Heliyon 9 (2023) e12746

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Antibody responses elicited by mRNA vaccination in firefighters persist six months and correlate inversely with age and directly with BMI

Caroline Holtkamp [a], Lara Schöler [a], Olympia E. Anastasiou [a], Bastian Brune [b,c], Kai Fessmann [b,c], Carina Elsner [a], Birte Möhlendick [d], Ieva Čiučiulkaitė [d], Marcel Dudda [b,c], Mirko Trilling [a], Ulf Dittmer [a], Jörg Spors [b], Vu Thuy Khanh Le-Trilling [a,*]

[a] Institute for Virology, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
[b] Emergency Medical Services, Fire Brigade Essen, Essen, Germany
[c] Department of Trauma, Hand, and Reconstructive Surgery, University Hospital Essen, University of Duisburg-Essen, Essen, Germany
[d] Institute of Pharmacogenetics, University Hospital Essen, University of Duisburg-Essen, Essen, Germany

ARTICLE INFO

Keywords:
COVID-19
SARS-CoV-2
Firefighters
Body mass index (BMI)
Vaccination
mRNA vaccine
Antibodies
Neutralization

ABSTRACT

Knowledge regarding the sustainability of immune responses after COVID-19 vaccination is important, e.g., to decide whom and when to booster. Thus, we analyzed antibody titers in firefighters six months after vaccination with the mRNA-based vaccine Comirnaty. SARS-CoV-2 spike-binding antibodies (bAb) were quantified and compared to peak responses determined in healthcare workers (HCW). For the firefighters, neutralizing antibodies (nAb) were also analyzed. Six months after the second vaccine dose, all analyzed firefighters had detectable bAb, and 91% exhibited nAb titers above 1:16. However, actual titers six months after vaccination were over 12-fold lower than in the HCW control group four weeks after vaccination. bAb and nAb responses showed a significant correlation, and age correlated inversely with antibody responses. Unexpectedly, participants with a body mass index over 25 had higher neutralization titers after six months. All participants with very low neutralization titers were offered booster vaccination. The booster vaccination improved the extent and sustainability of antibody responses.

1. Introduction

Since the end of December 2020 and just one year after the identification of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) as pathogen causing COVID-19, safe and effective vaccines are broadly available in Europe. In the European Union, five COVID-19 vaccines have been approved so far: (I) the messenger RNA (mRNA)-based SARS-CoV-2 vaccine Comirnaty/BNT162b2 (Pfizer/BioNTech; date of marketing authorization: 21/12/2020), (II) the mRNA vaccine Spikevax/mRNA-1273 (Moderna; date of marketing authorization: January 06, 2021), (III) the vector-based vaccine Vaxzevria (AstraZeneca; date of marketing authorization: 29/01/2021), (IV) the vector-based COVID-19 Vaccine Janssen (Johnson & Johnson; date of marketing authorization: 11/03/2021),

Abbreviations: COVID-19, Coronavirus disease 2019; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; BMI, Body mass index.

* Corresponding author. University Hospital Essen, University of Duisburg-Essen, Institute for Virology, Virchowstr. 179, 45147 Essen, Germany.
E-mail address: Khanh.Le@uk-essen.de (V.T.K. Le-Trilling).

https://doi.org/10.1016/j.heliyon.2022.e12746
Received 20 March 2022; Received in revised form 18 August 2022; Accepted 27 December 2022
Available online 30 December 2022

and (V) the adjuvanted recombinant protein vaccine Nuvaxovid/NVX-CoV2373 (Novavax; date of marketing authorization: 20/12/2021). Since the mRNA-based SARS-CoV-2 vaccine Comirnaty/BNT162b2 was the first vaccine available, we focused in this study on the Comirnaty vaccine. The effectiveness of two doses of Comirnaty is 93.7% against symptomatic infections with the Alpha variant of concern (VOC) and 88.0% against the Delta VOC [13]. The effectiveness of two doses against hospitalization due to infection with the currently predominant Omicron VOC has been reported to be 70% [1]. The sustainability of immune protection following vaccination against COVID-19 is gaining increasing interest as the first vaccinations already date back more than a year and the end of the pandemic or at least the transition to an endemic phase are not foreseeable, raising the question how long the protection of early vaccinees will last. During the first month after the second vaccination, patients exhibit peak antibody titers [15]. After this peak, antibody titers continuously wane over time [10]. Accordingly, a booster vaccination 3 months after the second vaccination is currently recommended by the permanent vaccination committee (STIKO) in Germany. Albeit with some country-specific aspects, all 30 EU/EEA countries currently recommend an additional dose as an extension of the primary vaccination series for immunocompromised individuals and as booster dose to different population groups to revert waning immunity [5]. This booster dose is usually offered regardless of the vaccinee's current antibody titer [17].

