ORIGINAL ARTICLE

Assessment of humoral and cellular immunity induced by the BNT162b2 SARS-CoV-2 vaccine in healthcare workers, elderly people, and immunosuppressed patients with autoimmune disease

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Abstract

The development of vaccines to prevent SARS-CoV-2 infection has mainly relied on the induction of neutralizing antibodies (nAbs) to the Spike protein of SARS-CoV-2, but there is growing evidence that T cell immune response can contribute to protection as well. In this study, an anti-receptor binding domain (RBD) antibody assay and an INFγ-release assay (IGRA) were used to detect humoral and cellular responses to the Pfizer-BioNTech BNT162b2 vaccine in three separate cohorts of COVID-19-naïve patients: 108 healthcare workers (HCWs), 15 elderly people, and 5 autoimmune patients treated with immunosuppressive agents. After the second dose of vaccine, the mean values of anti-RBD antibodies (Abs) and INFγ were 123.33 U/mL (range 27.55–464) and 1513 mIU/mL (range 145–2500) in HCWs and 210.7 U/mL (range 3–500) and 1167 mIU/mL (range 83–2500) in elderly people. No correlations between age and immune status were observed. On the contrary, a weak but significant positive correlation was found between INFγ and anti-RBD Abs values ($rho = 0.354$, $p = 0.003$). As to the autoimmune cohort, anti-RBD Abs were not detected in the two patients with absent peripheral CD19$^+$B cells, despite high INFγ levels being observed in all 5 patients after vaccination. Even though the clinical relevance of T cell response has not yet been established as a correlate of vaccine-induced protection, IGRA testing has showed optimal sensitivity and specificity to define vaccine responders, even in patients lacking a cognate antibody response to the vaccine.

Keywords Vaccine · INFγ · Antibodies · COVID-19 · SARS-CoV-2 · Immunogenicity

Abbreviations

Abs	Antibodies
BAU	Binding antibody unit
CLIA	Chemiluminescence immunoassay
HCWs	Healthcare workers
IGRA	Interferon gamma release assay
RBD	Receptor binding domain
nAbs	Neutralizing antibodies
VOC	Variants of concern

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Introduction

Vaccine-established herd immunity to SARS-CoV-2 is acknowledged as the straightforward way for controlling the trajectory of the present pandemic. However, effectiveness of vaccines for COVID-19 is hard to predict until results become available from large-scale time-consuming efficacy trials. Therefore, there is an urgent need for both reliable immune correlates of protection and suitable laboratory tools able to measure and to monitor them [1].

The role of Spike-specific nAbs in providing sterilizing immunity has been a natural avenue of investigation at the beginning of the pandemic [2], and induction of nAbs is

increasingly supported as a valuable surrogate endpoint in vaccine trials [3]. However, evidence is mounting that an early and coordinated T cell response, not necessarily accompanied by a detectable cognate antibody response [4, 5], is associated with less severe COVID-19 [6, 7] in both immune-competent and immune-compromised hosts [8, 9] and may provide longer-term protection [10–12], even against variants of concern (VOC) [13, 14]. To which extent these preliminary findings from naturally infected patients may translate into T cell surrogate endpoints of vaccine efficacy is poorly known, but preliminary studies are promising [14–16]. Moreover, cellular assays are both expensive and cumbersome compared to serological ones, even though they may be particularly useful in specific high-risk populations (e.g., humoral immunodeficiency, transplant recipients, neonatal period) [17].

As far as mRNA vaccines are concerned, published peer-reviewed trials showed that both mRNA-1273 (Moderna) and BNT162b2 (Pfizer-BioNTech) vaccines elicit Spike-targeted T cell responses as determined by means of indirect immunological assays such as ELISPOT, intracellular cytokine staining, and activation marker assays, with IFNγ emerging as a sensitive marker of responsiveness [18–22]. The diagnostic value of IGRA in COVID-19 has been explored in different cohorts [23, 24] and was more recently applied to investigate the persistence of T cell responses in Austrian patients recovered from SARS-CoV-2 infection [25].

