Combining SARS-CoV-2 interferon-gamma release assay with humoral response assessment to define immune memory profiles

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In the post-SARS-CoV-2 pandemic era, “breakthrough infections” are still documented, due to variants of concerns (VoCs) emergence and waning humoral immunity. Despite widespread utilization, the definition of the anti-Spike (S) immunoglobulin-G (IgG) threshold to define protection has unveiled several limitations. Here, we explore the advantages of incorporating T-cell response assessment to enhance the definition of immune memory profile. SARS-CoV-2 interferon-gamma release assay test (IGRA) was performed on samples collected longitudinally from immunocompetent healthcare workers throughout their immunization by infection and/or vaccination, anti-receptor-binding domain IgG levels were assessed in parallel. The risk of symptomatic infection according to cellular/humoral immune capacities during Omicron BA.1 wave was then estimated. Close to 40% of our samples were exclusively IGRA-positive, largely due to time elapsed since their last immunization. This suggests that individuals have sustained long-lasting cellular immunity, while they would have been classified as lacking protective immunity based solely on IgG threshold. Moreover, the Cox regression model highlighted that Omicron BA.1 circulation raises the risk of symptomatic infection while increased anti-receptor-binding domain IgG and IGRA levels tended to reduce it. The discrepancy between humoral and cellular responses highlights the significance of assessing the overall adaptive immune response. This integrated approach allows the identification of vulnerable subjects and can be of interest to guide antiviral prophylaxis at an individual level.

Keywords: Cell-mediated immunity · Interferon-gamma release assay · Immune memory profile

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

During the COVID-19 pandemic, the assessment of Correlates of Protection (CoPs) has predominantly centered on humoral
response assessment through the concentration of anti-Spike (S) immunoglobulin G (IgG), rapidly quantified using commercial serological assays.

These assays offer several advantages such as robustness, rapidity, and availability but also present limitations. Indeed, the kits currently deployed could be responsible for a misestimation of the humoral response capacities of individuals [1–4] as they mainly measure IgG targeting the ancestral S protein. While it is well-documented that humoral immunity decreases over time, experiencing a sharp decline postimmunization [5], an additional challenge arises from the emergence of variants of concern (VoCs) with substantial immune escape capacities exacerbating this effect [6–8]. Indeed, we are currently witnessing the circulation of heavily mutated VoCs such as the Omicron BA.1 strain presenting 37 mutations in the S protein including 15 in the receptor-binding domain (RBD). The significant antigenic shift in the RBD due to this evolution has led to a requirement for elevated anti-Spike IgG levels to achieve comparable neutralization capacities against emerging VoCs [9–11], challenging thus the relevance of anti-Spike IgG thresholds as a reliable correlate of protection, based solely on the correlation with serum antibody neutralization capacities [12–14].

Reflecting this observation, suggested antibody thresholds as CoPs in the literature vary widely, ranging from 154 binding antibody units (BAU)/mL for the ancestral lineage to over 1148 BAU/mL for the Omicron BA.4/5 variant [9, 12, 15, 16]. This points out the limitations of using antibody titer thresholds and a binary approach (above or below this threshold) to define CoPs.

This emphasizes the necessity to adjust strategies for assessing CoPs and, on a broader scale, define the immune memory profile over time. Notably, this entails considering other immunological mechanisms, particularly the T-cell response [17–20]. In addition to its important role in controlling SARS-CoV-2 infection and limiting disease severity [21, 22], the T-cell response appears more stable than the humoral one, as a virus-specific memory T-cell can be detected up to 22 months postsymptom onset [17]. Moreover, the T-cell response is less sensitive to immune escape caused by VOCs [23, 24] as the majority of SARS-CoV-2 epitopes are not mutated [25, 26]. Previous studies already highlighted the benefit of adaptive immune response assessment, notably in immunocompromised individuals [27] or in those treated using immunosuppressive therapies, for whom the humoral or cellular response may be partially impaired [28, 29].

