Clinical performance of a standardized SARS-CoV-2 interferon-γ release assay for simple detection of T-cell responses after infection or vaccination

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Summary: The SARS-CoV-2 IGRA assay used in this study accurately distinguished convalescent COVID-19 patients and vaccinated subjects of uninfected controls, and correlated with the presence of trimericS-IgG anti-SARS-CoV-2 and neutralizing antibodies. Overall, the test had high specificity and positive predictive value.

Abstract

Background: We evaluated a standardized interferon-γ (IFN-γ) release assay (IGRA) for detection of T-cell immune response after SARS-CoV-2 infection or vaccination.

Methods: This prospective study included COVID-19 patients with different severity of illness and follow-up (FU), vaccinated subjects, and healthy unvaccinated persons. SARS-CoV-2 T-cell response was measured using a specific quantitative IGRA in whole blood (Euroimmun, Germany) and TrimericS-IgG and neutralizing antibodies with validated serological platforms. Positivity of RT-PCR or vaccination was considered as reference standard.

Results: Two hundred and thirty nine individuals were included (152 convalescent, 54 vaccinated and 33 uninfected unvaccinated). Overall sensitivity, specificity, positive (PPV) and negative (NPV) predictive values (95% CI) of the IGRA were 81.1% (74.9%-86%), 90.9% (74.5%-97.6%), 98.2% (94.5%-99.5%), and 43.5% (31.8%-55.9%), respectively. All vaccinated SARS-CoV-2-naïve subjects had positive IGRA at 3 months. In convalescent subjects the magnitude of IFN-γ responses and IGRA accuracy varied according to disease severity and duration of FU, with the best performance in patients with severe COVID-19 at 3-month and the worst in those with mild disease at 12-month. The greatest contribution of IGRA to serological tests was observed in patients with mild disease and long-term FU (incremental difference, 30.4%).

Conclusion: The IGRA assessed was a reliable method of quantifying T-cell response after SARS-COV-2 infection or vaccination. In convalescent patients the sensitivity is largely dependent on disease severity and time since primary infection. The assay is more likely to add clinical value to serology in patients with mild infections.

Keywords: interferon-γ release assay, COVID-19, SARS-CoV-2, T-cell response, IGRA

Introduction

Whilst several studies have reported decreasing circulating antibodies over time in patients recovered from COVID-19 [1-4], recent investigations indicate a robust and durable T-cell immunity, suggesting that this may be a more reliable marker of prior infection than antibody response [5-8]. Therefore, to better characterize the magnitude and longevity of protective immunity against SARS-CoV-2 it may be important to measure both antibody production and T-cell response. Unlike serological assays to detect antibodies, the conventional methods of detecting T-cell immunity are complex and have not yet been standardized, requiring highly specialized facilities.

The interferon-γ (IFN-γ) release assays (IGRAs) are used in clinical laboratories to evaluate cell-mediated immune response against *Mycobacterium tuberculosis* or cytomegalovirus by measuring T-cell release of IFN-γ following stimulation with pathogen-specific peptides [9-11].

Published data on the performance of the IGRAs following COVID-19 or vaccination are scarce and inconsistent [12-14]. Preliminary studies using laboratory-developed assays with different SARS-CoV-2 peptides have reported excellent sensitivity and specificity when compared with a standardized ELISpot assay [15] and have found evidence of specific T-cell response in most or all convalescent COVID-19 patients [12,13]. However, positivity has also been reported in a significant number of healthy unexposed subjects, IgG seronegative for SARS-CoV-2, raising concerns on the specificity of these assays [14].

The IGRAs present practical advantages over conventional methods to evaluate T-cell immune response to SARS-CoV-2. However, to determine the real performance of these tests, studies evaluating standardized methods in subjects with a broad spectrum of exposure to SARS-CoV-2 and pre-existing immunity should ideally be conducted. The aim of the present work was to evaluate the accuracy of a commercial IGRA-based SARS-CoV-2 test system to detect T-cell response (SARS-CoV-2 IGRA, Euroimmun, Lübeck, Germany) in

a diverse population of convalescent COVID-19 and vaccinated subjects, household contacts of patients with COVID-19 and healthy unexposed individuals. We also measured SARS-CoV-2 specific IgG and neutralizing antibodies titers and compared antibody and T-cell responses in the different cohorts.