Recently, the diagnostic accuracy of IGRA to detect post-vaccination T cell antigen-specific responses was analyzed in a pilot study of 23 randomly selected subjects by Lange et al. [26]. In this study, we aimed at measuring the immunological cellular response to an mRNA-based SARS-CoV-2 vaccine (BNT162b2) by applying IGRA and correlating it to the humoral response in 3 different COVID-19-naïve cohorts: (1) HCWs; (2) elderly people (> 75 years old) from long-term residences; and (3) autoimmune patients on immunosuppressive treatment.

Materials and methods

Study population

One hundred and eight consecutive COVID-19-naïve HCWs (mean age, 51; range 23–69; male-to-female ratio, 27/81) receiving the mRNA-based BNT162b2 vaccine (Pfizer-BioNTech) had their blood drawn at three pre-fixed time points: T0, first shot of vaccine; T1, second shot (+ 21 days from T0); and T2, + 21 days from T1 (+ 42 days from T0). Abs assays were performed at T0, T1, and T2 and IGRA at T0 and T2. HCWs with a history of previous COVID-19, confirmed by positive polymerase chain reaction-based

molecular assay for SARS-COV-2 on nasopharyngeal swab, were excluded. No information about comorbidities and concomitant therapy was available.

A cohort of 15 COVID-19-naïve elderly patients randomly selected from a nursing home was enrolled (mean age, 91.8 years; range, 76–99; F/M 15/0). These patients were not affected by immune-mediated systemic diseases and were not on long-term immunosuppressive drugs. An additional limited cohort of five COVID-19-naïve patients affected by autoimmune diseases (four with rheumatoid arthritis and one with pemphigo) on disease-modifying antirheumatic drugs (methotrexate or mycophenolate) plus rituximab were also evaluated. Two of these patients were expressly selected because a validated SARS-CoV-2 serological assay, performed before enrollment, showed absent serological responses to the vaccine. The other three patients were randomly selected for comparison. In the latter two cohorts (elderly people and autoimmune patients), Abs assay and IGRA were performed between 60 and 120 days after the second dose of the vaccine, and peripheral CD19+B cell counting, in the autoimmune cohort only, was performed at the same time of IGRA.

INFγ-release assay

For any single patient, whole blood was collected in lithium heparin tubes, and within 2 h from venipuncture, five hundred μL each was placed into three separate tubes (Euroimmun, Lübeck, Germany): (1) IGRA TUBE, a stimulation tube coated with components of the S1 domain of SARS-CoV-2 Spike protein; (2) IGRA STIM, a stimulation tube coated with mitogen as an aspecific control; and (3) IGRA BLANK, a tube containing no activating components for immune system for the determination of individual background IFNγ levels. Plasma was harvested after 24 h of stimulation at 37 °C from each tube and evaluated for IFNγ levels on an IFNγ ELISA plate (Euroimmun, Lübeck, Germany), according to manufacturer's instructions. Briefly, the plate contains microplate strips each with 8 break-off reagent wells coated with a monoclonal anti-IFNγ antibody. In the first reaction step, undiluted lyophilized calibrators and plasma samples diluted in sample buffer were added to the coated reagent wells to bind IFNγ. In two further reaction steps, a biotin-labeled anti-IFNγ antibody was added, which was enzymatically detected by means of a streptavidin-bound horseradish peroxidase. The resulting color intensity is proportional to the concentration of IFNγ antigen in the sample. IFNγ concentration was expressed as milli-international units per milliliter (mIU/mL). The lower detection limit is 3.88 mIU/mL. The results above the concentration of the highest calibrator (2500 mIU/mL) were considered as equal to 2500 mIU/mL. A patient result was considered valid when IFNγ values were > 100 mIU/mL in IGRA STIM.

The actual IFNγ output for every patient was calculated by subtracting the BLANK value from the respective IGRA TUBE value. Unvalid results were not considered in the final analysis.

