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.

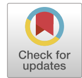
Diagnostic Microbiology and Infectious Disease 106 (2023) 115948

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Evaluation of T cell responses with the QuantiFERON SARS-CoV-2 assay in individuals with 3 doses of BNT162b2 vaccine, SARS-CoV-2 infection, or hybrid immunity



Maria-Myrto Dourdouna[a], Elizabeth-Barbara Tatsi[a,b], Vasiliki Syriopoulou[a], Athanasios Michos[a,*]

[a] *First Department of Pediatrics, Infectious Diseases and Chemotherapy Research Laboratory, Medical School, National and Kapodistrian University of Athens, "Aghia Sophia" Children's Hospital, Athens, Greece*
[b] *University Research Institute of Maternal and Child Health and Precision Medicine, Athens, Greece*

ARTICLE INFO

Article history:
Received 23 January 2023
Revised in revised form 14 March 2023
Accepted 26 March 2023
Available online 30 March 2023

Keywords:
SARS-CoV-2
mRNA vaccine
cellular immunity
QuantiFERON
interferon gamma release assay

ABSTRACT

Cellular immunity after SARS-CoV-2 infection or immunization may be important for long-lasting protection against severe COVID-19 disease. We investigated cellular immune responses after SARS-CoV-2 infection and/or vaccination with an interferon-γ release assay (QuantiFERON, QFN), in parallel, with humoral immunity assessment. We recruited 41 participants: unvaccinated convalescent children and adults and vaccinated uninfected or vaccinated convalescent adults. All vaccinated adults had received three doses of the BNT162b2 COVID-19 vaccine at 6.2 to 10.9 months prior to their inclusion to the study. All the unvaccinated participants were tested negative with QFN. Regarding the vaccinated population, 50% (8/16) of the vaccinated uninfected adults and 57.1% (8/14) of the vaccinated convalescent adults were tested positive. QFN did not detect T cell responses in unvaccinated individuals and in a significant number of vaccinated individuals. Further comparative studies with different immunoassays are required to elucidate whether this is the result of waning immunity or low sensitivity of the assay.

© 2023 Elsevier Inc. All rights reserved.

1. Introduction

Immunity after severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection or immunization is still under investigation [1]. SARS-CoV-2 infection induces B cell mediated humoral response and a $CD4^+$ and $CD8^+$ T cell response [2–5]. In patients with coronavirus disease 2019 (COVID-19), early and functional SARS-CoV-2 specific T cell response is associated with mild disease and accelerated viral clearance, whereas T cell lymphocytopenia is associated with severe clinical outcome [5–8]. Virus-specific T cell-mediated immunity was detected in SARS-CoV-2 exposed individuals, in the absence of seroconversion [9]. In addition, regarding the older coronavirus, SARS-CoV, it has been reported that a memory T cell response was detected up to 11 years after infection [8,10].

In parallel, it has been shown that the efficacy of a vaccine against SARS-CoV-2, is not only based on antibody production but also on a robust B and T cell response [11,12]. Also, synergy between memory B cells and T cells possibly plays a crucial role in protection against reinfection [11].

Various methods can be used to assess T cell-mediated immunity against SARS-CoV-2 [4]. Flow cytometry assay for intracellular cytokine staining (FC-ICS) and interferon-γ (IFN-γ) release assays (IGRA) are the most widespread [13]. QuantiFERON (QFN) SARS-CoV-2 assay is a commercially available IGRA, approved for research use for the assessment of cellular immunity after SARS-CoV-2 immunization. The QFN assay is less complex and time-consuming than other T cell assesment techniques, such as flow cytometry [14]. This method has also been used for the assessment of cellular immunity of other infectious diseases, such as cytomegalovirus (CMV) infection in immunocompromised patients and tuberculosis [15–17].

Limited data on the long-term duration of cell-mediated immunity have been reported after the third dose of the BNT162b2 mRNA vaccine [18]. Furthermore, the performance of IGRA in unvaccinated children and adults with SARS-CoV-2 infection is still under investigation, especially with the advent of new SARS-CoV-2 variants. The aim of this study is to investigate cellular immune responses elicited by infection and/or vaccination in adults and children, using a commercially available IGRA.

2. Materials and methods

2.1. Study design and participants

This was a prospective cohort study that was conducted at "Aghia Sophia" Children's Hospital, Athens, Greece. Healthcare workers

* Corresponding author. Tel.: +3021-0746-7478.
E-mail address: amichos@med.uoa.gr (A. Michos).

https://doi.org/10.1016/j.diagmicrobio.2023.115948
0732-8893/© 2023 Elsevier Inc. All rights reserved.

(HCWs) and children aged 5 to 16 years, who were admitted to the hospital between July 2022 to September 2022 were recruited. The study included participants with history of SARS-CoV-2 infection and/or vaccination with three doses of the BNT162b2 mRNA COVID-19 vaccine.

