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Research Article

Assessment of humoral and cellular immunity after bivalent BNT162b2 vaccination and potential association with reactogenicity

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Abstract

Background: This study investigated the feasibility and clinical value of using a novel, automated and high-throughput SARS-CoV-2 Interferon Gamma Release Assay (IGRA), combined with total anti-SARS-CoV-2 antibodies assessment, for evaluating the immune response after bivalent BNT162b2 vaccination.

Methods: A cohort of healthcare workers, who already underwent primary vaccination and boosting with monovalent BNT162b2 vaccine, received a booster dose of the new BNT162b2 bivalent formulation. Blood samples were taken immediately before vaccination (T0) and 1 month afterwards (T1). Humoral and cellular immunity were assayed with Roche Elecsys Anti-SARS-CoV-2 and Roche Elecsys IGRA SARS-CoV-2, respectively.

Results: The study population consisted of 51 subjects (median age: 43 years; 51% females). Total anti-SARS-CoV-2 antibodies and IGRA SARS-CoV-2 values increased at T1 from 9050 to 25000 BAU/mL (p<0.001), and from 0.44 to 0.78 IU/mL (p=0.385), accounting for median increase of 2.0 and 1.6 folds, respectively. Increased T1 values of total anti-SARS-CoV-2 antibodies and IGRA SARS-CoV-2 were recorded in 100% and 68.6% subjects, respectively. In those with baseline values below the median, post-vaccine levels displayed larger increases of 3.3 and 5.1 folds for anti-SARS-CoV-2 total antibodies and IGRA SARS-CoV-2, respectively. The variation of total anti-SARS-CoV-2 antibodies was inversely associated with their T0 values (r= -0.97; p<0.001), whilst that of IGRA SARS-CoV-2 was inversely associated with its T0 value (r= -0.58; p<0.001). No other associations were found with demographical or clinical variables, including side effects.

Conclusion: The bivalent BNT162b2 vaccine booster enhance humoral and cellular immunity against SARS-CoV-2, especially in recipients with a lower baseline biological protection.

Introduction

Nearly three years after coronavirus disease 2019 (COVID-19) was declared a pandemic by the World Health Organization (WHO), several features of the pathogenesis and of the pathways supporting the immune evasion strategies of SARS-CoV-2 (severe acute respiratory syndrome coronavirus disease 2) have now been clarified [1,2]. In particular, it has become increasingly evident that cellular immunity, probably more than the humoral response, is effective to prevent the risk of unfavorable disease progression and could more efficiently blunt the propensity to immune escape by new and highly mutated SARS-CoV-2 linages [3].

Although the precise mechanisms responsible for the protection conferred by cellular immunity remain to be fully elucidated, several lines of evidence now suggest that T cells are capable to efficiently kill virus-infected cells, thus limiting viral reproduction and spread, and support other immune-mediated mechanisms even in people infected by new viral variants, since they target a broader array of viral antigens compared to B cells [4]. In particular, not only is the physiological decline of neutralizing antibodies over time associated with enhanced risk of symptomatic infection, but the protection they provide against the risk of developing organic injuries also progressively fades as new and phylogenetically divergent lineages continues to emerge [5]. It has been demonstrated, for example, that the level of antigenic difference of the Omicron sublineage XBB.1.5 is nearly equivalent to the difference between SARS-CoV-2 Wuhan (ancestral strain) and SARS-CoV(-1) [6], and this is actually reflected by the dramatically reduced neutralizing potential against XBB lineages and other members of the BQ.1 lineage of the serum of patients with Omicron BA.5 breakthrough infection or receiving COVID-19 vaccines [7]. In this situation, one

positive aspect is that T cells can instead recognize many more epitopes throughout the sequence of the spike protein of SARS-CoV-2, including a remarkable number of those still present (almost unmodified) in many sublineages of the large Omicron family, thus making them more suited to raise a solid barrier against highly mutated SARS-CoV-2 variants [5,8]. As a result of this preserved recognition of heavily mutated viral linages, CD4+ T cells could still exert a kaleidoscope of antiviral functions, such as cytotoxic activity against infected cells, cytokine secretion (e.g., interferon gamma, transforming growth factor-β, Interleukin-6, Interleukin-12, Interleukin-22 and so forth), recruitment of innate cells, support of B cells during affinity maturation and antibody generation, support of CD8+ T cells during proliferation and differentiation, whilst CD8+ T cells are capable to highly efficiently kill infected cells (e.g., releasing substances like perforin and serine esterases), as well as they secrete a vast array of important cytokines such as interferon gamma, tumor necrosis factor-α, Interleukin 2 [9].

