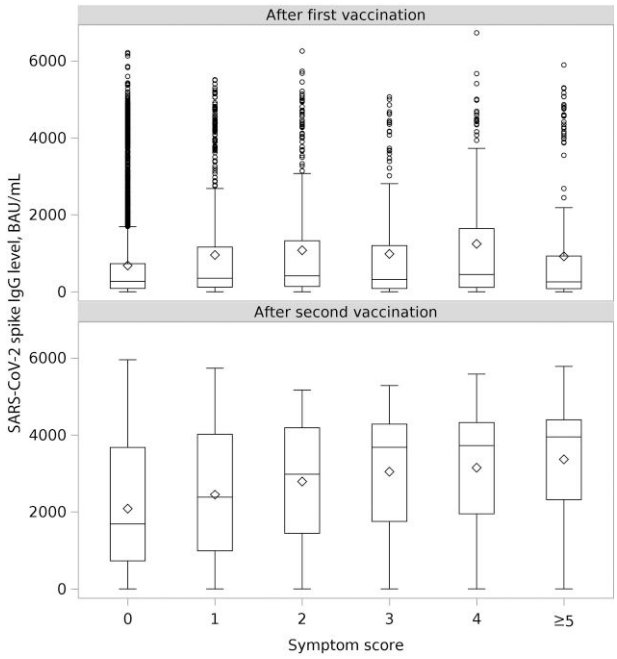

Figure 2. Post–vaccine dose SARS-CoV-2 spike IgG levels according to symptom severity scores. Symptom severity scores are based on self-reported systemic reactions within 1 week after vaccination, counting +1 for each moderate and +2 for each severe reaction. Abbreviations: IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Association Between Pre–Vaccine Dose SARS-CoV-2 Spike IgG and Systemic Reactions

Logistic regression models adjusted for age group, sex, CCI, and vaccine type identified a significant association between pre–vaccine dose spike IgG level and odds of reporting a moderate/severe systemic reaction after both the first and second vaccinations (global $P < .001$). Participants who had pre–vaccine dose spike IgG levels of ≥4 $\log_{10}$ AU/mL (=90.1 BAU/mL) were more likely to subsequently report ≥1 moderate/severe symptom (Table 2). Data for the booster vaccination are presented in the Supplementary Appendix (Supplementary Table 1).

Analyses were then stratified by vaccine type due to significant interactions with prior spike IgG levels ($P = .069$ and $P = .089$ at first and second vaccinations, respectively). The association between pre–vaccine dose spike IgG and likelihood of moderate/severe symptoms was present after the BTN162b2 and mRNA-1237 vaccines, but slightly stronger for mRNA-1237. In 341 participants who received ChAdOx1 as their first vaccine, there was no evidence of an association.