In this context, we hypothesize that incorporating T-cell response monitoring could offer additional insights to delineate a more comprehensive individual immune profile. This approach can aid in identifying vulnerable groups at risk of breakthrough infection or those who may benefit from enhancing their immune capacities, such as through the administration of a booster vaccine dose. An easy way to assess rapidly and efficiently the SARS-CoV-2 specific T-cell response is the use of an Interferon-Gamma Release Assay test (IGRA) [27, 30]. IGRA, performed on peripheral blood mononuclear cells (PBMC) or whole blood (WB) using either commercialized kits or not, are reliable and reproducible assays currently used to assess IFN-γ response to pathogens such as Mycobacterium tuberculosis or human cytomegalovirus [31]. Recent increases in standardization and, in some cases, in automation of such assays pave the way for widening their use for immune surveillance [32, 33].

Recognizing the limitations of defining long-term immune memory solely through a binary approach based on humoral thresholds, this study aimed to simultaneously explore the advantages of assessing T-cell responses.

**Methods**

**Study design, participants, and data collection**

The prospective longitudinal COVID-Ser cohort study, conducted in the Hospices Civils de Lyon (HCL), included healthcare workers (HCWs) who completed the following inclusion criteria: (1) to be ≥18 years old; (2) to have given their written consent and accepting a follow-up every 6 months for 24 months, followed by a final visit at 36 months, and (3) to be affiliated to or beneficiary of social security. The study excluded pregnant or lactating women. At inclusion, the demographical and clinical data of the HCWs were recorded by a trained clinical research associate using Clin-sight software (version _ Csonline 7.5.720.1), and clinical blood samples were processed and stored at the Centre de Ressources Biologiques Neurobiotec. Concerning naïve vaccinated HCWs, the absence of previous SARS-CoV-2 infection was confirmed using the Wantai Ab total assay in baseline pre-vaccine blood sample. The Wantai SARS-CoV-2 total antibody assay, designed to detect anti-RBD antibodies, was chosen for identifying prior infection prevaccination due to its superior sensitivity in infected individuals compared with other commercial qualitative assays assessed in a previously published study [34]. Concerning convalescents vaccinated HCWs, all of them have experienced a mild form of COVID-19 as they were symptomatic but did not require hospitalization. At each visit, a questionnaire recording information on vaccine schedule was completed by the participant. Furthermore, during the follow-up, patients displaying indicative symptoms of a SARS-CoV-2 infection or identified as a contact case have been systematically tested through an antigenic test or PCR. The included HCWs, having undergone frequent testing throughout our longitudinal follow-up study, in accordance with mandatory regulations set by the French authorities. The ethics approval of the protocol of research was obtained from the National Review Board for Biomedical Research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I; ID RCB 2020-A00932-37). The study was registered on ClinicalTrials.gov (NCT04341142).

**SARS-CoV-2-RBD-specific IgG antibodies**

Serum samples were immediately stored at −80°C after blood sampling. RBD-specific IgG were measured using bioMérieux Vidas SARS-CoV-2 IgG II (9COG) diagnosis kits (bioMérieux, #424114), according to the manufacturer’s recommendation. For
standardization of these assays to the first WHO international standard, the concentrations were transformed in BAU/mL using the conversion factor provided by the manufacturer. Given the well-established strong correlation between anti-RBD IgG titers and neutralization capacity [2, 12, 15], we defined a sufficient anti-RBD IgG threshold to achieve a neutralizing antibodies (nAbs) titer (PRNT_{50}) ≥ 80. This level is considered relevant for obtaining protection against symptomatic SARS-CoV-2 diseases [2, 34]. To do so, we used a previous dataset of 229 serum samples containing paired quantification of nAbs titers (plaque reduction neutralization test; PRNT_{50}) against Omicron BA.1 and anti-RBD IgG titers [4]. Using Youden’s J statistic approach (J = sensitivity + specificity − 1), the relevant anti-RBD IgG threshold was defined at ≥ 1557 BAU/mL (Fig. S1).