Methods

Setting and study subjects

This cross-sectional study was carried out at Hospital General Universitario de Elche, Spain. The study was approved by the Institutional Ethical Committee as part of the COVID-19 Elx/Spain project (PI19/2021). Patients were selected through our centralized prospective registry of adults (≥16 years) undergoing SARS-CoV-2 testing between 15th March and 8th July 2021. All subjects agreed to voluntarily participate in the study and gave written consent. We analysed blood samples and clinical data from the following independent cohorts of convalescent COVID-19 patients after recovery, vaccinated subjects and healthy unvaccinated persons:

1) Convalescent cohorts. We selected three cohorts of convalescent unvaccinated COVID-19 individuals, two of them comprising inpatients classified as having severe disease undergoing evaluation at 3 and 12 months after diagnosis, and a third cohort of outpatients evaluated 12 months after diagnosis.

2) Vaccination cohorts. We recruited two cohorts of vaccinated donors: (1) SARS-CoV-2-naïve subjects, comprising healthcare workers donors with no history of COVID-19 symptoms or positive SARS-CoV-2 test who had been fully vaccinated with two mRNA vaccine doses 12 weeks before evaluation; and (2) SARS-CoV-2-infected patients, with previous history of severe COVID-19, receiving one or two mRNA vaccine doses.

3) *Healthy unvaccinated cohorts.* Two groups of SARS-CoV-2-naïve unvaccinated subjects with negative serology were tested as controls: (1) Household uninfected contacts of COVID-19 patients; and (2) SARS-CoV-2-naive healthy donors with no history of SARS-CoV-2 infection or close contact.

Detailed information on cohorts' characteristics and laboratory measurements is provided in Supplemental data.

Specimen collection and processing

Serum, EDTA plasma and whole blood specimens were obtained for measuring SARS-CoV-2–specific antibodies, neutralizing antibodies and IFN-γ release assays, respectively. Whole blood from lithium heparin tube was used for IGRA incubation within 4 hours, although the manufacturer's instructions allow up to 16 hours at room temperature (Supplemental data).

SARS-CoV-2 IFN-γ release assay

SARS-CoV-2 cellular response was measured using a specific quantitative interferon-γ release assay in whole blood following the manufacturer´s instructions (SARS-CoV-2 IGRA stimulation tube set, Euroimmun, Lübeck, Germany). Briefly, lithium heparinized blood from each patient was incubated 21h at 37ºC in the three tubes supplied. The IFN-γ concentration released in the plasma fraction obtained after centrifugation of the three tubes was then measured by ELISA (Human interferon-gamma ELISA, Euroimmun, Lübeck, Germany) with an automated instrument (Dynex DS2® ELISA system). IFN-γ response was defined as stimulated minus unstimulated. Results were interpreted as follows: IFN-γ[SARS-CoV-2] -

IFN-γ[blank]<100 mIU/mL was considered negative, 100-200 borderline, and >200 positive. Upper limit of quantification achieved was 5000 mIU/mL.

Serologic Testing Methods

Detection of SARS-CoV-2–specific antibodies

IgG antibody serum levels against the trimeric spike protein (TrimericS-IgG) were quantified using a commercial quantitative immunoassay (LIAISON® SARS-CoV-2 TrimericS IgG assay, DiaSorin, Saluggia, Italy) in an automated platform; ≥33.8 BAU/mL was considered positive with a numeric value for quantitative measurement.

Detection of neutralizing antibodies

Detection of neutralizing antibodies against SARS-CoV-2 (NAb) was performed in an automated instrument (Dynex DS2® ELISA system) by means of a surrogate neutralizing antibody test (SARS-CoV-2 NeutraLISA, Euroimmun, Lübeck, Germany). Results were interpreted as inhibition percentage (%IH): <20 was considered negative, ≥20 to <35 borderline, and ≥35 positive.

Statistical analyses

Continuous variables are expressed as median ±25th and 75th percentiles (Q1-Q3), and categorical variables as percentages. The percent agreement (positive, negative and overall) for SARS-CoV-2 IGRA was calculated using the TrimericS-IgG and NAb assaysas reference standard. Borderline values for NAb and IGRA were considered negatives for these calculations. We determined IGRA sensitivity, specificity and positive (PPV) and negative

predictive values (NPV) in convalescent and vaccinated individuals considering positivity of SARS-CoV-2 RT-PCR or vaccination as reference standard. For accuracy calculation, we considered IGRA results according to the manufacturer's cutoffs, counting borderline values as negative. Accuracy was also evaluated considering borderline values as positive.

Statistical analyses were performed with R version 4.0.3 software. The Mann-Whitney U test was used to compare median IFN-γ and antibody responses between groups. Spearman's correlation coefficient (rho) was used to assess the correlation between IFN-γ and TrimericS-IgG concentrations.