We tested SARS-CoV-2 IgG titers in firefighters, who were among the first recipients of the COVID-19 vaccine in Essen, Germany, as essential critical infrastructure workforce, six months after the second vaccination with Comirnaty. The first vaccinations were administered on December 21, 2020, literally on the day of the marketing authorization by the European Medicines Agency (EMA). We compared the results with early immune responses as determined in a group of individuals four weeks after their second vaccination. To evaluate the influence of age on humoral immune responses, we included people of different age groups. Furthermore, we conducted a complementary questionnaire to evaluate potential associations between immune responses after vaccination and behavioral factors such as sport, smoking, and body mass index (BMI). Neutralizing antibodies are regarded as correlates of protection against SARS-CoV-2 infection and COVID-19 [6,7,11,14]. However, for routine diagnostics, the quantification of neutralizing antibodies (nAb) by neutralization tests (NT) is challenging, because such assays are time-consuming and need to be performed in biological safety level

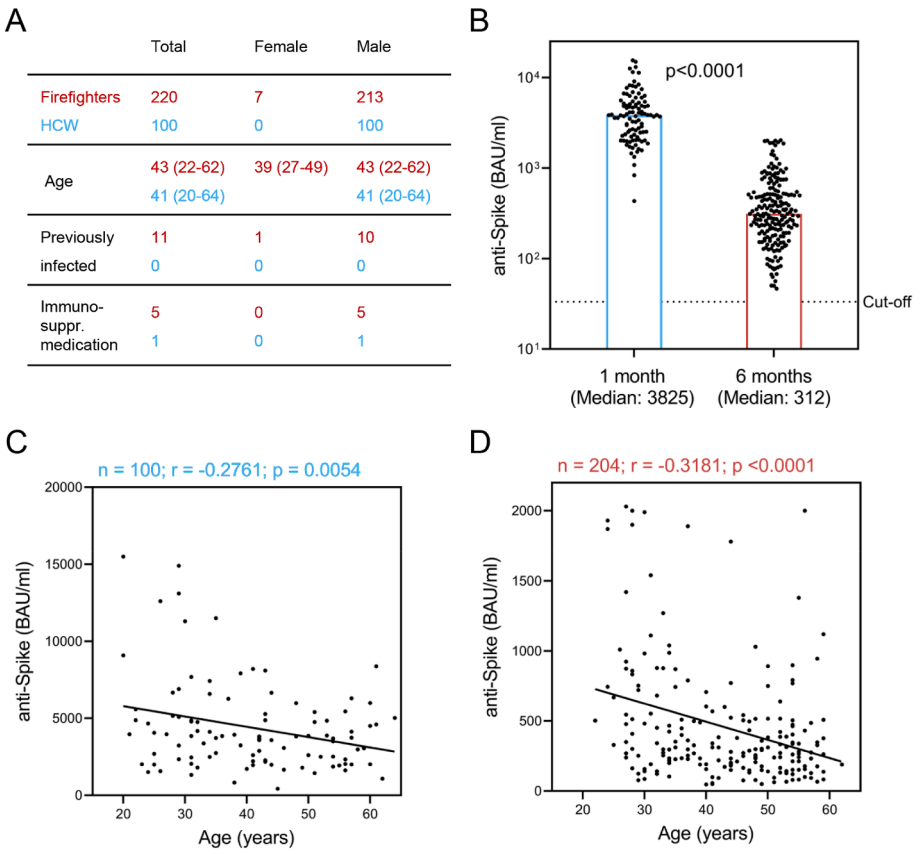

Fig. 1. Binding antibodies (bAb) recognizing SARS-CoV-2 spike decline but remain present six months after the second Comirnaty vaccine dose. (A) Characteristics of the firefighter and healthcare worker cohort. Age is shown as median with range in brackets. (B) Comparison of antibody responses determined from serum samples of 100 healthcare workers one month and 204 firefighters six months after the second Comirnaty vaccine dose. α-SARS-CoV-2 spike bAb titers were measured by the LIAISON SARS-CoV-2 TrimericS IgG assay. Bars depict the median values. Dots show the antibody titers of the individual subjects. (C) (D) Association between α-SARS-CoV-2 spike bAb titers one month (C) and six months (D) after the second Comirnaty vaccine dose and the age of participants. Pearson's correlation coefficient shows inverse linear correlation. Please note that the scales of the y-axis differ.

3 laboratories [18]. Therefore, we assessed the correlation between SARS-CoV-2 neutralization assays [16] with binding antibody (bAb) responses determined by the LIAISON SARS-CoV-2 TrimericS IgG. The latter can be applied in routine diagnostics, e.g., for immune surveillance programs and for decision making on the need for booster vaccinations.

2. Results

2.1. Binding antibodies recognizing SARS-CoV-2 spike decline but remain present six months after the second Comirnaty vaccine dose

To investigate whether the antibody responses induced by vaccination persist 6 months after vaccination, we tested 220 subjects from the professional fire department Essen, Germany. The seven female and 213 male firefighters were among the first who received vaccination in Essen, starting at the day of marketing authorization in the EU. The age of the participants ranged from 22 to 62 years (Fig. 1A). All study participants were immunized with two doses of the Comirnaty mRNA vaccine. Blood samples were collected approximately 6 months after the second vaccine dose. All tested subjects exhibited detectable anti-SARS-CoV-2 spike antibodies (>33.8 BAU/ml) six months after vaccination. Of these 220 participants, 16 subjects were excluded from further correlation analyses because of immunosuppressive medication or a subsided SARS-CoV-2 infection detected either by the presence of antibodies against the nucleocapsid antigen or by a positive qRT-PCR test in the past. 198 (97%) of the 204 included subjects were males and six (3%) females. The median antibody response 6 months after the second vaccination dose was 312 BAU/ml (interquartile range, IQR 191–516). These antibody titers were more than 12-fold lower than the titers of recently Comirnaty-vaccinated individuals (Fig. 1B), indicating a substantial decline over time. The reference group consisted of 100 male healthcare workers who were administered the Comirnaty vaccine twice and donated blood samples one month after the second dose. Here, the age ranged from 20 to 64 years (Fig. 1A), and the median antibody response was 3825 BAU/ml (IQR 2315–5190). When we correlated the antibody responses with the age of the participants, antibody titers correlated inversely with age at early and late time points after the second vaccination (Fig. 1C and D). The degree of age dependency was increased at 6 months after vaccination (Fig. 1D, $r = -0.3181$, $p < 0.0001$), suggesting that older vaccinees may particularly benefit from earlier booster immunization. Irrespective of this association with age, a high degree of variation was obvious in this rather homogenous group (active professional firefighters) with some individuals exhibiting bAb titers exceeding 2000 BAU/ml 6 months after second vaccination while others were close to the negative cut-off of the assay (Fig. 1B, D).