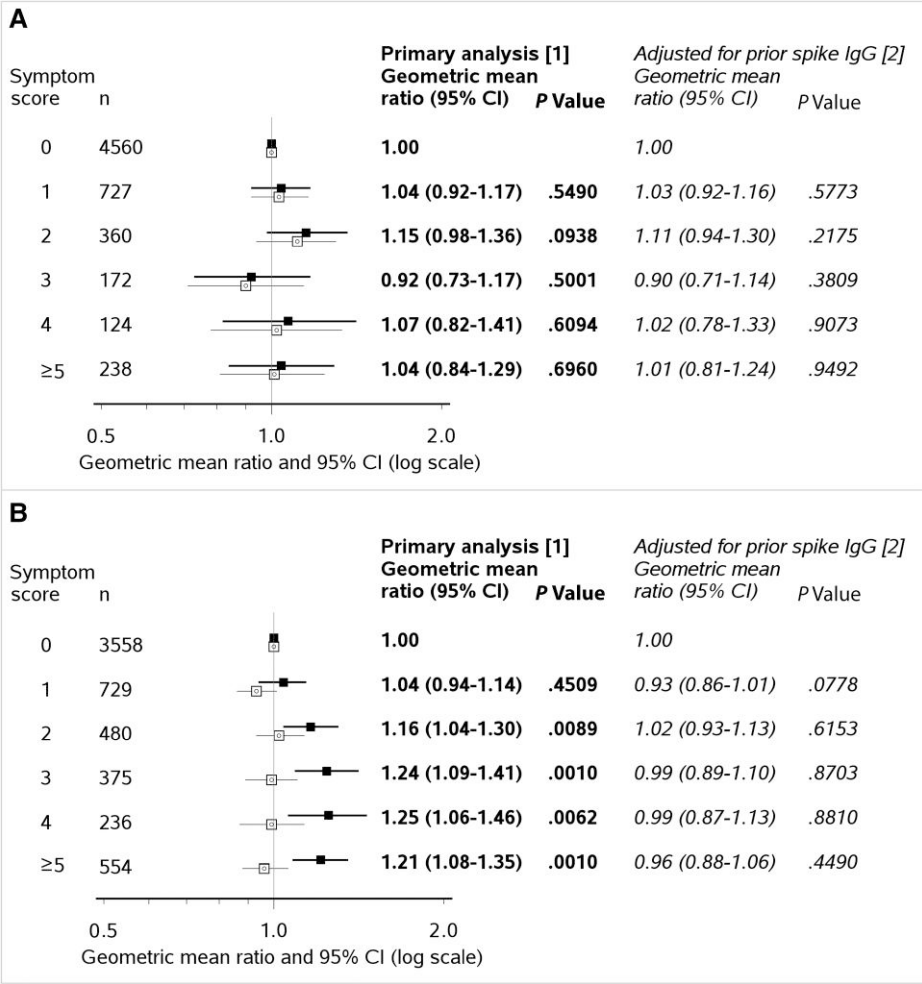

Figure 3. Comparison of post–vaccine dose SARS-CoV-2 spike IgG levels according to symptom severity score of self-reported systemic reactions following first vaccination (*A*) and second vaccination (*B*). [1] Primary: geometric mean ratios, 95% CIs, and *P* values are calculated from multivariable linear regression analysis adjusted for time from vaccination to spike IgG assessment, age, sex, CCI, vaccine type, and evidence of prior SARS-CoV-2 infection. [2] As per primary analysis but with additional adjustment for pre–vaccine dose SARS-CoV-2 spike IgG level. Abbreviations: CCI, Charlson Comorbidity Index; IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

DISCUSSION

In the present study including >6500 adult participants who received ≥1 SARS-CoV-2 vaccination in Denmark, we investigated the relationship between adverse reactions and immune response. More adverse reactions were observed after the second vaccination than after the first, consistent with previous research [7, 9, 23], whereas a lower rate was observed after the third vaccination compared with the second. Other studies have also reported similar results [24, 25]; however, there is variation between vaccine types and whether the same vaccine type was received at both doses [26]. Almost all participants (99.9%) in our analysis received a homologous booster schedule (58.4% BTN162b2% and 41.6% mRNA-1237).

Furthermore, those who reported moderate/severe reactions after the first vaccination were significantly more likely to report them after the second, likewise for the second and third vaccinations. This could reflect characteristics of individuals either based on different pain and discomfort sensations or who have different tendencies to report symptoms. For example, older participants >65 years have been found to report fewer symptoms [23]. It could also indicate higher preexisting immunity and ensuing exacerbated immune response. Our findings that higher pre–vaccine dose antibody levels both from prior SARS-CoV-2 infection and from vaccines were associated with moderate/severe symptoms after the first 2 vaccine doses support this as a potential explanation. However, the association between pre–vaccine dose antibody levels and symptoms after the third vaccination was less clear, with only the highest antibody level category (>901 BAU/mL) showing a significant association. This difference may be related to the longer time interval between the primary vaccination series and the booster, with less immune activation before the booster.

Table 2. Association Between Pre–Vaccine Dose SARS-CoV-2 Spike IgG Levels and Reporting at Least 1 Moderate or Severe Systemic Reaction Following Vaccination

	First Vaccination				Second Vaccination			
	No.	% With Reaction	Multivariable Odds Ratio (95% CI)	*P* Value	No.	% With Reaction	Multivariable Odds Ratio (95% CI)	*P* Value
Pre–vaccine dose SARS-CoV-2 spike IgG levels, BAU/mL								
≤0.901	3683	24.7	0.84 (0.60–1.17)	.2964	196	26.0	0.87 (0.60–1.25)	.4408
0.901–9.01	2069	25.7	0.82 (0.59–1.15)	.2544	279	22.6	0.78 (0.56–1.08)	.1403
9.01–90.1	218	28.9	1.00	…	1047	24.5	1.00	…
90.1–901	170	52.4	2.22 (1.41–3.50)	.0006	3027	38.5	1.33 (1.12–1.58)	.0010
>901	41	63.4	5.58 (2.66–11.72)	<.0001	1383	60.6	2.08 (1.70–2.55)	<.0001

Odds ratios, 95% CIs, and *P* values are calculated from multivariable logistic regression analysis adjusted for age, sex, CCI, and vaccine type. Categories defined according to $\log_{10}$-transformed increases in SARS-CoV-2 spike IgG levels (AU/mL), that is, ≤2, 2–3, 3–4, 4–5, and >5.