Results

Description of study groups

A total of 239 individuals were recruited into the study, including 152 convalescent patients, 54 vaccinated and 33 uninfected and unvaccinated healthy donors. Flowchart of study subjects is shown in Figure 1. The clinical and demographic characteristics of each study group are shown in Table 1. Median (Q1-Q3) age was 60 (47-70) years, 130 (54.4%) were male, and 98% self-identified as white.

Immune responses evaluated with the SARS-CoV-2 IFN-γ release assay and SARS-CoV-2-specific antibodies

Three (15.8%) of 19 healthy unvaccinated household contacts with negative serology for IgG anti-spike protein had positive results in the IGRA. None of 14 unexposed healthy controls gave a positive IGRA. Considering SARS-CoV-2 RT-PCR positive or vaccination as reference standard, sensitivity, specificity, PPV and NPV of the assay was 81.1% [95% confidence interval (CI): 74.9%-86%], 90.9% (95%CI: 74.5%-97.6%), 98.2% (95%CI: 94.5%-

99.5%), and 43.5% (95%CI: 31.8%-55.9%), respectively. When household contacts were excluded, specificity and NPV were 100% (95%CI: 73.2%-100%) and 26.4% (95%CI: 15.7%-40.6%), respectively (Table 2).

Levels of TrimericS-IgG correlated significantly with the magnitude of the SARS-CoV-2-specific T-cell response measured by the IGRA (Figure 2).

Table 3 illustrates the concordance between IGRA and SARS-CoV-2 antibodies in the convalescent and vaccinated cohorts. Overall agreement between the IGRA and both TrimericS-IgG and neutralizing antibodies was good, in particular between IGRA and TrimericS-IgG, with the lowest values found for negative percent agreement in convalescent cohorts.

SARS-CoV-2 IFN-γ release assay and antibody levels in convalescent subjects

Among all convalescent subjects, the median (Q1-Q3) age was 60 (48-70) years and 88 (57.9%) were males. Subjects with severe COVID-19 were significantly older (62 [55-71] years, p<0.001) and more often males compared with individuals with mild disease. The median (Q1-Q3) IFN-γ concentration for all convalescent subjects was 1178 (225-2383) mIU/ml. Thirty-one (20.4%) had IFN-γ negative, 6 (3.9%) borderline, and 115 (75.6%) positive concentrations. Overall, for convalescent subjects, sensitivity, PPV and NPV were 75.7% (95%CI: 67.9%-82.1%), 97.5% (95%CI: 92.2%-99.3%) and 44.8% (95%CI: 32.8%-57.4%) defining IFN-γ concentrations above 200 mIU/ml as true-positive, and 79.6% (95%CI: 72.2%-85.5%), 95.3% (95%CI: 89.6%-98.1%) and 46.6% (95%CI: 33.5%-60%) when borderline values were considered positive (Table 2).

A differential profile in immune responses was observed according to groups, with poorer antibody and T-cell responses in patients with longer follow-up (FU) after infection and milder disease (Table 1). All patients with severe COVID-19 and short-term follow-up

(median time from diagnosis, 84 [81-86] days), developed IGRA response (sensitivity, 100% [95%CI: 85.4%-100%]; PPV, 90.6% [95%CI: 73.8%-97.5]; NPV, 100% [95%CI: 85.9%-100%]), along with detectable TrimericS-IgG and neutralizing antibodies, whereas in those with longer follow-up (355 [351-357] FU days) the percentages of positivity of IGRA, TrimericS-IgG and neutralizing antibodies were 78%, 79% and 69%, respectively (Table 1). Sensitivity, PPV and NPV for IGRA are included in Table 2.

Compared with patients tested at 3 months, those with 12 months follow-up had significantly worse IFN-γ responses (median [Q1-Q3], 1073 [347-2027] vs 3158 [1744-4900] mIU/mL; p<0.001) and lower levels of TrimericS-IgG (172 [50-373] vs 812 [490-1210] BAU/mL; p<0.001) (Figure 3A).

In contrast to inpatients, only 8 (34.8%) of those with mild disease (median time from diagnosis of 336 [326-411] days) showed T-cell response in the IGRA, and 9 (39.1%) and 3 (13%) of 23 were serologically positive and had neutralizing antibodies, respectively (Table 1). The analysis of accuracy in this subgroup is detailed in table 2. The magnitude of T-cell and antibody responses was significantly poorer in subjects with mild disease, showing lower levels of IFN-γ (median [Q1-Q3], 109 [60-280] vs 1073 [347-2027] mIU/mL; p<0.001) and TrimericS-IgG (23 [7-88] vs 172 [50-373] BAU/mL; p<0.001) (Figure 3B).