**T-cells response after WB stimulation against SARS-CoV-2 peptides**

Fresh blood collected in heparinized tubes was stimulated for 22 h at 37°C under 5% of CO₂ with a peptide pool of 46 peptides targeting RBD synthetized by bioMérieux diluted in immune functional assays (IFA) solution (VIDAS STIMM BASIC Ruo, bioMérieux). The immune functional assay diluent was used as a negative control and a mitogen (VIDAS STIMM BASIC Ruo, bioMérieux) as a positive control. The peptides consist of 15 mer-sequences covering the entire RBD protein sequence and overlapped by 5 residues. The concentration of IFN-γ in the supernatant was measured using the VIDAS automated platform (VIDAS IFNγ RUO, bioMérieux). The measuring range was 0.08 to 8 IU/mL, and positivity thresholds were defined at 0.13 IU/mL, corresponding to LLoQ + 2 × standard deviation. For each sample, the IFN-γ response was defined as positive when the IFN-γ concentration was above 0.13 IU/mL and only if the negative and positive control were respectively below 0.08 IU/mL and above 8 IU/mL. Of note, performance of VIDAS COVIGRA (precision, linearity, sensitivity, specificity, and reproducibility) was assessed through and internal work performed by bioMérieux R&D department sensitivity and specificity [95CI] obtained were respectively 81.9% [77.7; 85.5] and 87.5% [76.4; 93.8].

**Stimulation with SARS-CoV-2 overlapping peptide pools and intracytoplasmic IFN-γ detection**

Intracytoplasmic IFN-γ detection was performed using intracellular cytokine staining (ICS) protocol. The overnight-rested PBMCs were stimulated using SARS-CoV-2 lyophilized peptides, consisting mainly of 15-mer peptides with 11-amino-acid overlaps, covering the entire Spike protein sequence. (PepTivator, Miltenyi Biotec) at a final concentration of 1 µg/mL for 1 h in the presence of 1 µg/mL monoclonal antibodies CD28 and CD49d, and then for an additional 5 h with GolgiPlug and GolgiStop (BD Biosciences). Dead cells were labeled using Fixable Viability eFluor 780 dye (Thermo Fischer Scientific). Surface markers, including BV786-conjugated anti-CD3 (BD Biosciences), BUV486-conjugated anti-CD4 (BD Biosciences), and PE-Cy7-conjugated anti-CD8 (BD Biosciences) were stained. Cells were then washed, fixed with Cytofix/Cytoperm (BD Biosciences), and stained with PE-conjugated anti-IFN-γ (BioLegend). Negative controls without peptide stimulation were run for each sample. All samples were acquired on a BD LSRRfortessa (BD Biosciences) flow cytometer and analyzed using FlowJo version 10 software.

**Immune profile group's affiliation**

All HCWs samples were subdivided into groups according to SARS-CoV-2 specific humoral and cellular immunity, respectively, quantified by the anti-RBD IgG level and the level of secreting IFN-γ post-RBD stimulation, using IGRA. Positivity thresholds were defined at an anti-RBD IgG level ≥ 1557 BAU/mL and an IGRA RBD level ≥ 0.13 IU/mL. Four groups (A to D) were then established, according to positive/negative status for both anti-RBD IgG and IGRA.

**Human leukocyte antigen typing**

Genomic DNA was purified from 5.10⁵ PBMCs using NucleoSpin Tissue, Mini kit from Macherey-Nagel (MN), according to the manufacturer’s instructions. DNA Purity was measured by the 260/280 nm ratio using NanoDrop 1000 spectrophotometer. Human leukocyte antigen (HLA) class I typing was performed using the Luminox head-based PCR SSO reverse method (Immucor). All tests were performed according to manufacturers’ instructions. The most probable allele was selected for each typing at loci HLA-A and HLA-B.