2.2. Binding and neutralizing antibody titers showed a significant correlation

The neutralizing antibody (nAb) titer was defined as the highest serum dilution at which 50% of input virus is neutralized. In the firefighter cohort, one participant receiving immunosuppressive medication had no detectable nAb and 18 healthy subjects showed a low nAb titer of ≤1:16. The median age of this subgroup of 18 participants was 49 years, higher than the overall cohort's median age of 43 years confirming that antibody responses inversely correlate with age. When we compared bAb titers with nAb titers, we observed significant linear correlation (Fig. 2A). This linear correlation applies in particular to samples below 200 BAU/ml and above 500 BAU/ml whereas intermediate bAb titers between 200 and 500 BAU/ml showed much lower correlation to the corresponding nAb titers (Fig. 2B). This finding implicates that bAb titer determination in routine diagnostics may be suitable to identify individuals with low nAb titers.

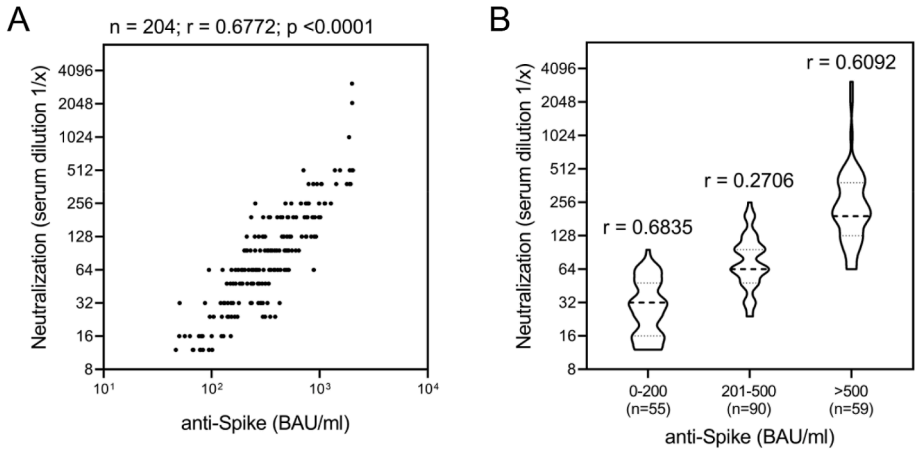

Fig. 2. Binding and neutralizing antibody titers showed a significant correlation. (A) The bAb and nAb titers of each of the 204 firefighter serum samples are depicted. The Pearson correlation coefficient of 0.6772 indicates linear correlation between bAb and nAb titers. (B) The serum samples were subgrouped with regard to their bAb titers. Violin plots with median of each subgroup are shown and the Pearson correlation coefficient of each subgroup is depicted.

2.3. Association between body mass index (BMI) and SARS-CoV-2 antibody responses six months after Comirnaty vaccination

To uncover additional factors associated with antibody responses, a questionnaire was distributed asking for information on behavioral factors such as smoking, sport, height, and weight. Of the 204 firefighters, 91 (45%) volunteers participated in this survey (although not all answered all questions). When integrating the information from the completed questionnaire, we observed that subjects with a BMI over 25 showed preferentially higher antibody responses 6 months after Comirnaty vaccination compared to their colleagues with a BMI below 25 (Fig. 3A). Whereas the trend did not reach statistical significance for α-SARS-CoV-2 spike bAb titers ($p = 0.149$, Fig. 3A and B), the effect was significant for neutralizing antibodies ($p = 0.019$, Fig. 3A, C). No significant differences were found for the other factors surveyed (physical training, smoking, Fig. 3A).

2.4. Booster vaccination improved the extent and durability of the antibody responses

Since several firefighters had low antibody titers 6 months after vaccination, participants with very low neutralization titers were offered booster vaccination. Two weeks after the boost, these individuals were asked to donate an additional sample. Analysis of these serum samples showed substantial increase of bAb (Fig. 4A) as well as nAb titers (Fig. 4B) in all tested subjects indicating that timely booster vaccinations are important instruments to avoid waning of immune responses. To analyze the sustainability of immune protection after booster vaccination, we invited the participants who had received the booster vaccination 6 months after the second vaccination to provide a follow-up sample. When comparing the samples taken at 6 months after the third vaccination dose with the samples taken at 6 months after the second vaccination dose, all tested vaccinees exhibited higher bAb and nAb titers after the booster vaccination (Fig. 4C and D). This finding showed that the booster vaccination improved the extent and sustainability of antibody responses in individuals with very low antibody titers 6 months after second vaccination.

