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Serological response in health care workers after a single dose of SARS-CoV-2 vaccine using six automated SARS-CoV-2 antibody assays

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ABSTRACT

Spike (S)- and nucleocapsid (N)-specific serological assay responses were determined before and/or after first dose SARS-CoV-2 vaccination in 22 individuals. S-specific assays quantified antibodies after vaccination with significant higher levels in participants with a previous infection. Be cautious combining N-/S-specific assay results, potentially differentiating post-infection/vaccination immunization as assay-specific N-antibody waning was observed.

Currently 2 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine forms have been approved by the European Medicines Agency involving messenger RNA (mRNA) (mRNA-1273, Moderna; BNT162b2, Pfizer) or a non-replicating viral vector (AZD1222, AstraZeneca; Ad26 Cov S1, Janssen Pharmaceutical). Efficacy at preventing severe infections and hospitalization was close to 100% in phase 3 trials of these vaccines after administration of 2 doses. These trials included mainly participants without previous SARS-CoV-2 infection (Kyriakidis et al., 2021; Poland et al., 2020). After a first wave of automated qualitative serological assays targeting spike (S-) or nucleocapsid (N-) antibodies, most manufacturers launched quantitative tests targeting S-antibodies, considering that all current vaccines trigger anti-SARS-CoV-2 S-antigen antibody formation (Kyriakidis et al., 2021; Poland et al., 2020; Tang and Farnsworth, 2021). Manufacturers evaluated clinical sensitivity using samples from subjects immunized through natural SARS-CoV-2 infection but not through vaccination. None of the manufacturers gives any information regarding the performance or capability to detect antibodies of the assay after vaccination. The first studies are emerging, shedding light on the antibody response after a single dose of SARS-CoV-2 vaccine (Bradley et al., 2021; Ebinger et al., 2021). By definition, vaccination-induced immunization for SARS-CoV-2 should give an 'anti-S positive' and 'anti-N negative' serological response whereas infection-induced immunization should give an 'anti-S positive' and 'anti-N positive' serological response.

This study investigated the following topics: Do automated quantitative S-antibody assays detect and objectify SARS-CoV-2 vaccination? Are there differences in antibody levels between persons with and without previous SARS-CoV-2 infection after vaccination? How specific is the "anti-S positive" and "anti-N negative" serological response for individuals with vaccine-induced immunization and no previous infection? Antibody levels were determined right before first dose (baseline) and pre-booster (= post-vaccination) (4-12 weeks after baseline) in 22 health care workers with (n = 12) and without (n = 10) a previous SARS-CoV-2 infection, vaccinated with either the Pfizer (n = 12) or AstraZeneca (n = 10) vaccine. The median time between the diagnosis of SARS-CoV-2 infection and baseline sampling prior to vaccination was 209 days 95%CI[192-278]. Six validated (cf. CLSI EP06, EP12-A2, EP15-A3 and EP17, data not shown) automated assays were performed on each sample of which 4 quantitative S-antibody assays, being the SARS-CoV-2 TrimericS IgG (Liaison anti-TriS IgG) (LIAISON® XL, DiaSorin), SARS-CoV-2 S IgG (Siemens anti-S IgG) (Atellica® IM, Siemens), total antibody Elecsys Anti-SARS-CoV-2 S (Roche anti-S tAb) (Cobas 8000, Roche), SARS-CoV-2 IgG II Quant (Abbott anti-S IgG) (Alinity i, Abbott) and 2 qualitative N-

List of abbreviations: SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; mRNA, Messenger RNA; S, Spike; N, Nucleocapsid; Liaison anti-TriS IgG, SARS-CoV-2 TrimericS IgG; Siemens anti-S IgG, SARS-CoV-2 S IgG; Roche anti-S tAb, Elecsys Anti-SARS-CoV-2 S; Abbott anti-S IgG, SARS-CoV-2 IgG II Quant; Roche anti-N tAb, Elecsys Anti-SARS-CoV-2; Abbott anti-N IgG, SARS-CoV-2 IgG assay