Specifically, the study included the following categories of participants: SARS-CoV-2 convalescent unvaccinated children or adults, at least one month after acute infection, uninfected vaccinated adults, and adults with hybrid immunity (immunization and breakthrough infection). Participants were classified as uninfected or convalescent from past infection based on the detection of SARS-CoV-2 nucleocapsid antibodies, which are produced only after SARS-CoV-2 natural infection. All vaccinated participants had received three doses of the COVID-19 BNT162b2 mRNA vaccine at least 6 months prior to their inclusion to the study. Participants were excluded from the study if they had a history of inborn/acquired immunodeficiency or blood transfusion.

From each participant, two samples of peripheral blood were collected. Heparinized whole blood sample was obtained, and the assessment of cellular immunity was performed the same day of blood collection. Additionally, a blood sample with clotting activator was obtained and, after centrifugation at 2000 x g for 10 minutes, serum was stored in -80 °C until humoral immunity measurement. Demographic and medical data, SARS-CoV-2 infection history, and vaccination status information were collected using a questionnaire.

All the laboratory assays were performed in the Infectious Diseases Laboratory of the Choremeion Research facility, First Department of Pediatrics, Medical School, National and Kapodistrian University of Athens, "Aghia Sophia" Children's Hospital.

2.2. SARS-CoV-2 antibody detection assay

Antibodies against SARS-CoV-2 spike (Abs-S) and nucleocapsid antigens (Abs-N) were measured from serum samples using Elecsys® Abs-SARS-CoV-2 S and Elecsys® Abs-SARS-CoV-2 (Roche Diagnostics; Switzerland) reagents, respectively. The antibody detection was performed with an electrochemiluminescence immunoassay (ECLIA), which is based on sandwich enzyme-linked immunosorbent assay (ELISA). Values ≥ 0.8 IU/mL are positive for antibodies against spike protein and values ≥1 COI (cut-off index) are positive for antibodies against nucleocapsid protein. These assays detects total antibodies (IgG, IgM, IgA) against both SARS-CoV-2 antigens.

2.3. SARS-CoV-2 neutralizing antibodies

SARS-CoV-2 neutralizing antibodies inhibit the binding between angiotensin converting enzyme 2 host receptor (ACE2) and SARS-CoV-2 Spike receptor binding domain (RBD). The blocking ELISA immunoassay, cPass™ SARS-CoV-2 Neutralization Antibody Detection Kit (GenScrip Biotech Corporation, Piscataway, New Jersey, USA), was used to assess the neutralizing capacity of antibodies against wild type and B.1.1.529 (Omicron) SARS-CoV-2 variant, according to the manufacturer's instructions. In this method horseradish recombinant SARS-CoV-2 receptor binding domain (HRP-RBD) fragment specific for wild type virus and Omicron variant is used. In this assay, the wells of the ELISA plate are coated with ACE2 and a substrate of purified HRP-RBD from the viral Spike protein is added. The test mimics the virus-host interaction in a way that if the serum sample does not include neutralizing antibodies, HRP-RBD will bind to the ACE2 and the solution will acquire yellow color, otherwise if the sample contains neutralizing antibodies the HRP-RBD and ACE2 binding is inhibited and the solution will remain colorless. The Labtech LT 4500 microtiter plate reader was used to estimate the absorbance of the final solution at 450 nm. The percentage of inhibition was calculated with the type: (%) = (1- OD value of sample/OD value of negative control) *100. Inhibition ≥30% is considered as positive result and is indicative of the presence of SARS-CoV-2 neutralizing antibodies.

2.4. SARS-CoV-2 T cell response

QuantiFERON SARS-CoV-2 (Qiagen, Hilden, Germany) assay, which is an Interferon-γ release assay (IGRA), was used to detect IFN-γ production from T cells after stimulation with SARS-CoV-2 spike subunits antigens (Ag1 and Ag2), according to manufacturer's instructions.

Heparinized whole blood samples were transferred to QuantiFERON blood collection tubes, which include the Nil tube (negative control), Ag1 tube, the Ag2 tube, and Mitogen tube (positive control). In Ag1 tube, IFN-γ was produced from stimulated $CD4^+$ T cells, while in Ag2 tube IFN-γ produced was from stimulated $CD4^+$ and $CD8^+$ T cells. The blood collection tubes were then incubated for 18 hours at 37 °C and centrifuged at 2000 rpm for 15 minutes. Plasma was harvested from the centrifuged tubes. Upon QFN SARS-CoV-2 ELISA Immunoassay was performed, the optical density was estimated at 450 nm using the microtiter plate reader Labtech LT 4500. The Nil tube value, which adjusts for background, was subtracted from the Ag1 and Ag2 values to estimate the final IFN-γ result. According to the manufacturer, a value of IFN-γ (Ag1-Nil or Ag2-Nil) ≥ 0.15 IU/mL is considered a positive response to the antigens. For the interpretation of the IFN-γ results, Qiagen QFN SARS-CoV-2 analysis software v.1.1.0.0 was used.