A

Behavorial factor	anti-Spike (BAU/ml)	p value	NT titer	p value
BMI <25 (n = 34)	232 (104-342)	0.149	56 (24-96)	**0.019**
BMI >25 (n = 53)	257 (173-400)		96 (48-192)	
Sport ≤3 times per week (n = 42)	260 (177-379)	0.176	64 (48-128)	0.463
Sport >3 times per week (n = 49)	213 (126-342)		64 (32-128)	
Smoker (n = 14)	203 (83-274)	0.092	64 (16-96)	0.273
Non-smoker (n = 77)	253 (157-362)		64 (32-192)	

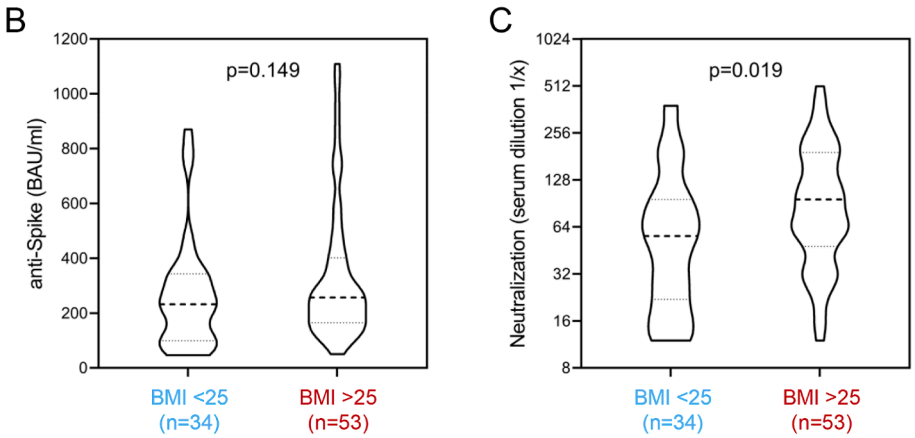

Fig. 3. Association between body mass index (BMI) and SARS-CoV-2 antibody responses 6 months after Comirnaty vaccination. (A) The influence of the behavioral factors smoking, sport, and body mass index (BMI) on SARS-CoV-2 antibody responses 6 months after Comirnaty vaccination. Median and interquartile range (IQR) are presented. Significance was calculated by Mann-Whitney *U* test. $P < 0.05$ was considered significant. (B) (C) Subjects were grouped regarding their BMI. Violin plots with median of each subgroup are shown. bAb titers (B) and nAb titers (C) are presented.

