Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Accuracy and real life performance of a novel interferon-γ release assay for the detection of SARS-CoV2 specific T cell response

Daniela Huzly a,1,*, Marcus Panning a,1, Franziska Smely a, Martin Enders b, Johanna Komp c, Valeria Falcone a, Daniel Steinmann d

a Institute of Virology, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany
b Laboratory Prof. Gisela Enders and Colleagues, Stuttgart, Germany
c Institute of Microbiology, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany
d Occupational Medical Service, Medical Center - University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany

ARTICLE INFO
Keywords:
COVID-19
Adaptive immune response
IFNγ release assay
T cell response
Test accuracy
Health care workers
Immunosuppressed patients

ABSTRACT

Background: The reliable detection of T cell response to COVID-19 or COVID-19 vaccination is important for individual patient care and for monitoring the immune response e.g. in COVID-19 vaccine trials in a standardized fashion.

Objectives and study design: We used blood samples from health care workers (HCW) with or without history of COVID-19 to define test accuracy of a novel interferon-γ release assay (IGRA). For a real-life performance evaluation, we analysed interferon-γ response to complete COVID-19 vaccination in HCW receiving homologous or heterologous vaccination regimens and in patients receiving immunosuppressive or immune modulating therapies.

Results: The assay had a specificity of 100%. Sensitivity of the IGRA to detect past infection was 72.2% after infection more than 5 months ago and 93.8% after COVID-19 up to 5 months ago. Quantitative results showed significant differences between first and second vaccine dose, but no difference between homologous and heterologous vaccination regimen. Immunocompromised patients often had no immune response or isolated T cell or antibody response to complete vaccination.

Conclusions: The novel IGRA proved to be a highly specific tool to detect SARS-CoV-2 specific T cell response to COVID-19 as well as COVID-19 vaccination, with sensitivity getting lower over time. In perspective, it may serve as a standardized tool in COVID-19 vaccine trials and in clinical care of immunosuppressed patients.

1. Background

The role of T cell immune response in SARS-CoV-2 infection is not entirely understood, but data from animal models show an important role for protection against coronavirus disease 2019 (COVID-19) mediated by CD4+ and CD8+ T cells [1, 2]. Since December 2020, the first vaccines against COVID-19 are available but the quality and duration of the immune response to vaccination remains unclear as of yet. Although numerous SARS-CoV-2 antibody detection assays were introduced with unprecedented speed and are now widely in use, the implementation of T cell assays lagged behind. However, besides analysing the antibody response, it will be of importance to investigate the T cell mediated immune response, e.g. in vaccine trials or in a clinical setting for individual patient care. Therefore, easy to perform, validated, and ideally standardized T cell assays are required [3].

Here we evaluated a novel commercially available IFN-γ release assay (IGRA) to analyse the SARS-CoV-2 specific T cell response. IGRA have revolutionized the diagnosis of tuberculosis [4] and several other pathogens [5, 6]. In experimental settings, in-house SARS-CoV-2 IGRA were introduced recently [7]. The main advantage of this type of assay is the possibility to perform them without special equipment and with very short hands-on time. The IFN-γ results are expressed in international units and are therefore truly quantitative.

* Corresponding author.
E-mail address: daniela.huzly@uniklinik-freiburg.de (D. Huzly).

https://doi.org/10.1016/j.jcv.2022.105098
Received 29 October 2021; Received in revised form 27 January 2022; Accepted 31 January 2022
Available online 2 February 2022
1386-6532/© 2022 Elsevier B.V. All rights reserved.
2. Objectives

For our test accuracy study, we asked immunocompetent healthcare workers (HCW) at Medical center - University of Freiburg to participate and offered measurement of SARS-CoV-2 antibodies. Recruitment was open to HCW who had experienced mild SARS-CoV-2 infection in 2020 as well as HCW without a history of COVID-19 prior to vaccination. After successful evaluation of the assay, we analysed the COVID-19 vaccine associated T cell response in HCW receiving two different vaccine regimens. Finally, the assay was applied in immunocompromised patients to evaluate the test performance in a real-life setting.

3. Study design

3.1. Study population

162 HCW agreed to participate in the accuracy study. Of these, 107 had previous SARS-CoV-2 infection (COVID-19 group) proven by a positive SARS-CoV-2 RT-PCR and seroconversion, and 55 were without history of SARS-CoV-2 infection or vaccination (no COVID-19 group). For performance evaluation 68 HCW were tested after vaccination. 38 had received two doses of BNT162b2 (Comirnaty®, BioNTech/Pfizer), 30 had been vaccinated with AZD1222 (Vaxzevria®, AstraZeneca) and 14 of these 27 received a heterologous vaccination scheme with a second dose of mRNA-1273 (Spikex®, Moderna Biotech). Blood was drawn two to three weeks after the first and second dose. Samples were tested consecutively without knowledge of SARS-CoV-2 antibody status or patient history. For the final real-life performance evaluation, patient samples (n = 149) sent to our routine diagnostic laboratory with the request for SARS-CoV-2 T cell analysis and antibody detection after COVID-19 vaccination were included.