Anti-SARS-CoV-2 antibodies

Anti-SARS-CoV-2 Abs were detected by a quantitative chemiluminescence immunoassay (CLIA) using the RBD of the Spike protein as capture antigen. All samples were processed according to the manufacturer's instruction using an automated CLIA platform (Advia Centaur, XPT, Siemens) with a cutoff level of 1.0 U/mL for a positive result (sensitivity 96.4%, CI95% 92.7–98.5%; specificity 99.9%, CI95% 99.6–100%). The conversion factor in binding antibody units (BAU) following the WHO International Standard (NIBCS 20/136) is 21.8.

Peripheral blood immunophenotyping

CD-19$^+$B lymphocytes were phenotypically analyzed by bead-based FACS CANTO II (BD Biosciences, San Jose, CA) using a multicolor flow cytometer, with three lasers, blue (488-nm, air-cooled, 20-mW solid state), red (633-nm, 17-mW HeNe), and violet (405-nm, 30-mW solid state). Whole blood was incubated with BD Multitest 6-color TBNK reagent and then lysed with BD FACS™ lysing solution. Lymphocyte subpopulations were acquired and analyzed with BD FACSCanto clinical software. Immune cells were first gated based on the combination of physical parameters and CD45 expression. The BD Multitest 6-color TBNK reagent contains antibodies to identify CD19$^+$B lymphocytes.

Statistical analysis

Statistical analysis was performed by using MedCalc software (Mariakerke; Belgium) and GraphPad (GraphPad Prism 8 XML ProjecT). Comparison of continuous variable was performed using Mann–Whitney U test. Correlation between continuous variables was performed by using Spearman's rho test. A p value less than 0.05 was considered statistically significant. The cutoff of INFγ to identify responders to the vaccine was calculated by using Youden index (i.e., cutoff at the highest sum of specificity and sensitivity) from a receiver operating characteristic (ROC) curve analysis.

Ethical issues

The study was approved by the local ethical committee and assigned the internal protocol number 034 2020H EPIDEMIOLOGIA COVID-19. Informed written consent was required to consecutively enroll patients by physicians. Demographic information was collected at enrollment.

Results

In the HCW cohort, the mean anti-RBD IgG Abs values at T0, T1, and T2 were 0.139 (range 0.00–1.25), 15.02 (range 1.33–71.27), and 123.33 U/mL (range 27.55–464), respectively (Fig. 1A). Overall, more than 50% of patients at T2
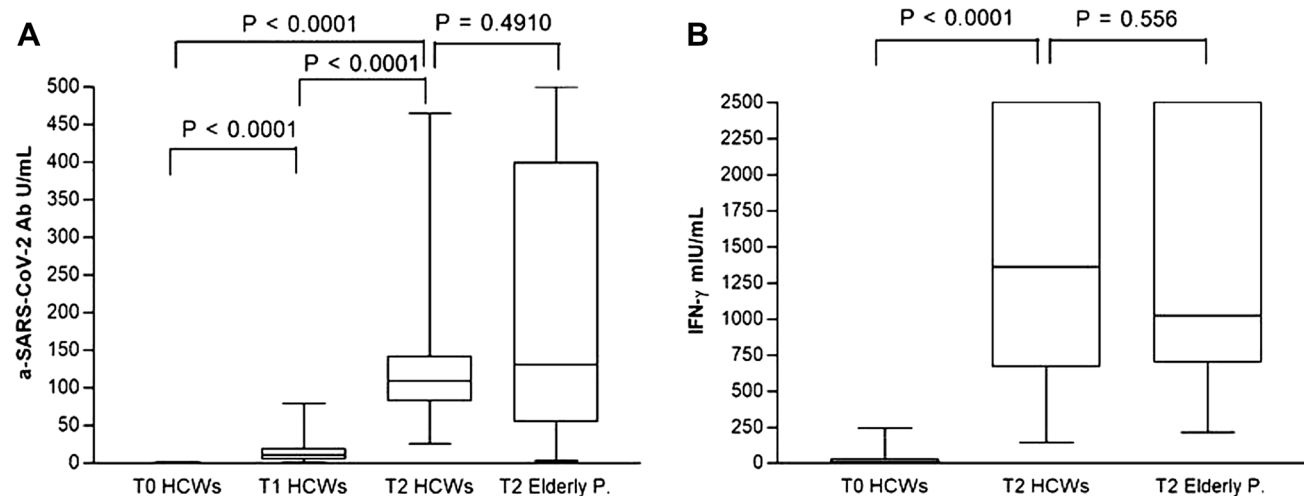
Fig. 1 **A** Anti-SARS-CoV-2 antibody levels in healthcare workers (HCWs, $n=108$) and in elderly people (Elderly P., $n=15$). **B** IFNγ levels in HCWs and in elderly people. Blood samples for immunological assays were collected at the time of the first dose of vaccine (T0 HCWs), of the second dose (T1 HCWs), and after 21 days from the second dose (T2 HCWs) in HCWs and in between 60 to 120 days after the second dose of vaccine (T2 Elderly P.) in elderly people. Comparisons were performed using Mann–Whitney U test