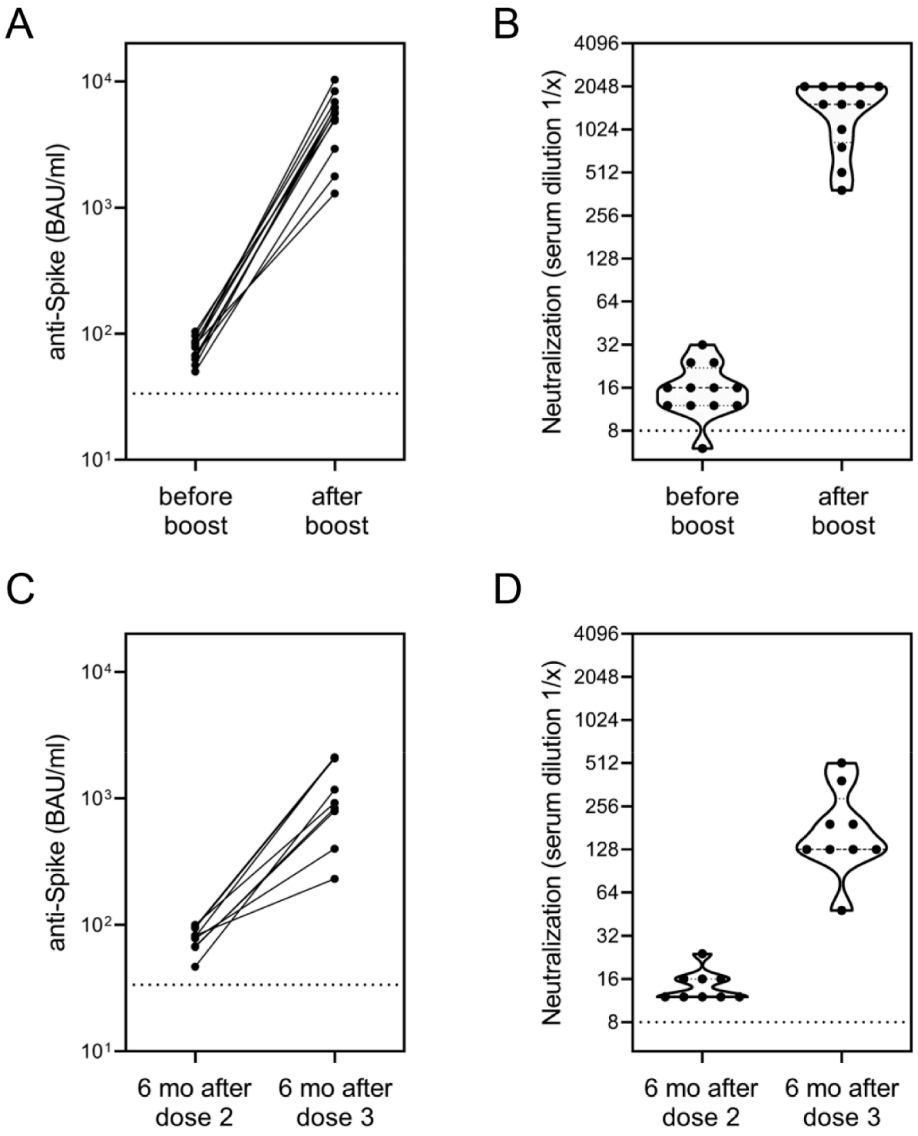

Fig. 4. Booster vaccination improved the extent and durability of the antibody responses. (A) (B) Serum samples from the same individual (n = 12) before and after booster vaccination were analyzed regarding bAb (A) and nAb (B) titers. (C) (D) Serum samples taken at 6 months after the second vaccination dose were compared to samples collected at 6 months after the third vaccination dose from the same individual (n = 9). bAb (C) and nAb (D) titers are depicted.

3. Discussion

We conducted a study addressing humoral immune responses among professional firefighters 6 months after Comirnaty vaccination against COVID-19. Firefighters belong to the essential workforce and contributed to the rollout of COVID-19 vaccines. Thus, we are convinced that data regarding the long-term sustainability of their immune protection are relevant. We are not aware of previous work addressing such issues. Accordingly, as of March 15, 2022, Pubmed, bioRxiv, and medRxiv queries with the terms "vaccine, COVID-19, and firefighter(s)" in abstract or title only yielded two publications that deal with vaccine acceptance and natural infections but not with vaccine responses. Our cohort is also special, because vaccinations started literally at the day of European marked authorization of Comirnaty so that long-term consequences of vaccination and booster immunization were assessable.

Six months after vaccination, all participants had detectable bAb recognizing the trimeric SARS-CoV-2 S protein (median titer 312 BAU/ml; IQR 191–516; cut-off for positivity: 33.8 BAU/ml). However, compared to a reference cohort of male healthcare workers also from Essen one month after Comirnaty vaccination, bAb responses were more than 12-fold reduced (Fig. 1B). Nevertheless, the vast majority (>91%) of firefighters were found to have nAb responses ≥1:16. Increased age was inversely associated with bAb responses at early and at late times after vaccination (Fig. 1C and D). Studies analyzing other groups came to similar conclusions. Both in

convalescent COVID-19 patients [21] and in vaccinated people [12], immune responses are preferentially higher in younger individuals. Vaccinations against diphtheria, hepatitis A, hepatitis B, pneumococcal disease (pneumococcal polysaccharide vaccine), and tetanus also show such associations [22], suggesting the existence of a broader age-associated immune senescence. Thus, strategies recommending vaccination and booster immunizations for elderly people, which are in place in several EU/EEA countries [5,17], are absolutely reasonable. Our data also underline the benefits of the third vaccine dose. Firefighters with exceptionally low antibody responses 6 months after the second vaccination experienced a substantial increase in bAb as well as nAb titers after the third booster dose of Comirnaty (Fig. 4A and B). These boostered humoral immune responses remained at high levels for at least 6 months (Fig. 4C and D), suggesting that booster immunizations not just reestablish the peak immune responses but may also increase the long-term sustainability of binding and neutralizing antibodies.

Across the entire range of BAU/ml titers, binding and neutralizing antibodies were significantly correlated ($r = 0.6772$; Fig. 2A). Other studies also observed this association [4,9]. Accordingly, individuals with low levels of bAb ($\leq$200 BAU/ml) also have very few nAb, and people with high bAb titers (>500 BAU/ml) almost without exception elicit strong neutralizing capacities. However, a close examination of the bAb-to-nAb relationship showed that it is far from being linear. This is reflected by the fact that the predictive value of the bAb determination regarding nAb titers is not very good at intermediate BAU/ml titers (201–500 BAU/ml) represented by a lower correlation coefficient ($r = 0.2706$; Fig. 2B). We concluded that bAb assays such as CLIA or ELISA can provide important clinical information by identifying individuals at risk of breakthrough infections due to waning immunity who would benefit from a booster immunization. However, such tests may fail to uncover definitive correlates of protections since the group of people with intermediate BAU/ml titers comprises both high and low neutralizers.

An intriguing observation of our study are the significantly higher nAb titers among the participants with a BMI above 25 six months after vaccination. Some studies dealing with long-term immune responses following natural SARS-CoV-2 infections observed similar effects [19,20]. At this point, we can only speculate regarding potential immunologic reasons. Interestingly, low serum levels of leptin - a hormone predominantly produced by adipose cells and enterocytes - have been associated with reduced vaccine responses [2]. If leptin would be beneficial for sustained nAb responses, this may explain the observed effect. It is noteworthy, that a higher BMI not necessarily indicates more body fat but may also result from more muscles. This may be particularly relevant when evaluating a male-dominated group with an occupation that comprises various physical activities, such as professional firefighters. However, we did not observe a significant association between nAb responses and regular sports activities. Irrespective of the molecular reasons, we think that our data suggest that people with a higher BMI may particularly benefit from COVID-19 vaccinations, firstly, because there is clear evidence that obese people have a higher likelihood of life-threatening COVID-19 disease (e.g. Ref. [3]) and, secondly, because their nAb responses appear to last longer.