**Linear regression and Cox proportional hazard model**

A linear regression was performed to evaluate the potential association between demographical, clinical, or biological data and the group defined by the immune parameters. A set of five explicative variables was used: age (years), sex of the patients, presence of comorbidity, time elapsed from last immunization (months), and type of immunization at each time point. Numerical variables (time and age) were centered by mean and reduced by SD to avoid a potential scale effect for the regression analysis. Of note, in this model, two variables were not time-dependent (sex and comorbidity) and, as several time points were available per patient, the analyses were conducted using repeated data model. Results from the logistic regression were represented as a forest plot according to the odds ratio (OR) and associated 95% confidence interval [95% CI] computed.

Then, in order to identify a correlation between explicative variables and an increased risk of symptomatic breakthrough infections, a univariate Cox proportional hazard model was performed. We first extracted the SARS-CoV-2 infection documented by positive antigen or RT-PCR tests that occurred between
September 9, 2020, and March 21, 2022. During this period, several variant waves occurred, including Alpha (lineage B.1.1.7), Delta (lineage B.1.1.529), and Omicron (lineage BA.1). A set of six explicative variables was used: age, sex, presence of comorbidity, time elapsed from last immunization, IgG/IGRA group affiliation, and delay up to Omicron BA.1 predominance (from date when the proportion of circulated SARS-CoV-2 in France was mainly (>50%) affiliated to Omicron BA.1; Fig. S2). At the initial visit (T0) for each participant, their assignment to an IgG/IGRA status was determined based on the IgG/IGRA status obtained immediately before the infection or vaccine administration, with the corresponding date serving as a new starting point for recalculating the time intervals. Such analysis has been performed up to a deadline set at March 3rd, 2022, which corresponds to the Omicron BA.2 predominance. Results from the Cox proportional hazard model were represented as a forest plot according to hazard ratios (HR) and associated 95% confidence interval [95%CI] computed.

**Results**

**Characteristics of study population**

A total of 630 blood samples from 235 HCWs were collected during the follow-up period, between September 9, 2020, and March 21, 2022. Among the participants, 77% \((n = 182)\) were female, the median [IQR] age was 40 [31–51] years, and 29% \((n = 67)\) presented at least one comorbidity. At inclusion, 52% \((n = 121)\) were SARS-CoV-2 naïve before vaccination. Of note, all SARS-CoV-2 naïve HCWs and three doses for convalescent HCWs; T3, 6 months after last immunization; cWhether post vaccine or initial SARS-CoV-2 infection; T4, at booster dose; dTwo doses for convalescent HCWs and three doses for naive HCWs; T5, 4 weeks after booster dose; T6, 6 months after booster dose.

**Statistical analyses**

The descriptive statistics generated appropriate figures and parameters according to the type of variable (i.e. continuous or categorical). Normality testing was performed using the Shapiro-Wilk normality test. The distribution of quantitative data was expressed as mean (range) or median [interquartile range, IQR]. The comparisons between groups were performed using the Chi-square test for categorical variables and the nonparametric test or student T-test according to the distribution for continuous variables. Data were plotted using GraphPad Prism software (version 9; GraphPad software) and statistical analyses were conducted using R (version 3.6.2). \(p\)-values adjusted or not were two-tailed and were considered statistically significant at \(p\)-value < 0.05.

**Group affiliation according to cellular and humoral responses**

The 630 samples from HCWs were subdivided according to the humoral and cellular response status over time. In total, 35.4% \((n = 223)\) of the samples were into group A (IgG+ /IGRA−); 3.5% \((n = 22)\) into group B (IgG+ /IGRA−); 38.6% \((n = 243)\) into group C (IgG− /IGRA+); and 22.5% \((n = 142)\) into group D (IgG− /IGRA−; Fig. 1A). Of note, the level of anti-RBD IgG was 7-fold higher in the 74% \((n = 466)\) of samples with a positive cellular response (1405 BAU/mL [272.6–3254]) compared with the 26% of samples \((n = 164)\) with a negative cellular response (186.4 [81.3–737], \(p < 0.001\); Fig. S3).