had anti-RBD IgG Abs values $\geq$ 100 U/mL. The mean IFNγ responses were 18.10 mIU/mL (range 0–242.5) at T0 and 1513 mIU/mL (range 145–2500) at T2 (Fig. 1B). No unvalid results were obtained. In the elderly cohort (mean age, 91.8; range, 76–99; female-to-male ratio, 15/0), mean anti-RDB IgG Abs and IFNγ values were 210.7 U/mL (range 3–500) and 1167 mIU/mL (range 83–2500), respectively (Fig. 1A and B).

In the HCW cohort, a ROC curve analysis was performed, and the cutoff for a vaccine-induced IFNγ T cell response, calculated by Youden index, was 72 mIU/mL with a sensitivity of 100% (CI95%, 96.5–100%) and a specificity of 98.9% (CI95%, 94.3–99.8%) (Fig. 2).

To explore the correlation between age and anti-RBD IgG Abs or IFNγ values, result values from the cohort of HCWs and elderly patients were joined. No significant correlation between age and Abs concentrations or IFNγ levels was found after vaccination (Fig. 3A and B).

When Abs and IFNγ levels after vaccination were compared, a weak but significant positive correlation was found: $rho = 0.354$, $p = 0.003$ (Fig. 3C).

In the cohort of rheumatic patients, INFγ values were all above 72 mIU/mL, while an Abs response was undetectable in 2 out of 5 patients. In these two patients, the peripheral CD19$^+$B cells were virtually absent (normal range, 200–400

cell/μL), while INFγ levels were > 2500 mIU/mL (Table 1). In the remaining patients, who showed both serological and cellular signs of vaccine immunogenicity, CD19$^+$B cell count was below the normal range but still detectable. Since time from last rituximab administration and the absolute number of rituximab administration may influence B and T cell immune-competency, we also provided this information in Table 1. Anyway, no apparent relationship was found between IFNγ or Abs levels and the number of rituximab administrations or the time lapse from last administration.

