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Evaluation of the QuantiFERON SARS-CoV-2 interferon-γ release assay in mRNA-1273 vaccinated health care workers

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Current studies focus on cellular and humoral immunity induced by novel SARS-CoV-2 vaccines. Non-responders to vaccinations are not uncommonly encountered in clinical medicine (e.g. in the field of hepatitis B). Whereas vaccine-induced humoral immunity against SARS-CoV-2 is compromised by emerging Variants of Concern (VOCs), cellular immunity against SARS-CoV-2 is emerging as resilient against VOCs. Thus commercially available test kits for diagnostic laboratories designed to evaluate cellular immune responses to SARS-CoV-2 are urgently needed. Here we evaluated the novel QuantiFERON SARS-CoV-2 assay (Qiagen) measuring INF-γ release induced by two spike-derived peptide pools (Ag1 and Ag2) in a cohort of health care workers vaccinated with the mRNA-1273 vaccine and confirmed humoral response. Our study indicates the usefulness of this novel assay for routine laboratories to evaluate cellular immunity against SARS-CoV-2 in response to mRNA-1273 vaccination.

1. Introduction

The new coronavirus SARS-CoV-2 emerged in 2019 causing a pandemic (Zhou et al., 2020; Zhu et al., 2020). Ongoing vaccination efforts are aimed at mitigating it. Non-responders to vaccinations are not uncommonly encountered in Clinical Medicine (e.g. in the field of Hepatitis B) (Heininger et al., 2010). Vaccinations may trigger cellular and humoral immune responses. Whereas vaccine-induced humoral immunity against SARS-CoV-2 is compromised by emerging Variants of Concern (VOCs), cellular immunity against SARS-CoV-2 is emerging as resilient against VOCs (Tarke et al., 2021; Woldemeskel et al., 2021). Whereas many commercially tests are available to evaluate humoral responses to SARS-CoV-2 vaccinations, reliable commercially available tests for vaccine-induced cellular immunity are urgently needed. IGRA Tests (INF-gamma Release Assays) such as the QuantiFERON test platform (Qiagen) are commonly used in Clinical Laboratories, e.g. to evaluate cellular immunity for Cytomegalovirus or Mycobacterium tuberculosis. Here we provide a first evaluation of the novel QuantiFERON SARS-CoV-2 assay.

2. Materials and methods

2.1. Study design

We examined serological responses to vaccination (performed with two doses of mRNA 1273 from Moderna applied within 4 weeks) for 18 health care workers (HCWs) in our Department. Written informed consent of all volunteers was obtained. Sample and data acquisition were approved by the Medical Ethics Committee of the University Hospital RWTH Aachen (EK 093/20). The median age in our cohort was 46.9 years (± 13.7 years) with 40 % male and 60 % female participants. Samples for serology and IGRA were taken 7–13 weeks after the second vaccination.

2.2. Assays

Successful humoral responses to vaccination were evaluated using the Liaison “SARS-CoV-2 S1/S2 IgG” assay from DiaSorin (Italy) and the “TECO SARS-CoV-2 Neutralization Antibody Assay” from TECOmedical (Switzerland) which were performed according the manufacturer’s instructions. The Teco surrogate virus neutralization assay measures the ability of serum samples to disrupt the interaction of the receptor
binding domain (RBD) of SARS-CoV-2 and its receptor ACE2 (angiotensin converting enzyme 2); diluted serum samples are incubated with HRP-conjugated RBD as a bait. Subsequently, this mix is added to ELISA plates coated with ACE2 and the resulting plate-retained complexes of ACE2 and RBD-HRP are incubated with a colorimetric HRP substrate. Inhibition of ACE2:RBD binding is calculated as % in comparison to negative control serum not contain neutralizing activity; a good correlation with classical cell culture virus neutralization tests was reported (Murray et al., 2021). Anti-Nucleocapsid IgG was determined using the “recomWell SARS-CoV-2 IgG ELISA” from Microgen (Germany). Also the novel QuantiFERON SARS-CoV-2 Interferon-γ release assay (so far for research use only) was performed according to the manufacturer’s recommendations. Background INF-γ levels (“Nil values” without stimulating peptides) were subtracted from the values of stimulations to obtain values depicted in the Figures.