| Table 1. Baseline characteristics of the study population |
|----------------------------------------------------------|
| SARS-CoV-2 convalescents | SARS-CoV-2 naïve | Total |
|---------------------------|------------------|-------|
| HCWs, \(n\) (%)           |                  |       |
| Female, \(n\) (%)         | 100 (83)         | 82 (72) | 182 (77) |
| Male, \(n\) (%)           | 21 (17)          | 32 (28) | 53 (23)  |
| Age (years), median [IQR] | 40 [31–51]       | 41 [32–52] | 40 [31–51] |
| Presence of at least one comorbidity \(^a\), \(n\) (%)   | 39 (32)          | 28 (25) | 67 (29)  |
| Sampling time, \(n\) (%) according to total sample     |                  |       |
| T1, \(n\) (%)             | 96 (15)          | /      | 96 (15)  |
| T2, \(n\) (%) \(^b\)      | 25 (4)           | 111 (18) | 136 (22) |
| T3, \(n\) (%) \(^c\)      | 18 (3)           | 105 (17) | 123 (20) |
| T4, \(n\) (%) \(^d\)      | 9 (1)            | 65 (10) | 74 (11)  |
| T5, \(n\) (%)             | 11 (2)           | 94 (15) | 105 (17) |
| T6, \(n\) (%)             | 8 (1)            | 88 (14) | 96 (15)  |

Note: Type of vaccines: BNT162b2 (Pfizer-BioNTech) and ChAdOx1 (Oxford-AstraZeneca); \(^a\)Neurologic or cardiovascular disorders, hypertension, heart failure, diabetes, immunodeficiency, liver or renal or rheumatic diseases, malignancy, hypothyroidism, chronic respiratory diseases, smoking and alcoholism. Sampling time: T1, before vaccination; T2, 4 weeks after vaccination; \(^b\)One dose for convalescent HCWs and two doses for naive HCWs; T3, 6 months after last immunization; \(^c\)Whether post vaccine or initial SARS-CoV-2 infection; T4, at booster dose; \(^d\)Two doses for convalescent HCWs and three doses for naive HCWs; T5, 4 weeks after booster dose; T6, 6 months after booster dose.
Interestingly, based on our defined cut-off, while 91.0% (223/245) of the IgG+ samples were also IGRA+, 63.1% (243/385) of the IgG− samples were still IGRA+. We next focused on this specific group (IgG+/IGRA−) to identify which factors were significantly associated with it. HR computed from the logistic regression’s coefficients and associated [95%CI] revealed that the time elapsed (in days) since the last immunization was the main factor explaining the affiliation to this specific group (2.32 [1.76–3.06], \(p < 0.001\)). Conversely, patients recently immunized with a third vaccine dose were unlikely to belong to this group, whether naive (0.29 [0.16–0.51], \(p < 0.001\)) or convalescent (0.03 [0.01–0.40], \(p < 0.008\); Fig. 1B).

Assessment of HLA typing and IFN-γ-secretion capacity by ICS

Surprisingly, as mentioned above, 3.5% (22/630) of our samples (\(n = 11\) HCWs) were affiliated with group B (IgG+/IGRA−), that is, presented a high anti-RBD IgG level but RBD peptide stimulation did not induce a detectable level of IFN-γ. Of note, the positive control using mitogen stimulation for these patients, as for all other patients, were ≥8 IU/mL confirming that T-cells were functional. To exclude an HLA bias of the IgG+/IGRA− group, we typed the HLA of 10 out of the 11 HCWs (as one refused the PBMC collection). No specific HLA profile was observed as 6 HLA-A and more than 8 HLA-B different profiles were observed among these 10 HCWs. Moreover, the identified alleles were also observed among a subset of 107 HCWs belonging to the other (IgG/IGRA) groups and were also largely represented within the French population, according to the allele frequency net database (Table 2). Finally, through ICS assessment we observed an increase of CD3+IFN-γ+ and CD4+IFN-γ+ T-cells induced by stimulation in 90% (9/10) of HCWs (Fig. S4).