Furthermore, direct and indirect occupational effects may occur. Irrespective of the professional activity, indirect occupational effects may apply to a group conducting a given duty (e.g., as result of gender-biased career choices or a selection for certain education levels based on defined requirements during the recruitment process). In addition to such indirect occupational influences such as gender and age, direct occupational effects could exist that result from the actual tasks (e.g., if firefighters work nightshifts, alterations of the circadian rhythm may influence immune responses). In follow-up studies, it would be interesting to investigate whether occupations in general may affect antibody responses after vaccination.

In conclusion, we provide relevant data regarding the correlation, sustainability, and increasibility of binding and neutralizing antibody responses in professional firefighters who were among the first to receive an mRNA vaccine against COVID-19.

4. Limitations of the study

We analyzed antibody responses in 220 firefighters after mRNA vaccination. However, there is an obvious limitation of our study: Since we purposely focused on a homogenous and in a certain way special and not generally representative group of vaccinees, it remains to be investigated whether or not the findings can be extrapolated to other parts of the population.

Author contribution statement

Caroline Holtkamp, Vu Thuy Khanh Le-Trilling: Performed the experiments; Analyzed and interpreted the data; Wrote the paper. Lara Schöler: Performed the experiments. Olympia E. Anastasiou: Analyzed and interpreted the data. Bastian Brune; Kai Fessmann; Carina Elsner; Birte Möhlendick; Ieva Čiučiulkaitė; Marcel Dudda: Contributed reagents, materials, analysis tools or data. Mirko Trilling: Analyzed and interpreted the data; Wrote the paper. Ulf Dittmer; Jörg Spors: Conceived and designed the experiments.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data included in article/supp. Material/referenced in article.

Additional information

No additional information is available for this paper.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Benjamin Katschinski and Kerstin Wohlgemuth for excellent technical assistance. We are also grateful for the support of the diagnostics department of the Institute for Virology Essen. We especially acknowledge the participating firefighters and healthcare workers for the contribution to the study.

STAR methods

Resource availability

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Vu Thuy Khanh Le-Trilling (Khanh.Le@uk-essen.de).

Materials availability

No new materials have been generated. All reagents are commercially available and all protocols are either presented here or are publicly available e.g., in previously published open-access papers. Technical questions should be directed and will be answered by the Lead Contact, Vu Thuy Khanh Le-Trilling (Khanh.Le@uk-essen.de).

Data and code availability

The published article includes all data sets generated or analyzed in this study. This study did not generate new algorithms.

Experimental model and subject details

Cells, viruses, and serum samples

Vero E6 cells (ATCC CRL-1586) were cultivated in high glucose Dulbecco's minimal essential medium (DMEM; Gibco 41,966-029) supplemented with 10% (v/v) FCS, penicillin, and streptomycin at 37 °C in an atmosphere of 5% CO_2. SARS-CoV-2 was isolated from a patient sample using Vero E6 and confirmed by SARS-CoV-2 diagnostic qRT-PCR. Briefly, swab samples were collected using Virocult vials (Sigma, Germany). Virocult medium from a SARS-CoV-2-positive sample was added to permissive Vero E6 cells. The next day, the medium was discarded and fresh medium was added to the cells. The cells were incubated until all cells were infected. The virus isolation and the collection of serum samples have been approved by the ethics committee of the Medical Faculty of the University of Duisburg-Essen (20-9511-BO, 20-9512-BO, 21-10176-BO, 21-10005-BO). Informed consent was obtained from all study participants. All work with infectious SARS-CoV-2 was conducted in a biosafety level 3 laboratory according to the regulations of the local authorities. Viral titers were determined by TCID50 titration.

50% tissue culture infectious dose (TCID50) endpoint dilution assay

To quantify the 50% tissue culture infective dose (TCID50) of SARS-CoV-2 stocks, permissive VeroE6 cells were seeded into 96-well plates one day before titration. Cells were then infected with 12 serial tenfold dilutions of the virus suspension in parallel octuplicates ($n = 8$). After 4 days, cell cultures were microscopically inspected and evaluated regarding the presence or absence of SARS-CoV-2 infection. TCID50 values were calculated by the Spearman & Kärber algorithm as previously described [8].

Sample collection

All study participants were immunized with two doses of the mRNA-based SARS-CoV-2 vaccine Comirnaty/BNT162b2 (Pfizer/BioNTech). Firefighters mostly received the first vaccine dose in December 2020. The second dose was usually administered 3 weeks after the first dose. Collection of blood samples was conducted approximately 6 months after the second vaccine dose. Six months after vaccination, participants with very low antibody titers were offered booster vaccination and two weeks after this boost, these individuals were asked to donate an additional sample. These participants were also asked to donate a follow-up sample 6 months after the third vaccine dose. The reference group of healthy healthcare workers was also vaccinated twice with Comirnaty. In June to July 2021, blood samples were taken one month after the second vaccine dose. Samples were centrifuged at 3500 g for 15 min. Sera were stored at 4 °C for few days or at −20 °C for longer time periods.