2.5. Statistical analysis

Statistical analysis was performed using SPSS v. 28 software (IBM SPSS Statistics for Windows; Version 28.0. Armonk, NY: IBM Corp) and a P value below 0.05 was considered statistically significant. Categorical variables were expressed as relative frequencies and percentages. Categorical variables were analyzed with Fisher's exact test and Fisher-Freeman-Halton Exact Test. The normal distribution of continuous variables was evaluated with the Kolmogorov-Smirnov test, the Shapiro-Wilk test and kurtosis and skewness. Continuous variables were described as median value (Mdn) and interquartile range (IQR: 75th, 25th percentile). Continuous variables were analyzed with the t test or Analysis of Variance (ANOVA) with the Bonferroni post hoc test if they were normally distributed and with Mann-Whitney or Kruskal-Wallis if they were not. Correlations between continuous variables were performed with the Spearman correlation coefficient.

2.6. Ethical issues

The study was carried out according to the Declaration of Helsinki and the study protocol was approved by the scientific and bioethics committee of "Aghia Sophia" Children's Hospital" (No 19546/2022). Written informed consent was obtained from each participant or the guardians of the children.

3. Results

3.1. Study population

A total of 41 participants were included in this study. Participants were divided into 4 groups depending on their immune status (vaccination and disease): 14.63% (6/41) convalescent unvaccinated children, 12.20% (5/41) convalescent unvaccinated adults, 39.02% (16/41) vaccinated uninfected adults, 34.14% (14/41) vaccinated convalescent adults.

The median age of the study population was 41.00 (IQR = 28.00−53.00) years and most of the participants were female (70.73%, 29/41). In vaccinated uninfected adults, the median time

between vaccination and measurement was 8.08 months (IQR=6.97 −8.97), while in the hybrid immunity group the median time was 9.55 months (IQR=7.93−10.14) (Table 1).

3.2. *Humoral response to SARS-CoV-2 infection and/or vaccination*

The levels of Abs-N, Abs-S, NAbs against the wild type and Omicron variants and the *P*-values are presented in Table 1.

All vaccinated uninfected adults were negative for Abs-N (range: 0.04−0.09 IU/mL). All vaccinated adults had a detectable Abs-S humoral response. Vaccinated convalescent adults had higher levels of Abs-S [Mdn = 17,336.50, (IQR =13,042.25−25,000)] compared to vaccinated uninfected adults [Mdn = 3185, (IQR = 1521.75 −12,991.5)], but the difference was not statistically significant (Table 1). Vaccinated convalescent adults had higher levels of Abs-S compared to convalescent unvaccinated adults and children ($P < 0.001$) (Table 1).

Neutralizing antibodies (NAbs) levels against wild type were higher in vaccinated uninfected adults than in convalescent unvaccinated adults and children ($P = 0.031$). Adults with hybrid immunity also demonstrated higher levels of NAbs [Mdn = 97.16, (IQR = 96.13−97.47)] against wild type than convalescent unvaccinated adults [Mdn = 58.44, (IQR = 7.13−96.75)] ($P = 0.031$). Higher NAbs levels against Omicron variant were observed in the hybrid immunity group [Mdn = 92.11, (IQR = 67.32−94.75)] compared to vaccinated uninfected adults [Mdn = 25.60, (IQR = 0.00−67.08)], ($P = 0.008$).

Among the 41 participants, Abs-S levels correlated with neutralizing capacity against wild type and Omicron variant ($r_s = 0.579$, $P < 0.001$ and $r_s = 0.517$, $P < 0.001$, respectively). Similarly, a statistically significant correlation was observed between Abs-N and NAbs against Omicron variant ($r_s = 0.456$, $P = 0.003$), whereas Abs-N did not statistically significantly correlate with NAbs against wild type ($r_s = -0.224$, $P = 0.160$).

3.3. *Cellular SARS-CoV-2 immunity*

Cellular immune responses that were evaluated with the QFN assay after the measurement of IFN-γ production after stimulation with SARS-CoV-2 Ag1 and Ag2 spike protein peptide mixes are presented in each separate group in Table 1 and Fig. 1.

In the total study population, IFN-γ Ag1-Nil (Ag1) and IFN-γ Ag2-Nil (Ag2) values were positively correlated ($r_s = 0.84$, $P < 0.001$).

Vaccinated convalescent adults had higher Ag1 values compared to convalescent unvaccinated children and adults ($P = 0.001$). Although, participants with hybrid immunity had higher Ag1 and Ag2 values than the vaccinated ones, this difference was not statistically significant (Table 1).

In the total sample, Abs-S values were positively correlated with Ag1 ($r_s = 0.52$, $P < 0.001$) and Ag2 ($r_s = 0.40$, $P = 0.009$) IFN-γ values. Additionally, a statistically significant correlation was detected between Ag1 and NAbs against wild type ($r_s = 0.324$, $P = 0.039$), but not with NAbs against Omicron variant ($r_s = 0.269$, $P = 0.09$). These correlations were not statistically significant regarding to Ag2 and in the separate group analysis.