Discussion

Besides tuberculosis, analysis of T cell response has been clinically applied in a very limited number of diseases, namely, cytomegalovirus (CMV) disease in transplant recipients [27] and *Toxoplasma* congenital disease [28]. T cell responses to SARS-CoV-2 or to vaccines for COVID-19 have been widely explored in observational studies and vaccine clinical trials, through techniques that span from direct HLA-tetramer-based immune assays to indirect assays that capture different targets and functional features of T cell activation [29]. Nonetheless, the clinical value of these markers as surrogates for immune protection is difficult to segregate from that of coincidental antibody-related neutralizing immunity [17]. Trying to figure out the most precocious immune correlates of protection induced by vaccination, a group from Singapore prospectively analyzed the development of immune cellular and humoral responses to BNT162b2 from the time of the first jab to the moment when effective immunity was clinically established, i.e., the moment when the rate of infection in the vaccinated group started to separate from that in the placebo group [16]. The authors found that, at this latter time point, a cellular immune response could be detected while neutralizing antibodies could not. A more recent study showed that the presence of a cellular immune response to a Spike-based vaccine was sufficient to confer protection in cancer patients in whom a cognate antibody response was not able to be detected [30]. These proof-of-principle studies suggested that T cell response is actively involved in early immune protection from SARS-CoV-2 and provided a solid background to introducing T cell assays in clinical research and eventually in clinical practice. As to IGRA, Lange et al. [26] were the first to provide some evidence on the validity of this assay to define vaccine responsiveness, although in a limited number of 20 healthy individuals. Similarly, in this study, we demonstrated that a standard cohort of COVID-19-naïve HCWs exhibited a measurable immune cellular response against the SARS-CoV-2 Spike 1 subunit on IGRA and a cutoff value of 72 mIU/mL with a 100% sensibility and 98.9% specificity could be identified. Not unexpectedly,
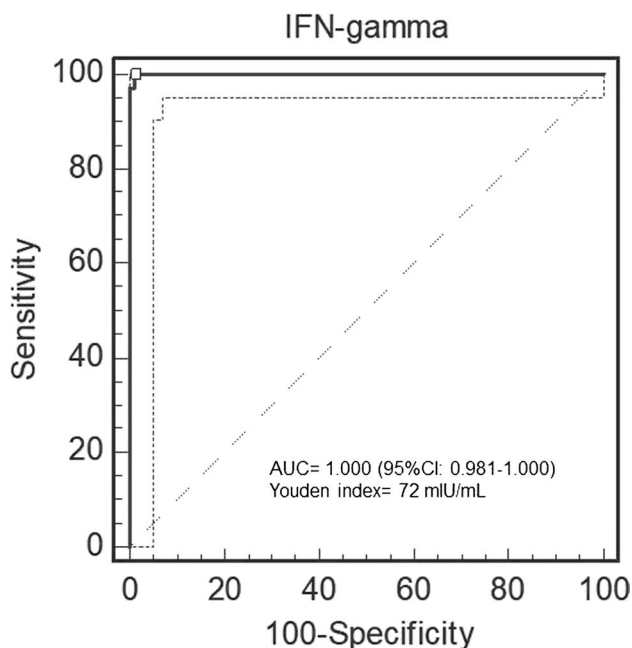
Fig. 2 Receiver operating characteristic (ROC) curve analysis for diagnostic performance of IGRA. The ROC analyzes the ability of IGRA to discriminate INFγ response in vaccinated HCWs (T2) versus baseline INFγ response (T0) to the Spike antigen of SARS-CoV-2. The Youden index (i.e., cutoff at the highest sum of specificity and sensitivity) is 72 mIU/mL. The area under the ROC curve (AUC) is 1.000 (95 CI, 0.981–1.000)
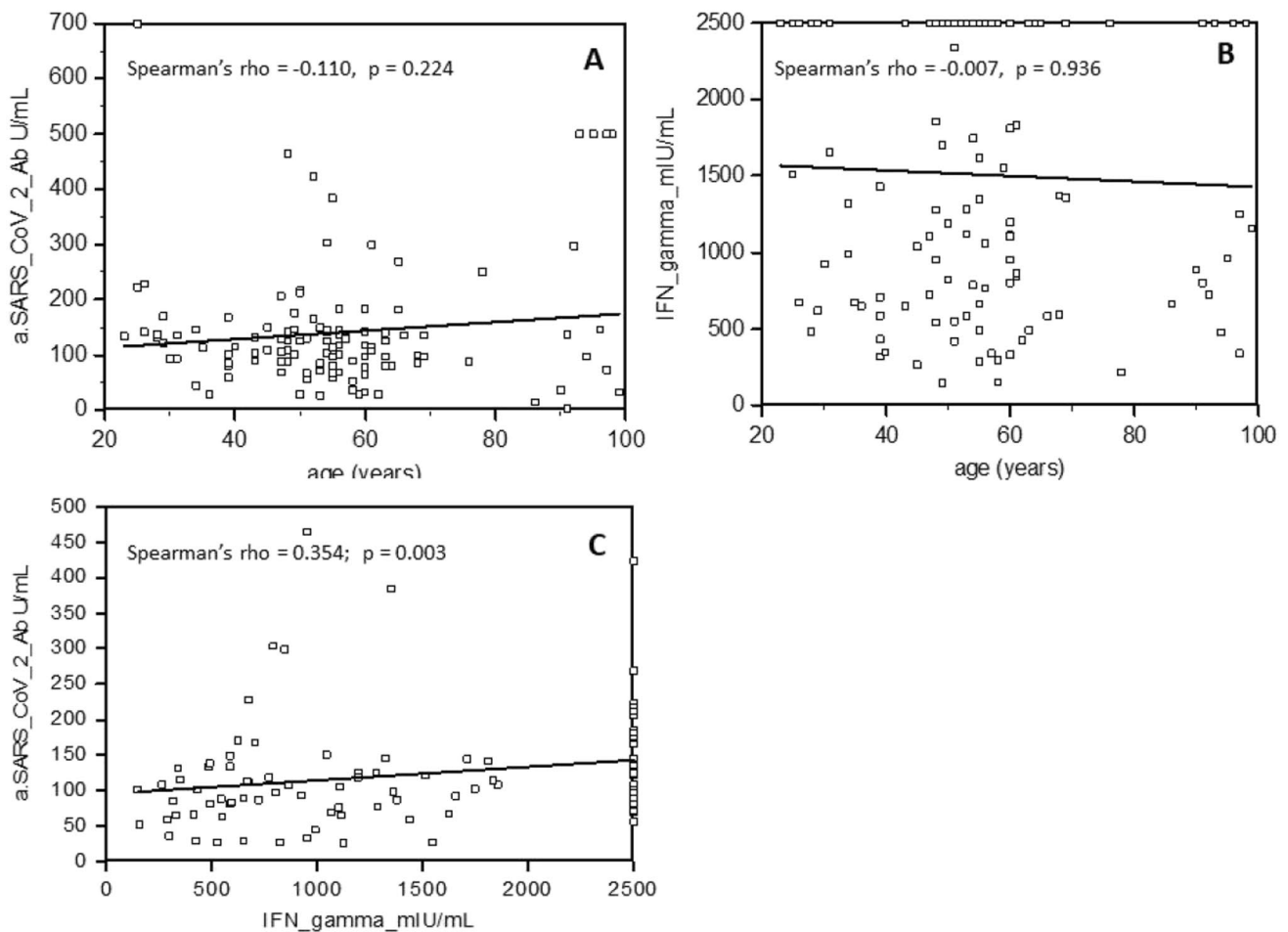
Fig. 3 Correlations between **A** anti-SARS-CoV2 antibody levels and patients' age; **B** INFγ levels and patients' age; **C** anti-SARS-CoV2 antibody levels and INFγ levels, in joined HCWs and elderly cohorts after the second dose ($n = 123$). Correlations between variables were performed by using Spearman's rho test. The Pearson's coefficient of correlation (r) and the level of significance (p) are indicated

