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Changes in interferon- γ release assay readout after COVID-19 vaccination: A prospective cohort study



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ABSTRACT

Objectives: Interferon- γ release assays (IGRAs) are widely used in public health practice to diagnose latent tuberculosis. During the COVID-19 pandemic and rollout of COVID-19 vaccination, it has remained unclear whether COVID-19 vaccines interfere with IGRA readouts.

Methods: We prospectively recruited healthcare workers during their annual occupational health examinations in 2021. Baseline IGRA readouts were compared with follow-up data after the participants had received two doses of COVID-19 vaccination.

Results: A total of 134 baseline IGRA-negative cases (92 with ChAdOx1 vaccine, 27 with mRNA-1273 vaccine, and 15 with heterologous vaccination) and seven baseline IGRA-positive cases were analyzed. Among the baseline IGRA-negative cases, there were decreased interferon- γ concentrations over the Nil (P = 0.005) and increased Mitogen-Nil (P < 0.001) values after vaccination. For TB2-Nil value, a similar trend (P = 0.057) of increase was observed. Compared with the 0.35 IU/ml threshold, the baseline and follow-up readout differences were less than $|\pm 0.10|$ IU/ml over the TB1-Nil and TB2-Nil values in >90% baseline IGRA-negative cases. No significant readout difference was observed among baseline IGRA-negative cases.

Conclusion: COVID-19 vaccination did not change IGRA interpretation in most cases. Cases showing conversion/borderline IGRA readouts should be given special consideration.

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Introduction

According to the World Health Organization (WHO), tuberculosis (TB) caused by *Mycobacterium tuberculosis* led to 1.5 million deaths worldwide in 2020 (2021). As TB is curable and preventable, many public health interventions have tried eliminating this infectious disease. To achieve the WHO's goal of ending the TB epidemic by 2030, public health approaches are underway to find and treat latent TB infections and active TB disease (World Health Organization World Health Organization WHO, 2018). The tuberculin skin test (TST) has conventionally been used to screen for TB infection. However, it is being increasingly replaced by interferon- γ release assays (IGRAs). IGRAs, which detect cell-mediated responses to *M. tuberculosis* antigens, are now widely used for TB contacts, healthcare workers, and immigrants because of their simpler procedure and interpretation compared with TSTs. However, like with TSTs, several vaccines, especially live virus vaccines (e.g., measles, mumps, rubella, varicella, yellow fever, and oral polio) may interfere with IGRA results. Therefore, testing should be performed either on the same day as vaccination or postponed until at least

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4 weeks after vaccination (Centers for Disease Control and Prevention CDC US, 2021). Currently, the COVID-19 pandemic is still underway, and COVID-19 vaccination programs have been rolled out worldwide; however, it is unknown whether COVID-19 vaccines interfere with IGRA results. Although none of the currently authorized SARS-CoV-2 vaccines are live virus vaccines (Creech et al., 2021) and IGRAs are designed to measure TB-specific cell responses, the effects of the COVID-19 vaccine on IGRA results cannot be excluded. Therefore, it was suggested that IGRAs should be performed 4 weeks after completing COVID-19 vaccination. However, this suggestion was quickly abandoned because issues in hospital logistics (e.g., new staff recruitment) have made it difficult to follow during the COVID-19 outbreak (Centers for Disease Control and Prevention [CDC] US, 2021; Chorba, 2021). Because of the lack of related evidence and the employment of new action mechanisms of currently used COVID-19 vaccines (e.g., chimpanzee adenovirus vaccine vector and mRNA vaccine), we examined serial IGRA readout changes in a cohort of healthcare workers before and after COVID-19 vaccination to clarify the feasibility of performing IGRA during COVID-19 vaccination rollout.