All the convalescent unvaccinated participants were tested negative with the QFN immunoassay (Table 2). Regarding the COVID-19 vaccinated population, 50% (8/16) of the vaccinated and 57.1% (8/14) of the hybrid immunity group were reactively tested (QFN (+)) ($P = 0.018$). The positivity of the QFN assay was statistically related to COVID-19 vaccination ($P = 0.003$), while SARS-CoV-2 infection (either in convalescent unvaccinated participants or in participants with hybrid immunity) was not statistically significant ($P = 0.33$).

Both in total sample population ($n = 41$) and in vaccinated adults (vaccinated uninfected and hybrid immunity), the age, time of vaccination, Abs-N, Abs-S, neutralizing capacity against wild type and

Table 1
Epidemiological characteristics and immunological responses in SARS-CoV-2 convalescent children and adults, mRNA BNT162b2 immunized and participants with hybrid immunity (immunization and breakthrough infection).

Status		Age Mdn (IQR)	Time from infection (months) Mdn (IQR)	Time from vaccination (months) Mdn (IQR)	Ab-N (COI) Mdn (IQR)	Ab-S (IU/mL) Mdn (IQR)	NAbs for wild type (%) Mdn (IQR)	NAbs for Omicron (%) Mdn (IQR)	IFN-γ Ag1 (IU / mL) Mdn (IQR)	IFN-γ Ag2 (IU/mL) Mdn (IQR)
SARS-CoV-2 Infection	Children ($n = 6$)	8.00 (6.50−13.50)	NA	-	43.26 (4.24−140.83)	39.93 (5.15−7,689)	19.37 (11.50−97.28)	52.73 (14.47−69.77)	0.00 (0.00−0.02)	0.02 (0.00−0.04)
	Adults ($n = 5$)	41.00 (31.00−51.50)	7.27 (1.43−7.88)	-	33.20 (14.41−98.17)	483.90 (2.64−3,103)	58.44 (7.13−96.75)	47.07 (25.47−90.76)	0.00 (0.00−0.05)	0.01 (0.00−0.02)
Vaccinated Uninfected Adults ($n = 16$)		42.00 (31.50−51.75)	-	8.08 (6.97−8.97)	0.07 (0.06−0.08)	3,185 (1,521.75−12,991.5)	97.06 (96.24− 98.03)	25.60 (0.00−67.08)	0.06 (0.02−0.17)	0.11 (0.00−0.28)
Hybrid Immunity Adults (n =14)		52.50 (40.25−56.00)	3.75 (1.41−6.86)	9.55 (7.93−10.14)	8.28 (4.86−31.40)	17,336.50 (13,042.25−25,000)	97.16 (96.13−97.47)	92.11 (67.32−94.75)	0.14 (0.04−0.56)	0.18 (0.09−0.67)
P-Value		<0.001[‡,a,b,c]	0.548[$]	0.047[$]	<0.001[‡,b,d,f]	<0.001[‡,c,e]	0.031[‡,b,d,e]	0.008[‡,f]	0.001[‡,c,e]	0.011[‡,e]

Notes: Ab-N = Antibodies against nucleocapsid protein; Ab-S = Antibodies against Spike protein; COI = Cutoff index; NAbs = neutralizing antibodies; INF-γ = Interferon-γ; Ag1 = Antigen 1; Ag2 = Antigen 2; Mdn = Median; IQR = interquartile range; NA = Not available.
P-value after conducting [†]Analysis of Variance (ANOVA).
[‡] Kruskal-Wallis H test.
[$] *t*-test.
Differences between
[a] SARS-CoV-2 Infection Children vs SARS-CoV-2 Infection Adults.
[b] SARS-CoV-2 Infection Children vs Vaccination Adults.
[c] SARS-CoV-2 Infection Children vs Hybrid Immunity Adults.
[d] SARS-CoV-2 Infection Adults vs Vaccination Adults.
[e] SARS-CoV-2 Infection Adults vs Hybrid Immunity Adults.
[f] Vaccination Adults vs Hybrid Immunity Adults