SARS-CoV-2 IFN-γ release assay and antibody levels in vaccinated subjects

The group of vaccinated SARS-CoV-2-naïve subjects comprised health care workers that were younger and with a higher number of females compared with those vaccinated after previous COVID-19 (Table 1). The median time from vaccination was of 77 (76-77), 31 (17-48) and 18 (7-19) days in SARS-CoV-2-naïve, SARS-CoV-2-infected subjects receiving two doses and SARS-CoV-2-infected subjects receiving one vaccine dose, respectively.

All vaccinated SARS-CoV-2-naïve subjects had positive T-cell responses in the IGRA assay (sensitivity, 100% [95%CI: 83.4%-100%]; PPV, 89.3% [95%CI: 70.6%-97.2]; NPV, 100% [95%CI: 85.9%-100%]), and nearly all of them had detectable TrimericS-IgG and neutralizing antibodies (Table 1). Interestingly, the IFN-γ levels were significantly lower than those seen in patients with severe COVID-19 tested at a similar timepoint (median [Q1-Q3], 1021 [516-1423] vs 3158 [1744-4900] mIU/mL; p<0.001) despite having comparable levels of TrimericS-IgG (median [Q1-Q3], 1040 [650-1380] vs 812 [490-1210] BAU/mL; p=0.302).

SARS-CoV-2-infected subjects receiving one or two vaccine doses showed strong humoral and cellular responses with median TrimericS-IgG and IFN-γ concentrations higher than those seen in vaccinated SARS-CoV-2-naïve individuals (Figure 3C). Of note, these subjects were tested much earlier post-vaccination. The magnitude of both antibody and T-cell responses observed in these groups were well above the observed in any of the unvaccinated convalescent cohorts (Table 1). Interestingly, one subject receiving one and another receiving two vaccine doses after prior COVID-19 had not developed T-cell response by the IGRA assay.

Contribution of the SARS-CoV-2 IFN-γ release assay to the assessment of pre-existing immunity in convalescent and vaccinated individuals

A total of 170 (82.5%) of the 206 convalescent and vaccinated subjects had positive TrimericS-IgG. Figure S1 shows how the IGRA and serological tests coincided in terms of positives in the convalescent and vaccinated cohorts. When we added positive IGRA results to serological tests, there were 175 (84.9%) individuals with pre-existing immunity (incremental difference: 2.4% [95%CI: -5%–+10%] [p=0.593]), i.e. positive antibody and/or IFN-γ response. When adding borderline IGRA results, the number of cases increased to 180 (87.4%) (incremental difference: 5.1% [95%CI: -3%–+12%] [p=0.215]). The greatest contribution of SARS-CoV-2 IGRA was observed in the cohort of patients with mild disease

and long-term follow-up. In this subgroup, there was an incremental difference of 13.0% [95%CI: -20%–+46%] [p=0.554] that increased to 30.4% [95%CI: -1%–+62%] [p=0.076] when adding borderline IGRA results to serological tests. In contrast, we did not find a significant contribution of IGRA in the vaccinated cohorts (Figure S1).

Discussion

The commercial SARS-CoV-2 IGRA used in this study accurately distinguished convalescent COVID-19 patients and vaccinated subjects of uninfected healthy controls, and correlated with the presence of IgG anti SARS-CoV-2 trimeric spike protein and neutralizing antibodies. Overall, the test had high specificity and positive predictive value, indicating that a positive result could be used for clinical and public health decision-making. The assay was also highly sensitive to detect a specific T-cell response in patients with severe COVID-19 and vaccinated individuals 3 months after SARS-CoV-2 infection or vaccination. However, in convalescent patients the sensitivity was largely dependent on the duration of follow-up and clinical course of primary infection, with low sensitivity and negative predictive value in patients with longer follow-up and mild COVID-19. Therefore, in this scenario, a negative result is a poor predictor of the absence of previous infection.