Abbreviations: CCI, Charlson Comorbidity Index; IgG, immunoglobulin G; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

While there are clear data showing that the vaccines are effective in generating a strong immune response against the SARS-CoV-2 virus regardless of occurrence of side effects, we found a significant association between severity of systemic reactions and magnitude of antibody response after the second vaccination. Most of our participants experienced a high antibody response by this time, but those with at least 2 moderate reactions or 1 severe adverse reaction had higher antibody levels than those with no systemic reactions or only mild reactions. We also found that adjusting for prevaccination antibody levels attenuated the association, suggesting that symptoms could be an indicator for an already reactive immune response that continues to improve following the second vaccination. This mechanism was also proposed by Uwamino et al., who reported similar findings [17].

Currently, there is limited research in this area. We identified a small number of studies that found some association between self-reported side effects and immune response to the SARS-CoV-2 vaccines [12, 14, 15, 17]. One of the larger studies (n = 954; Debes et al.) performed an adjusted analysis similar to our analysis and had comparable results, with significantly higher spike IgG levels after the second vaccination for those who had clinically significant symptoms (fatigue, fever, chills) [12]. Bauernfeind et al. found a relationship between adverse reactions and RBD-specific IgG and neutralizing antibodies after the second vaccination in men but not women, from an analysis of vaccinees who experienced the most severe reactions compared with sex- and age-matched controls with no/minor reactions [10]. Sex is known to affect immune response due to differences in genetic and hormonal factors as well as differences in environmental exposures that influence the microbiome [27]; however, in the present analysis, we did not find a significant interaction between sex and symptom score on spike IgG response. The contrast in results may be explained by the different measures of adverse reactions, with our scale representing the number of moderate as well as severe symptoms. In addition, our study population was older (median, 64 years compared with 43 years), and there was a longer median time between vaccine and antibody assessment.

While some studies did not find an association between side effects and immune response, the sample sizes were much smaller than in our analysis, so these studies may have had limited power to detect a difference [11, 13, 18–20]. Some noted a weak or nonsignificant trend after the second dose in those reporting reactions [13, 19]. Differences in study design may have contributed to alternative conclusions, such as study populations, definition of symptom severity (or reactions vs no reactions), vaccine types included, and factors adjusted for in the analysis. For example, although we adjusted for age group, nearly half of our study population was age >65 years, which was substantially older than some studies [11, 18, 19].

A significant interaction between severity of symptoms and comorbidities measured by CCI score was noted, and we did not find a consistent association between spike IgG and adverse reactions in those with a CCI score of ≥2. We considered that the effect may have been diluted by those with multiple comorbidities having a higher tolerability of symptoms, with these individuals being less likely to rate moderate or severe symptoms. The relatively small sample size in this subgroup may also have been a factor. In addition, there was a significant interaction observed for reporting a moderate/severe symptom between pre–vaccine dose spike IgG level and vaccine type, with no association found after the ChAdOx1 vaccine. Relatively few received the ChAdOx1 vaccine, thus reducing the power of this analysis. Also, the demographics within the vaccine groups varied greatly due to availability and prioritization of specific vaccines to risk groups during the rollout of the vaccination program, and although the analysis was adjusted for age, sex, and CCI, there is potential for unmeasured confounding.