Irrespective of this straightforward evidence, the global research on the important cellular arm of our immune system represented by specialized T cells has been very scarce so far in COVID-19, mostly because the methods used for assessment of their function, mostly represented by manual ELISpot (enzyme-linked immune absorbent spot) and flow cytometrical techniques, are plagued by low throughput, long turnaround time and relatively high costs compared to the more practical measurement of anti-SARS-CoV-2 antibodies [10,11].

Thus, following the very recent development and commercialization of a novel, automated and high-throughput SARS-CoV-2 Interferon Gamma Release Assay (IGRA) for assessing cellular immunity against SARS-CoV-2, we planned a clinical

study to investigate the feasibility and clinical value of this method, combined with anti-SARS-CoV-2 antibodies assessment, for evaluating the immune response after bivalent BNT162b2 vaccination.

Materials and Methods

Study population

The study population consisted in a cohort of ostensibly healthy healthcare workers of the Pederzoli Hospital in Peschiera del Garda, who previously underwent a primary vaccination cycle and a single homologous booster administration with the Pfizer/Biontech mRNA BNT162b2 monovalent vaccine (Comirnaty, Pfizer Inc, NY, USA). Between November and December 2022, a second booster dose with the new bivalent formulation (15 μg of RNA encoding for ancestral WA1/2020 spike protein plus 15 μg of RNA encoding for BA.5 spike protein) of the Pfizer/Biontech mRNA BNT162b2 vaccine (Comirnaty, Pfizer Inc, NY, USA) was offered on voluntary basis. The procedure for vaccine administration remained identical throughout the primary vaccination cycle and the administration of the two boosters, as comprehensively described elsewhere [12.]. Briefly, all BNT162b2 vaccine doses were prepared according to manufacturer's instruction and administered intramuscularly to the study participants within 30 min from resuspension, in a time interval between 8 and 11 AM, from Monday to Friday. As concerns this specific study, venous blood was collected by straight needle venipuncture immediately before the administration of the bivalent BNT162b2 vaccine (i.e., T0) into an evacuated blood tube containing gel and clot activator (Greiner Bio-One, Kremsmünster, Austria) and into the three Roche Cobas IGRA SARS-CoV-2 blood Tubes (PC, NC and AG; Roche Diagnostics, Basel,

Switzerland). A second identical blood drawn was conducted exactly 1 month (i.e., T1) after bivalent BNT162b2 vaccine administration. In both circumstances, the blood tubes were rapidly conveyed to the central hospital laboratory. The serum tube was centrifuged for 15 min at 1500×g at room temperature, serum was separated and used for testing anti-SARS-CoV-2 total antibodies, as described below. The Cobas IGRA SARS-CoV-2 blood Tubes were instead incubated in vertical position at 37°C for 20-24 hours, as recommended by the manufacturer. After this period of time the three blood tubes were centrifuged for 5 min at 1500×g and the supernatant was used for IGRA SARS-CoV-2 testing, as described below. At the T1 time point, a specific questionnaire aimed at collecting demographical data and post-vaccination side-effects (i.e., within 3 days from bivalent BNT162b2 vaccine administration) was administered to all recipients (Supplementary File 1). The entire hospital staff has been subjected to regular medical visits (annually) and mandatory molecular testing for diagnosing potential symptomatic or asymptomatic SARS-CoV-2 infections with 2-4 weeks intervals for over 2 years since now.

Total anti-SARS-CoV-2 total antibodies assessment

The serum levels of total anti-SARS-CoV-2 antibodies were assayed at both T0 and T1 using the Roche Elecsys Anti-SARS-CoV-2 electrochemiluminescence immunoassay (ECLIA) on a Roche Elecsys e411 (Roche Diagnostics, Basel, Switzerland). This test, based on a one-step double sandwich principle, enables quantitative assessment of total anti-SARS-CoV-2 receptor binding domain (RBD) antibodies in serum and plasma. Briefly, the first step encompasses the incubation of serum or plasma with a ruthenylated and biotinylated recombinant antigen mixture,