The poor IFN-γ responses in the SARS-CoV-2 IGRA observed in our convalescent patients with longer follow-up coincided with lower levels of antibodies and is in line with previous investigations showing that immune responses decay after initial peaks following infection [16]. Several studies have described declining levels of antibodies to SARS-CoV-2 spike over the first 3–6 months [17-20] of infection, while persistence of robust specific memory B-cells and T-cell responses at 5 and 9 months after symptom onset have been reported in moderate and severe COVID-19 patients [21,22]. Our results expand upon current knowledge confirming that most convalescent patients with severe COVID-19 retain T-cell responses one year after hospitalization, although the magnitude of IFN-γ response was

significantly lower compared with patients tested at 3 months. In contrast, more than half of patients with mild disease did not maintain IFN-γ responses nor anti-spike IgG antibodies at 12 months after diagnosis, suggesting weaker long-term protection against SARS-CoV-2 following mild primary infections. This finding is coherent with the IFN-γ decline observed in a small cohort of outpatients tested with IGRA on days 17 and 31 post–RT-PCR positivity [13], and with the rapid decay of anti-SARS-CoV-2 antibodies described in persons with mild COVID-19 [1].

As expected, we found that SARS-CoV-2-naïve subjects had positive responses in the IGRA assay after vaccination, although the IFN-γ levels were significantly lower than those seen in convalescent patients with severe COVID-19 tested at the same timepoint, that suggesting that natural T-cell immunity after severe infection may be stronger compared to vaccination. Noteworthy was the strong T-cell response observed following either one or two vaccine doses in previously infected subjects. This observation matches with previous studies demonstrating robust T-cell responses in previously infected individuals after one vaccine dose, equivalent to naïve individuals receiving two doses [23].

With the commercial assay and manufacturer's interpretation criteria used in this investigation, we found only three positive responses among healthy seronegative controls, all of them in household contacts of COVID-19 patients and none in unexposed healthy individuals. The significance of positive results of IGRA in healthy unvaccinated subjects with negative serology remains unclear. Using experimental techniques some studies have detected SARS-CoV-2-reactive T-cells in up to 60% of unexposed individuals, suggesting cross-reactive T-cell recognition between circulating "common cold" coronaviruses and SARS-CoV-2 [14,24], and, therefore, questioning their role as a potential diagnostic tool for assessing protective immunity against SARS-CoV-2. Interestingly, SARS-CoV-2-specific T-cells have been found in some convalescent individuals in the absence of seroconversion [6,8] and this may be more likely to occur in mild or asymptomatic infection [8,25,26]. Thus, the three positive IGRA results of our study, in household contacts, may indeed reflect

asymptomatic SARS-CoV-2 exposure and protective T-cell mediated immunity. If those were true-positive cases, the specificity of the assay would approach 100%. Nonetheless, either false-positive results or cross-reactivity with seasonal coronaviruses cannot be rule out.

To examine the potential contribution of the IGRA to conventional serological testing to assess immunity in clinical practice, we compared the presence of SARS-CoV-2 specific IgG and neutralizing antibodies with IFN-γ responses in patients recovered from the disease and vaccinated subjects. Overall, the IGRA increased only modestly the detection of immunity, but the contribution was substantial in convalescent patients with mild disease, where IGRA augmented the yield of serology by 13% (30% when including borderline values).

The study was limited by the small sample size in some subgroups limiting the precision of the estimations, and by the lack of demographic matching between the different cohorts with unbalanced sex distribution, reflecting the selection bias towards health care workers participating voluntarily in vaccinated cohorts. Unfortunately, the cross-sectional study design did not allow us evaluating the clinical impact of implementing IGRA to assess immunity memory after SARS-CoV-2 infection or vaccination nor the value of the test for predicting future SARS-CoV-2 infection.

This study has several notable strengths, including the utilization of standardized, high throughput IGRA and validated serological platforms to accurately measure antibody response in a diverse cohort of individuals with varying degrees of illness severity and follow-up, along with exposed and unexposed controls. To our knowledge this is the largest study to date to evaluate the performance of an easy-to-use assay for detection of cellular immune response in a clinical setting.

In conclusion, the commercial kit of whole blood SARS-CoV-2 IGRA assessed in this investigation is a simple and reliable method of quantifying T-cell response in convalescents and vaccinated subjects. In convalescent patients the sensitivity is largely dependent on the

duration of follow-up and severity of primary infection. The study suggests that the assay is more likely to add clinical value in patients with mild infections, a setting in which may increase the detection of immunity in subjects with negative serology. Therefore, combination of antibody and IGRA tests would help to assess more accurately SARS-CoV-2 immunity and might assist decision making for booster vaccine doses after previous natural infection or immunization in subjects in whom immunity is waning. Longitudinal studies are warranted to determine whether the IGRA truly identifies individuals with protective immunity against SARS-CoV-2.

NOTES

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Conflicts of Interest

All No conflict

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Table 1. Demographics and clinical characteristics of study groups.