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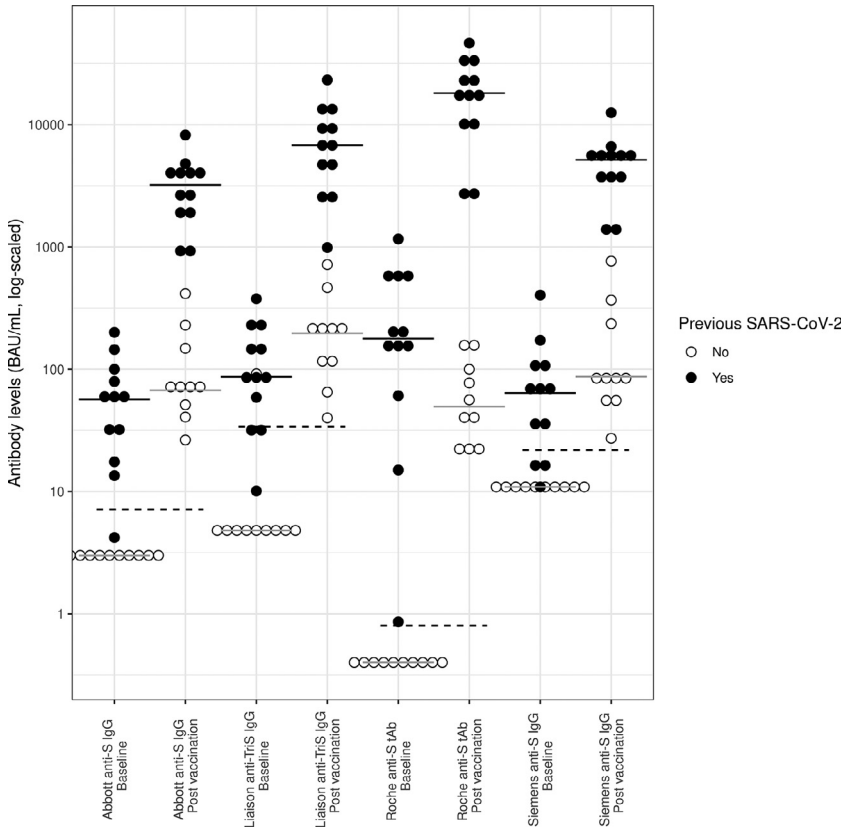

Fig. 1. Log-scaled baseline and post vaccination antibody levels for SARS-CoV-2. Dashed lines display the manufacturer's cut-off for presence of antibodies (Abbott anti-S IgG: ≥7.14 BAU/mL; Liaison anti-TriS IgG: ≥33.8 BAU/mL; Roche anti-S tAb: ≥0.80 BAU/mL; Siemens anti-S IgG: ≥21.8 BAU/mL). Full horizontal lines display the median baseline/pre-booster antibody levels in individuals without (Abbott anti-S IgG: <3.00/67 BAU/mL; Liaison anti-TriS IgG: <4.8/198 BAU/mL; Roche anti-S tAb: <0.40/49 BAU/mL and Siemens anti-S IgG: <10.9/87 BAU/mL) and with (Abbott anti-S IgG: 57/3215 BAU/mL; Liaison anti-TriS IgG: 86/6786 BAU/mL; Roche anti-S tAb: 178/18090 BAU/mL and Siemens anti-S IgG: 65/5167 BAU/mL) a previous SARS-CoV-2 infection.

antibody assays being the total antibody Elecsys Anti-SARS-CoV-2 (Roche anti-N tAb) (Cobas 8000, Roche) and SARS-CoV-2 IgG assay (Abbott anti-N IgG) (Alinity i, Abbott) assay. Sera were stored at -20° C awaiting analysis. Median anti-S levels were compared among individuals and vaccination time points using a Kruskal-Wallis test, followed by a 2-sided Wilcoxon post-hoc test with Bonferroni-Holm correction for multiplicity testing. P-values <0.05 were considered to be statistically significant. This study has approval of the Ethical Committee of the University Hospital Antwerp (reference number: 21/05/057) and has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration.