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which could then be followed by immunecomplexes generation when binding antibodies are present in the test sample. After adding streptavidin-coated microparticles, the immunecomplexes formed are captured by solid phase nanoparticles though biotin-streptavidin interaction. After transferring the reagent mixture within the measuring cell, the microparticles present in the test sample are magnetically captured at the electrode surface, the unbound material is washed out and electrochemiluminescence is finally applied and quantified. The generated signal is directly proportional to the concentration of total anti-SARS-CoV-2 antibodies in serum or plasma. Results of measurement are reported in Binding Antibody Units (BAU)/mL, since the values of total anti-SARS-CoV-2 antibodies measured with this immunoassay are traceable to the first World Health Organization (WHO) International Standard for anti-SARS-CoV-2 immunoglobulins (NIBSC code 20/136) [13.]. The limit of blank (LoB) of the assay is 0.30 U/mL, the limit of detection (LoD) is 0.40 U/mL, test results <0.8 BAU/mL are considered "non-reactive" (i.e., negative), whilst the range of linearity is comprised between 0.4-250 BAU/mL. Sample values >250 BAU/mL are automatically diluted with Diluent Universal 2 (Roche Diagnostics, Basel, Switzerland), reassessed and finally provided as undiluted values until reaching the upper quantification limit, set at 25000 BAU/mL. A recent evaluation of this immunoassay revealed excellent diagnostic performance for diagnosing COVID-19 in human samples collected ≥14 days after nucleic acid amplification test (NAAT) positivity (i.e., sensitivity and specificity of 100%), with within- and between-run imprecision comprised between 0.9-1.5% and 2.4-3.2%, respectively [14].

SARS-CoV-2 Interferon Gamma Release Assay (IGRA)

The Elecsys IGRA SARS-CoV-2 test combines IGRA SARS-CoV-2 Tubes for stimulating T cells in vitro with a fully-automated measurement of interferon gamma with ECLIA technique on Roche COBAS PRO (Roche Diagnostics, Basel, Switzerland). This assay has been specifically developed for qualitatively assaying T cell-mediated immune response against SARS-CoV-2 in human whole blood samples. Up to 189 different SARS-CoV-2 antigens are coated on the IGRA SARS-CoV-2 Ag Tube, enabling ample coverage of viral RNA and host HLA subtypes, and thus granting high sensitivity against a wide spectrum of viral sublineages. In brief, whole blood incubation at 37°C for 24 h enables the stimulation of T cells with specific peptides coated onto the blood tubes, which leads to interferon gamma generation in while blood samples of patients who have been in former contact with the coated antigens. The composition of the three tubes is as follows: negative control (NC; for establishing the baseline levels of interferon gamma in each set of individual samples), positive control (PC; for defining T cell fitness and assuring the reliability of the reaction measured in the set of three samples), and Ag (Antigen; for triggering the SARS-CoV-2-specific interferon gamma response). Interferon gamma concentration is finally measured in the supernatant of the three IGRA SARS-CoV-2 Tubes with double antibody sandwich ECLIA, with a procedure similar to that previously described for anti-SARS-CoV-2 antibodies. Test results are standardized against the first British interferon gamma (human, leukocyte-derived) standard (NIBSC code 82/587) [15]. According to manufacturer's specifications, the LoB of this assay is 0.03 IU/mL, the LOD is 0.07 IU/mL, the range of linearity is comprised between 0.07-160 IU/mL, whilst the total imprecision ranges between 1.4-1.9%. The assay is considered reliable when the value in the PC tube is ≥1.00 IU/mL and/or the value in the NC tube is <0.30 IU/mL.

The samples are considered "reactive" when the difference between interferon gamma values in the Ag and NC IGRA SARS-CoV-2 Tubes is >0.013 IU/mL.

Statistical analysis and ethical committee approval

The results of total anti-SARS-CoV-2 antibodies and IGRA SARS-CoV-2 measurements were expressed as median and interquartile range (IQR). Difference of values measured at T0 and T1 were analyzed with paired Mann-Whitney and chi squared tests. Univariate association between total anti-SARS-CoV-2 antibodies or IGRA SARS-CoV-2 levels and other variables was assayed with Spearman's correlation. The statistical analysis was conducted with Analyse-it (Analyse-it Software Ltd, Leeds, UK) and MedCalc Statistical Software (MedCalc Software Ltd., Ostend, Belgium).