Risk of symptomatic infection according to group affiliation

Finally, 30 episodes of symptomatic breakthrough infection were reported between November 15, 2020, and March 1, 2022. Cox regression analysis showed that the circulation of Omicron BA.1, was the main variable that influenced the risk of being infected, as depicted by an increase of adjusted HR [95%CI] (1.35 [1.23–1.49], \(p_{adj} < 0.001\)). Nevertheless, compared with the IgG+/IGRA− reference group, and regardless of the period, the risk of being infected was significantly lower for samples belonging to the IgG+/IGRA+ group (0.16 [0.006–0.44], \(p_{adj} < 0.001\)) while only a nonsignificant decreasing trend was observed for our group of interest IgG+/IGRA− (0.55 [0.19–1.62], \(p_{adj} = 0.276\), Fig. 2A). Nevertheless, these results were generated using our defined thresholds for anti-RBD IgG level (1557 BAU/mL) and IGRA (0.13 IU/mL), as usually employed for CoP definition. We then performed a nonbinary approach, assessing the risk of
Table 2. HLA class I typing according to patients humoral (anti-RBD IgG) and cellular (IGRA) response capacities

| HCWs alleles from Type HLA-A | Type HLA-A |
|-----------------------------|------------|
|                            | 02:01      | 03:01 | 11:01 | 24:02 | 25:01 | 29:02 | 02:06 | 23:01 | 24:02 | 26:01 | 31:01 |
| IgG+/IGRA− group (n = 10; %) | 50         | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 30    |
| Other groups (n = 107; %)    | 51.4       | 12.1  | 3.7   | 7.5   | 0.9   | 4.7   | 0.9   | 5.6   | 9.3   | 5.6   | 8.4   |
| French populationa (n = 130; %) | 38.5   | 23.8  | 8.5   | 20    | 1.5   | 10    | /     | 2.3   | 20    | 8.5   | 9.2   |

| HCWs alleles from Type HLA-B | Type HLA-B |
|-----------------------------|------------|
|                            | 07:02      | 07:05 | 08:01 | 15:01 | 18:01 | 27:05 | 35:01 | 39:01 | 40:02 | 18:01 | 35:03 | 40:01 | 44:02 | 44:03 | 49:01 | 51:01 | 55:01 |
| IgG+/IGRA− group (n = 10; %) | 10         | 10    | 10    | 20    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    | 10    |
| Other groups (n = 107; %)    | 18.7       | 3.5   | 12.1  | 5.6   | 11.2  | 5.6   | 8.4   | 0.9   | 2.8   | 2.8   | 8.4   | 3.7   | 10.3  | 10.3  | 6.5   | 18.7  | 1.9   |
| French populationa (n = 130; %) | 13.8  | 0.8   | 16.9  | 10.8  | 8.5   | 4.6   | 9.2   | 3.8   | 6.2   | 8.5   | 3.8   | 4.6   | 15.4  | 9.3   | 5.4   | 11.5  | 6.2   |

Note: Genotyping of HLA-A and HLA-B alleles from individuals belonging to the IgG+/IGRA− group (n = 10), from a subset of HCWs belonging to other groups (n = 107) and from French population (according to the Allele frequency net database). HLA typing are represented as frequencies (%). The most probable allele was selected for each typing at loci HLA-A and-B.

aMissing data for the allele HLA-A 02:06 within the French population.
symptomatic infection regarding anti-RBD IgG and IGRA quantitative values and still according to the delay from Omicron predominance. We observed that for each additional Log10 unit of anti-RBD the risk of symptomatic infection significantly decreased (HR [95%CI] 0.26 [0.13–0.55], \( p_{\text{adj}} < 0.001 \)), while once again, only a nonsignificant trend was observed regarding the IGRA increase effect (0.62 [0.29–1.31], \( p_{\text{adj}} = 0.208 \), Fig. 2B). In summary, our findings highlight that adaptive immune memory influences the susceptibility to symptomatic breakthrough infections among immunocompetent individuals, even during an active circulation period of variants with potent immune escape capabilities like Omicron BA.1.