SARS-CoV-2 Interferon-γ release assay

The SARS-CoV-2 IGRA (EUROIMMUN, Lübeck, Germany) is based on the SARS-CoV-2 spike protein and was done according to the manufacturer’s instructions. For a detailed description of the assay see suppl. 1. IFN-γ concentration was measured using the enzyme-linked immunosorbent assay (ELISA) delivered by the manufacturer in combination with the stimulation tubes. The manufacturer suggested cut-off is at >200 mIU/ml including a grey zone of 100 – 200 mIU/ml. IGRA Test kits for the first evaluation of the assay were provided by EUROIMMUN free of charge.

3.2. Detection of SARS-CoV-2 antibodies

We used three different SARS-CoV-2 antibody assays to rule out past infection in the no COVID-19 and in the vaccine cohorts: Details on the immunoassays are shown in Supplement 1.

3.3. Statistical analysis

Data were analysed using IBM SPSS Statistics version 27 software, GraphPad Prism version 9, and MedCalc version 19. For detailed description of statistical analysis see supplemental material. For test accuracy calculation, we used two different approaches: we defined grey zone results as either positive or negative [8]. As data had a non-Gaussian distribution, we used non-parametric tests throughout. In order to evaluate the manufacturers’ cut-off definition we performed receiver operating characteristic (ROC) analysis to define an optimum cut-off value at the Youden maximum index value.

4. Ethics

The study was approved by the ethics committee of Albert-Ludwigs University Freiburg (#20-1271, Nov 24, 2020 and #20-1271_1, Jan 18, 2021). Written informed consent was obtained from all participants.

5. Results

We enrolled 162 HCW for the validation study. All HCW in the COVID-19 group had referred mild symptoms. Baseline characteristics of the HCW study groups are shown in Table 1.

5.1. IFN-γ concentrations in the no COVID-19 group

Out of 55 samples from the no COVID-19 group, 50 had IFN-γ concentrations below 100 mIU/ml, two between 100 and 200 mIU/ml, and three above 200 mIU/ml. Serum samples of these three individuals were reactive in several antibody assays suggesting past infection. Thus, we excluded these samples from further analysis. Serum samples from the 52 remaining individuals of this cohort tested negative with three different SARS-CoV-2 antibody assays and were therefore defined as truly negative. Specificity of the assay using 200 mIU/ml and 100 mIU/ml was 100% [95% confidence interval (CI) 93.2%–100%] and 96.2% (95% CI 86.8%–99.5%), respectively.

5.2. IFN-γ concentrations in HCW with previous SARS-CoV-2 infection (COVID-19 group)

A total of 107 samples from HCW with previous SARS-CoV-2 infection were available to us. In one case, the assay was invalid, the sample was thus excluded from further analysis. 80 of the remaining 106 HCW (75.4%) yielded IFN-γ concentrations above 200 mIU/ml, 15 (14.2%) between 100 and 200 mIU/ml and 11 (10.3%) below 100 mIU/ml. Overall sensitivity to detect past infection was 75.4% using 200 mIU/ml as cut-off and 89.7% defining grey-zone results as positive. Of the 106 HCW, 90 had blood samples drawn more than six months after RT-PCR proven SARS-CoV-2 infection. Eleven (12.2%) yielded IFN-γ concentrations below 100 mIU/ml, 14 (15.6%) between 100 and 200 mIU/ml and 65 (72.2%) above 200 mIU/ml. In this subgroup, we calculated a sensitivity of 72.2% defining IFN-γ concentrations of 200 mIU/ml as true positive and 87.8% with grey-zone results defined as positive. Another 16 individuals were infected two to five months ago, 15 of which had IFN-γ concentrations above 200 mIU/ml and one was between 100 and 200 mIU/ml. Sensitivities for recent infection in this subgroup were 93.8% or 100% defining grey-zone results as negative or positive, respectively.