	Convalescent cohorts (n=152)				Vaccinated cohorts (n=54)				Healthy unvaccinated cohorts (n=33)		
	All	Inpatients, 3-month FU	Inpatients, 12-month FU	Outpatients, 12-month FU	All	SARS-CoV-2 naïve	SARS-CoV-2 infected-2 doses	SARS-CoV-2 infected-1 dose	All	Household contacts of COVID-19	Healthy controls
Patients, *n* (%)	152	29	100	23	54	25	17	12	33	19	14
Age, years	60 (48-70)	64 (57-68)	62 (54-72)	43 (39-45)	64 (48-79)	48 (40-60)	71 (67-76)	84 (83-87)	47 (42-52)	45 (39-49)	52 (45-65)
Gender male, *n* (%)	88 (57.9)	14 (48.3)	62 (62)	12 (52.2)	28 (51.9)	10 (40)	13 (76.5)	5 (41.7)	14 (42.4)	5 (26.3)	9 (64.3)
SARS-Cov-2 RNA PCR	Positive	Positive	Positive	Positive	_	Negative	Positive	Positive	_	Negative	_
Median (Q1-Q3) time since SARS-CoV-2 RNA PCR, months		2.8 (2.7-2.9)	11.8 (11.7-11.9)	11.2 (10.9-13.7)	_	6.6 (3.4-9.1)	11.4 (11.2-11.9)	11.8 (11.8-11.9)	_	5.0 (3.6-7.3)	_
Last dose of vaccine, days	_	_	_	_	51 (19-77)	77 (76-77)	31 (17-48)	18 (7-19)	_	_	_
Comorbidity*, *n* (%)		21 (72.4)	65 (65)	2 (8.7)	_	_	13 (76.5)	12 (100)	_	_	_
Charlson comorbidity index score		2 (1-3)	2 (1-4)	0 (0-0)	_	_	3 (2-4)	5.5 (5-7)			
Admission to ICU, *n* (%)		1 (3.4)	14 (14)	0	_	_	1 (5.9)	0	_	_	_
Symptomatic, *n* (%)		29 (100)	98 (98)	21 (91.3)	_	_	15 (88.2)	11 (91.7)	_	_	_
TrimericS-IgG Positive, *n* (%)	117 (77)	29 (100)	79 (79)	9 (39.1)	53 (98.1)	25 (100)	17 (100)	11 (91.7)	0 (0)	0 (0)	0 (0)
Negative, *n*	35	0	21	14	1	0	0	1	33	19	14
quantitative, BAU/mL	217 (46-514)	812 (490-1210)	172 (50-373)	23 (7-79)	1885 (758-10328)	1040 (650-1380)	11700 (2550-36100)	5930 (2505-14680)	<4.8 (<4.8-<4.8)	<4.8 (<4.8-<4.8)	<4.8 (<4.8-<4.8)
Neutralizing antibodies											

Positive, *n* (%)	100 (65.8)	28 (96.6)	69 (69)	3 (13)	51 (94.4)	24 (96)	16 (94.1)	11 (91.7)	0 (0)	0 (0)	0 (0)
Negative/Borderline, *n/n*	43/9	0/1	24/7	19/1	2/1	0/1	1/0	1/0	33/0	19/0	14/0
%IH	61.4 (11.2-86.5)	91.1 (76.6-95.5)	61.4 (21.5-79.8)	0.6 (0.1-11.1)	97.4 (80.9-99.4)	85.8 (70.5-92.1)	99.4 (98-99.4)	99.3 (99-99.4)	0.1 (0.1-0.1)	0.1 (0.1-0.1)	0.1 (0.1-0.1)
SARS-CoV-2 IGRA Positive, *n* (%)	115 (75.6)	29 (100)	78 (78)	8 (34.8)	52 (96.3)	25 (100)	16 (94.1)	11 (91.7)	3 (9.1)	3 (15.8)	0 (0)
Negative/Borderline, *n/n*	31/6	0/0	20/2	11/4	2/0	0/0	1/0	1/0	27/3	14/2	13/1
quantitative, mIU/mL	1178 (225-2383)	3158 (1743-4900)	1073 (347-2027)	109 (61-257)	1533 (922->5000)	1020 (516-1423)	4351 (1318->5000)	>5000 (4194->5000)	17 (0.5-64)	32 (8-124)	12 (0.5-34)

FU, follow-up; RT-PCR, real-time polymerase chain reaction; ICU, intensive care unit; TrimericS-IgG, IgG antibody serum levels against the trimeric spike protein; BAU, binding antibody units; %IH, inhibition percentage; IGRA, interferon gamma release assay.