Table 1 Clinical and laboratory characteristics of autoimmune patients

Patient	DMARDs	Weeks from last rituximab	n° RTX cycles	CD19+ (cells/μl)[#]	Anti-RBD IgG (U/mL)[*]	INFγ (mIU/mL)[**]
1. RA	MTX	37	8	2	0.08	> 2500
2. RA	MTX	26	5	3	0	> 2500
3. Pemphigo	MYC	54	2	36	3.4	357
4. RA	MTX	56	9	10	12.6	123
5. RA	MTX	36	9	31	282.6	> 2500

Legend: *RA*, rheumatoid arthritis; *DMARDs*, disease-modifying antirheumatic drugs; *MTX*, methotrexate; *MYC*, mycophenolate mofetil; *RTX*, rituximab. [#]CD-19-B cell reference range, 200–400 cells/μL; *anti-RBD IgG, > 1.0 U/mL; positive, ** INFγ > 72 mIU/mL. INFγ values from IGRA performed between 60 and 120 days after the second dose of vaccine

the intensity of T cell immune response was correlated, although weakly, to RBD-specific Abs titers. Apparently, no correlation between B and T cell immune response and age was observed, though it should be pointed out that the timing difference of Abs and IGRA testing in the HCWs and elderly people cohorts makes a definitive conclusion unobtainable. Moreover, the elderly cohort was composed only of females. Sex and gender have been acknowledged to play some role in COVID-19 susceptibility and outcome [31] as well as in susceptibility to vaccine adverse reactions [32]. Nonetheless, we are not aware of a sex-driven difference in vaccine humoral immunogenicity and would not

consider it a major limitation of the study. Since we lack information on other clinical and anthropometric features of the HCWs population, it was not possible to investigate other potential modulating factors (e.g., obesity). In the additional cohort of 15 elderly individuals, similar results were found although the limited number of patients and lack of clinical information prevented us from running comparisons with the HCW cohort.