5.3. ROC analysis, background IFN-γ concentrations and adapted performance calculation

Performing ROC analysis with the “no COVID-19 group” as true negative and the “COVID-19 group” as true positive cohort, highest Youden index was seen at 135 mIU/ml with a specificity of 98.1% (95% CI 89.8%–99.9%) and a sensitivity of 93.4% (95% CI 89.2%–96.3%). Background IFN-γ stimulation (BLANK values) was low in most participants (mean 35.2 mIU/ml, range 0–1520 mIU/ml), but 22/158 (13.9%) showed elevated background stimulation with IFN-γ concentrations above 100 mIU/ml including the two false positive samples (see above). We therefore aimed to include the BLANK values into the result interpretation and to circumvent the need of a grey-zone. Subsequently, we used a cut-off of equal or above 135 mIU/ml in individuals with

---

Table 1

Baseline characteristics of the 162 health care workers.

| Group                        | No. of HCW | COVID-19 ≥ 6 months ago | COVID-19 < 6 months ago | No COVID-19 group |
|------------------------------|------------|-------------------------|------------------------|-------------------|
| Age in years, mean (range)   |            | 43.9 (22 - 64)          | 38.1 (22 - 60)         | 42.58 (20 – 70)   |
| Sex                          |            |                         |                        |                   |
| Female%                      |            | 64.8                    | 50                     | 67.3              |
| Male%                        |            | 35.2                    | 50                     | 32.7              |
background stimulation below 100 mIU/ml and a cut-off of 200 mIU/ml in individuals with higher background stimulation. Using our BLANK-value adapted approach specificity reached 100% and overall sensitivity to detect past infection was 86.8% (100% for infection less than six months and 83.5% for infection more than 5 months ago). Predictive values and accuracy of the different cut-off strategies are shown in Table 2.

Of note, 20 out of 106 (18.9%) HCW several months after SARS-CoV-2 infection were negative for SARS-CoV-2 S1-IgG but tested positive for IFN-γ after vaccination. These patients (n = 149) had various underlying diseases and received immunosuppressive or immune modulating therapies or had variable immunodeficiency (Table 5). The majority of immunocompromised patients showed an inadequate immune response to vaccination.

5.4. Real life performance: IFN-γ concentrations in HCW after COVID-19 vaccination

Next, we analysed IFN-γ concentrations in HCW after COVID-19 vaccination (Table 2). In the BNT162b2 group, 36/38 (95%) had IFN-γ concentrations above 200 mIU/ml after the first dose and 38/38 (100%) after the second dose, respectively. In individuals receiving AZD1222, 28/30 (93%) had IFN-γ concentrations above 200 after the first dose, one had 190 mIU/ml (positive using the adapted cut-off definition) and one did not show specific IFN-γ release (64 mIU/ml). All 27 HCW who were vaccinated with the Moderna vaccine mRNA1273 after receiving a first dose with AZD1222 had IFN-γ concentrations clearly above 200 mIU/ml.

5.5. Comparison of IFN-γ concentrations in COVID-19 patients versus vaccinated HCW

Median IFN-γ concentration was significantly different between individuals with past infection more than 6 months vs. less than 6 months ago (Mann-Whitney U test, p = 0.001) (Fig. 1). IFN-γ concentrations after first dose of BNT162b2 vaccine were similar as those after recent infection and significantly lower than concentrations after the second dose (Wilcoxon test, p = 0.001). Median IFN-γ concentration after the first dose of AZD1222 was higher than after the first dose of BNT162b2 with a large range (Mann-Whitney U test, p = 0.04). Median concentration after heterologous booster immunization with mRNA1273 was similar to median concentration after second dose with BNT162b2. Mean, median, standard deviation and range are shown in Table 4.

5.6. Real life performance: IFN-γ response in immunocompromised patients

After validation and implementation of the assay in our routine diagnostic lab, we received test orders for analysing antibody and T-cell immune response of several immunocompromised patients after complete COVID-19 vaccination. These patients (n = 149) had various underlying diseases and received immunosuppressive or immune modulating therapies or had variable immunodeficiency (Table 5). The majority of immunocompromised patients showed an inadequate immune response to vaccination.
therefore of importance to measure T cell mediated reactivity to evaluate the described assay. Using the adapted cut-off strategy the IGRA was showed best accuracy with similar negative predictive values using our results and can also be used to confirm past infection in long-covid patients without PCR results.

For the use in vaccination studies quantification of the T cell response must be reliable and reproducible. We were able to show significant differences between the first and the second vaccination amongst groups as well as individuals and between different vaccines. Similar results have been reported recently in a study using the same IGRA [35]. Reproducibility was good and the assay showed high tolerance against variations of standard procedures (supplemental material). These properties make the assay highly suitable for routine diagnostic laboratories and opens up possibilities to analyse the immune response beyond using antibody detection assays in vaccinated patients especially under immunosuppressing or immune modulating medications. Importantly, we were able to show that B-cell depleted patients in most cases mount a strong T cell response to COVID-19 vaccination and are thus probably protected against severe disease [23]. However, this was not seen in most patients after organ transplantation confirming previous studies [36]. These patients can only be protected by vaccination of their close contacts and non-pharmaceutical intervention strategies.