All subjects recruited in this prospective observational study provided written informed consents for undergoing COVID-19 vaccination and for being included in the immunological prospective survey. The study was conducted in accordance with the Declaration of Helsinki and its protocol has been approved by the Ethics Committee of the Provinces of Verona and Rovigo (59COVIDCESC; November 8, 2021).

Results

The initial study population consisted of 82 subjects who voluntarily received the BNT162b2 bivalent vaccine booster. Twenty-seven of these were lost at follow-up (i.e., T1) and two others did not correctly complete the clinical survey. Two additional subjects were excluded because they reported to have taken immunosuppressive drugs at the time of vaccination (T0). Thus, the final study population included 51 ostensibly

healthcare workers (median age: 43 years; IQR: 33-57 years; 51% females). A previous diagnosis of SARS-CoV-2 infection by routine NAAT screening was officially recorded in 29/51 (56.8%) subjects (single infection: 22/51, 43.1%; double infection: 7/51, 13.7%). A total number of 39/51 (76.5%) subjects reported mild side effects after BNT162b2 bivalent vaccination, the most common were pain at injection site (35/51, 68.6%), fatigue (17/51, 33.3%), fever and headache (both 8/51; 15.7%), chills and musculoskeletal pain (both 6/51; 11.8%), followed by insomnia and vertigo (both 3/51; 5.9%).

As shown in figure 1, the values of both total anti-SARS-CoV-2 antibodies and IGRA SARS-CoV-2 increased from T0 to T1, specifically from 9050 (95%CI, 6623-17266) to 25000 (95%CI, 17652-25000; p<0.001) BAU/mL, and from 0.44 (95%CI, 0.13-1.15) to 0.78 (95%CI, 0.35-1.47; p=0.385) IU/mL, accounting for a median increase of 2.0 (IQR, 1.4-3.3) and 1.6 (IQR 0.8-4.7) folds, respectively. An increase of values from T0 to T1 was noticed in all subjects (51/51; 100%) for total anti-SARS-CoV-2 antibodies, and in 35/51 (68.6%) for IGRA SARS-CoV-2, respectively. The number of subjects with total anti-SARS-CoV-2 antibodies levels <6000 BAU/mL, i.e., the minimum threshold of protection against Omicron variants as recently estimated by Dimeglio et al. [16] decreased from 11/51 (22%) at T0 to 0/51 (0%) at T1 (odds ratio, 0.03; 95%CI, 0.002-0.60), whilst the number of subjects with non-reactive IGRA SARS-CoV-2 values decreased from 13.7% at T0 to 2.0% at T1 (odds ratio, 0.13; 95%CI, 0.01-1.06). In subjects with baseline (i.e., T0) total anti-SARS-CoV-2 antibodies and IGRA SARS-CoV-2 below the median value (i.e., <9050 BAU/mL and <0.44 IU/mL, respectively), the post-vaccine values (i.e., T1) displayed a larger increase, from 6623 (95%CI, 3652-7860) to 18337 (95%CI, 15006-24562; p<0.001) BAU/mL, and from 0.13 (95%CI, 0.01-0.24) to 0.56

(95%CI, 0.19-0.81; p<0.001) (Supplementary Figure 1), thus accounting for a median increase of 3.3 (IQR, 2.2-4.3) and 5.1 (IQR, 1.9-11.4) folds, respectively. In all but three of such subjects (i.e., 88.4%) the value of IGRA SARS-CoV-2 was found to be increased from T0 to T1.

In univariate analysis, the delta variation of total anti-SARS-CoV-2 antibodies [i.e., T1/T0] was inversely associated with total anti-SARS-CoV-2 antibodies values at T0 (r= -0.97; p<0.001) (Table 1), whilst the delta variation of IGRA SARS-CoV-2 was inversely associated with IGRA SARS-CoV-2 at T0 (r= -0.58; p<0.001) (Table 2). No other significant associations could be observed with other variables, neither with the single side effects (Supplementary Table 1).