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 27 for Windows (IBM Corporation, USA). Metric variables are given as arithmetic mean ± standard deviation. Correlation between metric variables was assessed by Spearman correlation analysis. Sensitivity was evaluated by agreement with the post vaccination serology status ( = 100 % in our cohort). Specificity could not be addressed because no vaccinee in our cohort lacked a serological response to vaccination.

3. Results

Using the DiaSorin “Liason SARS-CoV-2 S1/S2 IgG assay” (Krüttgen et al., 2021) and a surrogate virus neutralization assay (“TECO SARS-CoV-2 Neutralization Antibody Assay”, TEComedical) (Müller et al., 2021), we confirmed that all vaccinees (18/18) in our study had successfully mounted humoral immune responses after mRNA-1273 vaccination (data not shown). We also tested our vaccinees for anti-Nucleocapsid IgG (“recomWell SARS-CoV-2 IgG ELISA” from Microgen) which is a marker for past covid-infections. One individual in our cohort (vaccinee 15) with previous COVID-19 infection gave a positive result (data not shown).

Next blood from all 18 participants was subjected to the novel INF-γ Release Assay (IGRA) from Qiagen, measuring INF-γ release induced by two proprietary SARS-CoV-2 peptide pools (Ag1 and Ag2) encompassing the spike protein and designed to stimulate CD4 and CD8 T cells and induce the releases of INF-γ. The kit also contains a positive control antigen which induces robust INF-γ release from T cells. We first confirmed that this internal positive control works: all participants showed robust INF-γ release in response to positive control (mean of 3,27 IU/mL (± 0,068 IU/mL), data not shown, but see box chart in Fig. 2).

Next, we evaluated the INF-γ response to SARS-CoV-2 Ag1 and Ag2. As shown in Fig. 1, we found impressive individual differences in terms of INF-γ release induced by Ag1 ranging from 1,876 IU/mL (vaccinee 2) down to 0 IU/mL (vaccinee 17). In a similar way as for Ag1, we found large individual differences in terms of INF-γ release by Ag2 ranging from 2,442 IU/mL (vaccinee 4) down to 0,019 IU/mL (vaccinee 9). Fig. 2 shows a box chart of these data (for Ag1: mean 0,341 IU/mL (± 0,569 IU/mL); for Ag2: mean 0,477 IU/mL (± 0,778 IU/mL). Although there was a good correlation between the results for Ag1 and Ag2 (Spearman correlation coefficient 0.936 (p < 0.01); the higher mean value for Ag2 in comparison to Ag1 indicates that the Ag2 peptide mix is better suitable than the Ag1 peptide mix to evoke INF-γ responses.

Although clearly intended for use in clinical routine laboratories, the kit is so far for RUO (research use only); thus no official cutoff for clinical samples and no clinical sensitivity data for this assay has yet been released by the manufacturer. According to the data sheet provided by the manufacturer early data suggested an INF-γ cutoff for positivity between 0,15 IU/mL and 0,2 IU/mL. Therefore we simulated different scenarios regarding different cutoffs and calculated the resulting sensitivities: Under the assumption that all vaccinees with subsequently confirmed humoral immunity also developed cellular immunity, a cutoff of 015 IU/mL would lead to a sensitivity of 44 % (8/18). This scenario is visualized in Fig. 1 as indicated by an arrow denoting the cutoff of 0,15 IU/mL. In this scenario vaccinees 2, 3, 4, 10, 13, 14, 16 and 18 ( = 44 %) would have INF-γ levels above cutoff for either Ag1 or Ag2.