### Discussion

In this current study, we comprehensively delineated the immune memory profile by employing both cellular and humoral assays in immunocompetent HCWs followed longitudinally. A total of 630 paired samples were collected, and the analysis was conducted utilizing relevant thresholds as usually employed in CoP. Almost a quarter of the samples had an insufficient immune response capacity, both humoral (anti-i.e. RBD IgG <1557 BAU/mL) and cellular (i.e. IGRA < 0.13 IU/mL). Interestingly, we observed that among the samples with insufficient humoral response, approximately 60% exhibited a T-cell response after stimulation using SARS-CoV-2 RBD peptides. The time elapsed since the last immunization being the main factor significantly associated with this specific profile, the long-term nature of cellular immunity compared with humoral immunity was thus confirmed, as suggested by the previous findings of Hurme et al. and Naranbhai et al. who used respectively Luminex and ELISpot approach [35, 36]. These observations also suggested that those individuals therefore present a long-term cellular immunity, which could be protective from severe forms of the disease, whereas they would have been considered as nonresponders, that is, without protective immunity if the humoral response alone had been only considered. The present results were in line with observations made by Guo et al. [37] who described that T-cell responses could be detected 12 months after the initial infection even in case of nAbs loss; also highlighting the importance of cross-reactive SARS-CoV-2-specific T-cell responses in severe disease protection, especially post-VoCs emergence.

Moreover, despite it has been already demonstrated in immunocompromised individuals [38, 39], herein, the interest in combining cellular and humoral response assessment in a healthy population of HCWs was highlighted, regardless of the presence of risk factors, as also recently described by Graça et al. [40] and Vogrig et al. [27]. We furthermore confirmed that, as the IGRA tool yields results in less than 24 h with limited hands-on-time, it appears as an easier tool to assess cellular responses compared with other methods such as ELISpot or ICS, which are more expensive or time-consuming. Thus, this tool could enable the more precise identification of patients at risk for severe infection within specific populations. Such targeted identification may facilitate the adjustment of care management, whether in preventive or curative forms. As demonstrated by Muller et al., individuals who received booster vaccinations, regardless of underlying immunodeficiency, exhibited significant protection against severe disease. Moreover, they demonstrated heightened and diversified T-cell responses targeting both conserved and Omicron-specific epitopes [39].
Ultimately, our objective was to establish a connection between the efficacy of humoral and cellular memory profiles and the likelihood of developing symptomatic infections. We were able to report that the increased risk of symptomatic infection was related to Omicron BA.1 circulation, confirming observations made by Cao et al. [41]. Additionally, while we highlighted that an increase in anti-RBD IgG levels was significantly correlated with a decrease in infectious events, there was only a trend toward a correlation between the increase in the cellular response and the reduction of those events. However, it is important to note that this observed trend may be influenced by a potential lack of statistical power due to a relatively low number of breakthrough infection events. However, aligning with recent assertions presented in the review by Almendro-Vázquez et al. [17], we are firmly convinced of the importance of incorporating SARS-CoV-2-specific cellular immune response as a complementary element to humoral response for a more comprehensive definition of immune memory profile and protective capacity. We, therefore, emphasize the need to adapt future studies design to accurately confirm the interest of such a combined approach in the COP definition.

The present study has some limitations: (1) HCWs might differ slightly from the general population since they are repeatedly exposed to SARS-CoV-2 and closely monitored for vaccine coverage and COVID-19 incidence. Moreover, even though asymptomatic infections may have remained unnoticed, HCWs were heavily tested during the monitoring period of this study, as mandatory by French regulations during this period; (2) WB IGRA is not able to differentiate CD8⁺ and CD4⁺ T-cells response and moreover it was performed using peptides from one viral Spike protein, it would be of interest to renew our experiment using new pools of peptides that cover other SARS-CoV-2 proteins, as already described [42]; (3) WB IGRA sensitivity has to be considered, compared with other methods used to assess the T-cell response, as highlighted herein by IGRA negative samples despite a detectable poststimulation cellular response in ICS; (4) The binary classification for IgG and IGRA assays was determined using thresholds established through our prior laboratory experiences or provided by the manufacturer and requires confirmation/enhancement (5) The nature of this cohort, composed by HCWs presenting only an initial mild form of COVID-19 and the low numbers of infectious events recorded prevented to perform a robust statistical study regarding the risk of infection.