Discussion

The currently available information on the immune response in patients undergoing administration of the new bivalent COVID-19 vaccines is scarce, especially that concerning the potential effects on cellular immunity. The recent study published by Collier et al. showed that the administration of bivalent mRNA vaccine boosters is effective to elicit a considerable humoral response, though T-cell response was found to be only modestly affected [17]. More specifically, the authors found that the neutralizing titer of anti-SARS-CoV-2 antibodies increased between 11.1-17.8 folds after vaccination, while the CD4+ and CD8+ T cell response only increased between 1.4-1.7 and 1.6-2.6 folds, respectively. The above described variation in cellular immunity is similar to that found in our study, in that we observed a median IGRA SARS-CoV-2 increase of 1.6 (IQR, 0.8-4.7) folds, but compounded by a quite similar boosting of total anti-SARS-CoV-2 antibodies (i.e., median increase: 2.0; IQR, 1.4-3.3). In both

circumstances this relatively modest increase of humoral and cellular immunity against SARS-CoV-2 is perhaps attributable to the fact that our population of ostensibly healthcare workers had already received three standard doses of monovalent COVID-19 mRNA vaccine, while at least a single previous SARS-CoV-2 infection was diagnosed by NAAT in more than half of our study subjects. Thus, it is conceivable that most subjects already displayed a sustained immunity and a strong immunological memory before undergoing bivalent BNT162b2 vaccination, which may have ultimately contributed to attenuate the post-vaccine immune response. This hypothesis is also supported by the inverse correlation that we found between the post-vaccination variation of both total anti-SARS-CoV-2 antibodies and IGRA SARS-CoV-2 with their baseline (i.e., T0) values (Tables 1 and 2; Figure 1), which suggests that the higher is the basal immunity (both humoral and cellular), the lower is the immune response to the booster that could be triggered. Accordingly, we also found that healthcare workers with values below the median level at baseline (i.e., T0) of both total anti-SARS-CoV-2 antibodies and IGRA SARS-CoV-2 displayed a larger increase of values (3.3 vs 2.0 and 5.1 vs 1.6 folds, respectively) compared to the entire cohort (Supplementary Fig. 1). These biological findings are not really surprising, considering that the real-life efficacy of the new bivalent COVID-19 vaccines is somehow lower than predicted, conferring an only modestly incremented protection (i.e., between 28-56%) in fully vaccinated and boosted individuals [18]. Thus, it seems reasonable to envisage that monitoring both humoral and cellular immunity over time would enable to identify subjects with faster or more pronounced decline of SARS-CoV-2 immunity, who would need to be primarily boosted, but may also allow to detect those with higher SARS-CoV-2 protection, in whom the administration of additional vaccine

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boosters could probably be delayed. In support of this consideration, we found that the rate of subjects with total anti-SARS-CoV-2 antibodies values below the Omicron-specific protective threshold could be brought to zero after bivalent BNT162b2 vaccination (i.e., from 22% at T0 to 0% at T1) [16], while that of those with non-reactive IGRA SARS-CoV-2 values could also be remarkably decreased by 87% (odds ratio, 0.13), thus underpinning that bivalent BNT162b2 vaccination was effective to restore an excellent, perhaps optimal, level of humoral and cellular immunity in those with lower biological protection at baseline. Notably, the investigation of the single subject in whom the IGRA SARS-CoV-2 values remained below the limit of detection of the assay at both T0 and T1 did not reveal any clear factor that could explain the lack of cellular response (male, 42 years, no specific disease or ongoing treatment, 3.2-fold increase of total anti-SARS-CoV-2 antibodies after vaccination).

In conclusion, COVID-19 vaccines elicit robust B and T cell responses that are synergistically contributing, along with natural immunity, to reduce the clinical burden of SARS-CoV-2 infections, averting a considerable number of hospitalizations and deaths. Great expectations have been raised on novel COVID-19 bivalent vaccines, which come with the potential to further enhance the immune responses against new SARS-CoV-2 variants, thus including the T cell response [19]. According to our findings, the bivalent BNT162b2 vaccine booster seems effective to further boost both humoral and cellular immunity, virtually annulling the number of SARS-CoV-2 biologically vulnerable subjects, and ultimately confirming the crucial role that vaccines (especially if regularly updated) will have for the future management of this pandemic. We also demonstrated the feasibility of routinely assessing cellular immunity against SARS-CoV-2 with a fully-automated and high-throughput IGRA,

which would hence enable to broaden the assessment of this essential arm of the immunological response against COVID-19 to large parts of the general population [20,21].

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Author contributions

All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests

Authors state no conflict of interest.