* This category included at least one of the following: diabetes, cardiovascular (including hypertension) respiratory, kidney, neurological disease, cirrhosis, or malignant neoplasm.

Table 2. Sensitivity, specificity, positive (PPV) and negative (NPV) predictive values of the IFN-γ release assay (IGRA).

	All (n=239)	Convalescent cohorts				Vaccinated cohorts	
		All (n=152)	Inpatients, 3-month FU (n=29)	Inpatients, 12-month FU (n=100)	Outpatients, 12-month FU (n=23)	All (n=54)	SARS-CoV-2 naïve (n=25)
Sensitivity (% [95% CI])	81.1 (74.9-86) #81.1 (74.9-86)	75.7 (67.9-82.1) *79.6 (72.2-85.5)	100 (85.4-100)	78 (68.4-85.4) *80 (70.6-87.1)	34.8 (17.2-57.2) *52.2 (31.1-72.6)	96.3 (86.2-99.4)	100 (83.4-100)
Specificity (% [95% CI])	90.9 (74.5-97.6) #100 (73.2-100)	90.9 (74.5-97.6)	90.9 (74.5-97.6)	90.9 (74.5-97.6)	90.9 (74.5-97.6)	90.9 (74.5-97.6)	90.9 (74.5-97.6)
PPV (% [95% CI])	98.2 (94.5-99.5) #100 (97.2-100)	97.5 (92.2-99.3) *95.3 (89.6-98.1)	90.6 (73.8-97.5)	96.3 (88.8-99) *93 (84.9-97.1)	72.7 (39.3-92.7) *66.7 (41.2-85.6)	94.5 (83.9-98.6)	89.3 (70.6-97.2)
NPV (% [95% CI])	43.5 (31.8-55.9) #26.4 (15.7-40.6)	44.8 (32.8-57.4) *46.6 (33.5-60)	100 (85.9-100)	57.7 (43.3-71) *57.4 (42.3-71.4)	66.7 (50.9-79.6) *71.1 (53.9-84)	93.8 (77.8-98.9)	100 (85.9-100)

Healthy unvaccinated cohorts (N=33) were considered as reference standard for negative results.

#Subanalysis excluding household contacts of COVID-19 (All, n=220).

*Subanalysis considering IGRA borderline values (≥100 mIU/mL) as positive.

Table 3. Agreement of SARS-CoV-2 IGRA with IgG anti trimeric spike protein and neutralizing antibodies.

IgG anti-trimeric spike protein	**% agreement (95% CI)**			**Performance agreement (κ) (95% CI)**	
	Positive	**Negative**	**Overall**		
Global	95.3% (90.6-97.8)	88.4% (77.9-94.5)	93.3% (89.2-96.0)	0.84	(0.76-0.91)
Convalescent cohorts	94% (87.6-97.4)	85.7% (69-94.6)	92.1% (86.3-95.7)	0.78	(0.66-0.90)
Vaccinated cohorts	98.1% (88.6-99.9)	100% (5.5-100)	98.1% (88.8-99.9)	0.66	(0.04-1)
Neutralizing antibodies					
Global	88.8% (82.9-93%)	100% (93.4-100)	92.1% (87.7-95)	0.82	(0.75-0.90)
Convalescent cohorts	85.5% (77.5-91.1)	100% (87.7-100)	88.8% (82.4-93.2)	0.73	(0.61-0.85)
Vaccinated cohorts	96.2% (85.9-99.3)	100% (5.5-100)	96.3% (86.2-99.4)	0.49	(-0.11 – 1)

Figure Legends

Figure 1. Flowchart of participants.

Figure 2. Correlation between the concentrations of interferon-γ (IFN-γ) released in the SARS-CoV-2 IGRA and levels of IgG anti trimeric spike protein (TrimericS-IgG).

Figure 3. Specific T-cell responses evaluated with the SARS-CoV-2 IGRA and IgG anti trimeric spike protein levels (TrimericS-IgG). A: in convalescent patients with severe COVID-19 with 3 months and 12 months follow-up. B: in patients with severe and mild COVID-19 at 12 months follow-up. C: in vaccinated patients.