The present study had some limitations. First, adverse reactions were self-reported, so we cannot assume consistency in perspective of severity. However, these data provide a patient-centered view of feelings about symptoms, which could

influence attitude toward vaccines. Moreover, at the time of reporting adverse reactions, the results of serum spike IgG were unknown and thus did not influence the grading of symptoms. For our primary analysis, we excluded mild symptoms that could be more subjective, focusing only on moderate or severe symptoms. We also performed sensitivity analyses applying different ways of scoring symptoms, with broadly consistent findings. Second, we assessed immune response using SARS-CoV-2 spike IgG level and therefore cannot draw a conclusion regarding T-cell responses. We also recognize that antibody levels were assessed within a detectable range, so we investigated the effect of removing outliers, with consistent findings. After the booster dose, the data were skewed, with most participants having similar values at the top end of the assay range. This is likely due to reaching the maximum detectable limit, where participants had higher antibody levels than those detected. For this reason, the results are presented in a Supplementary Appendix, and we do not consider it possible to draw conclusions from this third vaccination analysis. Finally, although the statistical models were adjusted for known, measured potential confounders, residual confounding cannot be ruled out.

In conclusion, our data showed that systemic reactions following SARS-CoV-2 vaccination were more often reported as moderate or severe after the second vaccination than after the first or third (booster). Furthermore, there appears to be an association after the second vaccination between more severe reactions and immune response, as assessed by higher levels of antibodies to the SARS-CoV-2 spike protein. This may be attributed to higher levels of preexisting immunity, in most cases gained from response to the first vaccination. We did not find evidence of an association between severity of reactions and immune response after the first vaccination, although level of immunity before the vaccination was associated with reporting symptoms. Regardless of side effects, the vaccines generate an effective immune response in most people.

Our findings could help to encourage those who are vaccine-hesitant due to concerns about side effects by sending a positive message that more severe reactions could be a sign of preexisting immunity, likely acquired from the first immunization, and will associate with a stronger immune response.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

Author contributions. W.B., D.R., O.S.S., L.Ø., J.R., and J.L. developed the concept and design of the study. L.L., B.T., M.B.I., N.B.S., S.O.L., M.R.J., I.S.J., A.B.M., F.D.L., L.S.K., V.K., L.W., T.B., K.T.P., K.K.I., and H.N. contributed to the acquisition of data. M.T. was responsible for laboratory data. W.B. and M.L.J. performed data curation. W.B. performed the statistical analysis. W.B., D.R., P.V.-B., M.T., L.L., O.S.S., S.R.O., N.B.S., L.Ø., J.R., and J.L. contributed to interpretation of the results. W.B. and D.R. drafted the manuscript. All authors critically reviewed and approved the manuscript.

The ENFORCE Study Group. Sponsor/Data and Statistical Centre: CHIP, Rigshospitalet under the direction of Professor J. Lundgren. Principal Investigator: Professor L. J. Østergaard, Department of Infectious Diseases, Aarhus University and Aarhus University Hospital. Sites (alphabetical and regional coordinators in parentheses): Region Hovedstaden: (T. Benfield), L. Krohn-Dehli, D. K. Petersen, Copenhagen University Hospital—Amager and Hvidovre, Hvidovre. K. Fogh, E. H. Mikkelsen (K. Iversen), Copenhagen University Hospital—Gentofte and Herlev, Herlev. Region Midtjylland: P. Bek, V. Klastrup, F. Larsen, S. H. Rasmussen, M. H. Schleimann, S. Schieber (N. B. Stærke), A. Søndergaard, B. Tarp, M. Tousgaard, Y. Yehdego, Aarhus University Hospital, Aarhus. Region Nordjylland: J. Bodilsen (H. Nielsen), K. T. Petersen, M. Ruwald, R. K. Thisted, Aalborg University Hospital, Aalborg. Region Sjælland: S. F. Caspersen, M. Iversen, L. S. Knudsen, J. L. Meyerhoff, L. G. Sander (L. Wiese), Zealand University Hospital Roskilde, Roskilde. Region Syddanmark: C. Abildgaard, I. K. Holden, N. E. Johansen (I. S. Johansen), L. Larsen, S. O. Lindvig, L. W. Madsen, A. Øvrehus, Odense University Hospital, Odense. Scientific Steering Committee: N. A. Kruse, H. Lomholdt, Lægemiddelstyrelsen, T. G. Krause, P. Valentiner-Branth, Statens Seruminstitut, B. Søborg, Sundhedsstyrelsen, T. K. Fischer, Copenhagen University, C. Erikstrup, Aarhus University, S. R. Ostrowski, Rigshospitalet, H. Nielsen, Aalborg University Hospital, I. S. Johansen, Odense University Hospital, L. J. Østergaard (chair), M. Tolstrup, N. B. Stærke, O. S. Søgaard, Aarhus University Hospital, L. Wiese, Zealand University Hospital Roskilde, T. Benfield, Copenhagen University Hospital—Amager and Hvidovre, J. Lundgren, D. Raben, CHIP, Rigshospitalet. Operational group: H. Nielsen, Aalborg University Hospital, I. S. Johansen, Odense University Hospital, L. J. Østergaard, M. Tolstrup, N. B. Stærke, O. S. Søgaard, Aarhus University Hospital, L. Wiese, Zealand University Hospital Roskilde, T. Benfield, Copenhagen University Hospital—Amager and Hvidovre, J. Lundgren (chair), D. Raben, CHIP, Rigshospitalet, E. Jylling, Danske Regioner, D. Hougaard, Statens Seruminstitut. Coordinating Centre: Aarhus University and Aarhus University Hospital, S. D. Andersen, K. Lykkegaard, N. B. Stærke, O. S. Søgaard, M. Tolstrup, L. J. Østergaard. ENFORCE Lab: Aarhus University Hospital: S. R. Andreasen, E. Baerends, L. L. Dietz, A. K. Hvidt, A. K. Juhl, R. Olesen, M. Tolstrup. Data and Statistical Centre: CHIP, Rigshospitalet: K. K. Andersen, W. Bannister, C. Bjernved, T. W. Elsing, F. V. Esmann, M. A. Ghafari, E. Gravholdt, S. F. Jakobsen, M. L. Jakobsen, C. M. Jensen, T. Ø. Jensen, D. Kristensen, L. R. Kumar, J. Lundgren, C. Matthews, N. Normand, C. Olsson, D. Raben, J. Reekie, A. Traytel, T. Weide. Other contributors: A. M. Hvas, H. Støvring, Aarhus University Hospital, Statens Seruminstitut.