Several studies have already shown a reduced humoral immunogenicity of mRNA-based vaccines in acquired immunodeficiency states such as blood cancer [33, 34], end-stage renal disease [35], and transplant recipients [35, 36] as well as in patients treated with biological drugs that target discrete pathways of immune function [37, 38]. A number of observations have shown that vaccination against SARS-CoV-2 in patients with immune-mediated diseases treated with rituximab can result in the absence of production of Abs to the RBD [39]. A study of 126 patients demonstrated the detrimental effects of rituximab on immunogenicity of vaccines against SARS-CoV-2 [40], and another recent study showed that only patients who had repopulated for B lymphocytes exhibited an immune response to the vaccine against SARS-CoV-2 [41]. Anyway, despite a lacking cognate antibody response, a preserved and detectable T cell response can still be detected in patients with primary [42] or secondary immunodeficiency, i.e., end-stage renal disease patients on dialysis [43], kidney transplant recipients [44], and patients affected by rheumatic disease on CD20 depleting therapies [45, 46]. Although limited by the very small number of patients, this study provided further evidence that patients treated with immunosuppressive agents and rituximab can show a strong T cell Spike-specific immune response not accompanied by anti-RBD IgG Abs production when the vaccine was provided at times of low/absent CD19 + B cell counts. Even though reassuring from an immunological prospective, it is doubtful that, in the absence of nAbs, vaccine-induced T cell immunity can by itself provide full protection from SARS-CoV-2 infection (i.e., sterilizing immunity), and presently, our opinion is that vaccine administration should be postponed until B cell repopulation. Pending data demonstrate that the correct timing for vaccination in rituximab-treated patients with rheumatoid arthritis is 9 months after treatment, but data are needed to validate whether peripheral CD19^{+} B cell monitoring might help individualizing this time point [47].

Some limitations of IGRA applicability deserve further attention. The T cell response to a pathogen is multi-layered, and different subpopulations of cells and molecular pathways can be elicited by natural or vaccine-induced stimulation of T cells. This is mirrored by different profiles of cytokine functional expression and clinical outcomes [48]. Even though IFNγ is one of the main cytokines associated with adaptive immune response to SARS-CoV-2, it cannot be excluded that activation of T cells expressing other cytokines or activation markers (i.e., co-stimulatory or co-inhibitory molecules) might better predict vaccine-induced or naturally acquired protection. For example, it is known from vaccine trials that IL-2 producing T cells consistently dominated over IFNγ producing T cells in many of the experimental cohorts of patients [18, 19]. IGRA, which uses IFNγ as the only read-out, might thus not completely reflect the protection conferred by vaccination [49]. It cannot be excluded that IFNγ could also foster a maladaptive immune response in severe COVID-19 and it is not known whether, in the absence of humoral sterilizing immunity, an excessive IFNγ release primed by vaccination might be associated with worst clinical outcomes in case of breakthrough SARS-CoV-2 infections by the original strains or by more pathogenic variants. For all these reasons, human IFNγ release assays are still mainly used for research, and their value in the real life scenario will continue to be investigated before being used for diagnostic purposes [50].

In summary, IGRA has proved to be a reliable laboratory assay to study the cellular immune response to the Pfizer-BioNTech mRNA-based vaccine, especially in high-risk patients in whom antibody response is undetectable due to acquired humoral immune deficiency. More studies are needed to validate its use as a marker of vaccine responsiveness and disease protection in clinical practice and vaccine trials.

Declarations

Ethics approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the local ethics committee and assigned the internal protocol number 034 2020H EPIDEMIOLOGIA COVID-19.

Consent to participate Informed consent was obtained from all individual participants included in the study.

Consent for publication Patients provided informed consent regarding publishing their data.

Conflict of interest The authors declare no competing interests.

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