Figure 1

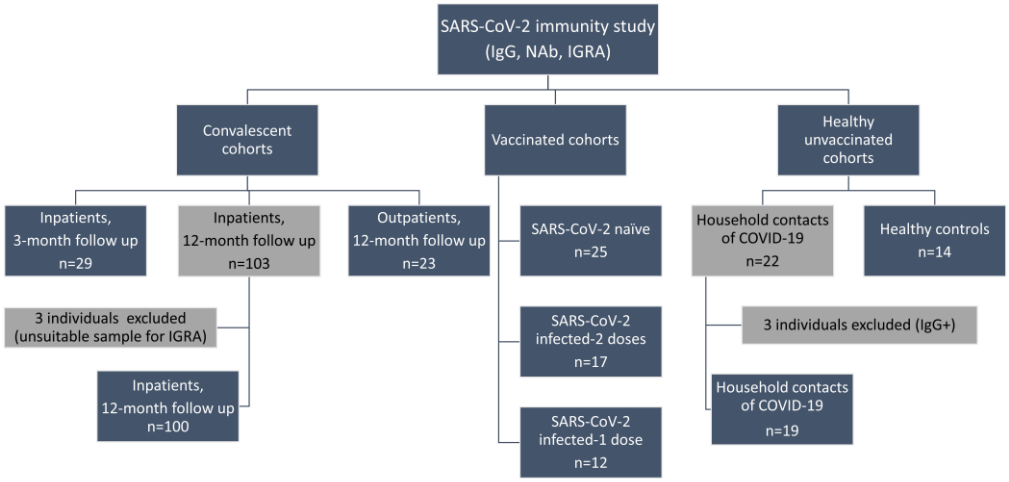

Figure 2

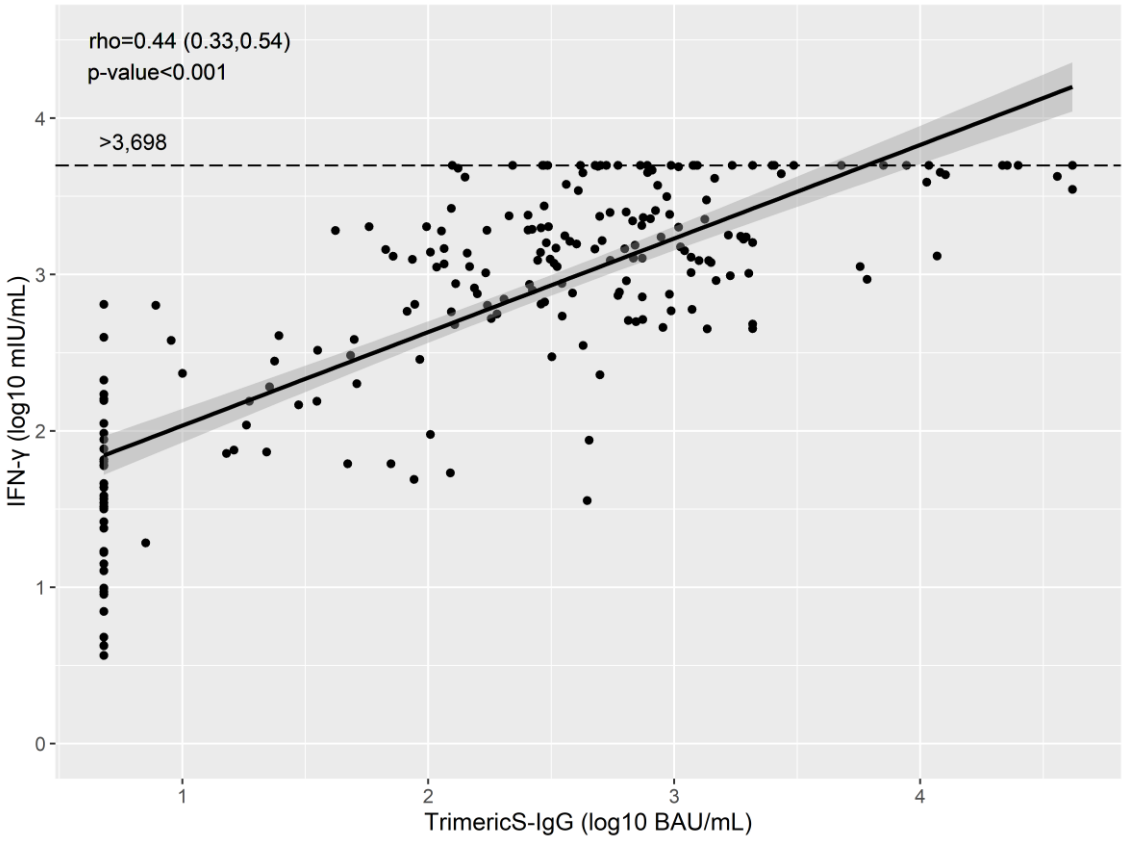

Figure 3