Financial support. This work was supported by the Danish Ministry of Health (legal deeds 150 [January 28, 2021] and 263 [June 3, 2021]).

Potential conflicts of interest. N.B.S. declares having served as primary investigator on clinical studies sponsored by Pfizer and Bavarian Nordic. T.B. declares receipt of unrestricted research or travel grants from GSK, Pfizer, Gilead Sciences, and MSD; being principal investigator on trials conducted by Boehringer Ingelheim, Roche, Novartis, Kancera, Pfizer, MSD, and Gilead; being a board member for Pentabase; and being an advisory board member for MSD, Gilead, Pfizer, GSK, Janssen, and AstraZeneca; receipt of consulting fees from GSK and Pfizer; receipt of a donation of study drug from Eli Lilly; and receipt of honoraria for lectures from GSK, Pfizer, Gilead Sciences, Boehringer Ingelheim, AbbVie, and AstraZeneca. All other authors report no potential conflicts.

References

1. Larson HJ, Jarrett C, Eckersberger E, Smith DM, Paterson P. Understanding vaccine hesitancy around vaccines and vaccination from a global perspective: a systematic review of published literature, 2007–2012. Vaccine **2014;** 32:2150–9.
2. Dubé E, Gagnon D, Nickels E, Jeram S, Schuster M. Mapping vaccine hesitancy—country-specific characteristics of a global phenomenon. Vaccine **2014;** 32: 6649–54.