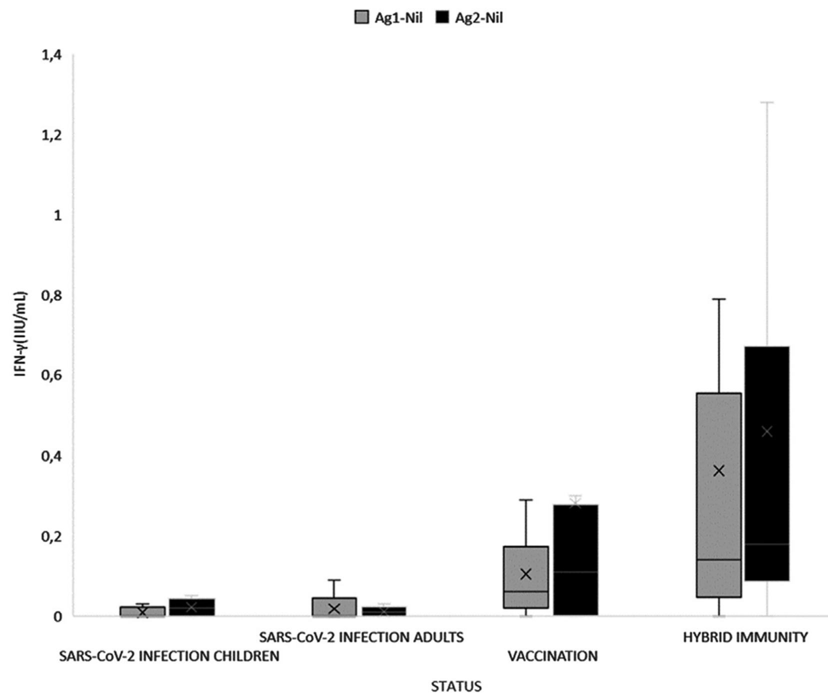


Fig. 1. Interferon-γ levels after stimulation of T cells with antigen 1 (Ag1) and antigen 2 (Ag2) of SARS-CoV-2 QuantiFERON immunoassay in SARS-CoV-2 convalescent children and adults, mRNA BNT162b2 immunized adults and adults with hybrid immunity (immunization and breakthrough infection).

Omicron did not correlate with the result of QFN. In vaccinated and convalescent unvaccinated participants, time from infection was not significantly associated with the result of the assay.

In QFN (+) individuals, the median value (IQR) for Ag1 was 0.25 IU/mL (IQR = 0.11–0.54) and for Ag2 was 0.36 IU/mL (IQR = 0.20–0.91), while in the QFN (-) group median value for Ag1 was 0.02 (IQR = 0.00–0.04) and for Ag2 was 0.01 (IQR = 0.00–0.07).

Among the QFN (+) individuals a reactive response to Ag1 ($CD4^+$ epitopes) and to Ag2 ($CD4^+$ and $CD8^+$ **e**pitopes), was detected in 68.8% (11/16) and 87.5% (14/16) respectively, while 56.3% (9/16) had a reactive response to both antigens (Ag1 and Ag2).

Among the COVID-19 vaccinated uninfected and hybrid immunity groups, SARS-CoV-2 infection status was not statistically important for the QFN result (P = 0.730).

4. Discussion

T cell immunity might play a crucial role in long-term protection from severe COVID-19, even in the context of emerging variants of convern (VOCs) [14]. Therefore, it is essential to access cellular immunity with reliable and scalable assays, so that we can monitor more accurately the duration of cell-mediated immune protection against SARS-CoV-2 [14]. In the present study, we evaluated T cell responses 8 to 9 months after the immunization with 3 doses of BN162b2 mRNA vaccine with or without SARS-CoV-2 infection.

In the study population, SARS-CoV-2 infection in convalescent unvaccinated children and adults did not stimulate IFN-γ production above the cut-off level of the assay. In accordance with these results, in a small pilot study, 4 out of 5 unvaccinated convalescent adults had negative QFN prior to vaccination [19]. In a small feasibility study, elevated IFN-γ responses in three out of four convalescent uninfected subjects were detected using QFN [8]. However, the above studies were conducted before the emergence of the Omicron variant.

Omicron variant has a high number of mutations in the Spike gene [20]. T cell epitopes are minimally affected, and the cell-mediated response is more resistant to VOCs than antibody response [20–22]. However, QFN may need validation in patients infected with other SARS-CoV-2 variants, such as Omicron, as it has been only validated against variant B.1.1.7 (Alpha) variant [22]. A test with a different group of peptides would probably be more appropriate for the detection of cellular immunity induced by the Omicron variant. A key question is if the cut-off of QFN that is set at 0.15 IU/mL can detect the cellular response after SARS-CoV-2 infection or if a lower cut-off could be proposed.

Using QFN, only 50% of COVID-19 naive vaccinated participants, were detected positive for IFN-γ 6-10.5 months after immunization

Table 2
SARS-CoV-2 QuantiFERON (QFN) immunoassay results in SARS-CoV-2 convalescent children and adults, mRNA BNT162b2 immunized and participants with hybrid immunity (immunization and breakthrough infection).