To conclude, the present study highlights the complementary value of assessing both humoral and cellular responses. This composite assay compared with a single COP definition based only on the humoral response, which remains a qualitative binary approach (above/below a specified cut-off), could allow to fulfill an unmet medical need, by offering complemental information. While other studies showed that this statement was valid for people presenting immunodepression [38, 39], herein we confirm that these findings can also be applied to immunocompetent individuals, which can be of interest to guide antiviral prophylaxis at an individual level.

Acknowledgements: The authors thank the staff members of the Occupational Health and Medicine Department, the clinical research associates for their excellent work, the members of the clinical research and innovation department for their reactivity (DRCI, Hospices Civils de Lyon); and all the HCWs for their participation in this clinical study. Human biological samples and associated data were obtained from NeuroBioTec (CRB HCL, Lyon France, Biobank BB-0033-00046). The authors also thank Shanez Haouari (DRS, HCL) for their help in manuscript preparation. This study has received funding from the Agence Nationale de Recherches sur le Sida et les Hépatites Virales (ANRS-0154), and bioMérieux kindly provided kits for humoral and cellular response assessment.

Conflict of interest: GO, CC, AF, XL, SD, FB, and KBP are bioMérieux employees. The remaining authors declare no commercial or financial conflict of interest.

Author contributions: All authors were involved in the analysis and interpretation of data as well as in drafting the manuscript or revising it critically for important intellectual content. William Mouton, Christelle Compagnon, Karen Brengel-Pesce, and Sophie Trouillet-Assant made substantial contributions to the conception and design of the study and designed the experiments. Xavier Lacoux, Soizic Daniel, and Franck Berthier realized the IGRA conceptualization for cellular response assessment. Christelle Compagnon, Carla Saade, Kahina Saker, and Bruno Pozzetto performed the humoral and cellular assay experiments. Guy Oriol and Priscille Franc performed the biostatistical analyses. Bouchra Mokedd, Cécile Barnel, and Jean-Baptiste Fassier analyzed the clinical data. William Mouton, Valérie Dubois, Sophia Djebali, Maxence Dubois, Thierry Walzer, Jacqueline Marvel, and Sophie Trouillet-Assant supervised and analyzed some of the experimental tasks. William Mouton and Sophie Trouillet-Assant wrote the article. Carla Saade, Bruno Pozzetto, Thierry Walzer, Jacqueline Marvel, and Karen Brengel-Pesce revised the manuscript content. All authors read and approved the final manuscript.

Ethics approval statement: The ethics approval of the protocol of research was obtained from the National Review Board for Biomedical Research in April 2020 (Comité de Protection des Personnes Sud Méditerranée I, Marseille, France; ID RCB 2020-A00932-37). The study was registered on ClinicalTrials.gov (NCT04341142).

Data availability statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Peer review: The peer review history for this article is available at https://publons.com/publon/10.1002/eji.202451035.
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Abbreviations: BAU: binding antibody units · CI: confidence interval · CoP: correlate of protection · HCWs: healthcare workers · HLA: human leukocyte antigen · HR: hazard ratios · ICS: intracellular cytokine staining · IFN-γ: interferon gamma · IGRA: interferon gamma releasing assay test · IQR: interquartile range · nAbs: neutralizing antibodies · OR: odd ratios · PBMC: peripheral blood mononuclear cells · PRNT50: plaque reduction neutralization testing · RBD: receptor-binding domain · S: spike · VoCs: variants of concern · WB: whole blood

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Received: 26/1/2024

Accepted: 22/3/2024

Accepted article online: 26/3/2024