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Figure 1. Variation of humoral (i.e., total anti-SARS-CoV-2 antibodies) and cellular (i.e., IGRA SARS-CoV-2) response after bivalent BNT162b2 vaccination in the entire cohort of ostensibly healthy healthcare workers.

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IGRA, interferon gamma release assay
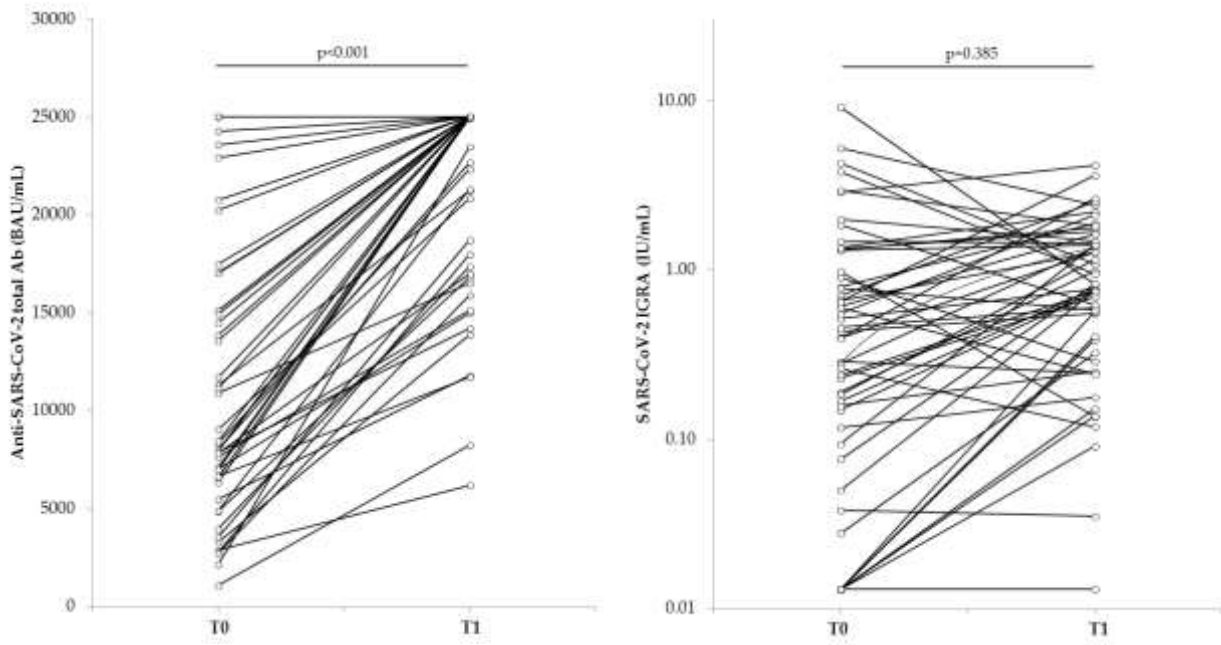
Table 1. Predictors of humoral response (i.e., total anti-SARS-CoV-2 antibodies) after bivalent BNT162b2 vaccination in ostensibly healthy healthcare workers.

Variable	Univariate analysis
Age	0.08 (-0.20 to 0.34; p=0.600)
Sex	0.06 (-0.22 to 0.33; p=0.669)
BMI	-0.16 (-0.42 to 0.12; p=0.250)
Previous SARS-CoV-2 infection (YES/NO)	0.04 (-0.24 to 0.31; p=0.793)
Side effects (YES/NO)	-0.16 (-0.42 to 0.12; p=0.252)
Number of side effects	-0.09 (-0.36 to 0.19; p=0.533)
Baseline (T0) anti-SARS-CoV-2 total Abs	-0.93 (-0.96 to -0.88; p<0.001)
Baseline (T0) IGRA SARS-CoV-2	-0.19 (-0.44 to 0.09; p=0.179)
IGRA SARS-CoV-2 variation	0.01 (-0.26 to 0.29; p=0.926)

BMI, body mass index; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IGRA, interferon gamma release assay

Table 2. Predictors of cellular response (i.e., IGRA SARS-CoV-2) after bivalent BNT162b2 vaccination in ostensibly healthy healthcare workers.