Limitation for the use of such a biological assay is the functionality of the T cell response. Even low-dose steroid treatment may interfere with IGRA testing, as has been shown for Quantiferon TB in immunocompetent children [34]. Further, the interpretation of grey zone results is always difficult, so we developed a double cut-off strategy including BLANK results in the cut-off definition. Diagnostic test accuracy analysis showed best accuracy with similar negative predictive values using our adapted cut-off strategy. However, the adapted cut-off should be validated independently in other cohorts.

In conclusion, the EUROMMUN IGRA is an easy to perform assay for the detection of SARS-CoV-2 specific T cell response with high sensitivity and specificity. During a performance evaluation of the assay we were able to show that patients on immunosuppressive regimens may have isolated T cell or antibody response or in several cases do not respond at all. We propose that measurement of immune response to vaccination in immunocompromised patients should always include an analysis of T cell response and the EUROMMUN IGRA proved to be highly suitable for this purpose.

### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.
Statement of meetings where the information has been previously presented

This work has not been presented at any meetings.

CREdIT author statement

Daniela Huzly, Marcus Panning: Conceptualization, Methodology, Software, Validation, Writing- Original draft preparation. Franziska Smely: Investigation, Data curation, Visualization. Martin Enders: Writing- Reviewing and Editing, Investigation. Johanna Komp: Data curation, Investigation. Valeria Falcone: Data curation, Software, Formal analysis. Daniel Steinmann: Resources, Writing- Reviewing and Editing, Project Administration.

Declarations of Competing Interest

All authors have nothing to declare.

Acknowledgement

We are grateful to Ingeborg Hanselmann for expert technical assistance. We would like to thank all health care workers and patients for their participation and support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jcv.2022.105098.