		QFN	Frequency n (%)
SARS-CoV-2 infection	Children (n = 6)	POSITIVE	0 (0)
		NEGATIVE	6 (100)
	Adults (n = 5)	POSITIVE	0 (0)
		NEGATIVE	5 (100)
Vaccinated uninfected adults (n = 16)		POSITIVE	8 (50)
		NEGATIVE	8 (50)
Hybrid immunity adults (n = 14)		POSITIVE	8 (57.1)
		NEGATIVE	6 (42.9)

with three doses of BN162b2 vaccine. In contrast, previous studies have shown a higher positivity of the method in vaccinated (with two doses of mRNA vaccines) uninfected individuals than in our study [8,19,23]. However, it is worth noting that these studies were conducted in a shorter time interval (1−2 months) after vaccination with two doses of mRNA vaccines than our study. In a recent study, 72.7% (8/11) participants were tested positive 6.6 to 9.4 months after immunization with two doses of BN162b2 vaccine [24]. This study used in addition to Ag1 and Ag2 QFN tubes, Ag3 QFN tube that contains $CD4^+$ and $CD8^+$ epitopes derived from S1 and S2 Spike subunits and immunodominant $CD8^+$ epitopes from the whole genome [24].

QFN results were positive in 56% of individuals with hybrid immunity. Interestingly, the percentage of QFN(+) results was not statistically significantly higher in the hybrid immunity versus the vaccinated group (50%) and there was no association between distance from vaccination or infection with QFN result in this group. On the contrary, higher IFN-γ levels, measured by QFN, have been observed in infected participants (with or without breakthrough infection) in other studies comparing the cellular response to vaccination (BNT162b2 mRNA vaccine) between uninfected and infected individuals [4,25]. Interestingly, breakthrough infections with Omicron and Delta variants resulted in similar levels of IFN-γ as measured by QFN, indicating conservation of T cell immunity [26].

In the present study, in almost half of the vaccinated (infected and uninfected) adults, the IFN-γ levels measured by QFN were not detectable 6 to 10.5 months after the third dose. All vaccinated participants had an adequate humoral response to vaccination. This could be attributed to the waning of the cellular immune response against SARS-CoV-2 after a substantial postvaccination time interval and could underscore the importance of a booster dose.

A possible pathophysiological explanation for these results is the fact that IFN-γ secreted from specific T cells was not detected due to migration of SARS-CoV-2 specific memory T cells to lymphoid tissues, after a certain period of time [23,27]. After their encounter with SARS-CoV-2 antigens, T cells circulate in the blood secreting IFN-γ [23]. Over time, specific T cells are located in lymph nodes, and regularly some of them circulate in the blood [23]. In the absence of antigenic stimuli, there is a possibility that QFN cannot detect IFN-γ secreted from these specific T cells [23]. Thus, re-evaluating the threshold of the assay at a certain time point after vaccination should be considered.

A decline in cellular responses measured with QFN 6 to 7 months postvaccination with two doses of BN162b2 mRNA vaccine has been observed in several studies [28−31]. Bonnet et al. [30] found that specific cell mediated responces, measured by QFN, 6 months after vaccination, decreased by 42% in 91 participants and increased by 33% in 17 others. In a cohort of HCWs, an increasing time interval between vaccination and time of measurement was associated with declining cellular response as measured by QFN [28].

There are studies indicating that QFN may be less sensitive than other laboratory methods for T cell measurements. Tormo et al. [13] reported that Flow Cytometry-Intracellular Staining assay (FC-ICS) demonstrated greater sensitivity in detecting T cell responses in vaccinated individuals with two doses of BN162b2 mRNA vaccine than the QFN assay. These differences may be attributed partially to the different nature of the Spike antigens used in the assays [13]. Furthermore, Busà et al. [11] observed that even though half of the participants did not develop a detectable with IGRAs (ELISpot, QFN) T cell effector response 3 weeks after the third dose with BN162b2 mRNA vaccine, all had specific Activated-Induced Marker (AIM) memory T cells, indicating the existence of memory T cells.

A limitation of our study is the limited number of participants included, especially in the convalescent unvaccinated group and the absence of vaccinated children. Another limitation of the study is that the used method for antibody detection does not discriminate between IgA, IgM, IgG response. However, most participants were immunized several months prior to their inclusion in the study, therefore the measurements were probably indicative of IgG humoral response. Furthermore, our study does not estimate the kinetics of T cell and humoral responses at different time points. However, to our knowledge, there are no previous published data with the QFN assay 6 months after vaccination, with three doses of the BNT162b2 mRNA vaccine.

5. Conclusion

In conclusion, SARS-CoV-2 QFN assay did not detect cellular immune responses in unvaccinated and a significant number of vaccinated or hybrid immunity individuals. The results of this assay, especially several months after immunization, must be interpreted with caution, as the clinical implications are not clear. More comparative studies with different immunological assays are required to elucidate whether this is the result of low sensitivity of the assay or waning immunity to SARS-CoV-2.

Author’s contribution

AM and VS was responsible for conceptualization, methodology, supervision, writing review & editing. EBT completed project administration, methodology, resources, investigation, formal analysis, writing - original draft preparation. MMD contributed to the resources investigation, formal analysis, writing - original draft preparation.

Funding

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

Declaration of Competing Interest

The authors report no conflicts of interest relevant to this article.