Supplementary Fig. 1 focuses on the “low responders” among our vaccinees (those vaccinees with INF-γ values below 0,15 IU/mL). As shown in this Figure, lowering the cutoff to 0,05 IU/mL (indicated by upper arrow) would lead to a sensitivity of 66 % (12/18) with vaccinees 5, 7, 11 and 15 reaching levels above this cutoff. Further lowering the cutoff to 0,015 IU/mL (indicated by lower arrow) would lead to a sensitivity of 100 % (18/18). Also 100 % of our cohort would be considered as positive if –alternatively- the cutoff was defined as mean value of negative controls plus one standard deviation ( = 0,004 IU/mL).
Subsequently we assessed a possible correlation between cellular responses (INF-γ release) and humoral responses (neutralizing activity of serum as measured by a surrogate virus neutralization assay). As shown in Fig. 3 all vaccinees possessed neutralizing activity in their sera; the highest levels were found in vaccinees 3, 4, 7, 15, whereas the lowest levels were found in vaccinees 6, 13. In comparison, the highest levels of INF-γ responses (compare Fig. 1) were observed in vaccinees 2, 4, 14 and the lowest levels in vaccinees 1, 8, 9, 12. Thus there was only one individual in our cohort who showed corresponding high qualitative responses in terms of both cellular and humoral immunity assays (vaccinee 4). Furthermore, there was no individual in our cohort who showed corresponding low qualitative responses in both cellular and humoral immunity assays. Accordingly, we found no significant statistical correlation between qualitative cellular [IU/mL of INF-γ] and qualitative humoral responses (% inhibition in a surrogate virus neutralization assay) in our cohort (for Ag1: Spearman correlation coefficient 0.170 (p < 0.499); for Ag2: Spearman correlation coefficient 0.259 (p < 0.099)). On the other hand, a perfect correlation would be found if qualitative (instead of quantitative) criteria were considered because all vaccinees showed qualitatively positive humoral responses above background and qualitatively positive INF-γ responses above background (INF-γ levels >0.004 IU/mL).

Next, we wanted to evaluate the specificity of the new QuantiFeron assay. (Un)fortunately we could not perform this analysis because all vaccinees in our cohort (100%) showed positive serological responses to vaccination.

4. Discussion

Whereas diagnostic laboratories have plenty of commercially available tests available to assess humoral immunity in response to SARS-CoV-2, there is an emerging need for commercially available validated assays to assess the cellular immunity against the novel pathogen SARS-CoV-2.

Assessing cellular immunity for SARS-CoV-2 (in addition to humoral immunity) by clinical diagnostic laboratories appears important for several reasons.

First, in case of vaccinated individuals undergoing immunosuppression because vaccinees with positive cellular immunity against SARS-CoV-2 should have a lower risk of developing disease following infection than those who do not have detectable cellular immunity (Kronbichler et al., 2021).

Secondly, because persistent infections with SARS-CoV-2 (Sahin et al., 2021) and persistent T cell abnormalities associated with “long COVID” syndrome have been described (Townsend et al., 2021). Also in these patients evaluating cellular immunity against SARS-CoV-2 might be informative for clinicians.

Thirdly, health authorities might also occasional request IGRAs in suspected secondary cases, because T-cell responses are reportedly sometimes more sensitive indicators of SARS-CoV-2 exposure than antibody assays (Gallais et al., 2021).

Lastly and most importantly, cellular immunity against SARS-CoV-2 is emerging as more resilient against mutated variants of SARS-CoV-2 featuring (humoral) immune escape phenotypes (Tarke et al., 2021; Woldemeskel et al., 2021). IGRAs for SARS-CoV-2 could fill this diagnostic gap. Such assays are commonly used in routine laboratories to assess the cellular immunity status of patients for other pathogens such as Mycobacterium tuberculosis or Cytomegalovirus (Pieterman et al., 2018). Using a mRNA-1273-vaccinated cohort with confirmed humoral immunity, we found a rather low sensitivity of 44% when using a cutoff of 0.15 IU/mL as suggested by the manufacturer in the current data sheet of the kit. In comparison, established Quantiferon assays feature similar cutoffs (0.2 IU/mL for the viral pathogen CMV (Ruan et al., 2019) or 0.35 IU/mL for the bacterial pathogen Mycobacterium tuberculosis (Fukushima et al., 2021)). Thus, adapting a similar cutoff for the novel assay could seem reasonable to consider. Of note, CMV and TB infections differ from SARS-CoV-2 infections because they cause permanent immune stimulation and thus more specific effector cells are found in circulation.