Methods

This study was performed from January 2021 to November 2021 at four study sites in Taiwan (Keelung, Taoyuan, Chiayi, and Kaohsiung Chang Gung Memorial Hospital). Healthcare workers at the study hospitals who consented to participate in the study during their annual occupational health examination underwent a baseline IGRA from January to April 2021. None of the study participants had been administered COVID-19 vaccines before sample collection because of a nationwide shortage of COVID-19 vaccines. The COVID-19 mass vaccination program was rolled out in the study hospitals in late March 2021. The AstraZeneca-Oxford ChAdOx1 vaccine was the first available COVID-19 vaccine in the study hospitals, followed by the Moderna mRNA-1273 vaccine, according to the vaccine supply. A heterologous ChAdOx1/mRNA-1273 prime-boost vaccination was approved by the Taiwan Centers for Disease Control on July 25, 2021; therefore, a few participants in the study received heterologous vaccination, mostly because of the intolerable side effects experienced by the participants after the first ChAdOx1 vaccination. A follow-up IGRA was performed from July to October 2021, 3-6 months after the baseline IGRA. By then, the participants had completed their COVID-19 vaccination; that is, two doses of the ChAdOx1 vaccine separated by at least an 8-week interval, two doses of the mRNA-1273 vaccine, separated by at least a 4-week interval, or heterologous vaccination with the two vaccines separated by at least an 8-week interval. Participants who received vaccines other than those for COVID-19 during the study period were excluded from analysis. IGRA-negative/positive cases were separately recruited according to their baseline IGRA results. QuantiFERON-TB Gold Plus (QIAGEN, Hilden, Germany) and QFT-Plus Analysis software were used according to the manufacturer's instructions. In short, fresh whole blood samples that were collected from participants in lithium-heparin tubes were transferred to QuantiFERON-TB Gold Plus tubes labeled Nil, TB1, TB2, or mitogen within 16 hours after blood collection. The tubes were then incubated at 37°C for 16-24 hours. After incubation, the tubes were centrifuged and the plasma fraction was taken out and stored at -70°C until use. Enzyme-linked immunosorbent assays were run in batches to determine the level of interferon- γ (IFN- γ) concentration in the plasma samples. The IGRA readouts included the IFN- γ concentrations in the Nil, TB1, TB2, and mitogen tubes and the IFN- γ concentration differences (TB1-Nil, TB2-Nil, Mitogen-Nil) before and after COVID-19 vaccination were then compared. For statistical analysis, an upper limit of 10 IU/ml was used for IGRA readouts >10 IU/ml. The t-test or Mann Whitney test or Wilcoxon signedrank test was used for numerical variables, and the chi-square test or Fisher's exact test was used to compare categorical variables. Statistical significance was set at P < 0.05 for all statistical tests.

Results

We recruited 144 baseline IGRA-negative cases and 32 baseline IGRA-positive cases during the study period. Figure 1 shows a flowchart of participant recruitment. Among the participants who were IGRA-negative at baseline, 134 cases-including 92 cases with the ChAdOx1 vaccine, 27 with the mRNA-1273 vaccine, and 15 with heterologous vaccination-were included in the final analysis. Only seven cases were included in the final analysis among the participants who were IGRA-positive at baseline. Table 1 shows the demographic characteristics of the participants. Compared with the IGRA-negative cases, the IGRA-positive cases were more likely to be male (P = 0.016), be older (median 51 vs 38 years old, P = 0.003), have diabetes (29% vs 2%, P = 0.02), and have renal disease (P = 0.05) or heart disease (P = 0.05). The follow-up period was shorter for IGRA-positive cases than for the IGRA-negative cases (median 43 vs. 62 days after the second dose of COVID-19 vaccine, P = 0.023).

We then compared the differences in the baseline and followup IGRA readouts among baseline IGRA-negative cases (Figure 2). These cases (n = 134) showed a significant increase in their IFN- γ concentrations after COVID-19 vaccination compared with changes in their Mitogen-Nil values; however, there was a subtle decrease of IFN- γ concentrations in the postvaccination Nil values. The TB2-Nil value revealed a probable trend (P = 0.057) of an elevated IFN- γ concentration in the follow-up test (Figure 2).

For the IGRA used in the study, the Nil tube served as the background control, and the mitogen tube served as the positive control; the values of TB1-Nil and TB2-Nil are directly related to the positive/negative interpretation of the IGRA (each with a cutoff value of 0.35 IU/ml), because the IFN- γ concentration had a trend of elevation over the TB2-Nil values among these COVID-19 vaccines; therefore, we compared the same-person serial IGRA readout differences for these participants (Figure 3). Among baseline IGRA-negative participants (n = 134), 83% and 80% of cases had a difference between the two IGRA readouts of less than $|\pm$ 0.05| IU/ml in TB1-Nil and TB2-Nil values, respectively. Moreover, in 92% and 93% of the participants who were IGRA-negative at baseline, the two IGRA readouts showed differences less than $|\pm$ 0.10 IU/ml in TB1-Nil and TB2-Nil values, respectively (Figure 3). Only 4.5% (6/134) of baseline IGRA-negative cases showed differences in either their TB1-Nil or TB2-Nil value of more than 0.20 IU/ml between the two IGRAs, and only 1.5% (2/134) of these cases showed differences of more than 0.20 IU/ml for both their TB1-Nil and TB2-Nil values between the two IGRAs. No significant IFN- γ concentration differences were observed between participants who underwent a follow-up IGRA test <7 weeks after completion of COVID-19 vaccination compared with those at 7-11 weeks or >11 weeks in baseline IGRA-negative cases (Figure 4). In baseline IGRApositive cases, there were no significant differences between the baseline and follow-up IGRA readouts after COVID-19 vaccination (Supplementary Figure 1).