Fig. 1 shows that all quantitative S-specific assays detected antibodies, using the manufacturer's cut-off, after a single vaccination in all individuals regardless from the vaccine administered or previous infection. Median anti-S levels for all four assays were significantly higher ($P< 0.001$) after a single vaccination in persons with a previous infection compared to those without a previous infection. A first dose vaccination antibody response lower than the 95th percentile of levels seen in individuals without a previous infection could designate individuals without a previous infection (<416 BAU/mL for Abbott anti-S IgG; <718 BAU/mL for Liaison anti-TriS IgG; <169 BAU/mL for Roche anti-S tAb and <766 BAU/mL for Siemens anti-S IgG assay). On the other hand, a first dose vaccination antibody response higher than the fifth percentile of levels seen in individuals with a previous infection could designate individuals with a previous infection (>858 BAU/mL for Abbott anti-S IgG; >1128 BAU/mL for Liaison anti-TriS IgG; >2493 BAU/mL for Roche anti-S tAb and >1328 BAU/mL for Siemens anti-S IgG assay), regardless from the anti-N antibody status. Median pre-booster levels from individuals without a previous SARS-CoV-2 infection were statistically not different from baseline median levels from individuals with a previous infection for the Abbott anti-S IgG, Liaison anti-TriS IgG and Siemens anti-S IgG assay (all $P> 0.15$) with the exception of the Roche anti-S tAb assay ($P= 0.03$). Vaccination did not trigger anti-N antibody response in any individual as negative anti-N baseline measurements remained negative pre-booster for Abbott anti-N IgG ($n = 18$) and Roche anti-N tAb ($n = 10$).

Using the Roche anti-N tAb assay, the "anti-S positive" and "anti-N negative" serological response was seen in 11 samples from individuals without a prior infection; 10 were prebooster samples (all S-specific assays were positive) and one was a baseline sample with a positive Liaison anti-TriS result (all other S-specific assays were negative). Using the Abbott anti-N IgG assay, this serological response was seen in 27 samples consisting of the same 11 samples as described for the Roche assay and 16 additional samples (8 baseline and 8 pre-booster) from eight participants with a previous infection with the "anti-S positive" and "anti-N negative" serological response. The anti-N IgG antibodies measured by the Abbott assay were reported to have a half-life of 106 days making this assay prone to antibody waning (Buss et al., 2021). This waning phenomenon is not seen in the Roche anti-N tAb assay (Mueksch et al., 2021). In our study, baseline sampling was performed a median 209 days (2 times the antibody half-life) after SARS-CoV-2 infection. All 12 individuals with a previous infection had a positive Roche N-antibody response of which eight had a negative Abbott N-antibody response.

The S-specific assays from Roche, Siemens, DiaSorin and Abbott detected and quantified anti-S antibodies pre-booster and/or infection; however, protective levels are not provided for any of these assays. Significantly higher pre-booster antibody levels were observed in persons with a previous SARS-CoV-2 infection for all S-

specific assays. Despite the small sample size and low statistical power, conclusions were statistically significant using non-parametric statistics. The findings of this study should be checked in larger population-based studies, increasing the statistical power, although we believe that similar conclusions will be drawn.

Clinicians should be aware of the phenomenon of assay-specific antibody waning when using the combination of N- and S-specific serological assays to differentiate individuals with post-infection or vaccination immunization and should use an N-specific assay with a robust antibody response (e.g. Roche anti-N tAb assay). One should also be aware of possible false positive results from S-specific assays and inter-individual differences in antibody responses after SARS-CoV-2 infection as 3% to 4% of individuals with a previous infection seemed to be serological non-responders in a large population-based study by Baron et al. (Baron et al., 2020). As previous infection is not a contraindication for vaccination and guidelines for apparent 'non-responders' are lacking, we conclude that serologic testing prior or post-vaccination is not standard clinical practice yet. Nonetheless, performant quantitative serological assays will play an important role in research to establish protective antibody levels.

Author contributions

Matthias Cuykx: Conceptualization; Data curation; Formal analysis; Investigation; Validation; Visualization; Revising the article; Final approval of the version to be submitted.

Olivier Mortelé: Acquisition of data; Revising the article; Final approval of the version to be submitted.

Hilde Jansens: Acquisition of data; Revising the article; Final approval of the version to be submitted.

Sofie Schouwers: Acquisition of data; Revising the article; Final approval of the version to be submitted.

Anissa Meskal: Acquisition of data; Revising the article; Final approval of the version to be submitted.

Ilse Hoffbauer: Acquisition of data; Revising the article; Final approval of the version to be submitted.

Bart Peeters: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing.

Declaration of competing interest

Matthias Cuykx: Declarations of interest: none.Olivier Mortelé: Declarations of interest: none. Hilde Jansens: Declarations of interest: none. Sofie Schouwers: Declarations of interest: none. Anissa Meskal: Declarations of interest: none. Ilse Hoffbauer: Declarations of interest: none. Bart Peeters: Roche, Siemens and DiaSorin provided the S-specific assay kits for this study. Roche, Siemens or DiaSorin played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation of manuscript, or final approval of manuscript.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.diagmicrobio.2021.115486.

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