Variable	Univariate analysis
Age	-0.03 (95%CI, -0.30 to 0.25; p=0.857)
Sex	-0.11 (95%CI, -0.38 to 0.17; p=0.434)
BMI	0.01 (95%CI, -0.27 to 0.28; p=0.948)
Previous SARS-CoV-2 infection (YES/NO)	-0.05 (95%CI, -0.32 to 0.23; p=0.750)
Side effects (YES/NO)	-0.04 (95%CI, -0.31 to 0.24; p=0.776)
Number of side effects	-0.16 (95%CI, -0.42 to 0.12; p=0.251)
Baseline (T0) IGRA SARS-CoV-2	-0.58 (95%CI, -0.74 to -0.36; p<0.001)
Baseline (T0) anti-SARS-CoV-2 total Abs	-0.09 (95%CI, -0.36 to 0.19; p=0.538)
Anti-SARS-CoV-2 total Abs variation	0.01 (95%CI, -0.26 to 0.29; p=0.926)

BMI, body mass index; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IGRA, interferon gamma release assay

SUPPLEMENTARY FILE 1. Anamnestic Form

MODULO ANAMNESTICO – Da compilare al secondo prelievo (1 mese post-vaccino bivalente booster)

Parametri demografici	
Cognome	
Nome	
Età (anni)	
Sesso (F/M)	
Peso (kg)	
Altezza (metri)	
Uso di immunosoppressori prima della vaccinazione (SI/NO)	
Anamnesi post-booster (si/no), con <u>sintomi comparsi entro 3 giorni dalla somministrazione</u>	**SI/NO**
Febbre (>37.5°C)	
Brividi	
Emicrania	
Spossatezza	
Insonnia	
Vertigini	
Nausea	
Dolore sito di iniezione	
Dolori muscolo-articolari	
Altro (descrivere)	

ANAMNESTIC FORM – To be filled during second sampling (1 month after bivalent booster vaccination)

Demographical data	
Last name	
First name	
Age (years)	
Sex (F/M)	
Weight (kg)	
Height (m)	
Use of immunosuppressive drugs before vaccination (YES/NO)	
Post-booster anamnesis (yes/no), with <u>symptoms onset within 3 days from vaccine administration</u>	**YES/NO**
Fever (>37.5°C)	
Chills	
Headache	
Fatigue	
Insomnia	
Vertigo	
Nausea	
Injection site pain	
Musculoskeletal pain	
Other (describe)	

Supplementary Table 1. Univariate correlation (with 95%CI) of specific side effects (YES/NO) with humoral (i.e., total anti-SARS-CoV-2 antibodies) and cellular (i.e., SARS-CoV-2 IGRA) response after bivalent BNT162b2 vaccination in ostensibly healthy healthcare workers.

Side Effect	Anti-SARS-CoV-2 antibodies	SARS-CoV-2 IGRA
Fever	-0.19 (-0.44 to 0.09; p=0.180)	-0.02 (-0.29 to 0.26; p=0.898)
Chills	-0.19 (-0.44 to 0.09; p=0.181)	-0.18 (-0.44 to 0.10; p=0.201)
Headache	-0.07 (-0.34 to 0.21; p=0.627)	-0.11 (-0.37 to 0.17; p=0.458)
Fatigue	0.03 (-0.24 to 0.31; p=0.813)	-0.19 (-0.45 to 0.09; p=0.170)
Insomnia	0.02 (-0.25 to 0.30; p=0.874)	0.07 (-0.21 to 0.34; p=0.608)
Dizziness	-0.14 (-0.40 to 0.14; p=0.321)	0.22 (-0.06 to 0.47; p=0.119)
Nausea	-0.21 (-0.46 to 0.07; p=0.136)	0.07 (-0.21 to 0.34; p=0.639)
Site injection pain	-0.27 (-0.51 to 0.00; p=0.052)	-0.15 (-0.41 to 0.13; p=0.305)
Musculoskeletal pain	-0.28 (-0.52 to 0.01; p=0.052)	-0.20 (-0.45 to 0.08, p=0.167)

Supplementary Figure 1. Variation of humoral (i.e., total anti-SARS-CoV-2 antibodies) and cellular (i.e., anti-SARS-CoV-2 IGRA) response after bivalent BNT162b2 vaccination in ostensibly healthy healthcare workers with values below the median level at baseline. T0: pre-administration of bivalent COVID-19 vaccine; T1: 1 month after vaccination.

SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; IGRA, interferon gamma release assay