There were four IGRA conversion cases: two baseline IGRAnegative cases that converted to a positive result at follow-up and two baseline IGRA-positive cases that converted to a negative result at follow-up (Supplementary Table 1). Because the range of IGRA readout differences in these cases was much greater than that in the above analysis, the conversion in these four cases was thought to be caused by factors other than the COVID-19 vaccines. The four cases were thus excluded from the final group analysis, as shown in the initial study flowchart (Figure 1). During the study period, COVID-19 was a notifiable infectious disease in Tai-



Figure 1. Flowchart of study participant recruitment from healthcare workers at the study sites with (A) baseline-negative or (B) baseline-positive IGRA results in their annual health exam. IGRA: interferon- γ release assay.

wan. None of the participants reported having a positive SARS-CoV-2 polymerase chain reaction in the study.

Discussion

We found that the currently employed major COVID-19 vaccines (ChAdOx1 vaccine and mRNA-1273 vaccine) may impact IGRA readouts but mostly on Nil and Mitogen-Nil values. The differences in TB1-Nil or TB2-Nil values were insignificant (less than \pm 0.10 IU/ml in more than 90% IGRA-negative cases than the 0.35 IU/ml cutoff). Two baseline IGRA-negative cases became positive at follow-up. However, it remains unclear whether this change was

caused by factors other than COVID-19 vaccines (e.g., recent infection with *M. tuberculosis* or assay variability). Even considering these two cases, the overall IGRA conversion rate after COVID-19 vaccination was as low as 1.47% (2/136) among baseline IGRA-negative cases. This value was even lower than the potential falsepositive rate of IGRA (Moses *et al.*, 2016; Slater *et al.*, 2013; Slater *et al.*, 2014). We do not know how COVID-19 vaccination impacted the Nil and Mitogen-Nil values in IGRA. However, it is known that IFN- γ as well as other cytokines were released by immune effector cells in natural SARS-CoV-2 infection (Tan and Tang, 2021). IFN- γ is part of human innate immunity against virus infection and has an immunoregulatory role in linking the innate immune response

Table 1

Demographic characteristics of the study participants.

	IGRA-negative	IGRA-positive	Dualua
	(II = 154)	$(\Pi = 7)$	P-Value
Sex (%, n)			0.016
Male	15% (20)	57% (4)	
Female	85% (114)	43% (3)	
Age			0.003
years, median (IQR)	38 (32-44)	51 (45-56)	
Underlying diseases (%, n)			
Diabetes mellitus	2% (3)	29% (2)	0.020
Renal disease	0% (0)	14% (1)	0.050
Liver disease	7% (9)	14% (1)	0.409
Malignancy	3% (4)	0% (0)	1.000
Autoimmune disease	0% (0)	0% (0)	
Heart disease	0% (0)	14% (1)	0.050
Smoking	0% (0)	0% (0)	
Alcoholism	0% (0)	0% (0)	
Others	7% (9)	0% (0)	1.000
Vaccine type (%, n)			
ChAdOx1	69% (92)	43% (3)	
mRNA-1273	20% (27)	57% (4)	
Heterologous	11% (15)	0% (0)	
Days after administration of second dose of COVID-19 vaccine			0.023
days, median (IQR)	62 (50-74)	43 (25-53)	

IGRA, interferon- γ release assay; IQR, interquartile range. Note: *P*-values in bold represent statistical significance.



Figure 2. Comparison of baseline and follow-up interferon- γ release assay (IGRA) readouts among baseline IGRA-negative participants. * or *P*-values in bold represent significance. *: P < 0.05; ***: P < 0.001. IFN- γ : interferon gamma.



Figure 3. Differences in (A) TB1-Nil and (B) TB2-Nil values between baseline and follow-up interferon- γ release assay (IGRA) in baseline IGRA-negative participants (n = 134) administered COVID-19 vaccines. IFN- γ : interferon gamma.



Figure 4. Distribution of interferon- γ release assay readout differences with time after administering the second dose of COVID-19 vaccine. Note: <7 weeks (n = 30); 7-11 weeks (n = 77); >11 weeks (n = 27). IQR: interquartile range.

and activation of adaptive immunity (Lee and Ashkar, 2018). Therefore, we reasoned that the COVID-19 vaccines, with the SARS-COV-2 antigen produced or the vector contained, may have activated or perturbed the immune cells (causing an IFN- γ change in the Nil tube). The activated immune cells may produce a stronger nonspecific immune response upon further stimulation by the mitogen in IGRA. There was also an intriguing finding that the impact of COVID-19 vaccines on IGRA lasted longer (>11 weeks) than previously expected (<4–6 weeks) (Centers for Disease Control and Prevention [CDC] US, 2021). Overall, the COVID-19 vaccine impacted IGRA readouts but did not change the interpretation of the results in most cases. However, special consideration (e.g., further testing) may be given in cases showing conversion or borderline IGRA readouts because changes caused by COVID-19 vaccines may influence the IGRA interpretation.