3. Ipsos World Economic Forum. Global attitudes: COVID-19 vaccines. **2021**. https://www.ipsos.com/en-ro/global-attitudes-covid-19-vaccine-january-2021. Accessed 13 June 2023.
4. Agosti F, Toffolutti V, Cavalli N, Nivakoski S, Mascherini M, Aassve A. Information and vaccine hesitancy: evidence from the early stage of the vaccine roll-out in 28 European countries. PLoS One **2022;** 17:e0273555.
5. Hervé C, Laupèze B, Del Giudice G, Didierlaurent AM, Tavares Da Silva F. The how's and what's of vaccine reactogenicity. NPJ Vaccines **2019;** 4:39.
6. Thomas SJ, Moreira ED Jr, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA COVID-19 vaccine through 6 months. N Engl J Med **2021;** 385:1761–73.
7. Walsh EE, Frenck RW Jr, Falsey AR, et al. Safety and immunogenicity of two RNA-based COVID-19 vaccine candidates. N Engl J Med **2020;** 383:2439–50.
8. Sobieszczyk ME, Maaske J, Falsey AR, et al. Durability of protection and immunogenicity of AZD1222 (ChAdOx1 nCoV-19) COVID-19 vaccine over 6 months. J Clin Invest **2022;** 132:e160565.
9. Baden LR, El Sahly HM, Essink B, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med **2021;** 384:403–16.
10. Bauernfeind S, Salzberger B, Hitzenbichler F, et al. Association between reactogenicity and immunogenicity after vaccination with BNT162b2. Vaccines (Basel) **2021;** 9:1089.
11. Coggins SA, Laing ED, Olsen CH, et al. Adverse effects and antibody titers in response to the BNT162b2 mRNA COVID-19 vaccine in a prospective study of healthcare workers. Open Forum Infect Dis **2022;** 9:XXX–XX.
12. Debes AK, Xiao S, Colantuoni E, et al. Association of vaccine type and prior SARS-CoV-2 infection with symptoms and antibody measurements following vaccination among health care workers. JAMA Intern Med **2021;** 181:1660–2.
13. Lapić I, Rogić D, Šegulja D, Zaninović L. Antibody response and self-reported adverse reactions following vaccination with comirnaty: a pilot study from a Croatian university hospital. J Clin Pathol **2022;** 75:782–6.
14. Oyebanji OA, Wilson B, Keresztesy D, et al. Does a lack of vaccine side effects correlate with reduced BNT162b2 mRNA vaccine response among healthcare workers and nursing home residents? Aging Clin Exp Res **2021;** 33:3151–60.
15. Rechavi Y, Shashar M, Lellouche J, Yana M, Yakubovich D, Sharon N. Occurrence of BNT162b2 vaccine adverse reactions is associated with enhanced SARS-CoV-2 IgG antibody response. Vaccines (Basel) **2021;** 9:977.
16. Takeuchi M, Higa Y, Esaki A, Nabeshima Y, Nakazono A. Does reactogenicity after a second injection of the BNT162b2 vaccine predict spike IgG antibody levels in healthy Japanese subjects? PLoS One **2021;** 16:e0257668.
17. Uwamino Y, Kurafuji T, Sato Y, et al. Young age, female sex, and presence of systemic adverse reactions are associated with high post-vaccination antibody titer after two doses of BNT162b2 mRNA SARS-CoV-2 vaccination: an observational study of 646 Japanese healthcare workers and university staff. Vaccine **2022;** 40: 1019–25.
18. Hwang YH, Song KH, Choi Y, et al. Can reactogenicity predict immunogenicity after COVID-19 vaccination? Korean J Intern Med **2021;** 36:1486–91.
19. Held J, Esse J, Tascilar K, et al. Reactogenicity correlates only weakly with humoral immunogenicity after COVID-19 vaccination with BNT162b2 mRNA (comirnaty®). Vaccines (Basel) **2021;** 9:1063.
20. Müller L, Andrée M, Moskorz W, et al. Age-dependent immune response to the BioNTech/Pfizer BNT162b2 coronavirus disease 2019 vaccination. Clin Infect Dis **2021;** 73:2065–72.
21. Stærke NB, Reekie J, Johansen IS, et al. Cohort profile: the Danish National Cohort Study of effectiveness and safety of SARS-CoV-2 vaccines (ENFORCE). BMJ Open **2022;** 12:e069065.
22. Quan H, Li B, Couris CM, et al. Updating and validating the Charlson Comorbidity Index and score for risk adjustment in hospital discharge abstracts using data from 6 countries. Am J Epidemiol **2011;** 173:676–82.
23. Chapin-Bardales J, Gee J, Myers T. Reactogenicity following receipt of mRNA-based COVID-19 vaccines. JAMA **2021;** 325:2201–2.
24. Hause AM, Baggs J, Marquez P, et al. Safety monitoring of COVID-19 vaccine booster doses among adults—United States, September 22, 2021-February 6, 2022. MMWR Morb Mortal Wkly Rep **2022;** 71:249–54.
25. Ferrara P, Ponticelli D, Losa L, et al. Risk of repeated adverse effects following booster dose of mRNA COVID-19 vaccine: results from the MOSAICO study. Vaccines (Basel) **2023;** 11:247.
26. Menni C, May A, Polidori L, et al. COVID-19 vaccine waning and effectiveness and side-effects of boosters: a prospective community study from the ZOE COVID study. Lancet Infect Dis **2022;** 22:1002–10.
27. Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol **2016;** 16:626–38.