However, assuming a positivity rate of 44% in our vaccinees using the proposed cut-off in the current manual of the kit leads to the question how such a low percentage rate could be explained. We envision two possible explanations: First, the novel test indeed has a low sensitivity and might need optimization before entering clinical use. Second, the sensitivity of the test is high but the mRNA-1273 vaccine did not efficiently trigger cellular immunity in most of our vaccinees.

Regarding the first possibility (“vaccine good, test needs optimization”): what could explain a low sensitivity of a novel IGRA assay for SARS-CoV-2? Due to the great diversity of MHC haplotypes, perhaps the Ag1 and Ag2 peptide pools of this kit might just not bind to all HLAs present in our cohort (Saull et al., 2021). This hypothesis is supported by clinical studies of another mRNA vaccine (BNT162b2) which found robust INF-γ production using a different IGRA platform (the ELISPOT format) using different peptide pools (Sahin et al., 2021). Alternatively, the Ag1 and Ag2 peptides of this kit are well-suited to measure past COVID-19 infections but may be not appropriate to measure the specific cellular immunity evoked by the Moderna vaccine. Of note, due to the antigens that were used the Quantiferon TB assay is not suitable at all to reveal individuals formerly vaccinated against TB but only suitable to detect individuals with previous infection (Pieterman et al., 2018). However, some of our mRNA-1273-vaccinees did show high INF-γ responses, indicating that the peptide pools of the kit work well for at least some vaccinees. Another possibility explaining low INF-γ responses in our cohort might be that we only examined one time point (12–13 weeks after first vaccination). Perhaps, different time points might have yielded higher responses in some vaccinees. Further studies should address this issue.

Regarding the second possibility (“test good, vaccine needs optimization”): one study with a small cohort of mRNA-1273 vaccinees found CD4 cytokine responses involving Th1 cells with TNF-α responses greater that interleukin-2 responses which in turn were greater than the interferon-γ responses. CD8 T-cell responses were only observed at low levels after the second mRNA-1273 vaccination (Anderson et al., 2020). According to this study, Interferon-γ might indeed not be the most sensitive parameter to assess cellular immune responses in mRNA-1273 vaccinees. However, some of our mRNA-1273-vaccinees in our study did...
show high INF-γ responses, indicating that the mRNA-1273 vaccination does induce sufficiently high responses in at least some vaccinees.

5. Conclusions

To our knowledge we provide the first evaluation of the new novel QuantiFERON SARS-CoV-2 Interferon-γ release assay. Our study indicates the usefulness of this rapid and convenient assay for routine laboratories to evaluate cellular immunity against SARS-CoV-2. The limitation of our study is a small number of participants (vaccinated with mRNA-1273) and the lack of negative controls (individuals who did not show humoral responses to vaccination) to evaluate the specificity of the assay. However, this does not alter the main conclusion of this study, an unexpected low sensitivity when using the cutoff of 0.15 IU/mL proposed by the current manual of the kit. However, further studies are necessary to validate this promising and important new assay before it can enter use in routine diagnostic.

Author contributions

Alexander Krüttgen: Conceptualization, investigations, data analysis, writing.
Hanna Klingel: sample collection, investigations, data analysis, review & editing.
Matthias Imöhü: sample collection, review & editing.
Helga Haefner: sample collection, review & editing.
Gerhard Haase: sample collection, review & editing.
Michael Kleines: Conceptualization, investigations, data analysis, review & editing.

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Declaration of Competing Interest

We declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi: https://doi.org/10.1016/j.jviromet.2021.114295.

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