The demographic characteristics of the study participants were similar to those in previous reports; for example, IGRA-positive cases were older (Katsenos *et al.*, 2011) and more likely to have chronic conditions, such as diabetes (Lee *et al.*, 2017) or renal diseases (Yuan *et al.*, 2005). There were also more male participants in the IGRA-positive group in this study; male sex is associated with an increased risk of TB incidence (Gao *et al.*, 2017). The type of

job and longer service years of the male healthcare workers in the study hospitals should also be considered. For example, more nursing staff in the study hospitals are female, and nursing staff generally had higher turnover rates and shorter service years in the study hospitals. The follow-up time of the baseline IGRA-positive cases after the second dose of COVID-19 vaccination (median 43 days) was shorter than that of the baseline IGRA-negative cases (median 62 days). This was mostly because of the clinical consideration of starting latent TB infection treatment in IGRA-positive cases, leading to a shorter waiting time before the second IGRA.

This study had several limitations. First, the sample size was small, particularly in the mRNA-1273 and heterologous vaccine groups; therefore, we could not delineate the individual effects of different COVID-19 vaccines on IGRA. In baseline IGRA-positive cases, we did not observe significant IGRA differences after COVID-19 vaccination; however, this may also be because of the small sample size. Second, all study participants underwent a follow-up IGRA after completing the second dose of COVID-19 vaccination. There was no IGRA readout comparison for samples collected immediately after the participants were administered the first dose of the COVID-19 vaccine. The short-term effect on IGRA immediately after COVID-19 vaccination was not evaluated because im-

mune reactions induced by vaccines are generally thought to be stronger after the booster shoot (Barrett et al., 2021; Chu et al., 2021; Ramasamy et al., 2021). Therefore, the IGRA readout differences immediately after COVID-19 vaccination were expected to be less pronounced than those observed in our study. Third, because of the shortage of the COVID-19 vaccine during the study period, more participants received the ChAdOx1 vaccine than the mRNA-1273 vaccine or heterologous vaccination. In addition, the follow-up time of the ChAdOx1 group was longer than those of the other groups. Fourth, we could not include a nonvaccinated control group in the study. Because the study participants were all first-line healthcare workers, it was difficult and potentially unethical to recruit nonvaccinated participants during the COVID-19 epidemic. However, to the best of our knowledge, we did not find literature that had reported a trend of IGRA readout elevation over time among the healthcare worker population. Finally, only the ChAdOx1, mRNA-1273, and ChAdOx1/mRNA-1273 heterologous schemes were compared; therefore, the results may not apply to other COVID-19 vaccines.

In summary, we observed serial IGRA readout differences among COVID-19 vaccinated healthcare workers. However, the TB1-Nil and TB2-Nil differences were considered minimal because the cutoff value (0.35 IU/ml) was much greater than the IGRA differences in most cases; TB antigen-specific reaction may outweigh the COVID-19 vaccine effect on IGRA. However, in cases with conversion or a borderline TB1-Nil, TB2-Nil readout that is close to the IGRA cutoff, the potential impact of COVID-19 vaccination may not be neglectable, and further testing may help distinguish the results. Because the COVID-19 vaccine rollout varies worldwide, concerns have been raised regarding postvaccine impacts on IGRAs. We believe this study addresses an important gap in patient management knowledge that can aid in implementing public health initiatives related to latent TB infection worldwide during the COVID-19 pandemic.

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Ethical approval

The study was approved by the institutional review board of the Chang Gung Medical Foundation in Taiwan (Approval number: 202002131B0C604).

Conflict of interest

The authors have no competing interests to declare.

CRediT authorship contribution statement

Nan-Yu Chen: Data curation, Investigation, Writing – original draft. **Zhuo-Hao Liu:** Data curation, Investigation, Writing – original draft. **Shu-Wei Kao:** Investigation, Formal analysis. **Huang-Shen Lin:** Supervision, Writing – review & editing. **Ing-Kit Lee:** Supervision, Writing – review & editing. **Jun-Yuan Zheng:** Supervision, Writing – review & editing. **Ssu-Wei Wang:** Formal analysis. **Yu-Hsiang Hsiao:** Investigation, Formal analysis. **Hui-Chin Lin:** Investigation. **Ting-Shu Wu:** Data curation, Supervision, Writing – review & editing.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2022.06.044.

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