References

[1] Altawalah H. Antibody responses to natural SARS-CoV-2 infection or after COVID-19 vaccination. Vaccines (Basel) 2021;9:910. doi: 10.3390/VACCINES9080910.
[2] Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. Cell 2020;181:1489–501. doi: 10.1016/J.CELL.2020.05.015.
[3] Sekine T, Perez-Potti A, Rivera-Ballesteros O, Strålin K, Gorin JB, Olsson A, et al. Robust T cell immunity in convalescent individuals with asymptomatic or mild COVID-19. Cell 2020;183:158–68 e14. doi: 10.1016/J.CELL.2020.08.017.
[4] Tormo N, Navalpotro D, Martínez-Serrano M, Moreno M, Grosson F, Tur I, et al. Commercial Interferon-gamma release assay to assess the immune response to first and second doses of mRNA vaccine in previously COVID-19 infected versus uninfected individuals. Diagn Microbiol Infect Dis 2022;102:115573. doi: 10.1016/J.DIAGMICROBIO.2021.115573.
[5] Vardhana S, Baldo L, Morice WG, Wherry EJ. Understanding T cell responses to COVID-19 is essential for informing public health strategies. Sci Immunol 2022;7:eabo1303. doi: 10.1126/SCIIMMUNOL.ABO1303.
[6] Tan AT, Linster M, Tan CW, le Bert N, Chia WN, Kunasegaran K, et al. Early induction of functional SARS-CoV-2-specific T cells associates with rapid viral clearance and mild disease in COVID-19 patients. Cell Rep 2021;34:108728. doi: 10.1016/J.CELREP.2021.108728.
[7] Moss P. The T cell immune response against SARS-CoV-2. Nat Immunol 2022;23:186–93. doi: 10.1038/S41590-021-01122-W.
[8] Jaganathan S, Stieber F, Rao SN, Nikolayevskyy V, Manissero D, Allen N, et al. Preliminary evaluation of QuantiFERON SARS-CoV-2 and QIAreach anti-SARS-CoV-2 total test in recently vaccinated individuals. Infect Dis Ther 2021;10:2765–76. doi: 10.1007/S40121-021-00521-8.
[9] Vallejo A, Vizcarra P, Quereda C, Moreno A, Casado JL. IFN-γ + cell response and IFN-γ release concordance after in vitro SARS-CoV-2 stimulation. Eur J Clin Invest 2021;51:e13636. doi: 10.1111/ECI.13636.
[10] Ng OW, Chia A, Tan AT, Jadi RS, Leong HN, Bertoletti A, et al. Memory T cell responses targeting the SARS coronavirus persist up to 11 years post-infection. Vaccine 2016;34:2008–14. doi: 10.1016/J.VACCINE.2016.02.063.
[11] Busà R, Sorrentino MC, Russelli G, Amico G, Miceli V, Miele M, et al. Specific Anti-SARS-CoV-2 humoral and cellular immune responses after booster dose of BNT162b2 Pfizer-BioNTech mRNA-based vaccine: integrated study of adaptive