Quantification of anti-SARS-CoV-2 antibodies

Determination of anti-SARS-CoV-2 bAb titers was performed using the diagnostic LIAISON SARS-CoV-2 TrimericS IgG assay (Diasorin, Saluggia, Italy) according to the manufacturer's instructions. This test is a Chemiluminescence Immunoassay (CLIA) for quantitative detection of SARS-CoV-2 antibodies against the Spike glycoprotein in human serum. Relative light units (RLUs) were automatically converted into quantitative values for binding antibody units (BAU/ml). The cut-off for positivity was $\geq$33.8 BAU/ml. Samples reaching more than 2080 BAU/ml (detection limit of the CLIA) were diluted 1:10 (v/v) with phosphate-buffered saline and subsequently retested.

All serum samples were also analyzed regarding SARS-CoV-2 IgG antibodies against the nucleocapsid antigen to test previous SARS-CoV-2 infections. This test was performed using the Architect i2000SR CoV-2 IgG assay (Abbott Diagnostics, IL, USA). Results with an index $\geq$1.4 were considered positive for previous infections, an index between 0.4 and 1.4 was regarded as indication for mild infections or infections dating back longer ago. All measurements were conducted according to the manufacturer's instructions.

Neutralization assay

Neutralizing antibodies were analyzed by an in-cell-ELISA-based neutralization test (icNT) as previously described [16]. Briefly, serum samples were inactivated at 56 °C for 30 min. 2000 PFU of SARS-CoV-2 (B.1) were incubated with serial dilutions of serum samples for 1 h at 37 °C prior to infection of Vero E6 cells. At 20–24 h post-infection, cells were fixed with 4% (w/v) paraformaldehyde/PBS. Cells were permeabilized with 1% (v/v) Triton-X-100/PBS and blocked with 3% (v/v) FCS/PBS. The primary antibody (α-N mAb; ABIN6952435) was added and incubated for 2 h at room temperature or overnight at 4 °C. Peroxidase-labelled secondary antibody (Dianova) was incubated for 1–2 h. Washing steps were performed with 0.05% (v/v) Tween-20/PBS. Tetramethylbenzidin (TMB) substrate was added to visualize the enzyme reaction. The reaction was stopped with 0.5 M HCl. The absorbance was measured using a microplate multireader (Mithras2 LB 943; Berthold Technologies). The nAb titers represent the highest serum dilution at which 50% of input virus was neutralized.

Statistical analysis

Statistical analyses were performed using SPSS software (v23, SPSS Inc., Chicago, IL, USA) and GraphPad Prism 8.0.1 (GraphPad, CA, USA). Two-tailed *p* values less than 0.05 were considered statistically significant. Comparison between two groups was performed using Mann-Whitney *U* test. Correlations were analyzed using Pearson test. Shapiro-Wilk test was used to test normality.

References

[1] S. Collie, J. Champion, H. Moultrie, L.G. Bekker, G. Gray, Effectiveness of BNT162b2 vaccine against Omicron variant in South Africa, N. Engl. J. Med. 386 (2022) 494–496, https://doi.org/10.1056/NEJMc2119270.

[2] J. Deng, Q. Chen, Z. Chen, K. Liang, X. Gao, X. Wang, F.V. Makota, H.S. Ong, Y. Wan, K. Luo, et al., The metabolic hormone leptin promotes the function of T (FH) cells and supports vaccine responses, Nat. Commun. 12 (2021) 3073, https://doi.org/10.1038/s41467-021-23220-x.

[3] A.B. Docherty, E.M. Harrison, C.A. Green, H.E. Hardwick, R. Pius, L. Norman, K.A. Holden, J.M. Read, F. Dondelinger, G. Carson, et al., Features of 20 133 UK patients in hospital with covid-19 using the ISARIC WHO Clinical Characterisation Protocol: prospective observational cohort study, BMJ 369 (2020) m1985, https://doi.org/10.1136/bmj.m1985.

[4] R. Dolscheid-Pommerich, E. Bartok, M. Renn, B.M. Kümmerer, B. Schulte, R.M. Schmithausen, B. Stoffel-Wagner, H. Streeck, S. Saschenbrecker, K. Steinhagen, G. Hartmann, Correlation between a quantitative anti-SARS-CoV-2 IgG ELISA and neutralization activity, J. Med. Virol. (2021), https://doi.org/10.1002/jmv.27287.

[5] European Centre for Disease Prevention and Control, Overview of the Implementation of COVID-19 Vaccination Strategies and Deployment Plans in the EU/EEA, ECDC, 2021.

[6] S. Feng, D.J. Phillips, T. White, H. Sayal, P.K. Aley, S. Bibi, C. Dold, M. Fuskova, S.C. Gilbert, I. Hirsch, et al., Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection, Nat. Med. (2021), https://doi.org/10.1038/s41591-021-01540-1.

[7] W.F. Garcia-Beltran, E.C. Lam, M.G. Astudillo, D. Yang, T.E. Miller, J. Feldman, B.M. Hauser, T.M. Caradonna, K.L. Clayton, A.D. Nitido, et al., COVID-19-neutralizing antibodies predict disease severity and survival, Cell 184 (2021) 476–488, https://doi.org/10.1016/j.cell.2020.12.015, e411.

[8] J. Hierholzer, R. Killington, Virus isolation and quantitation, Virol. Methods Manual (1996) 25–46, 1996 Jan.

[9] K. Jung, S. Shin, M. Nam, Y.J. Hong, E.Y. Roh, K.U. Park, E.Y. Song, Performance evaluation of three automated quantitative immunoassays and their correlation with a surrogate virus neutralization test in coronavirus disease 19 patients and pre-pandemic controls, J. Clin. Lab. Anal. 35 (2021), e23921, https://doi.org/10.1002/jcla.23921.