References

[1] K. McMahan, J. Yu, N.B. Mercado, et al., Correlates of protection against SARS-CoV-2 in rhesus macaques, Nature 590 (2021) 630-634.
[2] A. Bertolotti, A.T. Tan, N Le Bert, The T cell response to SARS-CoV-2: kinetic and quantitative aspects and the case for their protective role, Oxford Open Immunol. (2021) igb0086.
[3] M. Hellerstein, What are the roles of antibodies versus a durable, high quality T-cell response in protective immunity against SARS-CoV-2? Vaccine 6 (2020), 100076.
[4] M. Sester, G. Soitguy, C. Lange, et al., Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis, Eur. Respir. J. 37 (2011) 100-111.
[5] B. Paouri, A. Soldatou, E. Petrakou, et al., Quantiferon-Cytomegalovirus assay: a potentially useful tool in the evaluation of CMV-specific CD8+ T-cell reconstitution in pediatric hematopoietic stem cell transplant patients, Pediatr. Transplant. 22 (2018) e13202.
[6] K. Terada, Y. Itoh, A. Fujii, S. Kitagawa, S. Ogita, K. Ouchi, Varicella-zoster virus-specific, cell-mediated immunity with interferon-gamma release assay after vaccination of college students with no or intermediate IgG antibody response, J. Med. Virol. 87 (2015) 350-356.
[7] K. Murugesan, P. Jagannathan, T.D. Pham, et al., Interferon-gamma release assay for accurate detection of SARS-CoV-2 T cell response, Clin. Infect. Dis. (2020).
[8] P.M. Bossuyt, J.B. Reitsma, D.E. Bruns, et al., STARD 2015: an updated list of essential items for reporting diagnostic accuracy studies, BMJ 351 (2015) h5527 (Clinical research ed).
[9] E.J. Haas, F.J. Angulo, J.M. McLaughlin, et al., Impact and effectiveness of mRNA SARS-CoV-2 infections among skilled nursing facility residents and staff members - Chicago, Illinois, December 2020-March 2021, Am. J. Transplant 21 (2021) 2290-2297.
[10] E. Hacisuleyman, C. Hale, Y. Saito, et al., Vaccine breakthrough infections with SARS-CoV-2 variants, N. Engl. J. Med. 384 (2021) 2212-2218.
[11] C.C. Song, J. Christensen, D. Kumar, N. Vinchelob, M. Morales, G. Gupta, Early experience with SARS-CoV-2 mRNA vaccine breakthrough among kidney transplant recipients, Transpl. Infect. Dis. (2021) e13654.
[12] S. Schwarzkopf, A. Kowczuk, D. Knop, et al., Cellular immunity in COVID-19 convalescents with PCR-confirmed infection but with undetectable SARS-CoV-2-specific IgG, Emerg Infect Dis (2021) 27.
[13] C.J. Reynolds, L. Swadling, J.M. Gibbons, et al., Discordant neutralizing antibody and T cell responses in asymptomatic and mild SARS-CoV-2 infection, Sci. Immunol. 5 (2020).
[14] B.A. Woldegonenkl, C.C. Garli, J.N. Blankson, SARS-CoV-2 mRNA vaccines induce broad CD4+ T cell responses that recognize SARS-CoV-2 variants and HCV-NL63, J. Clin. Invest. (2021) 131.
[15] D. Geers, M.C. Shamiyer, S. Borges, et al., SARS-CoV-2 variants of concern partially escape humoral but not T-cell responses in COVID-19 convalesce donors and vaccinees, Sci. Immunol. 6 (2021).
[16] Z. Wang, F. Schmidt, Y. Weihslim, et al., mRNA vaccine-elicited antibodies to SARS-CoV-2 and circulating variants, Open Forum Infect. Dis. (2021) ofab143.
[17] A. Tarke, J. Sidney, N. Methot, et al., Negligible impact of SARS-CoV-2 variants on CD4 (+) and CD8 (–) T cell reactivity in COVID-19 exposed donors and vaccinees, bioRxiv (2021).
[18] U. Sahin, A. Murtk, E. Dohvanessianen, et al., COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses, Nature 586 (2020) 594-599.
[19] A.W. Rutjes, J.B. Reitsma, A. Coomarasamy, K.S. Khan, P.M. Bossuyt, Evaluation of diagnostic tests when there is no gold standard. A review of methods, Health Technol. Assess. 11 (2007) iii-ix.
[20] A. Grifoni, D. Weihslim, S.I. Ramirez, J.M. Dan, et al., Antigen-specific adaptive immunity to SARS-CoV-2 and COVID-19, Cell 184 (2021) 861-880.
[21] K. Tyagi, A. Ghosh, D. Nair, et al., Breakthrough COVID19 infections after vaccinations in healthcare and other workers in a chronic care medical facility in New Delhi, India, Diabetes Metab. Syndr. 15 (2021) 1007-1008.
[22] R.A. Teran, K.A. Walblay, E.L. Shane, et al., Postvaccination SARS-CoV-2 infections among skilled nursing facility residents and staff members - Chicago, Illinois, December 2020-March 2021, Am. J. Transplant 21 (2021) 2290-2297.
[23] A. Sette, S. Crotty, Adaptive immunity to SARS-CoV-2 and COVID-19, Cell 184 (2021) 861-880.
[24] C. Pyrdymski, M. Moroder, S.I. Ramirez, et al., Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity, Cell 183 (2020) 996-1012, e19.
[25] A. Soresina, D. Moratto, M. Chiarchi, et al., Two X-linked agammaglobulinemia patients develop pneumonia as COVID-19 manifestation but recover, Pediatr. Allergy Immunol. 31 (2020) 565-569.
[26] K. Tyagi, A. Ghosh, D. Nair, et al., Breakthrough COVID19 infections after vaccinations in healthcare and other workers in a chronic care medical facility in New Delhi, India, Diabetes Metab. Syndr. 15 (2021) 1007-1008.
[27] K. Tyagi, A. Ghosh, D. Nair, et al., Breakthrough COVID19 infections after vaccinations in healthcare and other workers in a chronic care medical facility in New Delhi, India, Diabetes Metab. Syndr. 15 (2021) 1007-1008.
[28] K. Tyagi, A. Ghosh, D. Nair, et al., Breakthrough COVID19 infections after vaccinations in healthcare and other workers in a chronic care medical facility in New Delhi, India, Diabetes Metab. Syndr. 15 (2021) 1007-1008.
[29] K. Tyagi, A. Ghosh, D. Nair, et al., Breakthrough COVID19 infections after vaccinations in healthcare and other workers in a chronic care medical facility in New Delhi, India, Diabetes Metab. Syndr. 15 (2021) 1007-1008.
[30] K. Tyagi, A. Ghosh, D. Nair, et al., Breakthrough COVID19 infections after vaccinations in healthcare and other workers in a chronic care medical facility in New Delhi, India, Diabetes Metab. Syndr. 15 (2021) 1007-1008.
[31] K. Tyagi, A. Ghosh, D. Nair, et al., Breakthrough COVID19 infections after vaccinations in healthcare and other workers in a chronic care medical facility in New Delhi, India, Diabetes Metab. Syndr. 15 (2021) 1007-1008.