immune system components. Front Immunol 2022;13:856657. doi: 10.3389/FIMMU.2022.856657/FULL.
[12] Sauer K, Harris T. An effective COVID-19 vaccine needs to engage T cells. Front Immunol 2020;11:581807. doi: 10.3389/FIMMU.2020.581807.
[13] Tormo N, Giménez E, Martínez-Navarro M, Albert E, Navalpotro D, Torres I, et al. Performance comparison of a flow cytometry immunoassay for intracellular cytokine staining and the QuantiFERON® SARS-CoV-2 test for detection and quantification of SARS-CoV-2-Spike-reactive-IFN-γ-producing T cells after COVID-19 vaccination. Eur J Clin Microbiol Infect Dis 2022;41:657–62. doi: 10.1007/S10096-022-04422-7.
[14] Schwarz M, Mzoughi S, Lozano-Ojalvo D, Tan AT, Bertoletti A, Guccione E. T cell immunity is key to the pandemic endgame: How to measure and monitor it. Curr Res Immunol 2022;3:215–21. doi: 10.1016/J.CRIMMU.2022.08.004.
[15] Aiello A, Najafi Fard S, Petruccioli E, Petrone L, Vanini V, Farroni C, et al. Spike is the most recognized antigen in the whole-blood platform in both acute and convalescent COVID-19 patients. International J Infect Dis 2021;106:338–47. doi: 10.1016/J.IJID.2021.04.034.
[16] Kim SH. Interferon-γ release assay for cytomegalovirus (IGRA-CMV) for risk stratification of posttransplant CMV infection: is it time to apply IGRA-CMV in routine clinical practice? Clin Infect Dis 2020;71:2386–8. doi: 10.1093/CID/CIZ1211.
[17] Goletti D, Lindestam Arlehamn CS, Scriba TJ, Anthony R, Cirillo DM, Alonzi T, et al. Can we predict tuberculosis cure? What tools are available? Eur Respir J 2018;52:1801089. doi: 10.1183/13993003.01089-2018.
[18] Almendro-Vázquez P, Chivite-Lacaba M, Utrero-Rico A, González-Cuadrado C, Laguna-Goya R, Moreno-Batanero M, et al. Cellular and humoral immune responses and breakthrough infections after three SARS-CoV-2 mRNA vaccine doses. Front Immunol 2022;13:981350. doi: 10.3389/FIMMU.2022.981350/FULL.
[19] Martínez-Gallo M, Esperalba J, Pujol-Borrell R, Sandá V, Arrese-Muñoz I, Fernández-Naval C, et al. Commercialized kits to assess T-cell responses against SARS-CoV-2 S peptides. A pilot study in health care workers. Med Clin (Barc) 2022;159:116. doi: 10.1016/J.MEDCLI.2021.09.013.
[20] GeurtsvanKessel CH, Geers D, Schmitz KS, Mykytyn AZ, Lamers MM, Bogers S, et al. Divergent SARS-CoV-2 Omicron-reactive T and B cell responses in COVID-19 vaccine recipients. Sci Immunol 2022;7:eabo2202. doi: 10.1126/SCIIMMUNOL.ABO2202.
[21] Tarke A, Sidney J, Methot N, Yu ED, Zhang Y, Dan JM, et al. Impact of SARS-CoV-2 variants on the total CD4 + and CD8 + T cell reactivity in infected or vaccinated individuals. Cell Rep Med 2021;2:100355. doi: 10.1016/J.XCRM.2021.100355.
[22] Barreiro P, Sanz JC, Román JS, Pérez-Abeledo M, Carretero M, Megías G, et al. A pilot study for the evaluation of an interferon gamma release assay (IGRA) to measure T-cell immune responses after SARS-CoV-2 infection or vaccination in a unique cloistered cohort. J Clin Microbiol 2022;60 e02199-21. doi: 10.1128/JCM.02199-21.
[23] Kurteva E, Vasilev G, Tumangelova-Yuzeir K, Ivanova I, Ivanova-Todorova E, Velikova T, et al. Interferon-gamma release assays outcomes in healthy subjects following BNT162b2 mRNA COVID-19 vaccination. Rheumatol Int 2022;42:449–56. doi: 10.1007/S00296-022-05091-7.
[24] Stieber F, Allen N, Carpenter K, Hu P, Alagna R, Rao S, et al. Durability of COVID-19 vaccine induced T-cell mediated immune responses measured using the QuantiFERON SARS-CoV-2 assay. Pulmonology 2023;29:151–3. doi: 10.1016/j.pulmoe.2022.09.006.
[25] Desmecht S, Tashkeev A, el Moussaoui M, Marechal N, Perée H, Tokunaga Y, et al. Kinetics and persistence of the cellular and humoral immune responses to BNT162b2 mRNA vaccine in SARS-CoV-2-naive and -experienced subjects: impact of booster dose and breakthrough infections. Front Immunol 2022; 13:863554. doi: 10.3389/FIMMU.2022.863554.
[26] Søraas A, Grødeland G, Granerud BK, Ueland T, Lind A, Fevang B, et al. Breakthrough infections with the omicron and delta variants of SARS-CoV-2 result in similar re-activation of vaccine-induced immunity. Front Immunol 2022; 13:848099. doi: 10.3389/FIMMU.2022.964525.
[27] Klingel H, Lauen M, Krüttgen A, Imöhl M, Kleines M. Severity of adverse reactions is associated with T-cell response in mRNA-1273 vaccinated health care workers. Clin Exp Vaccine Res 2022;11:121–4. doi: 10.7774/CEVR.2022.11.1.121.
[28] Costa C, Scozzari G, Migliore E, Galassi C, Ciccone G, Ricciardelli G, et al. Cellular immune response to BNT162b2 mRNA COVID-19 vaccine in a large cohort of healthcare workers in a Tertiary Care University Hospital. Vaccines (Basel) 2022;10:1031. doi: 10.3390/VACCINES10071031/S1.
[29] Takeuchi JS, Fukunaga A, Yamamoto S, Tanaka A, Matsuda K, Kimura M, et al. SARS-CoV-2 specific T cell and humoral immune responses upon vaccination with BNT162b2: a 9 months longitudinal study. Sci Rep 2022;12:15447. doi: 10.1038/S41598-022-19581-Y.
[30] Bonnet B, Chabrolles H, Archimbaud C, Brebion A, Cosme J, Dutheil F, et al. Decline of humoral and cellular immune responses against SARS-CoV-2 6 months after full BNT162b2 vaccination in hospital healthcare workers. Front Immunol 2022;13:842912. doi: 10.3389/FIMMU.2022.842912.
[31] Uwamino Y, Kurafuji T, Takato K, Sakai A, Tanabe A, Noguchi M, et al. Dynamics of antibody titers and cellular immunity among Japanese healthcare workers during the 6 months after receiving two doses of BNT162b2 mRNA vaccine. Vaccine 2022;40:4538–43. doi: 10.1016/J.VACCINE.2022.06.016.