[10] S.L. Kwok, S.M. Cheng, J.N. Leung, K. Leung, C.K. Lee, J.M. Peiris, J.T. Wu, Waning antibody levels after COVID-19 vaccination with mRNA Comirnaty and inactivated CoronaVac vaccines in blood donors, Hong Kong, April 2020 to October 2021, Euro Surveill. 27 (2022), https://doi.org/10.2807/1560-7917.Es.2022.27.2.2101197.

[11] E.H.Y. Lau, O.T.Y. Tsang, D.S.C. Hui, M.Y.W. Kwan, W.H. Chan, S.S. Chiu, R.L.W. Ko, K.H. Chan, S.M.S. Cheng, R. Perera, et al., Neutralizing antibody titres in SARS-CoV-2 infections, Nat. Commun. 12 (2021) 63, https://doi.org/10.1038/s41467-020-20247-4.

[12] J. Li, A. Hui, X. Zhang, Y. Yang, R. Tang, H. Ye, R. Ji, M. Lin, Z. Zhu, Ö. Türeci, et al., Safety and immunogenicity of the SARS-CoV-2 BNT162b1 mRNA vaccine in younger and older Chinese adults: a randomized, placebo-controlled, double-blind phase 1 study, Nat. Med. 27 (2021) 1062–1070, https://doi.org/10.1038/s41591-021-01330-9.

[13] J. Lopez Bernal, N. Andrews, C. Gower, E. Gallagher, R. Simmons, S. Thelwall, J. Stowe, E. Tessier, N. Groves, G. Dabrera, et al., Effectiveness of covid-19 vaccines against the B.1.617.2 (Delta) variant, N. Engl. J. Med. 385 (2021) 585–594, https://doi.org/10.1056/NEJMoa2108891.

[14] C. Lucas, J. Klein, M.E. Sundaram, F. Liu, P. Wong, J. Silva, T. Mao, J.E. Oh, S. Mohanty, J. Huang, et al., Delayed production of neutralizing antibodies correlates with fatal COVID-19, Nat. Med. 27 (2021) 1178–1186, https://doi.org/10.1038/s41591-021-01355-0.

[15] F.P. Polack, S.J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J.L. Perez, G. Pérez Marc, E.D. Moreira, C. Zerbini, et al., Safety and efficacy of the BNT162b2 mRNA covid-19 vaccine, N. Engl. J. Med. 383 (2020) 2603–2615, https://doi.org/10.1056/NEJMoa2034577.

[16] L. Schöler, V.T.K. Le-Trilling, M. Eilbrecht, D. Mennerich, O.E. Anastasiou, A. Krawczyk, A. Herrmann, U. Dittmer, M. Trilling, A novel in-cell ELISA assay allows rapid and automated quantification of SARS-CoV-2 to analyze neutralizing antibodies and antiviral compounds, Front. Immunol. 11 (2020), 573526, https://doi.org/10.3389/fimmu.2020.573526.

[17] STIKO, Ständige Impfkommission: beschluss der STIKO zur 18. Aktualisierung der COVID-19-Impfempfehlung, Epidemiol. Bull. 2022 *7* (2022) 3–18, https://doi.org/10.25646/9735.2.
[18] C.W. Tan, W.N. Chia, X. Qin, P. Liu, M.I. Chen, C. Tiu, Z. Hu, V.C. Chen, B.E. Young, W.R. Sia, et al., A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2-spike protein-protein interaction, Nat. Biotechnol. 38 (2020) 1073–1078, https://doi.org/10.1038/s41587-020-0631-z.
[19] S. Wendel, R. Fontão-Wendel, R. Fachini, G. Candelaria, P. Scuracchio, R. Achkar, M. Brito, L.F. Reis, A. Camargo, M. Amano, et al., A longitudinal study of convalescent plasma (CCP) donors and correlation of ABO group, initial neutralizing antibodies (nAb), and body mass index (BMI) with nAb and anti-nucleocapsid (NP) SARS-CoV-2 antibody kinetics: proposals for better quality of CCP collections, Transfusion 61 (2021) 1447–1460, https://doi.org/10.1111/trf.16323.
[20] S. Wendel, J.M. Kutner, R. Machado, R. Fontão-Wendel, C. Bub, R. Fachini, A. Yokoyama, G. Candelaria, A. Sakashita, R. Achkar, et al., Screening for SARS-CoV-2 antibodies in convalescent plasma in Brazil: preliminary lessons from a voluntary convalescent donor program, Transfusion 60 (2020) 2938–2951, https://doi.org/10.1111/trf.16065.
[21] T. Xiang, B. Liang, Y. Fang, S. Lu, S. Li, H. Wang, H. Li, X. Yang, S. Shen, B. Zhu, et al., Declining levels of neutralizing antibodies against SARS-CoV-2 in convalescent COVID-19 patients one year post symptom onset, Front. Immunol. 12 (2021), 708523, https://doi.org/10.3389/fimmu.2021.708523.
[22] P. Zimmermann, N. Curtis, Factors that influence the immune response to vaccination, Clin. Microbiol. Rev. 32 (2019), https://doi.org/10.1128/cmr.00084-18.

DOI: 10.1016/j.heliyon.2022.e12746
URN: urn:nbn:de:hbz:465-20230626-144036-7